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Case Series

Role of Minimally Invasive Autopsy in Identifying Histopathological Abnormalities in Brain among COVID-19 Deceased Cases: An Interesting Case Series

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Abstract

Objective: To explore the role of minimally invasive trucut autopsy as a safe and rapid approach to obtain brain tissues and to identify histopathological spectrum of brain injury among COVID-19 deceased cases.

Methods: Minimally invasive trucut autopsy was done via bilateral transorbital approach through medial canthus to obtain single cores of brain tissues from each side among COVID-19 deceased cases after obtaining written informed consent from the attendants of the deceased. Histopathological examination was done by two independent pathologists after tissue processing and findings were recorded.

Results: Out of ten trucut autopsies, three were excluded due to loss of tissue during processing and four out of seven were showing histopathological features of acute cerebral injury, viral encephalitis and cerebrovascular thrombotic microangiopathy. Rest three brain tissues were normal histologically. All cases were critically ill with COVID-19, have comorbidities and poor compliance with COVID-19 specific management.

Conclusion: We conclude that MIA is a rapid, cost-effective, safer technique with higher diagnostic yield. It can provide significant histopathological findings for confirmative diagnosis among histopathologically unknown highly infectious diseases. We also observed that brain tissues can be affected in COVID-19 by direct cytotropic effect of virus through olfactory bulb or indirectly by systemic response. Presence of vacuolations and inclusions in brain biopsy must be further examined for RNA isolation for further confirmation. These findings strengthen our hypothesis of long COVID-19 associated Neurological complications resulting from virus induced brain injury.

Keywords: Minimally Invasive Brain Autopsy; Histopathological Spectrum; Covid-19 Deceased; Conventional Autopsy

Abbreviations

CNS: Central Nervous System; ARDS: Acute Respiratory Distress Syndrome; CKD: Chronic Kidney Disease; CLD: Chronic Liver Disease; MODS: Multiorgan Dysfunction Syndrome

Introduction

COVID-19 associated neuronal diseases were often reported during the pandemic. The CNS related symptoms include headache, loss of taste, loss of smell, generalized weakness, fatigue, seizures, cognitive functional defects, cerebrovascular accidents etc. [1]. Incidence of thrombotic events like ischemic stroke, intracerebral hemorrhage, acute cerebrovascular disorders, deep vein thrombosis was reported in approximately 6% of cases [2]. In addition to this demyelinating disorders like acute demyelinating encephalomyelitis (ADEM), transverse myelitis, neuromyelitis optica, sensory motor polyneuropathy, Guillain- Barre syndrome were also reported among COVID-19 patients [3]. Increased risk of depression, dementia, stroke, encephalopathy and vasculopathy were also reported as delayed or late neuronal consequences among survivors. This had increased morbidity and burden on socio-economic and health care systems of under-resourced countries.

Diagnosis of CNS complications require radiological and histopathological evaluation of cases. Histopathological examination of the neuronal tissues is the gold standard for confirmative diagnosis. The surgical approach to obtain brain tissues varies among live or deceased patients. In living patients radio imaging guided stereotactic trucut biopsy and surgical excision through craniotomy is the option whereas in deceased patients open full brain autopsy, *In Situ* autopsy and minimally invasive autopsy are the methods. Minimally invasive autopsies (MIA) was practiced by Dr Howard Kelly in Baltimore, Maryland, in the late 1800s for determination of cause of death [4]. Later on it's become more popular to obtain tissues in some special circumstances like highly contagious diseases, to minimize time for autopsy or for definitive diagnosis among mid or low income countries where full autopsy is time consuming, tedious and relatively costly practice.

With time long COVID-19 associated complications were also reported that involves vital organs and are responsible for sudden death among apparently young healthy individuals. The effect of viral replication and its association with interaction at histological, cellular and molecular level resulting in post COVID impact in the long run is yet to be an area of research.

This study was aimed to determine significance of MIA in obtaining brain tissue from COVID -19 deceased cases in search of histopathological abnormalities in the brain that may be responsible for delayed onset of neurological complications.

Material and Methods

Subject selection

This was a prospective observational study conducted during December 2020 to March 2021 in a single COVID-19 dedicated center of the state of Jharkhand after taking permission from the Institutional Ethics Committee. We had performed minimally invasive autopsy from RT-PCR confirmed deceased COVID-19 cases fulfilling the eligibility criteria.

