Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

The research works for which award is being claimed are related to Clinical Neurosciences. The first research work is related to Ischemic Stroke whose brief citation is as under:

1. Stroke is one of the leading causes of mortality in India and around the world and is usually caused by a clot in an artery. A clot in an artery supplying blood to the brain is the most frequent cause of acute stroke that is one of the leading causes of death worldwide. My prime contribution is towards establishing safety of Mechanical Thrombectomy (Stent Retrievers) in the management of Acute Ischemic Stroke. Myself along with other co-investigators also provided systematic histological analysis of fresh thrombi/clots retrieved from patients with acute ischemic stroke undergoing mechanical thrombectomy with stent-retriever. Predictive value for successful mechanical thrombectomy and the potentially damaging effect of mechanical thrombectomy with stent-retrievers on the target vessel intima were provided. It may be pertinent to mention that knowledge of the composition of the fresh brain clots holds a great promise by not only helping to improve the existing medical and interventional treatment modalities for stroke but also aiding in introduction of newer treatment modalities as well as improved prevention strategies. Brief citation is as under:

INTRODUCTION

A unique feature of endovascular mechanical thrombectomy devices is the opportunity to directly investigate fresh pathological thrombi from patients with acute ischemic stroke, which till recently were inaccessible. Acquiring more knowledge about the composition of thrombus retrieved from patients with acute ischemic stroke may facilitate the development of new reperfusion treatment strategies and improving upon existing reperfusion strategies. We analysed whether withdrawal of the unfolded stent by mechanical force to perform thrombectomy in acute ischemic stroke causes intimal injury.

MATERIAL AND METHODS

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 with Solitaire FR (Solitaire 4 mm, Covidien) which was used in 39 patients, 9 patients were treated with the Aperio device (Aperio 4.5 mm,

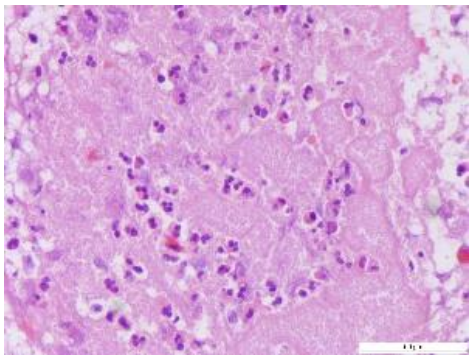
Acandis). Recanalization was classified according to the Thrombosis in Cerebral Infaction (TICI) criteria. TICI 2b and 3 were considered a successful recanalization.

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. Histopathological analysis included qualitative rating of proportion composed of red blood cells (RBC) and fibrin on microscopy of sectioned thrombi. We also correlated histological findings with clinical data of the patients.

Histopathological and Clinical Outcomes

The majority of thromboemboli shared architectural features of random fibrin:platelet deposits interspersed with nucleated cells (figure) and confined erythrocyte-rich regions. There was a great variety in overall appearances of retrieved thrombi. Of the retrieved clots, 13 were classified as fibrin dominant, 15 red blood cell dominant, and 20 were mixed. Correlation of thrombus histology with presumed etiology, vessel location, and target-vessel response showed no prevalence of histological structure with cardioembolic or arteriopathic etiology, no relation with ICA or MCA occlusion, and no predictive attribute for successful extraction. 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. We did not identify any subendothelial vessel components during our histological evaluations of the examined specimens. Despite the fact that 20/48 patients experienced vasospasm as a result of the stresses placed on the target vessels during retrieval of thrombi from wall, we were unable to identify any meaningful intimal damage histopathologically. We were able to confirm findings that thrombus histology does not seem to be of predictive value for successful mechanical thrombectomy.



Polymorphonuclear cell infiltration with eosinophilic granulocyts (arrows), higher Magnification 400X

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2. The second study is an ongoing investigation of Radio-genomics in Indian participants with major depressive disorder whose brief citation is as under:

