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Immunohistochemical Analysis of Thrombi Retrieved During Treatment of Acute Ischemic Stroke

Does Stent-Retriever Cause Intimal Damage?

Paramdeep Singh, MD; Soroush Doostkam, MD; Matthias Reinhard, MD; Vera Ivanovas, MD; Christian A. Taschner, MD

Background and Purpose—To provide evidence whether mechanical thrombectomy with stent-retrievers in the treatment of acute ischemic stroke causes intimal damage.

Methods—This study analyzed thrombi retrieved by mechanical thrombectomy from cerebral arteries in 48 consecutive patients with acute ischemic stroke for the presence of endothelial cells using CD34 antibodies.

Results—Of 48 thrombi analyzed, CD34-positive cells were absent in 20, present as isolated cells in 21, and found in clusters in 7 thrombi. We did not find any subendothelial vessel wall structures.

Conclusions—Our findings suggest that mechanical thrombectomy with stent-retrievers does not cause relevant intimal damage in acute ischemic stroke treatment.

Clinical Trial Registration—URL: http://www.germanctr.de. Unique identifier: DRKS00004695 **(*Stroke*. 2013;44:1720-1722.)**

Key Words: immunohistochemistry ■ ischemic stroke ■ thrombectomy ■ thrombus

Thrombolysis with intravenous administration of recombinant tissue-type plasminogen activator has proven effective in randomized controlled trials.[1] However, in large-vessel occlusion with high thrombus burden, poor recanalization rates with limited patient outcome have been reported.[2] Most recently, a new class of mechanical thrombectomy devices has emerged with reported recanalization rates ranging from 67% to 100%.[3,4] However, the stress to the vessel wall related to stent-retrieval seems important. During retraction of the stent-retriever, the vascular anatomy may be distorted and considerable vasospasm of the target vessel and along the course of the stent-retriever are observed on a regular basis.[5,6] To date no histopathologic data on the effect of mechanical thrombectomy with stent-retrievers on the initima in men are available.

In this study, we analyzed whether withdrawal of the unfolded stent by mechanical force to perform thrombectomy in acute ischemic stroke causes intimal injury. We, therefore, analyzed thrombi retrieved from acutely occluded vessels with stent-retrievers, for occurrence of denuded endothelial cells and subendothelial connective tissue.

Methods

Patient Selection

This study was approved by the institutional review board of the University Hospital Freiburg. All interventional procedures were in accordance with institutional guidelines.

We included 48 patients admitted for acute ischemic stroke in which we were able to retrieve thrombus material by means of mechanical thrombectomy with stent-retrievers. Routine baseline investigations included neurological and physical examination, assessment of National Institutes of Health Stroke Scale (NIHSS), brain imaging with either computed tomography or MRI, and vascular imaging with either computed tomography angiography or MR angiography. The presumed ischemic stroke mechanism was determined with use of the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria by the stroke neurologist. Patient neurological status was scored with a further NIHSS at discharge. Good neurological outcome was defined as a NIHSS score of 0 to 4 or a NIHSS score improvement of >9 points.

Thrombus Retrieval Procedure

The thrombectomy procedure was performed as described elsewhere.[6] The Solitaire FR (Solitaire 4 mm, Covidien) was used in 39 patients, 9 patients were treated with the Aperio device (Aperio 4.5 mm, Acandis). Recanalization was classified according to the Thrombosis in Cerebral Infaction (TICI) criteria.[7] TICI 2b and 3 were considered a successful recanalization. A case example of a successful mechanical thrombectomy is displayed in Figure 1.

Processing of Thrombi and Analysis

Thrombi were mostly retrieved in multiple fragments, completely removed from the stent-retrievers, and fixed in phosphate buffered formalin within the angiosuite. Thrombus material was embedded in paraffin, sectioned at 4-µm thickness and stained with hematoxylin and eosin, Prussian-blue, Elastica-van-Gieson, Kossa, and Periodic acid-Schiff reaction. All thrombi specimens were in addition tested immunohistochemically for CD34 (monoclonal, class II, clone QBEnd10, DAKO) to detect and quantify endothelial cells. An

