

The Use of Proton MR Spectroscopy in Epilepsy: A Methodological Review

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Abstract

Magnetic Resonance Spectroscopy (MRS) has at least two major roles in the evaluation of epileptic patients. First, MRS can help to understand the interaction between seizures and metabolic function. Thus, MRS is particularly interesting for basic science studies of seizures and epilepsy. Second, MRS can explain the nature of seizure control and/or provide localization information by measuring metabolic changes. So MRS can be used as a powerful complementary technique to structural MRI for diagnosis and assessment of response to therapy, and measurement of disease progression. The aim of this paper is to review the methodological aspects of ¹H-MRS publications between 1994 to 2016, which utilized ¹H-MRS in lateralizing the epileptogenic zone in lesional and non-lesional Temporal Lobe Epilepsy (TLE), Extra-Temporal Lobe Epilepsy (ETLE), and generalized epileptic patients to help the spectroscopist, magnetic resonance imaging technologists, and radiologists to improve the overall diagnostic sensitivity in epileptic patients.

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1. Introduction

According to the World Health Organization (WHO), more than 50 million individuals are living with epilepsy, of whom nearly 80% are in middle- and low-income countries and do not get the proper treatment. Approximately 2.4 million individuals are diagnosed with epilepsy annually, and about 0.2% of all deaths in the world occur among this group of patients every year [1].

The first suggested treatment line for patients with epilepsy is Antiepileptic Drugs (AEDs). Although most

patients with generalized seizures and a considerable number of patients with partial epilepsy respond well to antiepileptic drugs, 20-30% of patients in the focal epilepsy category are resistant to the current medications [2]. Surgical resection of seizure foci is frequently one of the most effective treatments for this group of patients. Defining the exact resection margin is crucial in the surgical treatment of epilepsy to remove the epileptogenic zone as much as possible.

Pre-surgical planning for epilepsy surgery includes an extensive medical history, physical exam,

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Electroencephalogram (EEG) findings, and neuroimaging information. Structural and functional neuroimaging methods, including Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) have a key role in evaluating and selecting patients for surgery. However, advancements in imaging techniques such as Diffusion Tensor Imaging (DTI), MRI morphometry, Magnetic Resonance Spectroscopy (MRS), EEG-fMRI (functional MRI), Magnetoencephalography (MEG)/Magnetic Source Imaging (MSI), and EEG/ESI (Electronic Source Imaging) can improve surgical decision making noticeably [3].

Cellular and biochemical changes occur before structural damage can be detectable by conventional imaging. In addition, there are cases where conventional MRI cannot demonstrate structural changes. Methods such as MRS that discover biochemical changes in the tissue are perfectly suited to assessing such pathological changes even in the absence of morphological alterations. Therefore, MRS can be used as a powerful complementary technique to structural MRI for diagnosis, response to therapy and measurement of disease progression.

Proton Magnetic Resonance Spectroscopy (^1H -MRS) identifies hydrogen atoms and uses the same principles of physics as MRI to obtain the biochemical information of tissue metabolites by detecting their resonance frequencies, referred to as chemical shift. MRS can measure concentrations of different metabolites in various structures and different pathological situations of the brain to generate sensible and reliable results.

MRS has at least two major roles in the evaluation of epileptic patients. First, MRS can help to understand the interaction between seizures and metabolic function through the study of the relationship between the clinical characteristics of seizures and ^1H -MRS alternations. Therefore, MRS is particularly interesting for basic science studies of seizures and epilepsy [4, 5]. Second, MRS can explain the nature of seizure control and/or provide localization information by measuring metabolic changes [6, 7]. On the other hand, no study has reviewed the literature based on methodology and its contribution to ^1H -MRS results in epilepsy although there is

significant methodological variability among the relevant studies. Therefore, this paper is designed in such a way that it can provide an overview of the findings of 99 ^1H -MRS studies on patients with epilepsy based on various methodological and technical aspects, including the strength of the magnetic field, MRS sequence, and absolute concentration versus metabolite ratios.

2. Methodological Considerations for ^1H -MRS Studies

2.1. Literature Review

We obtained 250 papers through the electronic databases of Medline/PubMed and ScienceDirect from 1994 to 2016 (Table 1, 2, 3). The search terms were epilepsy, Temporal Lobe Epilepsy (TLE), generalized epilepsy, Juvenile Myoclonic Epilepsy (JME), hippocampus, Frontal Lobe Epilepsy (FLE), Hippocampal Sclerosis (HS), and correlation, which were also combined with proton magnetic resonance spectroscopy, proton MR spectroscopy, ^1H MRS, neuroimaging, and EEG. We excluded studies which included children or had already been published in pediatric journals and included studies on patients with HS lesions on MR imaging. Two reviewers studied the titles and abstracts of the studies and excluded some papers because of a lack of necessary criteria for patients or methodology and studied 107 papers based on methodological aspects. A summary of the details of all included studies was tabulated into a spreadsheet. Then, these studies were classified into three groups (generalized epilepsy, TLE, ETLE researches) and seven methodological factors were extracted from them.

