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Surgical outcome in patients with mesial temporal sclerosis, with and without associated temporal lobe pathology: A clinicopathological study

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Abstract

Objective: Mesial temporal sclerosis with associated extra hippocampal pathology is considered 'dual pathology' that could influence the progression and clinical outcome of complex partial seizures. This study is undertaken to evaluate and compare the clinical outcome and pathology of the temporal lobe and hippocampus in cases of mesial temporal sclerosis (MTS) alone and MTS with associated pathological changes in the extrahippocampal temporal lobe (dual pathology). *Methods*: The clinical and presurgical evaluation data and post surgical follow up (2 years) were reviewed retrospectively from medical records of 15 cases with MTS alone and 11 cases having dual pathology. Specific pathological changes were recorded after reviewing the material from hippocampus and temporal lobe and immunostaining with antibodies to synaptophysin, and neurofilament to delineate dystrophic neurons and synaptic pathology and S-100 protein for glial elements. *Results*: Among the 11 patients with dual pathology, 2 patients had mild cortical dysplasia (MCD) and 9 had focal cortical dysplasia (FCD) in the adjacent temporal cortex, as described by Palmini *et al*. High resolution MRI (1.5 Tesla) did not detect the presence of the second pathology reported in this series. Thirteen of the 15 patients with MTS alone and 6 of the 11 patients with dual pathology had good post surgical outcome. Six (2 MTS + 4 dual pathology) out of 7 patients who failed to show good clinical outcome had significant loss of neurons in CA3 sub-field of Ammon's horn, whereas only 12 out of 19 patients who had good outcome had CA3 neuronal loss. Various types of cytoskeletal and synaptic pathology are found in the dysplastic neurons in the zones of cortical dysplasia.
Conclusion: Two types of structural lesions underlie complex partial seizures, MTS with or without associated extrahippocampal lesions of neuronal cytoarchitectural abnormality may influence the prognosis. Neuronal loss in CA3 subfield of Ammon's horn seems to have a role in negative clinical outcome, though this feature needs to be further validated.

INTRODUCTION

An important step in the management of epilepsy is the recognition that among the medically refractory patients, are an identifiable subgroup who have surgically remediable syndromes. The most common pathological finding in resected temporal lobe among the patients suffering from complex partial epilepsy (CPS) is hippocampal sclerosis, followed by cortical dysgenesis, tumours and vascular malformations.[1] Mesial temporal lobe syndrome is the most common syndrome associated with CPS and its distinct clinical, electrophysiological, MRI and pathological features define this syndrome. Hippocampal sclerosis can be diagnosed in vivo by volumetric study and signal intensity changes on MRI. Mesial temporal sclerosis (MTS) or hippocampal sclerosis refers to neuronal loss and gliosis primarily involving the hippocampus but also occasionally the uncus, amygdala and parahippocampal gyrus. Cortical dysplasia, vascular malformation and primary benign tumours are identified as lesional pathology for epileptogenesis in patients with CPS in approximately 30% of cases.[2]

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The term 'single pathology' refers to either MTS or any of the above mentioned as the single lesion responsible for epileptogenesis. The term 'dual pathology' refers to the co-existence of MTS with another lesion involving the extrahippocampal temporal lobe.[3-5] The second lesion may be developmental abnormalities, cortical dysgenesis or tumours that by themselves are capable of seizure initiation. Though high resolution imaging techniques can detect the dual pathology to some extent, there are certain forms whose presence as a possible source of epileptogenesis can be confirmed only after histological evaluation of the resected specimen. Approximately 5-30% of patients who have undergone surgical resection of temporal lobe for intractable CPS were shown to harbor dual pathology.[3] The inclusion of microscopic abnormalities like neuronal heterotopia and microdysgenesis raise the incidence of dual pathology to 30% or even higher.[2]

The pathophysiological relationship between the two lesions in cases of dual pathology remains unclear. It has been suggested that repeated epileptiform discharges may produce hippocampal injury mediated by excitotoxin release and/or calcium influx from adjacent anatomical structures, which explains higher association of lesions in temporal lobe itself rather than extratemporal lesions with MTS.[6] It appears that lesions that underlie CPS, probably have an origin during the development of brain.

Recognition of the underlying lesion and the extent of epileptogenic area are the key issues in presurgical evaluation of the patients with refractory CPS. Assessment of possible dual pathology in patients with lesional epilepsy is important because their management and surgical outcomes are distinct.[7,8] The surgical outcome of epilepsy caused by a single lesion is good when the lesion can be completely resected. In patients with dual pathology, surgery should be targeted at both the lesion and the mesial temporal structures in order to achieve a good post operative outcome.[9,10] A comparative study evaluating the clinical presentation, pathological, and neuropsychological features and surgical outcome among patients with single versus dual pathology with CPS has not been reported from India so far. In the present study an attempt has been made to understand the evolution of temporal lobe epilepsy with single and/or dual pathology and their role in the final outcome.

METHODS

This retrospective study was carried out on 26 patients who attended the 'Refractory Epilepsy Clinic' at the National Institute of Mental Health and Neurosciences (NIMHANS) Bangalore, with clinical features of intractable temporal lobe epilepsy. All of them had undergone a uniform type of surgery, anterior temporal lobectomy with amygdalohippocampectomy. In 19 cases enblock resection of hippocampus and temporal lobe were achieved while in 7 the tissue was fragmented, though the general anatomical architecture could be easily oriented. All the patients have been evaluated by the same team and the surgery has been carried out by a single neurosurgeon as a part of 'Comprehensive Epilepsy Surgical Programme'. The pathological evaluation was carried out by single neuropathologist throughout the study period.