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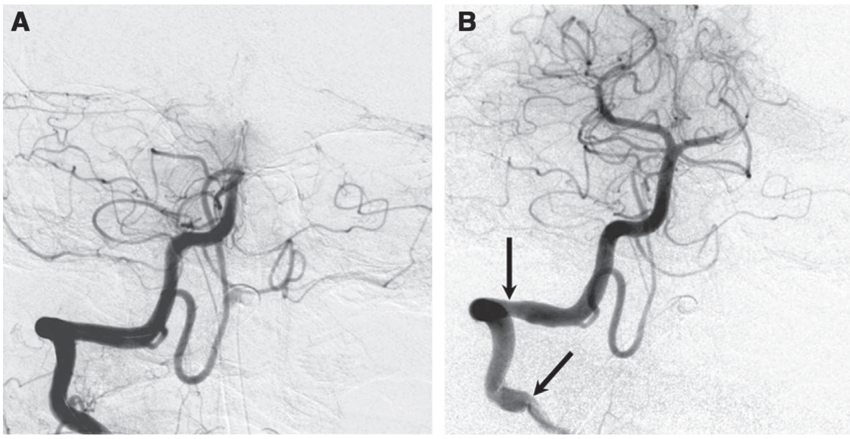

Figure 1. Case example: a 56-year-old patient presenting with an acute basilar occlusion and an underlying high-grade stenosis at the origin of the right vertebral artery. The National Institutes of Health Stroke Scale (NIHSS) on presentation was 17. The stenosis was treated with percutaneous transluminal angioplasty and stenting (not shown). An intermediate catheter was placed through the stent into the V2 segment of the right vertebral artery. Digital subtraction angiography confirms occlusion of the basilar artery (**A**). After 2 passages with a stent-retriever, the basilar artery was recanalized (Thrombosis in Cerebral Infaction 2b; **B**). Mind the moderate vasospasm along the course of the fully deployed stent-retriever (arrows). The patient was discharged with an NIHSS of 3.

example for positive CD34 immunohistochemistry is shown in Figure 2. The CD34 assays were performed retrospectively. The slides were analyzed by a board-certified neuropathologist (S.D.) without knowledge of the clinical findings. The degree of underlying intimal damage was categorized into 3 grades: (1) absence of endothelial cells: intimal damage unlikely; (2) single or clustered endothelial cells: minor intimal damage cannot be excluded; (3) endothelial cells and subendothelial connective tissue: relevant intimal damage confirmed.

Results

Patient Data

From July 2011 to October 2012, 48 consecutive patients with acute ischemic stroke were included into the study. There were 29 men and 19 women, the mean age was 67 years (range, 43–91 years; SD, 12). Mean NIHSS score on admission was 19 (range, 7–34; SD, 5). Twenty seven patients received intravenous recombinant tissue-type plasminogen activator before the endovascular intervention. The mean time interval from stroke onset to start of cerebral angiography was 4 hours 8 minutes (range, 1 hour 53 minutes to 8 hours 6 minutes; SD, 1 hour 53 minutes).

Angiographic and Clinical Outcome

Angiography demonstrated combined occlusion of internal carotid artery (ICA) and M1 in 25 patients, occlusion of M1 in 18, and the basilar artery in 5. Percutaneous transluminal angioplasty and stenting were performed before stent-retrieval in 12 patients with an underlying high-grade stenosis of the ICA and 1 patient with a high-grade stenosis of the proximal vertebral artery. TICI 2b/3 results were achieved in 35/48 patients (73%). The average number of passes required was 2 (range, 1–5). Vasospasm of the target vessel and along the course of the stent-retriever was observed in 20/48 patients. Dissections of the ICA occurred in 4 patients. These were limited to the extracranial ICA and related to either the balloon catheter or ICA stenting. Mean time from symptom onset to successful recanalization/end of procedure was 5 hours 43 minutes (range, 3 hours 7 minutes to 12 hours 23 minutes; SD, 1 hour 54 minutes). Presumed origins of thrombi were athero-embolic in 18, cardio-embolic in 29, and remained cryptogenic in 1. On discharge, 18/48 patients (38%) had a good clinical outcome.

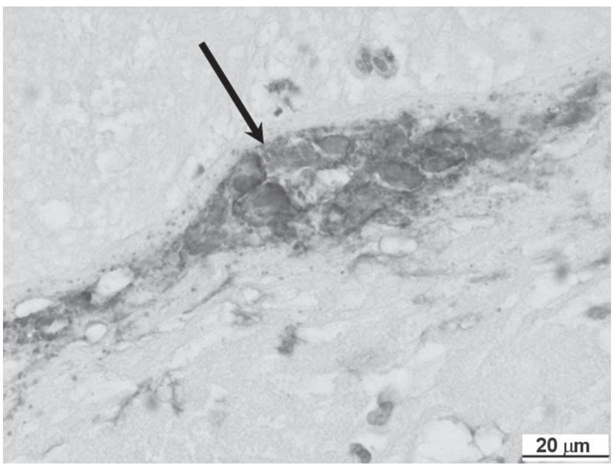

Figure 2. Corresponding thrombus histology. Grouped, CD34-positive cells (arrow) of endothelial origin in a fibrin-dominant thrombus. No subintimal vessel wall structures are found.