2.2. MRS in Epilepsy

The most popular metabolites studied in epileptic patients include N-Acetyl Aspartate (NAA, 2.01 p.p.m.), Creatine (Cr, 3.0 p.p.m.), and Choline (Cho, 3.2 p.p.m.). NAA is considered as a neuronal and axonal integrity marker; Cr and Cho are found in neurons and glia. On the other hand, Cr is a key component of Phosphocreatine (PCr) and it can be considered a normalization factor for the bioenergetics parameter [8].

Several groups have suggested that MRS can be used as a biomarker to assess the mechanism of treatment failure and may have a role in the pre-surgical evaluation of refractory Temporal Lobe Epilepsy (TLE), especially when conventional MRI cannot reveal any abnormality [9-11]. Cendes *et al.* performed Magnetic Resonance Spectroscopy Imaging (MRSI) and volumetric analysis in 100 refractory TLE patients. They found that the combination of these two methods can lateralize TLE accurately in the great majority of patients (90% by combining the two methods, 86% using MRSI, 83% using MR volumetry, and 93% by EEG) [10]. Moreover, ¹H-MRS studies on MRI-negative patients have provided evidence of reduced NAA/Cr and/or NAA/Cho ratios in the seizure focus, e.g. thalamic and frontal lobe in Idiopathic Generalized Epilepsy (IGE) and Frontal Lobe Epilepsy (FLE) patients, respectively [5, 12, 13].

On the other hand, ¹H-MRS is a non-invasive and sensitive method to help detecting subtle variations for Extra-Temporal Lobe Epilepsy (ETLE) patients even in the absence of structural alterations [10, 11]. Moreover, it has been shown that ¹H-MRS has successfully identified processes of epileptogenesis in Juvenile Myoclonic Epilepsy (JME), which was characterized by a reduction of NAA levels in the thalami and prefrontal cortex of this group of patients [14]. ¹H-MRS was able to reveal elevated levels of Glutamate (Glu) and Glutamine (Gln) (Glu+ Gln= Glx) and Gamma-Aminobutyric Acid (GABA) in the frontal and occipital lobes of IGE patients [15, 16].

2.3. General Considerations

There are only a few factors which affect the information content of the proton spectra of the brain, including the type of the pulse sequence, TE, and field strength. Despite the use of ¹H-MRS as a non-invasive method for investigating brain neurochemistry for understanding psychiatric and neurological diseases, a low Signal-to-Noise Ratio (SNR) has limited its use in clinical investigations. On the other hand, due to an approximately linear increase in the noise voltage with frequency, it is expected that SNR increases linearly with B₀. In recent years, thanks to the increased field strength of scanners and improved acquisition techniques and analysis approaches, ¹H-MRS has gained the support and

interest of the MR and clinical neuroscience communities [8].

At 1.5 T field strength with long TE, signals from Cr, Cho, and NAA are easily detectable in a normal brain where other chemical compounds such as Alanine and Lactate (Lac) may be observable due to some pathological processes. At short TE, chemical compounds such as Glutamate (Glu), Glutamine (Gln), GABA, Myo-Inositol (mI), lipids and macromolecules can be observed. After increasing the field strength to 3, 4, and 7 T, spectral resolution increases and more components can be recognized with high confidence [8].

Currently, multi-voxel MRS (Chemical Shift Imaging (CSI) or MRSI) and Single-Voxel Spectroscopy (SVS) are two methods of obtaining the spatially localized biochemical information in epilepsy. SVS is a simple and rapid image acquisition technique (2-4 min), but has some limitations such as the voxel position. The SVS voxel should contain the lesion correctly during patient examination. In other words, it should be carefully positioned on the tissue. MRSI can help overcome this limitation by changing the grid to fit precisely over the desired lesion any time during data acquisition. MRSI is important in obtaining spatial information on small structures like amygdala or hippocampus, and SVS is preferred when comparing a pathological zone with a contralateral region. Therefore, whether to choose SVS or MRSI depends heavily on the aim of the study (See [8] for more details).

Both types of localization techniques can use various types of pulse sequences. Point Resolved Surface coil Spectroscopy (PRESS) and Stimulated Emission Acquisition Mode (STEAM) are the two most common pulse sequences that the single-voxel technique uses. PRESS excitation is the most common method for two or three-dimensional MRSI. For more information about PRESS, see [17], and for a detailed comparison between STEAM and PRESS, see [18].

Two parameters, TE and TR are effective on the ¹H-MRS results and should be determined based on the desired metabolite. MR spectra at short TE (based on our knowledge from ¹H-MRS studies in epilepsy “20-72 ms”) involve broad macromolecule resonances signals that decay completely after 60-80 ms because of their

short T2 relaxation time. However, spectra obtained at short TE values are richer in information because MR spectra obtained using short TE provide more metabolite signals, including Glx or mI (increased Glx levels may reflect seizure activity). The quantification of long TE (107-272 ms) acquisitions is less sensitive to measurement and tissue conditions compared to signals measured at short TE values. SNR and then quantification shall be improved by using a long TR (>3 seconds, about 3000-6000 ms), whereas examination time increases. The typical in-vivo TR value for clinical MRS is in the range of 1-3 sec. Nevertheless, it is necessary to use long TR by keeping TE as short as possible, so that T1 and T2 relaxation do not affect signal intensities. Therefore, choosing between short and long TE along with appropriate TR depends on the aim of the study; for example, if only NAA, Cho, and Cr need to be quantified, ¹H-MRS must be performed at long TE to prevent the effects of macromolecular signals on the quantification procedure [8].