Sample extraction

Minimally invasive autopsy from CNS was performed on 10 deceased COVID -19 cases in the form of trucut biopsy. Since COVID-19 was a highly infectious disease with no definitive treatment and vaccine as well at that point of time hence the entire procedure was performed in an isolated sanitized room. The pathologist wore a PPE kit and followed entire COVID-19 related precautionary guidelines for self protection. Neuronal tissue cores were collected through bilateral transorbital approach after obtaining written consent from the relatives or attendants of the deceased cases. We used disposable trucut biopsy gun with 22 mm penetration depth and 16 Guaze needle size (Make Bard, India) to obtain single tissue core from right and left cerebral lobes. The pathologist pierced the conjunctiva at the medial canthus of the eye with the biopsy gun needle which was then negotiated downward and laterally to enter through the orbital canal into the frontal lobe. Once the needle entered into the brain pathologist felt loss of resistance after that they hit the gun and the needle was retracted along with the tissue core. The tissue core was immediately dipped into 10% formol buffered saline and then container capped tightly. Same was repeated on the opposite side. These tissues were then transported and processed to prepare Hematoxylin and Eosin stained smears which were examined microscopically by two independent pathologists. The histopathological findings were reported, analyzed and compared with the existing data available in literature.

Results

The MIA was performed in ten deceased cases out of which seven samples were adequate for histopathological examination. Out of seven cases three were showing normal brain tissues on histopathological examination and four had abnormalities. The other three cases were excluded from the evaluation because of poor processing resulting in loss of tissues. (Figure 1)The demographic details, clinical symptoms, pre-existing comorbidities and laboratory investigations were described in table 1. Abnormal histopathological analyses of four cases are reported in table 2.

Case Description

- **Case 1:** A 58 years old male was admitted to intensive care unit (ICU) with features of acute respiratory distress syndrome (ARDS). He had multiple co - morbidities since past 12 years for which he was on medication. He had fever, dry cough, anosmia and dysguesia from past one week and markedly reduced oxygen saturation from past two days. He was unable to maintain saturation on nasal canula hence admitted in ICU for ventilator support. He was clinically managed according to existing COVID-19 management guidelines but deteriorated gradually and on day three of admission succumbed to death. MIA was performed immediately and two cores of brain tissues were examined microscopically. Histo-

pathological evaluation shows edema, vacuolar degeneration, intravascular microthrombi, calcium clefts and cytoplasmic inclusions suggestive of diffuse neuronal injury and coagulopathies of acute onset. No necrosis or inflammatory infiltrates were found in the core biopsy. The final diagnosis of cerebrovascular thrombotic microangiopathy was reported (Figure 2,3).

- **Case 2:** A 71 years old male admitted to ICU with complaints of fever, Breathlessness since four days and altered sensorium since one day. He had multiple comorbidity and was critically ill thus put on ventilator immediately. In spite of all efforts we failed to revive him and he succumb to death after seven days of admission. MIA was performed. Histopathological examination shows features of viral encephalitis (Figure 4).
- **Case 3**: A 26 years old known case of hepatic abscess admitted in ICU with acute onset of COVID-19 associated ARDS. He had dry cough, breathlessness and pain abdomen. He was managed with clinically managed according to existing COVID-19 management guidelines but deteriorated gradually and on day six of admission succumbed to death. MIA was performed immediately and two cores of brain tissues were examined microscopically. Histopathological evaluation shows nonspecific features of acute cerebral injury.
- **Case 4:** A 52 years old male admitted to ICU with complaints of fever and headache since three days and altered sensorium since one day. He was known case of diabetes and hypertension with poor therapeutic compliance. His saturation was less than 60%, thus put on ventilator immediately. He had dyselectrolytemia, hyperlactatemia and hyperglycemia. He was managed according to existing COVID-19 management guidelines but in spite of all efforts we failed to revive him and he succumbed to death after five days of admission. MIA was performed immediately. Histopathological examination shows features of cerebrovascular thrombotic microangiopathy.