Histopathologic Outcome

Of the retrieved clots, 13 were classified as fibrin dominant, 15 red blood cell dominant, and 20 were mixed. CD34-positive endothelial cells were absent in 20 thrombi (grade I) and present in 28 (grade II). In 21 thrombi those endothelial cells were isolated, in 17 of these CD34-positive cells were considered to represent surface endothelialization. Clustered endothelial cells of >1 cell were found in 7 cases. We did not detect any associated subendothelial connective tissue (grade III). Polymorphonuclear cell infiltration was present in 33 thrombi (in 3 red, 11 white, and 19 mixed thrombi) of which 30 thrombi (91%) were either white or mixed subtype.

In a univariate analysis, the presence of clustered endothelial cells was not associated with a poorer clinical outcome, the occurrence of vasospasm, or a TICI score 0-2a. Moreover, there was no significant association between thrombus histology (red, white, and mixed) and procedure time for mechanical thrombectomy, TICI score 0-2a, and poor clinical outcome.

Discussion

This study provides systematic histological analysis of thrombi retrieved from 48 consecutive patients with acute ischemic stroke undergoing mechanical thrombectomy with stent-retriever. Retrieved thrombi varied between fibrin dominant, red blood cell dominant, and mixed thrombi. Our observations on the great variety in overall appearances of retrieved thrombi corroborate earlier findings reported in the literature.[8–11] In addition, we were able to confirm findings by Marder et al[8] that thrombus histology does not seem to be of predictive value for successful mechanical thrombectomy.

The aim of this study was to analyze the potentially damaging effect of mechanical thrombectomy with stent-retrievers on the target vessel intima. In our histological assessments, we did not find any subendothelial components of the vessel intima within analyzed specimens. Although the forces applied to the target vessels and the vessels along the course of the stent-retriever were sufficient to induce vasospasm in 20/48 patients, we did not find histopathologic evidence of relevant intimal damage. Single endothelial cells or small clusters of endothelial cells might belong to the physiological turnover of the endothelium. In addition, these endothelial cells might correspond to a surface endothelialization—a sign of beginning thrombus organization.[9]

Nogueira et al[12] reported on severe disruption of the intima observed in a preclinical study of a thrombectomy device in 2 different animal models of arterial thrombo-occlusive disease. Yet, the histopathologic analysis showed no hemorrhage of media or adventitia. These findings might not lead to long-term vessel damage. The authors did not find any microscopic evidence of dissection, pseudoaneurysm formation, or inflammation in the vessel wall.[9]

In the literature on various different thrombectomy devices, vasospasm is regularly reported.[5,6] Yet dissections or subarachnoid hemorrhage associated with the procedure remains exceptional. To date no histopathologic data on the effect of thrombectomy with stent-retrievers on the initima in men are available. Kurre et al[5] analyzed the long-term side effects of mechanical thrombectomy with stent-retrievers on intracranial vessels in 116 patients. On follow-up, digital subtraction angiography target vessel occlusion was present in 1, and de novo stenosis occurred in 4 vessel segments.

As a limitation, our study was conducted in a single center. CD34 is not specific for endothelial cells and may be expressed on early hematopoietic cells as well. Two different stent-retrievers were used in this trial.

Conclusion

Our findings suggest that mechanical thrombectomy with stent-retrievers does not cause relevant intimal damage in acute ischemic stroke treatment.

Disclosures

Dr Singh has received a grant of the European Society of Radiology for a research stay at the Department of Neuroradiology, University Hospital Freiburg, Germany. The other authors have no conflicts to report.

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Original Article

Assessment of Resting-state functional Magnetic Resonance Imaging Connectivity Among Patients with Major Depressive Disorder: A Comparative Study

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Abstract

Background: Resting-state functional connectivity analysis has a potential to unearth the putative neuronal underpinnings of various disorders of the brain. Major depressive disorder (MDD) is regarded as a disorder arising from alterations in functional networks of the brain.

Purpose: There is paucity of literature on resting-state functional magnetic resonance imaging (Rs-fMRI) in MDD, especially from the Indian subcontinent. The purpose of our study was to elucidate the differences in Rs-fMRI connectivity between MDD patients and age and gender matched healthy controls (HC).

Methods: In this prospective single institute-based study, the patients were recruited consecutively based on Hamilton depression rating scale (HAM-D). Age and gender matched HC were also recruited. Rs-fMRI and anatomical MRI images were acquired for all the subjects (MDD and HC group) and subsequent analysis was done using the CONN toolbox.