Major Depressive Disorder (MDD) has a number of adverse consequences, both medically and sociologically, and has a substantial influence on quality of life and the ability to adapt. Death on account of suicides in these patients is a serious concern for the family as well as society. The contribution of genetic factors to depression risk has been well documented in family and twin studies. These studies provided a strong proof of the contribution of genetic risk factors in susceptibility of depression. Several hypothesis have been postulated regarding the pathogenesis of MDD. These include, the monamine theory, cytokine theory, circadian rhythm theory, and disturbances in neurogenesis/neuroplasticity. In order to identify a proper mechanism for the diagnosis of MDD, genes associated with stress, circadian rhythm, and some other candidates should be screened for variation in association with behavioural changes and magnetic resonance imaging (MRI) findings. Diagnosing MDD can be challenging because a variety of other diseases can mimic it such as obsessive-compulsive disorder (OCD), or post-traumatic stress disorder, schizophrenia and also MDD sometimes accompanied by seasonal affective disorders. In worst cases, it can lead to suicide if depression is not correctly diagnosed and treated. Diagnosis of MDD is mainly based on the behavioural screening of the patients and patient-reported symptoms, and ultimately confirmed by the Hamilton Scale for Depression and DSM-V criteria. Currently there are no molecular diagnostic markers or tools to diagnose the depression at molecular or functional level. However, physicians around the world are using imaging techniques to diagnose and study the brain activities during the MDD. Structural and functional MRI (fMRI) studies have shown excellent potential to aid in the diagnosis and treatment planning of MDD. However, these important findings have not been translated into the clinical practice. Therefore, there is an urgent need to develop a precise diagnostic strategy at molecular level which can endorse the findings of fMRI in patients with MDD. The proposed study is first of its kind to explore the altered genes involved in different pathways implicated in the pathogenesis of depression and correlate the significant variants with fMRI findings and behavioural symptoms in patients with MDD. The outcome of the study has a potential to develop a quick diagnostic strategy based on molecular markers, fMRI findings and behavioural symptoms.

Objectives i. To analyse the brain of patients showing depression like symptoms using fMRI in comparison with controls (Published)

ii. To explore the significant gene variants (including their functional evaluation) involved in various pathways like brain development, neuroplasticity, circadian rhythm, stress, and anxiety in association with development of the disease (ongoing)

iii. To establish a diagnostic criterion for MDD (ongoing)

In the portion of the current work that has been recently published, we basically sought to identify changes in resting state functional MRI (rs-fMRI) connectivity between the healthy controls (HC) group and major depressive disorder (MDD) patients. The study, which was conducted at a hospital at a tertiary care facility in North India, is the first of its type to assess the variations in functional connection between MDD and HC from the Indian Subcontinent. The Hamilton Depression Rating Scale (HDRS) was used to diagnose MDD, and patients who scored higher than 17 were placed in the MDD group. For more than 40 years, HDRS has been frequently utilized in previously published research on MDD and has been regarded as the gold standard for evaluating MDD. On a cutting-edge 3T MRI equipment, we used conventional image capture techniques for rs-fMRI data as well as structural data. The CONN toolbox, which offers a graphical user interface for processing rs-fMRI data, was used for data processing. For rs-fMRI analysis, CONN is a commonly used and well-validated program that provides a user-friendly setting for interpreting and presenting results. To compute the differences in whole-brain rs-fMRI functional connectivity between the MDD and HC groups, we used ROI-ROI seed-based analysis.

The baseline demographic characteristics of the MDD group and HC group were comparable. Between the left paracingulate gyrus and the left posterior middle temporal gyrus, as well as between the left paracingulate gyrus and the right posterior middle temporal gyrus, we found a considerably decreased connection. The mesolimbic dopaminergic pathway, which includes the nucleus accumbens, is essential to reward-related experiences. The insular cortex performs a wide range of tasks, including the processing of sensory and emotional information, self-awareness, high-level cognition, and additional tasks including the reaction to fear, anxiety, and happiness, among others. It has been demonstrated that the paracingulate cortex is essential for the control of cognition and emotion. The retrieval of both semantic and non-semantic information, including visuo-spatial perception and multimodal sensory integration, has been linked to the posterior middle temporal gyrus, which connects to the DMN and ventral regions of the inferior frontal gyrus.

To summarize, this is ongoing research on Radio-genomics in MDD patients from the Indian subcontinent whose preliminary results we have published. The authors have discovered decreased connectivity between two key brain regions in MDD patients, specifically between the left paracingulate gyrus and the bilateral posterior middle temporal gyrus, and between the left insular cortex and left nucleus accumbens. These results potentially provide an explanation for the underlying causes of the clinical characteristics of MDD, including anhedonia, ruminative thinking, diminished visual-spatial comprehension, diminished language function, and reactivity to external stimuli.

Figure: Connectome ring showing the results of ROI-ROI functional connectivity differences between MDD and HC with contrast MDD<HC. 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).

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