2.4. Methodological Review of Hippocampal ¹H-MRS Studies in TLE

There are methodological differences in the hippocampal ¹H-MRS in TLE studies published to date. Our investigation of 78 papers showed that they had all been carried out with different MR scanners (e.g. Philips, General Electric, Siemens) running at a field strength of 1.5 Tesla (T), except for 18 of them: four studies used 2 T equipment [4, 19-21], seven studies used 3 T [22-28], three studies used 4 T [29-31], four studies used 4.1 T [9, 32-34], and one study used 7 T [35]. Most of these studies used the acquisition technique of SVS with the PRESS sequence and long TE in the range of 107-288 ms and TR between 1500 and 5000 ms, whereas four studies preferred the STEAM sequence [21, 23, 24, 36] and one study used 3D Echo Planar whole brain Spectroscopic Imaging (EPSI) [30].

One of the most important issues in hippocampal ¹H-MRS studies in TLE patients is the size of investigated voxels, ranging from 1 cm³ to 6 cm³. Because of the hippocampal structure in which the middle part and the tail end are considerably thinner than the edge lengths, the voxel size often contains extra tissue beyond the hippocampus, which can lead to partial volume effects.

Chang *et al.* obtained spectra from hippocampal areas bilaterally with VOIs of 2.25 cm³ and 6 cm³ in healthy and patients groups. They found that the overall lateralization ability of MRS was 72% and the correct lateralization rate was 82% for the VOI of 2.25 cm³ (1.5×1.5×1 cm³) and 62% for the VOI of 6 cm³ [37]. Moreover, tissue heterogeneity in the selected VOIs is also another important factor which has been addressed in several studies. The differences in the concentration of metabolites between Gray Matter (GM) and White Matter (WM) [38], in medial and lateral temporal lobe [39], and at different positions along the hippocampus [39-41] are those relevant examples. Table 1 shows a comparison of methodological aspects for hippocampus in TLE patients. Based on our current knowledge, the two most popular voxel sizes are 2×2×2 cm³ and 1.5×1.5×1.5 cm³, and the most popular sequence is PRESS with long TE but as discussed above, the choice between short and long TE along with appropriate TR depends on the aim of the study.

On the other hand, the literature has usually suggested ¹H-MRS as a supplementary tool in the interictal localization of seizure foci. In six studies the possible role of this modality was studied in the ictal and postictal phases [42-47]. F. Fadaie *et al.* showed significant decreases in the NAA/Cr, NAA/Cho, and NAA/Cr+Cho ratios immediately after ictus in the ipsilateral hippocampus compared with the contralateral hippocampus of patients and controls. They showed that the metabolite ratios in the interictal phase of the three groups were not significantly different [42]. Maton *et al.* showed that in patients with TLE, there were no significant differences in metabolite ratios in the ictal versus the interictal phase [44]. The post-ictal period of MRS acquisition might be one factor that affects the results in such studies. Simister *et al.* showed that change in Cr144/Cr30 and NAA144/Cr144 ratios correlated with the post-ictal interval [46].

Mohammadi *et al.* suggested an interesting approach for the quantification of MRS data. They employed a dimension reduction technique for the spectral fitting of the MR spectroscopy data with short TE (=30 ms) and showed that the sensitivity and specificity of this modality in TLE patients are 60% and 82%, respectively [48].

Table 1. Comparison of methodological aspects (¹H-MRS protocols, analysis software) for hippocampus in TLE patients

#	Study Author (year)	Strength of Magnetic Field/Vendor (Tesla)	TE/TR (ms)	¹ H-MRS Sequence	Localization Techniques	Metabolic Result	Quantification Software
1	F. Riederer et al. (2006)	3 Bruker Medspec	20/2500	STEAM	SVS	NAA, Glx, Glu, Gln, mI, mI/Cr, Cho, tCr	LCModel
2	F. Riederer et al. (2007)	3 Bruker Medspec	20/2500	STEAM	SVS	NAA	LCModel
3	M. Brazdil et al. (2009)	1.5 Siemens	80/1500	PRESS	SVS/CSI	NAA, NAA/Cr, NAA/Cr+Cho	LCModel
4	D. Fojtiková et al. (2007)	1.5 Siemens	80/1500	-/PRESS	SVS/CSI	NAA, NAA/Cr, NAA/Cr+Cho	LCModel
5	M. Hajek et al. (2009)	1.5 Siemens	135/5000	PRESS	SVS	NAA/Cr, NAA/Cho, NAA/Cr+Cho, Cho/Cr	LCModel
6	L. Hanoglu et al. (2004)	1.5 GE	144/1500	PRESS	SVS	NAA/Cho+Cr	-
7	H. Aydin et al. (2012)	1.5 Philips	26, 144/1500	PRESS	CSI	Lac, phosphocreatine, NAA, Cr, Cho, mI, Glx, NAA/Cr, NAA/Cho+Cr, Cho/Cr	Philips software
8	E. Achten et al. (1998)	1.5	135/1600	-	SVS	NAA/Cho+Cr	-
9	E. Achten et al. (1997)	1.5	135/1600	-	SVS	NAA/Cho+Cr, NAA/Cr, NAA/Cho, Cho/Cr, NAA	-
10	G. Lantz et al. (2006)	1.5 Eclipse, Philips	288/1600	PRESS	SVS	NAA/Cho+Cr, NAA/Cr,	SAGE spectroscopy