Results: A total of 49 subjects were included in the final analysis (MDD = 28 patients, HC = 21). HAM-D score was noted to be 24.4 ± 4.8 in the MDD group. There was no significant difference between MDD and HC groups as far as age, gender, employment status, and level of education is concerned. Region-of-interest-based analysis of Rs-fMRI data showed a significantly lower connectivity between the left insula and left nucleus accumbens and between left paracingulate gyrus and bilateral posterior middle temporal gyri in MDD group as compared to HC group.

Conclusion: There is reduced connectivity between certain key regions of the brain in MDD patients, that is, between the left insular cortex and the left nucleus accumbens and between the left paracingulate gyrus and the bilateral posterior middle temporal gyrus. These findings could explain the basis of clinical features of MDD such as anhedonia, rumination of thoughts, reduced visuo-spatial comprehension, reduced language function, and response to external stimuli.

Keywords

Resting-state fMRI, connectivity, major depressive disorder, MRI

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Introduction

Depression among the leading mental health problems across the world with an estimated prevalence of approximately 5% among adults.[1] It has been estimated that one in every 20 adults in India suffers from major depressive disorder (MDD).[2] Major depression is characterized by a wide range of clinical features including the lack of interest in work (anhedonia), behavioral changes, sleep disturbances, cognitive disturbances, changes in appetite, alterations in body weight and suicidal thoughts.[3] Typically, these clinical

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features last for six months or longer. MDD is primarily diagnosed on the basis of clinical assessment of symptoms in accordance with the international classification of disease or diagnostic and statistical manual criteria.[4] Many different scores have been developed for assessment of severity of depression, which also help in the recruitment of patients into clinical trials.[5] While many studies have attempted to explore the alterations in structural and functional magnetic resonance imaging (fMRI) in patients with depression, the results have been varied across the studies. This may largely be due to the overlapping nature of symptoms in depression and other neuropsychiatric conditions and the differences in study design and case definitions. Also, attempts towards developing neuroimaging biomarkers for diagnosis of depression have been largely futile, largely due to the reverse inference fallacy, where the particular imaging parameter may not necessarily be specific for depression.[6] While many authors have attempted to study the role of fMRI in MDD,[7] no study exploring the changes in Rs-fMRI connectivity among patients diagnosed with major depression is available from the Indian subcontinent.

In this paper, we present our findings regarding the changes in resting-state functional connectivity between different region of interests in patients diagnosed with MDD as compared to the healthy controls (HC). We hypothesize that MDD patients may have specific changes in Rs-fMRI connectivity, which may help explaining the basis of clinical symptoms. We further hypothesize that the regions of the brain concerned with perception of reward, pleasure as well as processing of visuo-spatial information, may show altered resting-state functional connectivity in MDD as compared to HC.

Methods

This was a single center, hospital-based study, funded by the Science and Engineering research board (SERB), Department of Science and Technology, Government of India (Approval number: SRG/2022/001306). The study was approved by the institutional ethical committee (Approval number – IEC/AIIMS/BTI/222, dated: 10/10/2022). We included patients with a clinical diagnosis of MDD according to the ICD-10 (International Classification of disease – 10th edition) criteria,[8] and a score >17 based on Hamilton Depression Rating Scale (HAM-D).[9] We included only those patients who had not been exposed to any antidepressant medication. Age and gender matched healthy subjects with HAM-D scores less than 4 and no other diagnosed co-morbid medical or psychiatric illness were included in the HC group. Informed written consent was obtained from all the participants. The patients were included consecutively from the outpatient department and were explained about the procedure for undergoing the fMRI scan. Only right-hand dominant participants were included in the study. The exclusion criteria of the study included:

1. Any history of co-morbid illness
2. Any history of intake of antidepressant medication
3. Left-handed
4. Pregnancy and active breastfeeding mother
5. Substance abuse/addiction of drugs
6. Refusal to consent
7. Any other general contraindication for undergoing MRI scan.

The inclusion criteria for recruitment of MDD patients are mentioned as follows:

1. HAM-D score >17
2. Age (18−65)
3. Right-handed
4. No substance abuse.

MRI Acquisition

All the MRI scans were acquired on 3T MRI (Skyra, Siemens Healthineers, Germany) using a 32-channel head coil. The patients as well as the HC were asked to relax during the scan and keep their eyes closed. All the standard protocols were followed to maximize the patient comfort and minimize motion-related artifacts. The functional MRI data was acquired using the T2* Echoplanar imaging BOLD sequence and 3D MPRAGE sequence was acquired for anatomical co-registration. The imaging parameters for these sequences are shown in Table 1.