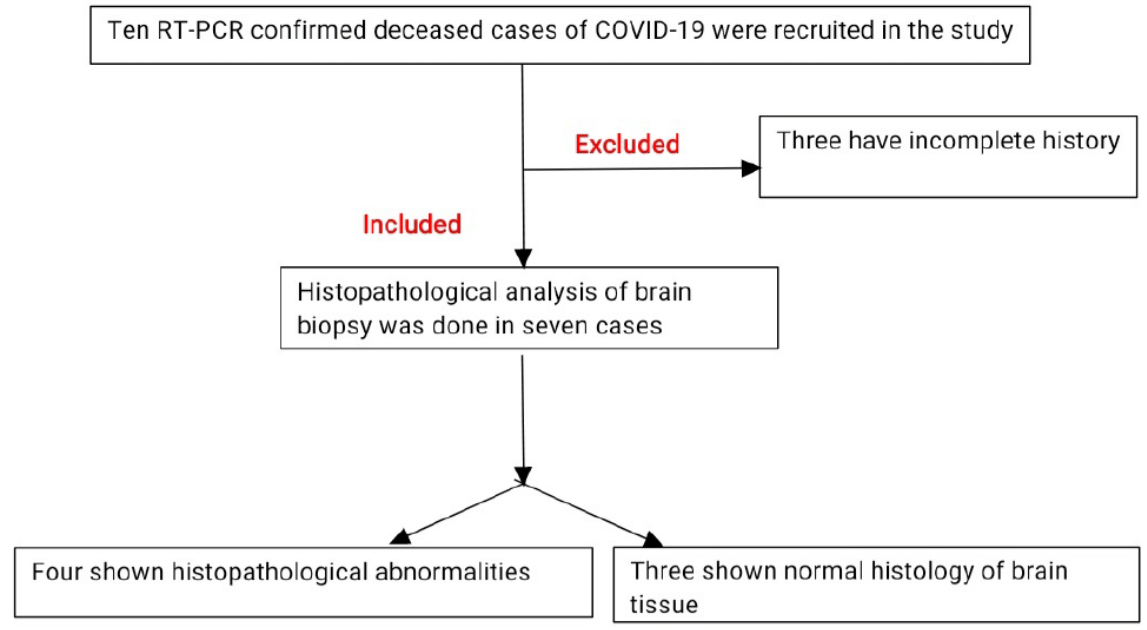

Figure 1: Flow Diagram of selection of cases.

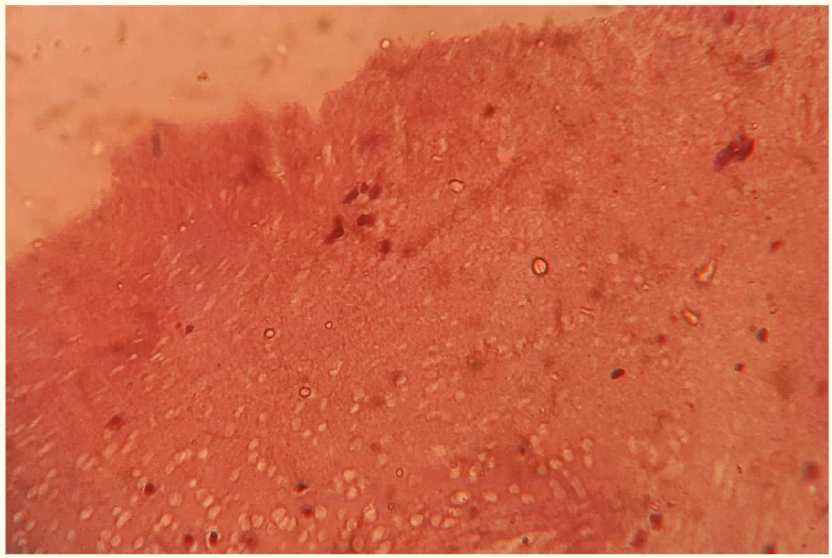

Figure 2: Hematoxylin and Eosin-stained brain biopsy at 40x magnification: Cerebral edema, dilated capillaries with microthrombi and calcium clefts.