						NAA/Cho, Cho, Cr, NAA	analysis software
11	F. Zubler <i>et al.</i> (2003)	1.5 Marconi Medical	270/1600	PRESS	SVS	NAA/Cho+Cr	SAGE spectroscopy analysis software
12	C. O. Duc <i>et al.</i> (1998)	1.5 Philips	136/6000	PRESS	SVS	NAA, Cr, Cho	DEC workstations using a home-built processing software based on DI 3000
13	G. R. Ende <i>et al.</i> (1997)	1.5 GE	135/1800	PRESS	CSI	NAA/Cho+Cr, NAA/Cr, NAA/Cho, Cho, Cr, NAA	software provided by Siemens (LUISE)
14	P. Vermathen <i>et al.</i> (2002)	1.5 Siemens	135/1800	PRESS 2D	CSI	NAA, Cr, Cho	-
15	P.A. Garcia <i>et al.</i> (1997)	2 Philips	-	3D	CSI	NAA/Cr	NMR1(NewMethods research, Syracuse,NY, USA)
16	S. G. Mueller <i>et al.</i> (2011)	4 Siemens	45/1750	3D echo planar whole brain spectroscopic imaging (EPSI)		NAA/Cho+Cr	SPM2
17	J. W. Hugg <i>et al.</i> (1993)	2 Philips	272/1700	3D PRESS	CSI	NAA, Cho, Lac	-
18	A. A. Capizzano <i>et al.</i> (2002)	1.5 Siemens	135/1800	PRESS 2D,3D	CSI	NAA, NAA/Cr+Cho	-
19	A. A. Capizzano <i>et al.</i> (2001)	1.5 Siemens	135/1800	PRESS	CSI	NAA	software developed in our laboratory
20	P. Vermathen <i>et al.</i> (2000)	1.5 Siemens	-		CSI	NAA/Cho+Cr, NAA, Cr, Cho	LUISE; Siemens
21	P. Vermathen <i>et al.</i> (2003)	1.5 Siemens	140/1800	-	CSI	NAA/Cho+Cr	Luise Siemens

22	J. W. Pan <i>et al.</i> (2013)	7 Varian	40/1500	gradient-based slice-selective excitation (10-mm thick slices)	CSI	NAA/Cr	-
23	D. C. Spencer <i>et al.</i> (2005)	1.5 GE	144/1500	PRESS	CSI	NAA/Cr	AGE (SAGE Dev 2000.3, Minneapolis, MN, USA)
24	J.G. Burneo <i>et al.</i> (2004)	4.1	50/2000	-	CSI	NAA/Cr	NMR1 (New Methods Research, Syracuse, NY, USA)
25	J. J. Shih <i>et al.</i> (2011)	1.5	135/1500	PRESS	SVS	NAA/Cho	Levenberg–Marquardt algorithm
26	J.J. Shih <i>et al.</i> (2004)	1.5 Picker edge	135/1500	PRESS	SVS	NAA/Cho	-
27	A. A. Cohen-Gadol <i>et al.</i> (2004)	4 Varian Inova	-	-	CSI	NAA/Cr	-
28	D. Lu <i>et al.</i> (1997)	1.5 GE	136/1600	PRESS	SVS	NAA/Cr+Cho	-
29	Sun-Won Park <i>et al.</i> (2001)	1.5	144,288/1500-2000	PRESS	SVS	NAA/Cr, NAA/Cho	-
30	Kee-Hyun Chang <i>et al.</i> (2000)	1.5 GE	136/1500	PRESS	SVS	NAA/Cr, NAA/Cho	-
31	S. K. Lee <i>et al.</i> (2005)	1.5 GE	136/1500	PRESS	SVS	NAA/Cr, NAA/Cho	In house using Levenberg–Marquardt algorithm
32	L. C. Meiners <i>et al.</i> (2000)	1.5	136/2000	PRESS	SVS	NAA/Cr, NAA/Cho	MRUI software
33	M.T. Doelken <i>et al.</i> (2008)	1.5 Siemens	30/3000	PRESS	SVS	tNAA, Cho, Cr, Glx, mI	LCModel