Data Analysis

All the imaging data was converted from DICOM format to Nifti format and further analyzed using the CONN toolbox Version 21a based on SPM12 (Wellcomecentre for human Neuroimaging, United Kingdom) and Matlab version 2021a (Mathworks, United States) for iMac (Apple Inc, United States).

Table 1. Data Acquisition Parameters for 3D MPRAGE and T2* EPI BOLD Sequences on 3T MRI.

Parameters	3D-MPRAGE	T2* EPI BOLD
TR (ms)	1700	1500
TE (ms)	2.3	30
FOV (mm × mm)	256 × 230	192 × 192
Flip angle (degree)	8	70
Time of acquisition	3 min 12 sec	9 min 11 sec
Slice thickness (mm)	0.5	3
Size of voxel (mm × mm × mm)	0.5 × 0.5 × 0.5	2 × 2 × 3
Number of volumes acquired	1	192

Preprocessing Pipeline

A default preprocessing pipeline based on volume-based analyses was utilized and normalization was done directly to the MNI space. Furthermore, we removed the initial 10 volumes of the Rs-fMRI data from each patient so that a steady state of longitudinal magnetization could be reached. Also, this step allows for equilibrium in the signal and also allows time for the participants to adapt to the environment within the scanner, particularly the noise. The pipeline includes various pre-processing steps, which are summarized as follows:

1. Function labeling of the functional files, which are a part of list of secondary datasets (labeled as "Original Data").
2. Realignment and unwrapping of functional data (for estimation of subject motion and correction accordingly).
3. Translation of the data so that the center lies at (0,0,0) co-ordinate.
4. Slice-timing correction of the functional data (STC; this step corrects for the differences in the time of acquisition of individual slices).
5. Detection of the outliers in fMRI data (the identification of the outlier scans is done using ART-based algorithm for scrubbing of the data).
6. Direct segmentation and normalization of functional data (simultaneous segmentation of grey matter, white matter and CSF, and normalization to MNI space).
7. Labeling of the files of fMRI data as a component of secondary datasets (data is labeled as "MNI-space data").
8. Translation of the structural MRI data to (0,0,0) co-ordinates.
9. Segmentation and normalization of the structural MRI data (simultaneous segmentation of gray matter, white matter and CSF, and normalization to MNI space).
10. Smoothing of fMRI data (Gaussian kernel was used for spatial convolution).
11. Labeling of fMRI data files as components of secondary datasets (labeled as "smoothed data").

The slice order was selected as interleaved and functional outlier detection setting was kept at intermediate (i.e., 97th percentile). The smoothing Kernel was selected at 8 mm (default selection based on full-width half maximum criteria).

At successful completion of preprocessing, quality assurance plots were reviewed, and the data was subjected to denoising using linear regression of potential confounding factors (white matter, CSF, realignment, scrubbing, and effect of rest). The band-pass filter was set at a frequency range of 0.008−0.09 Hz. Subsequently, the preprocessed data was subjected to seed-based connectivity analyses and a connectome ring was derived to visualize the results. We selected 132 ROIs based on the Harvard Oxford atlas, which is inbuilt within the CONN toolbox.

Statistical Analysis

The tabulation of data was carried out in the form of a spreadsheet using MS office (Microsoft Corporation, USA) and statistical analysis done using MedCalc software (Belgium). Mean and standard deviation were used as measures of central tendency and measures of dispersion, respectively, for continuous data. Percentages and proportions were used for the representation of categorical data. T-test was used to determine the difference between MDD and HC groups for continuous variables. Fischer Exact test was used for the analysis of dichotomous variables. The normality of data was tested using the Shapiro-Wilk test. A p-value of <.05 was considered as significant.

For functional connectivity analysis, standard setting for cluster-based inferences was used in CONN toolbox. False discovery rate (FDR) method (using the multivariable pattern analysis [MVPA]) was used for the correction of p-value. Corrected p-FDR of <.05 was considered statistically significant.

Results

Clinical and Demographic Characteristics

Final analysis was done on 49 subjects (male – 26, female – 23). Table 2 summarizes the clinical and demographic

Table 2. Summary of the Differences in Baseline Characteristics and Demographics Between MDD and HC.

Characteristics	MDD Group (N = 28)	HC Group (N = 21)	p-Value
Age (years)	45.85 ± 10.53	42.69 ± 9.56	.09
Gender (male)	53.5%	50%	.93
Level of education (N)			
Less than secondary	19	14	.924
Secondary (but not post-secondary)	06	04	
Post-secondary, graduation, university diploma	03	03	
Employment status (N)			
Employed	14	14	.2433
Unemployed	14	07	
HAM-D score (Mean + SD)	24.4 ± 4.8	–	–

Abbreviations: MDD, major depressive disorder; HC, healthy control; HAM-D, Hamilton rating scale for depression.

characteristics of the patients (MDD group) and healthy controls (HC group).