Histopathology

Hippocampus and temporal lobe specimens from all the 26 patients received fresh from the operation theatre within 30 minutes of resection were sliced into 5 mm thick segments maintaining the neuroanatomical orientation and fixed in 10% buffered formation for 48 hours. Two slices from anterior and posterior hippocampus and three slices from different levels of temporal lobe were processed for paraffin embedding in an automated tissue processor with a standard protocol for uniformity and histological sectioning. Five micro thick sections were stained with Haematoxylin-Eosin and Luxol-Fast-Blue-Cresylviolet for routine evaluation. All sections of the hippocampus and selected ones from temporal lobe were immunnostained by indirect immunoperoxidase technique, using the following antibodies, after antigen retrieval in microwave.[11]

Immunohistochemistry

Antibody and source	Antigen labelled	Dilution
1. SMI-33 (monoclonal)	Non phosphorilated Neurofilament (NF)	1:1000
2. SMI-31 (monoclonal) sternberger Inc.USA	Phosphorilated H&M peptides of NF	1:1000
3. Synaptophysin (polyclonal) DAKO, USA	Synaptic vesicle protein	1:50
4. S-100 polyclonal (immunotech. California, USA)	Calcium binding protein in glial cells	1:200

Histopathological evaluation of hippocampal sclerosis was based on semiquantitative visual analysis and grading into three grades; mild, moderate and severe, depending on the degree of neuronal loss and astrogliosis. Neuronal loss visually assessed as less than 30% in each anatomical subfields of hippocampus was considered *mild* compared to five control specimens of hippocampi from adult (20-30 years age) collected at medicolegal autopsies with informed consent (6-8 hrs postmortem delay, with no damage to the cerebral hemispheres). Loss of 30-60% was graded as *moderate* and above 60% as *severe*. The semiquantitative grading of Wyler[12] was not used as the range of neuronal loss in this classification was very wide and hence grades of 30% increment was considered for semiquantitative evaluation of neuronal depletion and gliosis. Dual pathology was diagnosed, when the lesions were essentially extrahippocampal, but still located in the temporal lobe. These lesions included cortical dyslamination, heterotopic neurons, dysplastic neurons/balloned neurons, neuronal clusters in subcortical white matter, immature neuronal clusters close to fimbia or in white matter, gliotic nodules extending beyond the pia glial interface in the temporal cortex, vascular malformation or neoplastic lesions (similar pathological changes if present in hippocampus were also recorded in both groups of cases). Because of variation in size of histological section and anatomical extent of the temporal gyri resected, (though 19 cases received enblock following surgery), no quantitative assessment of neuronal loss in this area has been attempted. The immunostaining was a complementary tool to identify and characterize the neuronal and glial pathology. The cortical dysplastic lesions were graded following the system of Palmini *et al*.[13,14] As the study is a retrospective one using archival material, Timm's silver stain to demonstrate mossy fibre sprouting could be carried out. All the slides were reviewed blind to the clinical history and epilepsy semiology.

The clinical data was collated after reviewing the medical records of 15 patients with single pathology and 11 patients with dual pathology by the clinician who was blinded to the pathological diagnosis. The clinical history included the age of onset, duration of epilepsy, semiology of epilepsy, history of febrile convulsions, birth injury and family history of epilepsy. A comprehensive presurgical evaluation included serial 10-20 scalp EEG recording, Video EEG for confirming the localization and the semiology of seizure, MRI (1.5 Tesla T1, T2, FLAIR-CPS protocol) along with volumetry in relevant cases, detailed neuropsychological evaluation and other routine blood investigations. The neuropsychological test scores of the patients were compared with age, education and gender matched Indian norms and the deficits were marked for each patient in each of the functions assessed.[15] Patients who scored below the 15th percentile on the scores of accuracy and who scored above the 85th percentile on 'time and error' scores were considered deficient in performance. Post surgical outcome at 2 years, indicating the grade of seizure freedom, change in the dose of antiepileptic drugs, cognitive/ neuropsychological change (if any), post operative EEG, and neuropsychological assessment were noted. Postoperative seizure outcome has been assessed according to Engel's outcome scale (Class 1: free of disabling seizures, Class 2: rare disabling

seizures, Class 3: worthwhile improvement, Class 4: no worthwhile improvement). Only descriptive method has been used to describe the findings and limited statistical correlation has been attempted, as the sample size is small in each of the groups in this study.

RESULTS

Fifteen patients had pure MTS and 11 patients, along with MTS, had focal cortical dysplasia (9 patients) or microdysgenesis/mild cortical dysplasia (2 patients) and focal subcortical ectopic collections of neurons (5 patients) in the white matter of the adjacent temporal cortex (away from amygdala complex) reflecting dual pathology. Ten out of 15 (66.6%) patients with pure MTS had febrile convulsion in early childhood compared to 3/11 (27.3%) patients with dual pathology, which is statistically significant. The patients who had febrile convulsion in early childhood, had early onset of CPS. (<12 years of age in 6/10 patients with MTS and 2/3 patients with dual pathology).

Electrophysiological tests (Video EEG and sphenoidal EEG) demonstrated concordant EEG in all patients with single pathology (MTS only) and among 9 out 11 with dual pathology. Only 2 patients (No.4 and 9, Table 1B) had bilateral discharges though one side distinctly more than the other concordant to MRI changes. MR imaging (1.5T) revealed hippocampal atrophy with signal changes suggestive of MTS in all the 26 patients. However in two of the dual pathology groups, patient No.7 had in addition diffuse cerebral atrophy and patient No.11 had right temporal cystic lesion in addition to MTS.

Detailed neuropsychological assessment revealed visual/verbal memory deficit along with poor visuaospatial performance in 10 out of 15 (66.6%) with pure MTS and 4 out of 11 (36.4%) in those with dual pathology. Remaining 5 (MTS only) and 5 (dual pathology) had bitemporal deficit though one side more than the other appropriate to MRI changes. Only one patient (Case 9) with dual pathology had no deficit on neuropsychological testing.

All the 26 patients had undergone the same surgical procedure, i.e. anterior temporal lobectomy with amygdalohippocampectomy. They were followed up post operatively; single pathology patients for 8-40 months and dual pathology patients for 5-48 months. The postoperative outcome at the last follow up revealed that in single pathology group 86% patients had an Engel's class 1 outcome whereas 50% patients with dual pathology could not attain an Engel's class 1 outcome (Table 2).