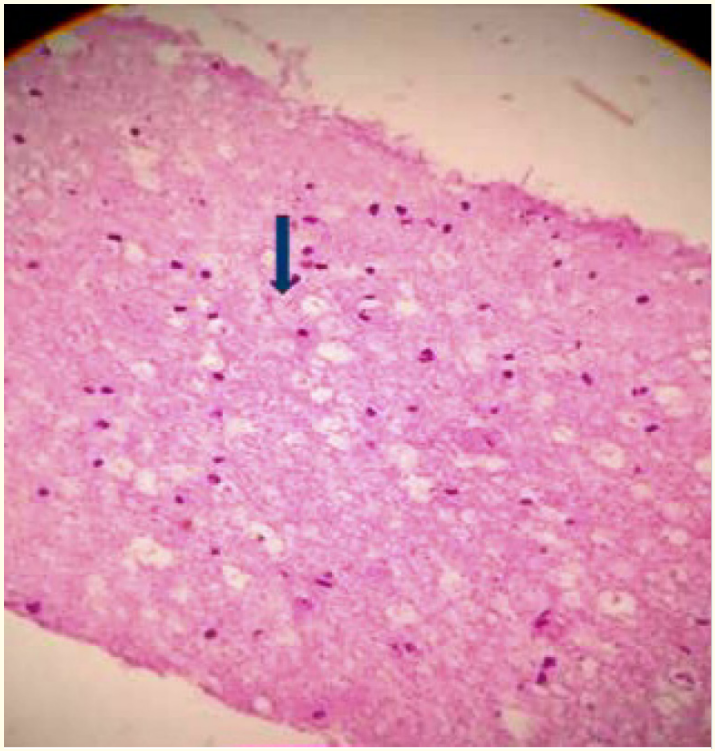

Figure 3: Hematoxylin and Eosin-stained brain biopsy at 40x magnification: Cytoplasmic inclusions (blue arrow).

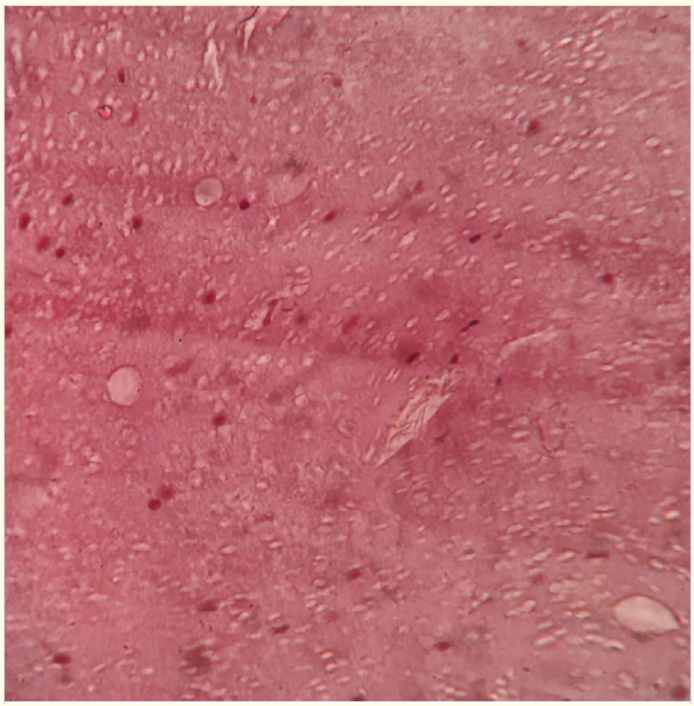

Figure 4: Hematoxylin and Eosin-stained brain biopsy at 40x magnification: Cerebral edema, foci of inflammatory infiltrates (lymphocytes).

Case No.	Age	Gender	Clinical symptoms	Pre-existing comorbidity	WHO Grading of severity of illness	Hematological abnormalities	Biochemical abnormalities	Duration of-hospitalstay
1	58	M	Fever, Dry cough, Anosmia, Dysgeusia, Breathless ness, ARDS,	DM Hypertension Alcoholic CKD CLD	Critically ill	Microcytic hypo-chromic anemia Neutrophilia NLR 30.67	RFT deranged Elevated trans-aminases conjugate d hyperbilirubinemia Hy-poproteinemia Elevated D-Dimer Elevated CRP Dyselectrolytemia Hyper-lactatemia	8 days
2	71	M	ARDS, Fever, Altered sensorium	DM Hypertension	Critically ill	Microcytic hypo-chromic anemia Neutrophilia NLR 21.02	Dyselectrolytemia Hyper-glycemia RFT deranged Elevated CRP Elevated D-Dimer	7 days
3	26	M	Cough, pain abdomen,	Hepatic abscess	Critically ill	Neutrophilia NLR 12.86 Thr-mobocytopenia	Hyperglycemia RFT-deranged Dyselectroly-temia Hyperlactatemia Hyperbilirubinemia LFT deranged	6 days
4	52	M	Fever, headache, altered senso-rium	DM, hyperten-sion	Critically ill	Microcytic hypo-chromic anemia, Neutrophilia, Thrombocytope-nia, NLR 24.32	Hyperglycemia, Elevat-edb HbA1c,Elevated D-Dimer and CRP, hy-perlactatemia, Dyselec-trolytemia	5 days

Table 1: Demographic characteristics of cases.