No statistically significant difference was found between the MDD group and the HC group with regards to age, gender, and employment status (employed vs. unemployed at the time of recruitment into the study). The mean of HAM-D score for MDD group was 24.4 ± 4.8. No statistically significant difference was noted between MDD and HC group with regards to educational status (i.e., pre-secondary, secondary, and post-secondary education)

ROI-ROI Functional Connectivity Analyses

The results of ROI-based connectivity analysis in HC and MDD groups are represented as a connectome ring in Figure 1. The MDD group shows more negative correlations between the ROIs and reduced number of positive connections between the ROIs. When controlling for age and gender, MDD group showed lower functional connectivity as compared to HC group between left insular cortex and left nucleus accumbens and between left paracingulate gyrus and bilateral posterior middle temporal gyri as shown in Table 3 and Figure 2.

There was no significant correlation between ROI-ROI functional connectivity among MDD group and HAM-D score, episodes of depression, and number of episodes of depression.

Discussion

MDD has been considered as a disorder arising from the alterations in functional connectivity within the brain networks.[10] To this end, many studies have attempted to unearth the putative alterations in whole brain functional connectivity among MDD patients as compared with HC.[11–13] The analysis of structural MRI data from a large consortium (known as the Enhancing Neuroimaging Genetics through Meta-Analysis [ENIGMA]) observed a significantly reduced hippocampal volume and cortical thickness among MDD as compared to HC groups.[14] However, these results may not explain the spectrum of symptomology in MDD. In recent years, functional MRI has emerged as a potential tool for determining the neurocognitive biomarkers in MDD as well as identification of the predictors of treatment response.[15] Task-based fMRI studies, which include the performance of

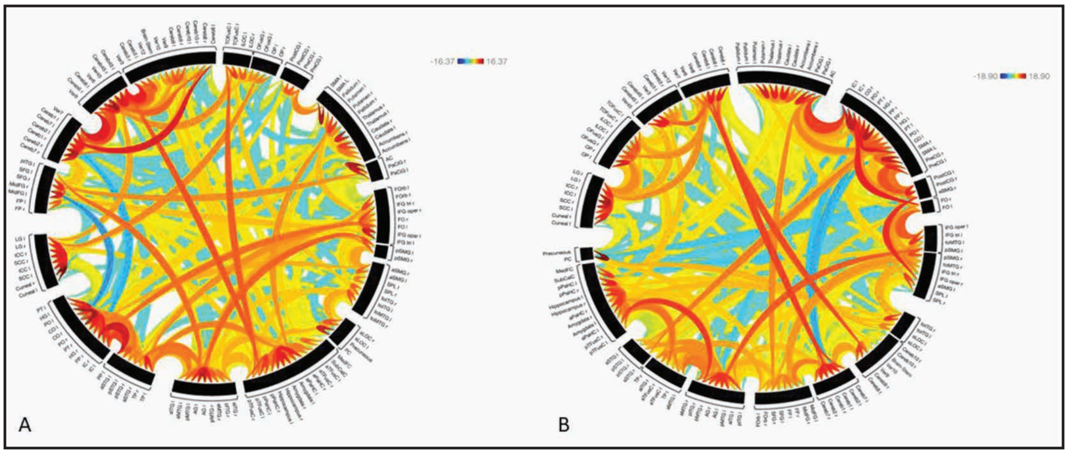

Figure 1. Results of ROI-based Connectivity Analysis in HC Group.

Note: (A) MDD group and (B) the connectivity between the ROIs (132 ROIs based on Harvard Oxford atlas) is represented by red (strong positive connectivity) and blue (strong negative connectivity) lines. There is more positive correlations (red lines) between ROIs in HC group, whereas in MDD group, there is reduced number of positive correlations and more of negative correlations between the ROIs.

Abbreviations: HC, healthy controls; MDD, major depressive disorder.

Table 3. Results of ROI-ROI Functional Connectivity Analysis with Contrast MDD < HC Showing ROIs with Significant Differences Between the Two Groups.

Unit of Analysis	T-Statistic	Uncorrected *p*-Value	*p*-FDR
Insular Cortex L-Accumbens l	T (49) = 3.91	.00029	0.0374
Paracingulate Gyrus L-Posterior Middle Temporal Gyrus Right	T (49) = 3.93	.00027	0.0262
Paracingulate Gyrus L-posterior Middle Temporal Gyrus Left	T (49) = 3.80	.0004	0.0262

Abbreviations: HC, healthy controls; MDD, major depressive disorder.