Pathological changes in the dentate gyrus, Ammon's horn, subiculum and temporal isocortex between the two groups are presented in Table 3 and 4. In patients with single pathology, neuronal loss in CA1 area was moderate to severe with gliosis in all the cases representing hippocampal sclerosis (Figure 2A, 2B). The neuronal loss in anterior hippocampus was essentially similar to posterior hippocampus in CA1 zone, but ability to characterize the lesions to various hippocampal sub area was less precise and the neuronal depletion extended to the subicular zone. In the dentate gyrus, in addition to depletion of granule cells of variable degree, lamination, dispersal of granule cells and numerous clusters of corpora amylacea were seen in stratum moleculare, in both the groups with no distinct difference in the pattern and degree.

The severity of cell loss and the pattern of synaptophysin immunolabelling did not differ significantly, in the hippocampal subfields though the pattern of cell loss was different between the single and dual pathology groups (Figure 2,3). Varying degrees of neuronal depletion in CA3 zone was seen in all patients of dual pathology group, in addition to CA1 zone (Figure 2C), while it was less evident in patients with single pathology (Table 3). Some of the pyramidal neurons in CA1 revealed malorientation (Figure 3 C). Dentate gyrus granule cell dispersion and extra lamination was noted in nearly 40% of cases in both the groups (Figure 2B).

In patients with only hippocampal sclerosis (single pathology group), altered lamination, and abnormal cytological architecture of neurons in temporal cortex was negligible but was clearly discernable in the group with dual pathology (Figure 4A). Mild cortical dysplasia (microdysgenesis) was observed in addition to clusters of ectopic neurons (away from amygdala complex) in the white matter of the temporal cortex indicating nodular haterotopia MD type II (Figure 4B,C). The MRI in this patient could not highlight this feature.

Immunostaining with an antibody to neurofilament (phosphorylated epitopes H and M of neurofilament protein – SMI-31) highlighted dystrophic neurons in hilar zone (CA 4), CA 1, subiculum and temporal cortex in both the groups (Table 3). In addition many abnormal meganeurons labeled by neurofilament antibody were found in the white matter of subiculum and

Table 1A: Electrophysiological, neuroimaging, and neuropsychological data of patients with intractable epilepsy with single pathology

Case no	Scalp EEG	Video EEG	Sphenoidal EEG	MRI	Neuropsychology	Concordance between neuroimaging and electro-physiology Yes / No
1	Lt anterior temporal focus	Lt anterior temporal focus	Lt anterior and mid temporal focus	Gross atrophy of the left amygdala and hippocampus with signal intensity change	Impairment in verbal learning and memory suggestive of left focus	Yes
2	Rt fronto temporal focus	–	Rt anterior and mid temporal	Gross atrophy of Rt hippocampus, dilated Rt temporal horn	Impairment in visual learning and memory suggestive of Rt focus	Yes
3	Rt anterior temporal	Rt temporal	Rt anterior and mid temporal	Rt mesial temporal sclerosis	Impaired visual and verbal learning and memory, Rt > Lt	Yes
4	Normal	Rt temporal focus	Rt anterior temporal foci Occasional Lt temporal focal discharges	Rt hippocampal atrophy altered signal intensity in T2W images	IQ-58, Impairment in visual learning and memory suggestive of Rt focus	Yes
5	Lt anterior temporal foci	Rt anterior temporal foci	Lt anterior and mid temporal foci, occasional Rt anterior temporal focal discharges +	Lt mesial temporal volume loss, signal intensity changes in T2, Flair loss of architecture	Phenomic fluency, verbal and visual learning and memory of bitemporal, Rt > Lt	Yes
6	Lt temporal	–	Lt anterior and mid temporal	Lt hippocampal sclerosis, T2 hyperintensity	Lt temporal dysfunction	Yes
7	Predominantly Rt temporal	–	Rt anterior temporal	Rt medial temporal atrophy with hyperintense signals	Impaired visual learning and memory, Rt focus	Yes
8	Rt > Lt temporal activity	Rt mid temporal	Rt temporal	Rt mesial temporal sclerosis with volume loss 50% T2W signal intensity	Bilateral frontotemporal involvement Rt > Lt Impaired visual learning and memory	Yes
9	Lt temporal foci	–	Lt temporal foci	Lt hippocampus altered architecture and signal changes MRS NAA/CR peak	Bilateral pre frontal involvement with impaired verbal and design fluency Bilateral minimum temporal involvement	Yes

Case no	Scalp EEG	Video EEG	Sphenoidal EEG	MRI	Neuropsychology	Concordance between neuroimaging and electro-physiology Yes / No
10	Normal	–	Lt temporal	Atrophy of Lt hippocampus, dilated Lt temporal horn	Impaired verbal and visual learning and memory bitemporal, Lt > Rt	Yes
11	Normal	Rt anterior and mid temporal	Rt anterior and mid temporal	Rt temporal lobe atrophy, loss of architecture with mild diffuse cortical atrophy	Impaired visual learning and memory, Rt focus	Yes
12	Rt fronto temporal	–	Lt anterior and mid temporal	Lt hippocampal atrophy, Lt temporal horn dilated	Verbal learning and memory impaired also bilateral frontal, parietal, temporal lobe dysfunction	Yes
13	Lt anterior temporal	–	Bilateral temporal spikes Lt > Rt	Lt mesial temporal sclerosis	Impaired verbal and visual learning and memory bitemporal, Lt > Rt	No
14	Rt anterior and mid temporal	–	Rt anterior and mid temporal occasional Lt temporal focal discharges	Bilateral medial temporal lobe atrophy and Rt hippocampal sclerosis	Bilateral frontal and Lt temporal deficits	Yes
15	Lt fronto temporal	–	Lt anterior and mid temporal	Lt medial temporal and hippocampal atrophy	Impairment in verbal learning and memory suggestive of left focus	Yes

Rt: right, Lt: left

entorhinal cortex (Figure 5A). In the temporal cortex white matter, some of the neurons were oriented parallel to glial cell columns in white matter suggesting arrested migration along the radial glia (Figure 5C). One patient had Type II B focal cortical dysplasia with ballooned neurons in the entorhinal cortex (Figure 5B). In the hilar zone of Ammon's horn (CA 4) and temporal cortex in a single patient (Case 8) large dysplastic neurons with neurofibrillary tangles (FCD-Type II A) and 'neuritic plaque like structures' without amyloid core were noted (Figure D,E) while in three other cases aberrant axonal bundles were noted in the hippocampus (Figure 5D). Persistence of horizontal Cajal Retzeus neurons and neuronal clusters in stratum oriens and alvial white matter was more frequent in the dual pathology group. On immunolabeling with antibody to synaptophysin, the supragranular layer of dentate gyrus, especially along the dorsal lip was labeled in focal densities (Figure 3B) reflecting mossy fibre sprouting and synaptic remodeling, (single pathology group 4/15 patients (26%) dual pathology group 1/11 patients (9%). CA3 and CA1 zones revealed laminar