Case No.	Post- mortality time of mini autopsy	Approach of minimally invasive trucutbiopsy	Histopathological evaluation	Final diagnosis
1	4 hours	Bilateral transorbital	Intravascular microthrombi Cerebral edema Vacuolations	Cerebrovascular thrombotic microangiopathy
2	5 hours	Bilateral transorbital	Cerebral edema, Vacuolations, foci of lymphocytic infiltration, singly scattered inflammatory cells	Viral Encephalitis
3	4 hours	Bilateral transorbital	Cerebral edema, vacuolations, inclusions, dilated capillaries	Acute Cerebral injury
4	3 hours	Bilateral transorbital	Cerebral edema, dilated capillaries, microthrombi	Cerebrovascular thrombotic microangiopathy

Table 2: Histopathological analysis of MIA samples.

Discussion

MIA is a very useful tool to obtain tissues for confirmative diagnosis among highly infectious diseases like viral infections, rickettsial diseases etc and infectious diseases of unknown histopathology like COVID-19 pandemic. MIA was found to be equally effective in detection of immediate cause of death as the conventional full autopsy (92%) than conventional full autopsy. MIA has a higher diagnostic yield.[5] Conventional full autopsies are time consuming, tedious work that require long hours of tissue processing, are costly, cosmetically disgraceful for the attendants of deceased and are responsible for delayed disposal of bodies for rituals by the family members of the deceased. Decrease in rate of conventional full autopsies is observed globally from 50% in 1960s to < 10% at present [6]. These techniques are also having a limited role in highly infectious diseases. MIA is effective in reducing the risk of infection to the health care personnel and the surrounding area by minimizing the formation of aerosols. The tissues extracted were immediately dipped into formalin which is a powerful disinfectant for viruses. None of the healthcare personnel involved in transfer of the deceased to an isolated area for procedure or laboratory personnel involved in processing of tissues or pathologists who were involved in diagnosing the disease were contracted with COVID-19 disease within 24 days of the last case studied. Tissues obtained can be further utilized to perform histopathological, cytological, immunological, molecular, microbiological or biochemical examination for confirmation of diseases.

The histopathological spectrum of brain autopsy findings among COVID-19 deceased cases are scant in number. We found in our study, acute cerebral injury, cerebrovascular thrombotic microangiopathy and viral encephalitis similar to those described by Fisicaro F., *et al.* study. The more interesting thing we observed in our study was the presence of vacuolations and inclusions in all the four cases. We failed to perform RNA detection for the virus from the tissues. We assume that these vacuolations and inclusions may contain viral particles and remain dormant resulting in long term neurological consequences but further study is still required to prove this assumption [7].

The COVID-19 induced axonal injury, leukomalacia and astroglial injury (astrogliosis, microgliosis and immune cell infiltration) involves spike-NRP1 interaction and results in persistent or long term (>4 weeks) neurological complications. Viral RNA are isolated from brain cells and cerebrospinal fluids among COVID-19 infected patients [8].

Limitations

This study was limited in few aspects like single center study performed on a small population of COVID-19 deceased cases and poor tissue processing resulting in loss of tissues. We were unable to analyze viral presence by molecular methods. A large-scale experimental study is further required to confirm these findings and to prove that these injuries may be responsible for long term COVID-19 associated neuropsychiatric illnesses.

Conclusion

We conclude that MIA is a rapid, cost-effective, safer technique with higher diagnostic yield. It can provide significant histopathological findings for confirmative diagnosis among histopathologically unknown highly infectious diseases. We also observed that brain tissues can be affected in COVID-19 by direct cytotropic effect of virus through olfactory bulb or indirectly by systemic response. Presence of vacuolations and inclusions in brain biopsy must be further examined for RNA isolation for further confirmation. These

findings strengthen our hypothesis of long COVID-19 associated Neurological complications resulting from virus induced brain injury.

Conflict of Interest

There is no conflict of interest among authors.
Disclaimer: No.

Acknowledgement

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