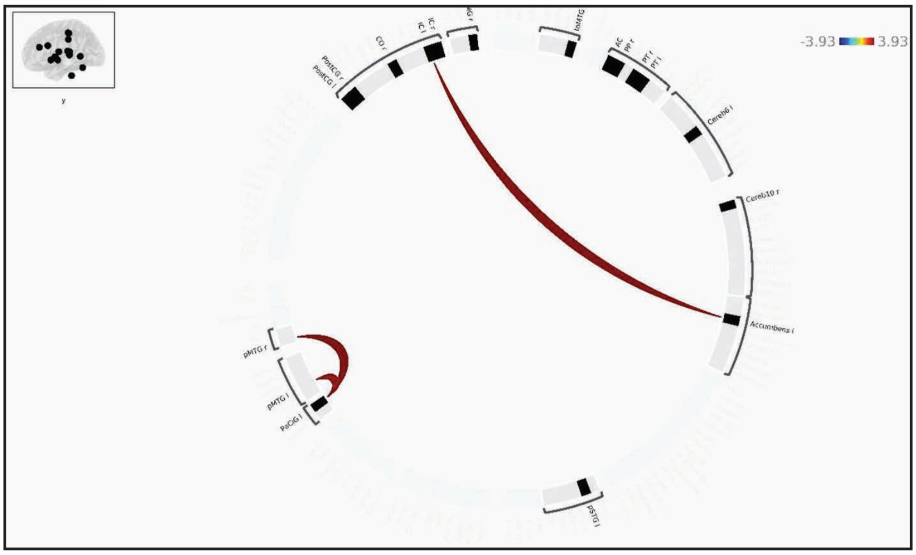

Figure 2. Connectome Ring Showing the Results of ROI-ROI Functional Connectivity Differences with Contrast MDD < HC.

Note: HC group shows a significantly higher connectivity between left paracingulate gyrus (PaCG l) and bilateral posterior middle temporal gyri (pMTG r and pMTG l), and between left nucleus accumbens (Accumbens l) and left insular cortex (IC l).

Abbreviations: HC, healthy controls; MDD, major depressive disorder.

specific cognitive tasks, have found a reduced activity in frontal regions and an increased activity in limbic regions among the MDD patients. Also, the rostral anterior cingulate gyrus has been observed to show an increase in activity during tasks involving the viewing of faces showing negative emotion, and this finding has been shown to be predictive of favorable response to antidepressants.[16–18] Recently, Rs-fMRI studies have been a focus among researchers for identifying the neural correlates of MDD. Rs-fMRI is an attractive study design in the evaluation of biomarkers of MDD due to the ease of performance and an independence from task-based design, which is desirable in MDD patients; many of whom may not be interested in task performance, and it may be difficult to solicit co-operation from them.[19] Various brain networks have shown changes in functional connectivity using Rs-fMRI in MDD.[6,20] The default mode network (DMN) has received most of the attention in Rs-fMRI studies in MDD. Most of the studies have found hyperconnectivity within the DMN in MDD group, correlating with the rumination of thought seen in patients with MDD.[10] However, variations in results in different studies have been observed largely due to variations in sample size and differences in data processing pipelines used. A recent large consortium from China (REST-meta-MDD) showed hypoconnectivity within the DMN in patients with MDD.[21] Although the literature is replete with many conflicting results concerning resting-state functional connectivity in MDD, it is clear that chiefly three major resting-state functional networks, that is, DMN, salience network (SN), and central executive network (CEN) play a major role in the pathophysiology of MDD and its treatment response.[10]

In the present study, we have attempted to determine the differences in the Rs-fMRI functional connectivity among MDD patients and HC group. The study is a hospital-based study from a tertiary care center in North India and is the first study of its kind, evaluating the differences in functional connectivity among MDD and HC from the Indian subcontinent. We utilized HAM-D for diagnosis of MDD and a score of more than 17 was included in MDD group. HAM-D has been widely used in previously published studies on MDD and has been considered as a gold standard for the evaluation of MDD for more than four decades.[22] We have utilized standard image acquisition protocols for Rs-fMRI data as well as structural data on a state of 3T MRI unit. For data processing, CONN toolbox was utilized, which has a graphical user interface for processing of Rs-fMRI data. CONN is a well validated and widely used application for Rs-fMRI analyses and offers an intuitive working environment for data interpretation and presentation.[23]