34	M.T. Doelken et al. (2007)	1.5 Siemens	30/3000	PRESS	SVS	tNAA, Glx, Cho, Cr, mI, NAA/Cho+Cr	LCModel
35	Th. Hammen et al. (2003)	1.5 Siemens	135/1500	-	CSI	NAA/Cho+Cr	MAGNETOM Vision system
36	E. Pauli et al. (2000)	1.5 Siemens	135/1500	2D CSI spin-echo protocol	CSI	NAA/Cho	Magnetom Vision system
37	E. Duzel et al. (2004)	1.5 GE	35/1500	PRESS	SVS	NAA/Cho+Cr	AMARES program
38	C. G. Choi et al. (1999)	2 Siemens	20/6000	STEAM	SVS	tNAA, tCr, Cho, mI	LCModel
39	Th. Hammen et al. (2007)	1.5 Siemens	30/3000	PRESS	SVS	tNAA, Cho, Cr, Glx, mI, NAA/Cho+Cr, NAA/Cr, tNAA/Cr	LCModel
40	Th. Hammen et al. (2008)	1.5 Siemens	30/3000	PRESS	SVS	tNAA mI, tNAA/Cr	LCModel
41	T. C. Ng et al. (1994)	1.5 Siemens	135/1500	double-echo pulse sequence	CSI	NAA/Cho	SA/GE software; GE Medical Systems, Milwaukee, Wis
42	Th. Hammen et al. (2006)	1.5 Siemens	30/3000	PRESS	SVS	Glx, mI, Cho, Cr, tNAA	LCModel
43	R. J. Simister et al. (2009)	1.5 GE	30/3000	PRESS	SVS	tNAA/Cr, Glx/Cr, GABA+/Cr	LCModel
44	F. G. Woermann et al. (1999)	1.5 GE	30/3000	PRESS	SVS	NAA, Cr, Cho, mI, Glx, NAA/Cr+Cho, NAA/mI, NAA/Glx	LCModel
45	A. Connelly et al. (1998)	1.5 Siemens	135/1600	PRESS	SVS	NAA, Cho, Cr, NAA/Cho+Cr	-
46	R. J. Simister et al. (2008)	1.5 GE	30,144/3000	PRESS	SVS	tNAA, Cr, Lac, Cho, mI, Glx, tNAA/Cr,	LCModel

						Glx/Cr, Cr144/Cr30	
47	R. J. Simister et al. (2002)	1.5 GE	30/3000	PRESS	CSI	tNAA, Glx, Cho, Cr, mI, tNAA/Cr, tNAA/Cho+Cr, tNAA/mI	LCModel
48	W. Serles et al. (1999)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
49	F. Cendes et al. (1997)	1.5 Philips	272/2000	PRESS	CSI	NAA/Cr	-
50	F. Cendes et al. (1995)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
51	F. Cendes et al. (1994)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
52	A. Bernasconi et al. (1999)	1.5 Philips	-	-	CSI	NAA/Cr	-
53	L. M. Li et al. (2000)	1.5 Philips	272/2000	-	CSI	NAA/Cr	locally developed software
54	L. M. Li et al. (2000)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
55	J. Zhang et al. (2014)	1.5 Siemens	107/4500	PRESS	SVS	NAA, Cho, Cr, NAA/Cr, NAA/Cho+Cr	software provided by Siemens on the Siemens MRI scanner
56	J Shen et al. (2009)	3 Philips	31/2000	2D PRESS	CSI	NAA/Cho+Cr, mI/Cr, Glx/Cr	the Intera Achieva 3.0 T MRI system
57	J. Qi et al. (2007)	2 Gyrex SGR	135/2000	PRESS	SVS	NAA/Cho+Cr, Cho/Cr, NAA/Cr	-
58	M. F. Chernov et al. (2009)	1.5 Toshiba	128,136/2000	-	SVS	NAA, Cho, Cr	software provided by the supplier (MRS-PRO/PX; Toshiba Medical Systems)

59	Y. Someya et al. (2000)	1.5 Philips	136/1500	-	-	NAA/Cho+Cr	-
60	S. Kikuchi et al. (2001)	1.5 GE	30/2000	PRESS	SVS	NAA/Cr	SA/GE, General Electric, WI, USA
61	T. Obata et al. (2004)	1.5 Philips	136/1500	-	SVS	NAA/Cho+Cr	-
62	M. Guye et al. (2002)	1.5 Siemens	136/1500/// 135/1600	2D in-house-designed acquisition-weighted/// PRESS	CSI/SVS	NAA/Cho+Cr	AMARES program
63	I. J. Namer et al. (1999)	3 Bruker	135/2000	PRESS	SVS	NAA/Cho+Cr	software package Paravision/UX NMR (Bruker)
64	M. A. S. Mantoan et al. (2009)	1.5 Siemens	135/1500	PRESS	SVS	NAA/Cho+Cr	(Leonardo, Syngo MR 2004 A
65	R. S. Briellmann et al. (2007)	3 GE	30/3000	PRESS	SVS	NAA, Cr, Cho, mI, Glx	LCModel
66	P. T. Meyer et al. (2001)	1.5 GE	35/2000	PRESS	SVS	NAA/Cr	-
67	H. Hetherington et al. (1995)	4.1	50/2000	-	CSI	Cr/NA, Cho/NA	NMR1 (Tripos, Syracuse, NY)
68	R. Kuzniecky et al. (1998)	4.1	50/2000\\ 58/2000	2D PRESS	SVS/CSI	Cr/NA	-
69	S. M. Sawrie et al. (2001)	4.1	50/2000	PRESS	CSI	Cr/NA	-
70	B. Maton et al. (2001)	1.5 Philips	272/1500	-	CSI	NAA/Cho+Cr	NMR1 (Tripos, Syracuse, NY)
71	J. W. Pan et al. (2012)	4 Varian Inova	72/2000	-	CSI	NAA/Cr	-