Table 1B: Electrophysiological, Neuroimaging, and neuropsychological data of subjects with intractable epilepsy with Dual pathology

Case no	Scalp EEG	Video EEG	Sphenoidal EEG	MRI	Neuropsychology	Concordance between neuroimaging and electro-physiology Yes / No
1	Rt temporal foci	–	Rt anterior temporal foci	Rt hippocampus amygdala signal intensity changes, Rt temporal horn dilated	Impairment of visual learning and memory Rt temporal involvement	Yes
2	Normal	Lt anterior and mid temporal foci	Lt anterior and mid temporal foci	Hyperdense signal changes in Left parahippocampus T2 and flair	Impaired verbal and visual learning and memory bitemporal, Lt > Rt	Yes
3	Lt anterior and mid temporal foci	–	–	Signal changes in Lt medial temporal region with volume loss Lt temporal horn dilated	Slower motor speed of Rt hand, Impaired verbal learning and memory Lt MTLS	Yes
4	Lt mid temporal foci	Lt temporal	Bilateral anterior temporal leads Lt > Rt	Hyperintense signal changes in Left medial temporal, dilated Lt temporal horn	Bilateral MTL involvement	Yes
5	Rt anterior temporal	Rt anterior and mid temporal foci	Rt anterior and mid temporal foci	Rt hippocampus altered architecture Vol Lt 1.5% Vol Rt 1.2%	Impaired verbal and visual learning and memory bitemporal, Rt > Lt	Yes
6	Normal	–	Lt anterior and mid temporal foci	Gross atrophy of the Lt hippocampus	Impaired verbal and visual learning and memory bitemporal, Lt > Rt	Yes
7	Rt temporal	Rt and Lt anterior temporal spikes Rt > Lt	Rt and Lt anterior temporal spikes Rt > Lt	Rt medial temporal atrophy, Rt temporal horn dilatation, cerebral and cerebellar atrophy	Impaired visual learning and memory Rt MTLS	No
8	Rt anterior temporal	Generalized seizures	Rt anterior temporal	Rt hippocampal hyperintensity T2 with volume loss	Impaired visual learning and memory Rt focus	Yes
9	Rt temporal	Rt temporal	Bilateral anterior temporal	Rt medial temporal sclerosis	No learning and memory deficits	Yes
10	Lt mid and anterior temporal	–	Rt anterior and mid temporal, occasional Lt temporal discharges	Rt mesial temporal sclerosis with significant volume loss	Pre frontal and bitemporal involvement Rt > Lt	No
11	Rt temporal	Rt temporal	–	Rt temporal lobe cystic lesion + Rt MTLS	Impaired verbal and visual learning & me mory bitemporal, Rt > Lt	Yes

Rt: right, Lt:left, MTLS: medial temporal lobe sclerosis, MTL: medial temporal lobe

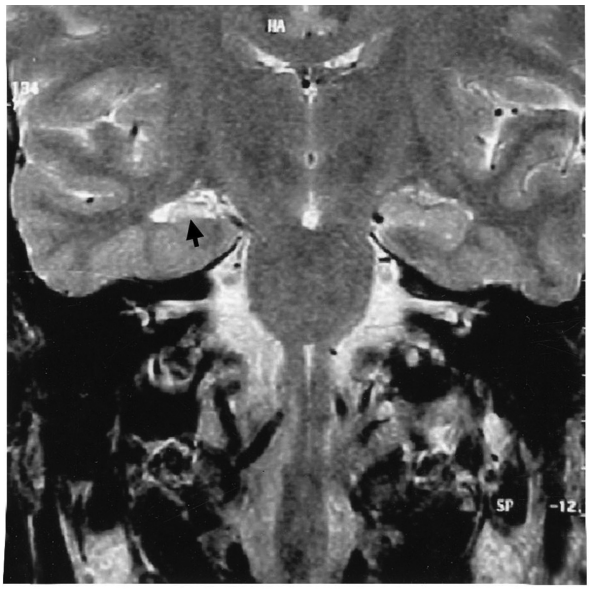

Figure 1: MRI T2WI shows unilateral hippocampal atrophy with mesial temporal sclerosis.

Table 2: Relationship between post surgical outcome and pathology

Outcome Engel's grading	Single pathology N=15 (%)	Dual pathology N=11 (%)	P value
Class I (Excellent outcome)	13 (86)	6 (54)	NS
Class II (Fair outcome)	1 (6)	3 (27)	NS
Class III (Worthwhile improvement)	–	1 (9)	NS
Class IV (No worthwhile improvement)	1 (6)	1 (9)	NS

NS- Not significant

Table 3: Single/dual pathology – hippocampal changes

Hippocampus	Single pathology N =15 (%)	Dual pathology N = 11 (%)	P Value
Mild ⟶ Moderate Ammon's horn cell loss	4 (26)	3 (27)	NS
Severe Ammon's horn cell loss	11 (73)	8 (72)	NS
CA3 neuronal loss – CA3 zone	5 (33)	11 (100)	<0.001
Horizontal neurons in stratum oriens	8 (53)	9 (81)	NS
Dystrophic neurons with cytoskeletal accumulation	8 (53)	3 (27)	NS
Reactive astrocytosis	11 (73)	8 (72)	NS
Neuronal cell loss in subiculum	7 (46)	1 (9) (DNT/Protoplasmic astrocytoma-1 patient)	NS

Cell loss was graded visually as mild (+), moderate (++), severe (+++)
Mild loss: <30% neuronal loss; Moderate: 30-60% neuronal loss; Severe: >60% to total loss in different anatomical fields.
Neuronal loss continued from CA1, into subiculum in cases with exclusive hippocampal sclerosis in contracts to those with dual pathology.
NS: not significant

Table 4: Pathological changes in temporal cortex

	Single pathology N=15	Dual pathology N=11
1	No MCD; astrocytosis in white matter perivascular space widened	MCD Type II (Heterotopic/excess neurons outside layer 1) 2 patients
2	No focal cortical dysplasia	FCD (9 patients) Type IA 5 patients Type II 4 patients (IIA-3, IIB-1)
3	No subcortical ectopias. Perivascular space widened	Focal gyral fusion – 2 Subcortical focal neuronal nodular heterotopias in white matter 5/11 patients

MCD: Mild cortical dysplasia or microdysgenesis, FCD: Focal cortical dysplasia (Classification according to Palmini and Lunders, 2000[13]).
Focal nodular heterotopias found were away from amygdala cortex in the subcortical white matter in temporal lobe.