We employed ROI-ROI seed-based analyses for computation of Rs-fMRI functional connectivity differences between MDD group in comparison with HC group. Both the groups were matched as far as the baseline demographic features are concerned. Our data shows that there is reduced overall positive connectivity between the ROIs in MDD group as compared to HC, while the number of negative correlations between the ROIs is greater in MDD as compared with HC. This suggests an alteration of brain networks in MDD. We observed a significantly decreased connectivity left paracingulate gyrus and left posterior middle temporal gyrus, between the left insular cortex and left nucleus accumbens, between and between left paracingulate gyrus and right posterior middle temporal gyrus. The nucleus accumbens is regarded as a key player in the mesolimbic dopaminergic pathway and plays an important role in reward-related experiences.[24] The insular cortex has a wide range of functions including sensory and affective processing, self-awareness, high-level cognition, and other functions such as response to fear, anxiety, and happiness.[25] The paracingulate cortex has been shown to play a crucial role in cognitive and affective regulation.[26] The posterior middle temporal gyrus has connections with the DMN and ventral parts of inferior frontal gyrus and has been shown to play a key role in the retrieval of semantic and non-semantic information, including visuo-spatial perception and multimodal sensory integration.[27]

Several studies on depression have shown altered connectivity of the nucleus accumbens with prefrontal cortex, lateral hypothalamus, ventral tegmental area, and hippocampus.[28] Furthermore, the anterior insula, thalamus, and nucleus accumbens are a part of reward neurocircuitry.[29] In our study, decreased functional connectivity between the nucleus accumbens and insula may explain some of the key symptoms of MDD such as anhedonia, where the patients lack the desire to obtain reward or pleasure. The paracingulate cortex, which includes the region of the cortex around the anterior cingulate gyrus, is a part of the DMN, and so is the posterior aspect of the middle temporal gyrus. It has been shown that the volume of anterior cingulate gyrus has been shown to be reduced in MDD as compared with controls.[30] The paracingulate cortex, which may exhibit anatomical variations among subjects, has a crucial role to play in cognitive tasks and affect.[26] The findings of our study, that the paracingulate cortex shows a reduced resting-state functional connectivity with the posterior medial temporal gyrus bilaterally, may be the underlying neural correlate for symptoms such as feelings of sadness, impairment of concentration, and feeling of low self-esteem. Additionally, reduced connectivity between paracingulate cortex and posterior medial temporal gyrus may explain few of the features of MDD, such as reduced language output, altered vision and spatial perception, and response to external noxious stimuli (such as pain and heat), as have been systematically evaluated in previous publications.[31–33] While previously published studies have demonstrated hyperconnectivity within the DMN in MDD,[34,35] our study and few recent publications form large consortium and meta-analyses have shown hypoconnectivity within the DMN.[27] While hyperconnectivity within the DMN may be correlated with heightened self-rumination of thoughts in MDD, the conflicting findings of hypoconnectivity have questioned the role of DMN as a biomarker for MDD.[36] However, it must be acknowledged that the DMN remains a major brain network in the pathophysiology of MDD and further studies are necessitated in order to reliably establish the patterns of the changes in Rs-fMRI connectivity within the DMN in patients with MDD.

Limitations

We acknowledge a few limitations in the present study. This is a preliminary study based on a convenient sample size of 49 subjects (28 in MDD group and 21 in HC group) in a hospital-based practice. Due to limited sample size study, finding may have poor generalizability. A larger community-based sample is envisaged by our group in future to derive a more data-driven network-based analysis of functional connectivity in MDD. Functional connectivity analysis on a larger sample size could better evaluate the correlations between various symptoms of MDD and functional connectivity.

Conclusion

In this preliminary study on functional connectivity in MDD from the Indian subcontinent, we found a reduced connectivity between certain key regions of the brain in MDD patients, that is, between left insular cortex and left nucleus accumbens and between left paracingulate gyrus and bilateral posterior middle temporal gyrus. These findings could explain the underpinnings of the key clinical features of MDD such as anhedonia, rumination of thoughts, reduced visuo-spatial comprehension, reduced language function, and response to external stimuli; however, further studies with larger sample size are warranted to better understand the correlations between the functional connectivity alterations and the symptoms of MDD.

Authors' Contribution

All authors contributed to the preparation of the manuscript. PS and SP designed and conceptualized the study. PS, MJ, and JS recruited the participants and completed the screening assessments. SP, AM, SK, and AL analyzed the data and performed the statistical analysis. SP, PS, and MJ wrote the initial draft of this manuscript. PS acquired funding and the final manuscript was approved by all the authors.

Statement of Ethics

The study received an approval from the institutional ethics committee (IEC) (Approval number − IEC/AIIMS/BTI/222, dated: 10/10/2022).
An informed and written consent were obtained from all the participants.

Declaration of Conflicting Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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