72	K. N. Fountas et al. (2012)	3 GE	35/1500	PRESS	SVS	NAA/Cr, NAA/Cho, NAA/Cho+Cr	-
73	M. Castillo et al. (2001)	1.5	270,135/1500	PRESS	SVS	NAA/Cr	-
74	J. A. Mendes-Ribeiro et al. (1998)	1.5 Siemens	270/3000	STEAM	SVS	NAA/Cr, NAA/Cho, Cho/Cr	lack the appropriate software
75	F. Fadaie et al. (2016)	1.5 Siemens	135/1200	PRESS	CSI	NAA/Cr+Cho, NAA/Cho, NAA/Cr	Subtract_QUESTION algorithm
76	J. Zhang et al. (2014)	1.5 Siemens	107/4500	PRESS	SVS	NAA, Cr, Cho, NAA/(Cho + Cr), NAA/Cr	software provided by Siemens on the Siemens MRI scanner
77	M. Y. Xu et al. (2015)	3 GE	35/1500	PRESS	SVS	NAA, Cr, Cho, NAA/(Cho + Cr), NAA/Cr	-
78	R. MarkWeldlard et al. (2003)	1.5 GE	35/1500	PRESS	SVS	NA, Cho, Cr, Glx, ml	LCModel

Table 2. Comparison of methodological aspects (¹H-MRS protocols, analysis software) in ETLE patients

#	Study Author (year)	Strength of Magnetic Field/Vendor (Tesla)	TE/TR (ms)	¹ H-MRS Sequence	Localization Techniques	Metabolic Result	Quantification Software
1	P. Krsek et al. (2007)	1.5 Siemens	135/1500	PRESS	CSI	NAA/Cho, NAA/Cr, Cho/Cr	LC-Model
2	M. Guye et al. (2005)	1.5 Siemens	136/1500	2D, 1D in-house-designed acquisition	CSI	NAA/Cr, NAA/Cho+Cr	AMARES
3	J. W. Pan et al. (2013)	7 Varian	40/1500	n-weighted gradient-based slice-	CSI	NAA/Cr	-

				selective excitation			
4	P.A. Garcia at al. (1997)	2 Philips	-	3D	CSI	NAA/Cr	NMR1(NewMethods research, Syracuse,NY, USA)
5	P. A. Garcia at al. (1995)	2 Philips	-	-	CSI	NAA/Cr, Cho/Cr	NMR1(NewMethods research, Syracuse,NY, USA)
6	S. G. Mueller at al. (2004)	1.5 Siemens	135/1800	-	CSI	NAA/Cr, NAA/Cho	A fully automated spectral-fitting software package developed in this laboratory
7	R. J. Simister at al. (2003)	1.5 GE	30/3000, 68/2000	PRESS, DQF sequence was applied to the same PRESS-localised volume	SVS	tNAA, Glx, Cho, Cr, mI, GABA+, tNAA/Cr, Glx/mI	LCModel
8	R. J. Simister at al. (2008)	1.5 GE	144,30/3000	PRESS	SVS	tNAA, Cr,Lac, Cho, mI, Glx, tNAA/Cr, Glx/Cr, Cr144/Cr30	LC-Model
9	J. W. Pan at al. (2012)	4 Varian Inova	72\2000	-	CSI	NAA/Cr	-
10	W. Serles at al. (1999)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
11	L. M. Li at al. (2000)	1.5 Philips	272/2000	-	CSI	NAA/Cr	-
12	J. A. Stanley at al. (1998)	1.5 Philips	272/	2D	CSI	NAA/Cho, NAA/Cr, Cho/Cr, Lac/Cr, NAA/Cr+Cho	Fitmasters program, Philips Medical Systems, Best, The Netherlands
13	R. A. A. Leite at al. (2013)	1.5 GE	135/1500	PRESS	CSI	NAA/Cho, NAA/Cr, NAA/Cho+Cr, Cho/Cr	work station Sun 60–Ultrasparc

14	D. Fojtiková et al. (2007)	1.5 Siemens	80/1500	-/PRESS	SVS/CSI	NAA, NAA/Cr, NAA/Cr+Cho	LCModel
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Table 3. Comparison of methodological aspects (¹H-MRS protocols, analysis software) in generalized epileptic patients

#	Study Author (year)	strength of magnetic field/vendor (Tesla)	TE/TR (ms)	¹ H-MRS sequence	localization techniques	Metabolic result	Quantification software
1	A. J. Ristić et al. (2011)	1.5 Siemens	135/1500	3D PRESS	CSI	Cho/tCr, NAA/tCr, NAA/Cho, NAA/Cho+tCr	Syngo Multi-Modality Workplace version VE23A
2	A. Bernasconi et al. (2003)	1.5 Philips	272/2000	-	CSI	NAA/Cr	in-house software
3	D. Fojtikova et al. (2006)	1.5 Siemens	80/1360	-	CSI	NAA/Cr	software supplied by the scanner producer
4	S. B. Mory et al. (2003)	2 Elscint Prestige	135/1500	PRESS	SVS	NAA/Cr	software supplied by the machine manufacturer
5	K. Lin et al. (2009)	1.5 Siemens	30/1500	2D PRESS	CSI	NAA/Cr, Glx/Cr	Syngo MR Spectroscopy Evaluation Programme—Syngo v.2004A
6	I. Savic et al. (2000)	1.5 GE	30/6000	STEAM	SVS	NAA, Cho, Cr, mI	LCModel
7	I. Savic et al. (2004)	1.5 GE	30/6000	STEAM	SVS	NAA, Cho, Cr, mI	LCModel
8	M.T. Doelken et al. (2010)	3 Siemens	30/1700	2D PRESS	CSI	tNAA, Glx, Cho, Glu, mI	LCModel
9	C. Haki et al. (2007)	1.5 Siemens	135,270/1500	PRESS	SVS	NAA, NAA/Cr	software provided by Siemens
10	S. C. Kabay et al. (2010)	1.5 GE	144/1000	2D PRESS	CSI	NAA, NAA/Cr	Advanced function software by GE Healthcare