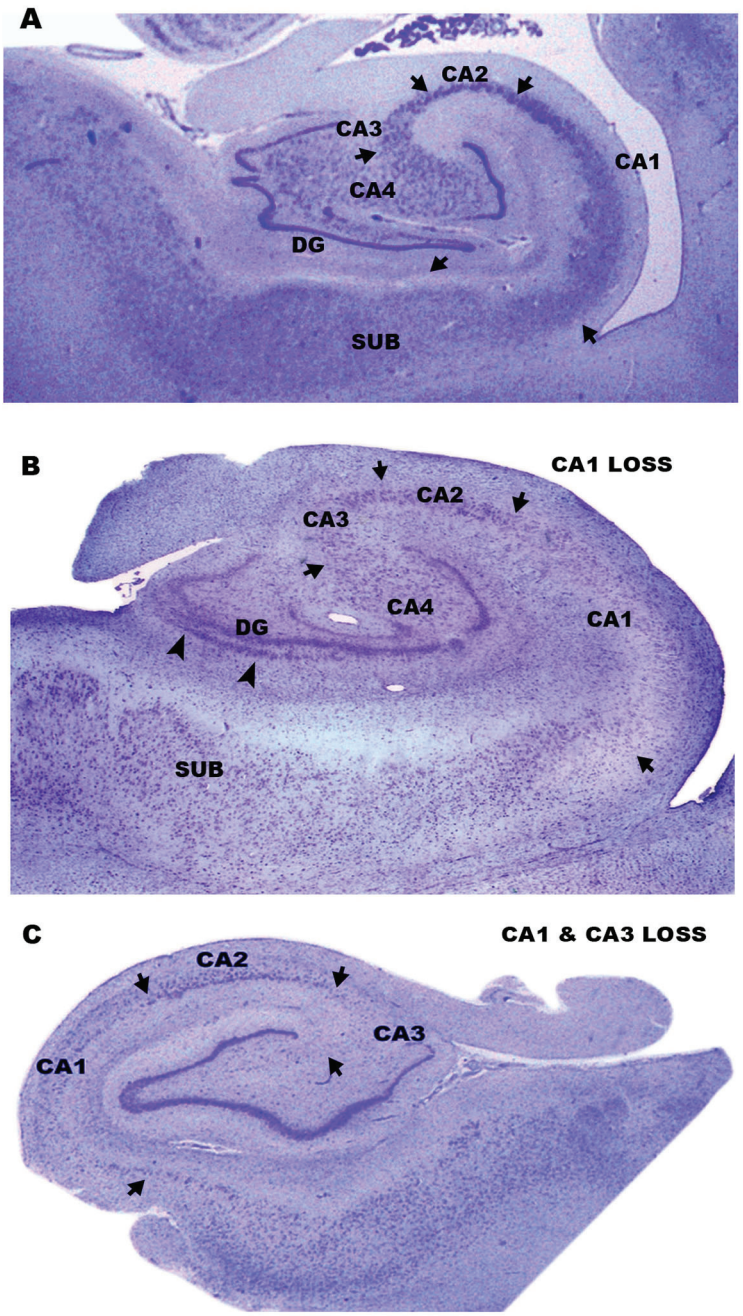

Figure 2: **A**. Normal hippocampal formation showing various zones of Ammon's horn. DG-Dentate gyrus; CA1, CA2, CA3 (arrows indicate the boundaries) subfields; Sub-subiculum. **B**. Hippocampus from a case of mesial temporal sclerosis (single pathology group showing dentate gyrus lamination, (arrow heads), significant loss of neurons in CA1, mild loss in CA3 and relative preservation of CA2 (single pathology group, case 7). **C**. Mesial temporal sclerosis with dual pathology. Hippocampus showing significant loss of neurons in CA3 and CA1 zone. DG granule cells were focally dispersed. (Case 4). **A**, **B**, **C** cresyl violet, magnification: X 30