11	R. J. Simister et al. (2003)	1.5 GE	30/3000	PRESS, the DQF sequence	SVS	tNAA, Glx, Cho, Glu, mI, tNAA/Cr, Glx/tNAA, Glx/mI	LCModel
12	D. Flügel et al. (2006)	1.5 GE	30/3000	PRESS	SVS	Cho, Cr, mI, NAA, Glx, Macromolecules	LCModel
13	R. MarkWellard et al. (2003)	1.5 GE	35/1500	PRESS	SVS	NA, Cho, Cr, Glx, mI	LCModel

2.5. Methodological Review of Extra-hippocampal ¹H-MRS Studies in TLE

We studied 16 papers in this area. In some studies, authors have examined a set of brain structures and regions that are anatomically and functionally associated with the epileptogenic zone (hippocampus) in TLE patients. This network includes the thalamus, temporal pole, basal ganglia, and insula. There are no significant differences in the reported methodology of ¹H-MRS studies regarding extra-hippocampal metabolite abnormalities in patients with TLE. All studies have used a 1.5 T scanner except for three [26, 29, 30]. One study has employed the EPSI sequence [30] whereas the others have used the PRESS sequence to obtain spectra from regions. The values of TE and TR used here are TE = 30-270 ms, and TR is between 1500-2000 ms.

2.6. Methodological Review of ¹H-MRS Studies in Extra-Temporal Lobe Epilepsy

In all patients in such studies (13 papers), no structural lesions were found on the MRI scans, but based on EEG the diagnosis was lateralized and localized in the parietal lobe or the frontal lobe and in two studies the occipital lobe (Table 2 # 7, 8).

All studies were conducted at 1.5 T [16, 46, 49-55] except four [4, 5, 29, 35]. Four of thirteen examinations used short TE in the range of 30-72 ms (Table 2 # 3, 9, 7,

8). All studies obtained spectra from the MRSI method except two of them (SVS with voxel size of 4×3.5×2.5 cm) (Table 2 # 7, 8).

2.7. Methodological Review of ¹H-MRS Studies in Generalized Epilepsy

All studies (13 papers) in this section examined subjects with IGE, including nine studies which examined JME patients [12, 14, 15, 56-61], three which examined Juvenile Absence Epilepsy (JAE) patients [15, 60, 62], and four which examined patients with Generalized Tonic-Clonic Seizures (GTCS) [12, 13, 15, 56].

There are some methodological differences in ¹H-MRS studies in generalized epilepsy studies. All studies were performed at 1.5 T, except two which used a 2 T scanner and a 3 T scanner [13, 59]. Two out of eleven studies used the STEAM sequence [56, 57], six used the MRSI technique and five studies obtained spectra from the SVS technique to examine patients. The values of TE and TR used here are: short TE: 30 and 80 ms, long TE: 135-272 ms, and TR: between 1000-6000 ms. Table 3 shows a comparison of methodological aspects in generalized epileptic patients. According to current knowledge, the most popular sequence for these studies is PRESS.

2.8. Review of Alterations of Neuro-metabolites in Patient with Epilepsy

The most important metabolite ratios in the study of TLE patients are NAA/Cr, NAA/Cr+Cho, and NAA/Cho [42, 63-85]. These three ratios decrease in the hippocampal and extra-hippocampal areas in TLE patients, which show neuronal loss or damage in that area, and may signify structural abnormality. In the second level, alterations of neuro-metabolite ratios are Cho/Cr [36, 65], (total N-acetyl aspartate) tNAA/Cr [39, 46, 86], tNAA/Cr+Cho, tNAA/mI [39], GABA plus homocarnosine (GABA+)/Cr [87], Glx/Cr [46, 87], NAA/mI, NAA/Glx [88], mI/Cr [22, 23]. Studies regarding ¹H-MRS in TLE patients with HS showed that there is a decrease in NAA and an increase in Cho, Cr, and mI concentrations ipsilateral to the seizure focus. Reduced hippocampal NAA and elevated Glx values have also been reported in MRI-negative patients [89-93].

The most common metabolite ratio in patients with ETLE is NAA/Cr (Table 2 # 1-6, 9-13). Other metabolite ratios in such patients are: Cho/Cr (Table 2 # 1, 5, 12, 13), NAA/Cho (Table 2 # 1, 6, 12, 13), NAA/Cr+Cho (Table 2 # 2, 12, 13), tNAA/Cr, Glx/mI and Glx/Cr (Table 2 # 7, 8), and Lac/Cr (Table 2 # 12). Reduced NAA/Cr or NAA/Cho ratios have been found in the seizure focus in FLE patients, which may help to lateralize the epileptogenic zone in MRI-negative FLE patients. Metabolic abnormalities in ¹H-MRS have also been identified in areas away from the seizure focus where there is no evidence of a lesion on MRI, and it is not clear whether this reflects widespread pathology without an MRI-evident lesion or a functional consequence of a seizure focus. tNAA, Glx, Cho, Cr, mI, GABA+, Lac concentrations in ETLE patients has been reported by R. J. Simister and his colleagues (Table 2 # 7, 8). In this study, IGE patients showed increased Glx and GABA+.