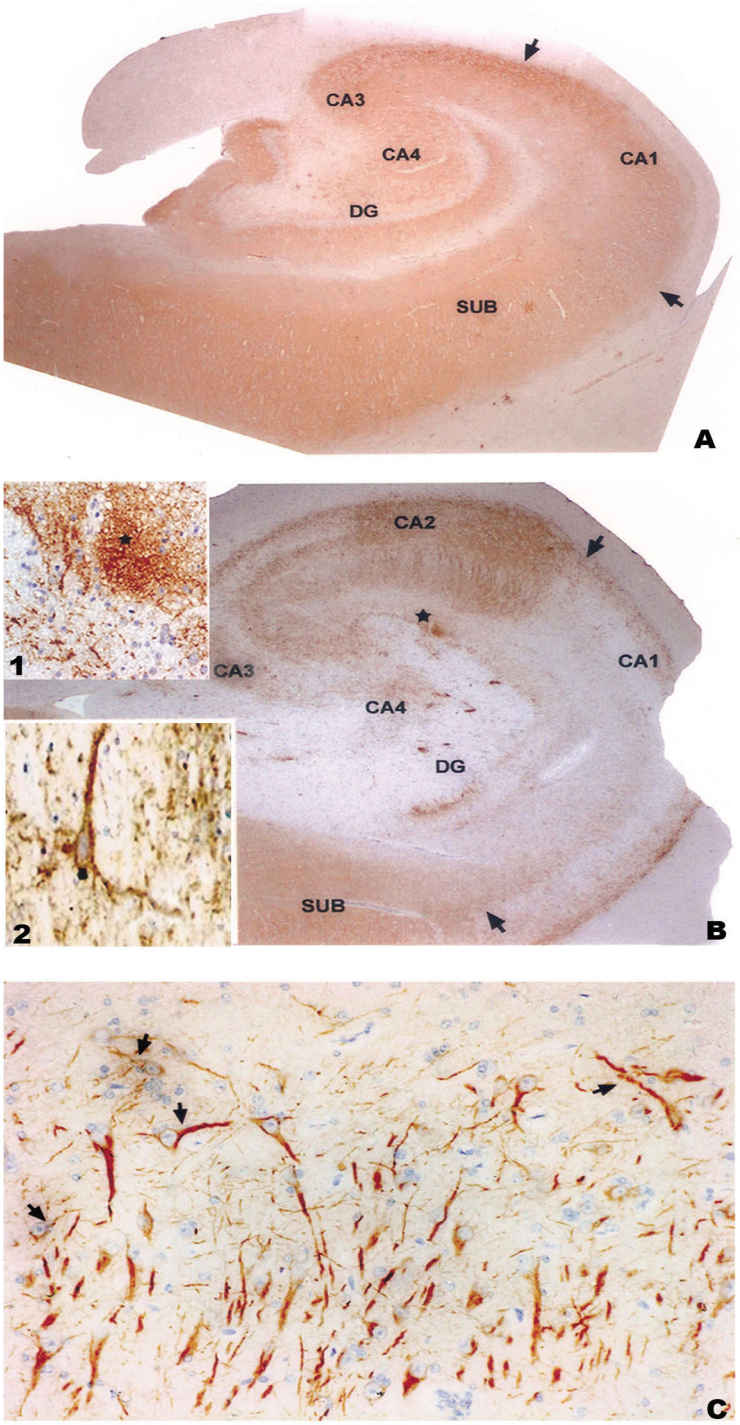

Figure 3: **A**. Normal and uniform synaptophysin immunolabelling of various subfields of Ammon's horn in hippocampus, normal control from an adult. DG: Dentate gyrus; CA1- CA2- CA3- CA4- Ammon's horn subfields; Sub: subiculum. Arrows mark the boundaries of subfields. **B**. Case of mesial temporal sclerosis (Dual pathology) showing patchy depletion of synaptophysin labeling along the supragranular molecular layer, (arrow heads) laminar labeling along CA3, dense staining along CA2 and depletion in CA1 zone. In addition focal dense labeling is seen (★) in supra granular area dorsal tip of DG. Note depletion of DG neurons focally (Case 4). Inset 1: High power view of **B**. ★: highlighting the increased synaptic labeling in supragranular zone of dentate gyrus focally. Inset 2: Dense perineuronal synaptophysin labeling around a pyramidal neuron in entorhinal cortex in a case of MTS of long duration (Dual pathology, Case 7). Synaptophysin immunostaining. Magnification: **A**, **B**: X 60, Inset 1, 2 X 320. **C**. Malorientation of pyramidal neurons (arrows) in CA1 zone, a case of mesial temporal sclerosis (single pathology group, Case 1). Immunoperoxidase SMI-31, magnification X 160.

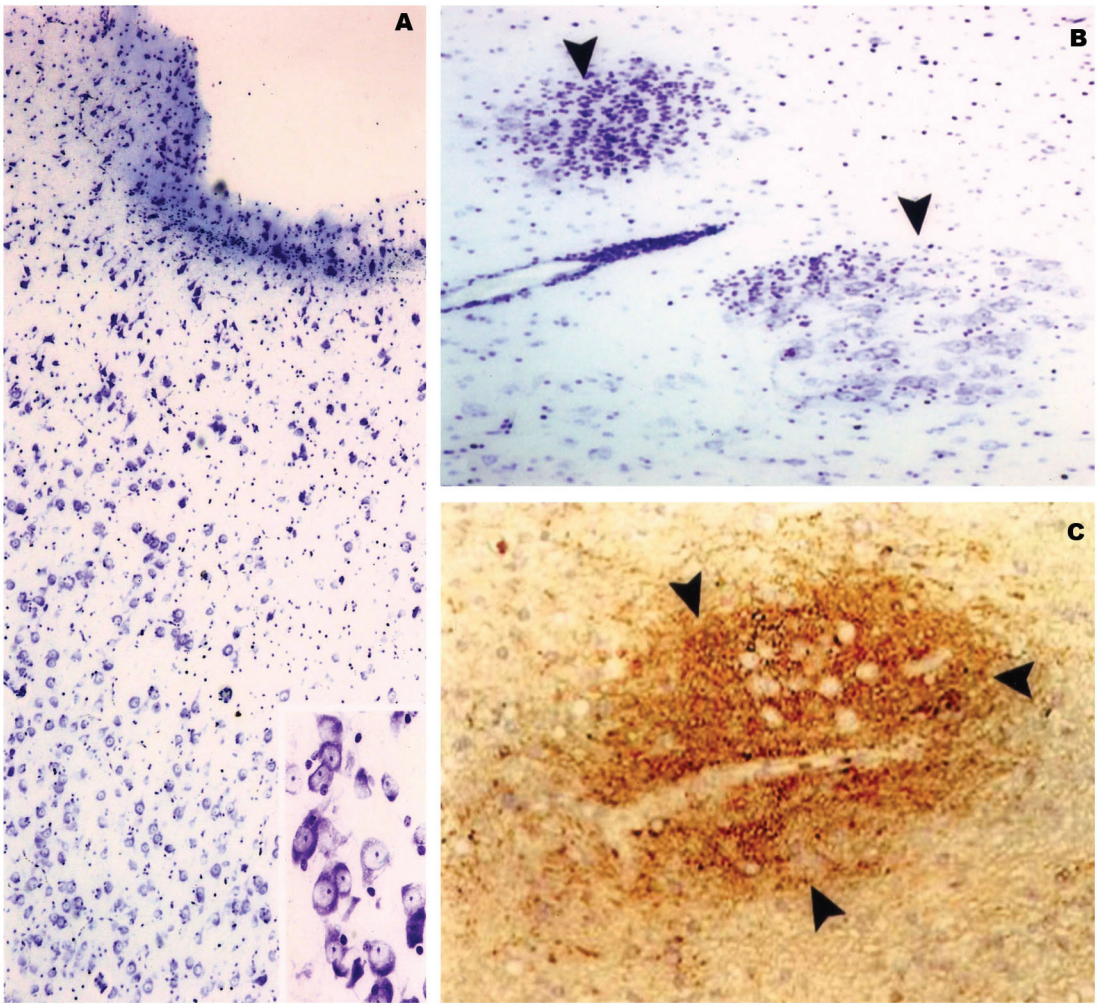

Figure 4: Cortical dysplasia in temporal cortex – dual pathology group. **A**. Mild cortical dysplasia with significant cortical dyslamination and excess of large neurons (MCD Type II). (Dual pathology, Case 4). Inset: large neurons in clusters. Cresyl violet. Magnification: X 60, Inset X280. **B**. Clusters of ectopic neurons in the subcortical white matter of temporal cortex, away from amygdala complex (Focal nodular heterotopia) (Case 5). Cresyl violet; magnification X 60. **C**. Synaptophysin immunolabelling highlighting a large ectopic cluster (Case 5). Immunoperoxidase synaptophysin, magnification X 180

labeling of outer and inner layers of stratum pyramidale (with compact and relatively normal CA2 zone) and merging with single broad zone of subiculum (Figure 3A,B) in both the groups, but more frequent in patients with dual pathology (Table 3). Similarly perineuronal dense synaptic labeling reflecting synaptic remodeling was noted in CA4 zones of hippocampus and occasionally in entorhinal cortex (Figure 3B inset 1) in patients of MTS of long duration in both groups (86% and 81% of patients in single and dual pathology groups respectively). In one patient with dual pathology a large zone of laminary synaptophysin labeling in fimbria extending to fornix (Figure 5F) was found. In another patient of the same group of dual pathology, a focus of cells resembling DNT/protoplasmic astrocytoma (cells negative for synaptophysin and S-100) was noted in the subiculum. No other neuroglial hamartomas or vascular lesions were observed in any of the patients studied in this series.

Neuronal loss in CA3 zone of Ammon's horn and presence of neuronal ectopias and focal cortical dysplasia of varying grades were observed in patients with dual pathology who did not have freedom from disabling seizures even after surgery

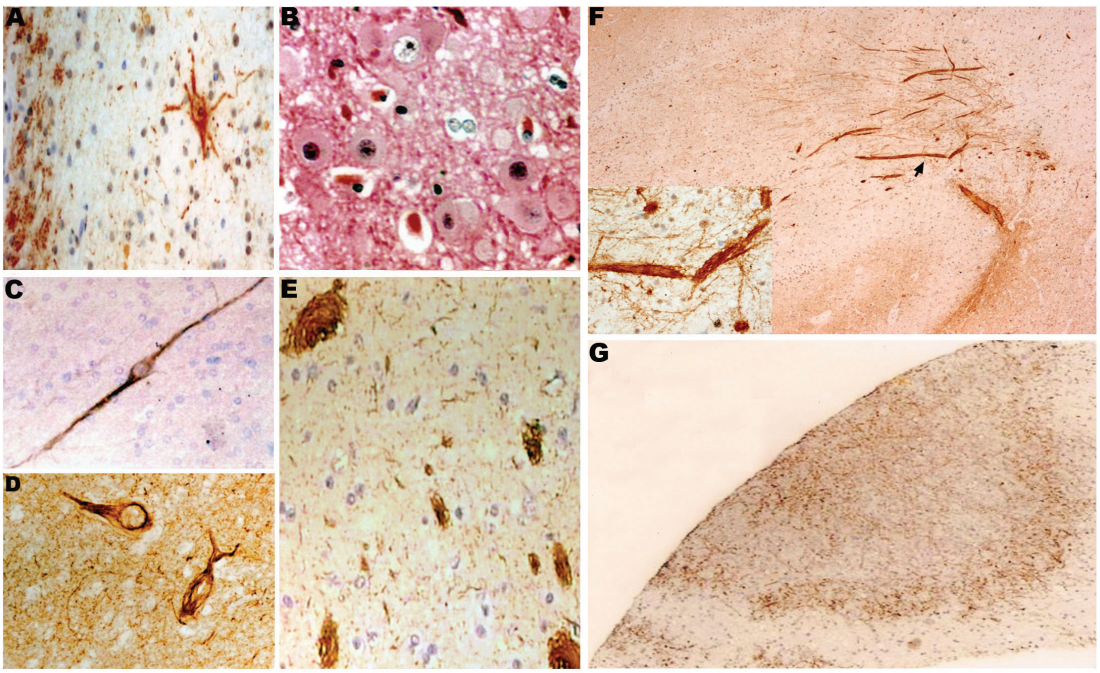

Figure 5: Spectrum of pathologies noted in cases with dual pathology. **A**. Abnormal meganeuron in the white matter in entorhinal cortex (Case 4). Immunoperoxidase SMI-31; magnification: X 280. **B**. Cluster of ballooned cells in entorhinal cortex with pale cytoplasm and vasicular nucleus. (FCD type II; Case 6). Haematoxylin-Eosin; magnification X 120. **C**. Large Bipolar microdysgenetic neuron in deep white matter of temporal cortex parallel to glial cell columns suggesting migration disorder (Case 11). Immunoperoxidase SMI-31, magnification: X 200. **D, E**. Large dysplastic neurons with neurofibrillary tangles (FCD Type II A) inside the neuronal soma (**D**), and compact neuritic plaque like structures in neuropil of the hilum of hippocampus (**E**), (Dual pathology group, Case 8). Immunoperoxidase SMI 31, magnification **D**, **E** X 240. **F**. Aberrant axonal bundles in hippocampus adjacent to CA3 zone, extending to molecular layer. Inset: Higher magnification showing floral form and packets of axonal bundles. (Case 1). Immunoperoxidase SMI-31, magnification X 100; Inset X 280. **G**. Abnormal laminar synaptophysin labeling in the white matter of fibria (Case 1). Immunoperoxidase synaptophysin, magnification X 100.

(Cases 2, 4, 10, 11). In single pathology group, following surgery 13/15 patients were free from disabling seizures, one reached Engel class 2 (Case 13) and one patient did not attain worthwhile improvement (Case 5) necessitating increase in dosage of the antiepileptic drugs. In dual pathology group 6/11 had freedom from seizures even after tapering drug dosage. Five cases attained Engel class 2-4 at 1 year after surgery (Cases 1, 3, 4, 10, 11) needing additional antiepileptic drugs or increase in dosage of antiepileptic drugs (Table 2). Clinical status maintained even at the end of 2 years in majority of the patients.

DISCUSSION

In patients with refractory CPS, recognition of the underlying epileptogenic lesion/foci is the key step in presurgical evaluation of the patients.[16] The existence of second pathology in the vicinity of temporal lobe might materially alter the clinical outcome to surgical resection. Surgical resection of the epileptogenic area can be curative or can provide significant amelioration of the seizure frequency in majority of individuals. The most common pathological findings in surgical specimens with refractory CPS are hippocampal sclerosis, with or without associated neoplastic or vascular lesions or cortical dysgenesis.[1] In a Meta analysis of 14 surgical series based on 876 patients with temporal or extra temporal lesions, Cascino *et al*[3] noted variable success rate following surgery. The variability of the surgical results probably reflect differing nature of the underlying lesions, the extent of their surgical resection and associated wide electro-physiological abnormalities. Surgical resection of a unilateral atrophic hippocampus renders more than 80% of

patients seizure free[16] while bilateral atrophy or lack of atrophy is found to be less favourable.[16-18] Favorable surgical outcome in lesional epilepsy is variable ranging in literature from 39 to 83%[3], incomplete removal of the epileptic focus being the main reason for poor surgical outcome.[3] Complete removal of the macroscopic lesion leads to seizure freedom or reduction in seizure frequency (Engel class I-II). In nearly 5-30% of patients with refractory partial epilepsy, MRI is found to disclose co-existence of hippocampal atrophy in addition to extrahippocampal lesion, defined as "dual pathology".[10,19-21] The most common forms of extrahippocampal lesions found in dual pathology are developmental abnormalities such as cortical dysgenesis of subtle to gross forms and gliotic lesions acquired during early child hood[21] and likely to be involved in seizure generation.[9] In various series favorable therapeutic results were obtained when both the atrophic hippocampal and extra hippocampal lesions were resected.[9,21-26] However, as the mesial temporal structures are involved in memory function, it is possible that just lesionectomy without extensive anatomical resection might offer potentially better neuropsychological outcome. Hence it is imperative that the influence of more extensive resection of mesial temporal structures needs to be assessed based on evaluation of neuropsychology, lifestyle needs of the subject and goals of surgery in each of the subject.

The present study focused on comparison of clinical, radiological and pathological features in patients with single and dual pathology. The association with early febrile seizures in both the groups did not alter the seizure onset or progression phenomenon. The neuroimaging revealed the hippocampal atrophy readily, but could not highlight dual pathology in the majority. This might have led to discordance with the electrophysiological studies. Neuropsychological assessment also pointed to bilateral involvement while MRI lateralized to one lobe. The surgical resection has not materially enhanced the neuropsychological score, when presurgical score was poor. Looney *et al* found that resection of left hippocampus without sclerosis was associated with greater risk of memory impairment post operatively while resection of markedly sclerosed hippocampus was less likely to have decreased memory post operatively.[27]

One interesting observation in our study is that in hippocampal subfields, CA3 zone is more severely affected in the dual pathology group. In CA3 monosynaptic recurrent excitatory circuits exist, normally controlled by more powerful recurrent inhibition. In temporal lobe epilepsy, depletion of these inhibitory neurons in CA3 region probably predispose to the risk of lower seizure threshold and recurrence.[28] Many animal studies have indicated that selective neuronal loss in hippocampus leads to synaptic re-organisation of the remaining neurons and play an important role in epileptogenesis.[29-31] Dystrophic neurons in the Ammon's horn and subcortical white matter were found in nearly half the patients with either single or dual pathology, while persistence of horizontal Ritzius neurons were more frequently found in patients with dual pathology.[32,33] These anomalous neurons could be contributing to the epileptogenesis. The presence of aberrant white matter tracts in hippocampus and temporal cortex as reported in literature[34,35] and noted in 3 of our patients could also be contributing to altered circuitry and seizure transmission.

The reactive astrocytosis, both in hippocampus and temporal lobe could be a reparative response to altered neurotransmitter environment, than causally related to seizure initiation and propagation in view of its important role in potassium and glutamate homeostasis.[36] Synaptophysin immunostaining has highlighted the synaptic reorganization in CA4 and CA1 sub fields and indirectly the mossy fiber sprouting in the supragranular molecular layer of the dentate gyrus as suggested by Proper *et al*.[37] These correlate with the degree of sclerosis, but may not reflect the exact duration of the disease. The clinical significance of synaptophysin immunostaining pattern and its quantitative assessment is not yet clear, though synaptic reorganization and propagation of electrical activity and kindling[37-39] has been suggested. Similarly immunoreactivity to dynorphin, an opoid neuropeptide in the granule cells of dentate gyrus has also been suggested to indirectly reflect synaptic re-organization in the mossy fiber pathway and sprouting[40] though they do not assist to trace individual recurrent mossy fibers. In the present study in the single pathology group almost 86% of patients became seizure free, while it is around 50% in dual pathology group, although both extra hippocampal lesion and the sclerosed hippocampus have been resected enblock in all the cases. The possible explanation could be that pathologically subtle neuronal dysplasias are electrically more extensive with ramification and synaptic connectivity and hence 'total' resection may not be possible with the current surgical procedure. However, even in patients with dual pathology, the currently used

surgical procedure provided reasonable degree of amelioration of seizure related morbidity. Further studies on a larger sample size correlating the hippocampal sclerosis with spectrum of extra hippocampal and extra temporal focal dysplastic, neoplastic and non-neoplastic lesions and various clinical parameters can provide more insight into the evolution of epileptogenesis, keeping in mind geographic variations and genetic polymorphism in patient population in different parts of the world.

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