The NAA/Cr ratio is the most important neuro-metabolite ratio in patients with generalized epilepsy (Table 3 # 2-5, 9, 10). A. J. Ristić and colleagues reported Cho/tCr (Creatine-Phosphocreatine), NAA/tCr, NAA/Cho, and NAA/Cho+tCr ratios (Table 3 # 1). tNAA/Cr, Glx/tNAA, Glx/mI ratios have been reported by R. J. Simister and colleagues (Table 3 # 11). K. Lin and his colleagues reported Glx/Cr ratio (Table 3 # 5). Absolute neuro-metabolite concentrations in patients

with generalized epilepsy are NAA (Table 3 # 6, 7, 9, 10), Cho (Table 3 # 6-8, 11), Cr (Table 3 # 6, 7), mI (Table 3 # 6-8, 11), tNAA, Glx, and Glu (Table 3 # 8, 11).

3. Correlations Studies

3.1. The Relationship between Histopathologic Finding and ¹H-MRS Alterations

MRS studies regarding histopathologic correlations in TLE showed that the observed NAA, NAA/Cr, tNAA, mI, NAA/Cho changes have a linear regression relationship with reduction in neuronal populations [31, 49, 94-96]. To address this, T. Hammen *et al.* studied 23 patients with unilateral TLE. They found a positive correlation between tNAA reduction and neuronal density in the dentate gyrus ($p=0.006$), hippocampal Cornu Ammonis (CA)1, CA3, and CA4 subfields ($p<0.001$, 0.015, 0.031, respectively) and a negative correlation between mI and neuronal loss in the hippocampal CA4 subfield ($p=0.028$) [95].

3.2. The Relationship between EEG Finding and ¹H-MRS Alterations

The results regarding correlations between the distribution of metabolic alterations and interictal EEG spikes showed a moderate level of concordance in the lateralization of TLE and ETLE patients [10, 44, 52, 53, 86, 97, 98]. T. Hammen *et al.* found that tNAA values and the degree of Interictal Epileptiform Discharges (IEDs) in EEG ($p=0.04$) have a negative correlation. However, there was a positive correlation between Cr and the duration of the seizure ($p=0.01$), which represents the relationship between the duration of the seizure and increased levels of Cr. The results of this study showed that the degree of tNAA reduction is probably indicative of neuronal dysfunction [86]. H. Aydin *et al.* also suggested the MRSI sensitivity index for lateralization in the left and right hippocampi, which were 100% and 92% respectively according to EEG findings [98].

3.3. The Relationship between Cognitive Performance and ¹H-MRS Alterations

The underlying pathology, seizures, medication, and IEDs are a number of factors that correlate with cognitive dysfunction in refractory TLE. Some studies have been carried out to investigate the correlation between memory function and $^1\text{H-MRS}$ findings and to characterize cognitive dysfunction in TLE patients with HS [25, 32, 64, 99-102]. To address this, M. A. Mantoan *et al.* assessed 29 patients and 24 controls and found a negative correlation between Intelligence Quotient (IQ), Rey Auditory Verbal Learning Test results, NAA/(Cho+Cr) ratio and epilepsy duration ($r=0.551$, $p=0.031$; $r=0.585$, $p=0.022$, $r=0.569$, $p=0.027$, respectively) in the left mesial temporal sclerosis group [101]. I. J. Namer *et al.* showed that there is no correlation between the NAA/(Cho+Cr) ratio or the T2 relaxation measurement alone and memory performances, whereas it has been declared that the combination of T2 relaxation time value and NAA can be used to examine the relationship between hippocampal dysfunction and memory performances before surgery for the evaluation of patients with TLE with MRI signs of HS [25].

3.4. The Relationship between MR Volumetry and $^1\text{H-MRS}$ Alterations

MR volumetry demonstrates volume loss in the hippocampus, which is an MRI abnormality in TLE patients with HS. Six studies investigated the relation between $^1\text{H-MRS}$ neuro-metabolites and MR volumetry in TLE patients with or without HS [9, 10, 32, 103-105]. E. Duzel *et al.* examined 22 patients with MR abnormality and normal MR. They found that hippocampal volumetry results did not correlate with the Apparent Diffusion Coefficient (ADC) value, NAA/(Cho+Cr), and the duration of the disease in any of the patients (with or without HS) or controls. There was a negative correlation between the NAA/(Cho+Cr) ratio and ADCs in the ipsilateral hippocampus (based on EEG recordings) and MR-positive patients ($r= -0.45$, $p=0.05$ and $r= -0.94$, $p=0.001$, respectively). In contrast, these two measurements did not have a significant correlation in patients without HS. According to the results, they suggested that these three techniques show the complementary aspects of hippocampal pathology [103]. G. Helms *et al.* examined 43 IGE patients and 38 healthy controls. They used the quantitative single voxel proton

MR spectroscopy, thalamic volumetry, and Voxel Based Morphometry (VBM), to measure concentrations of Glx and NAA in the thalamus and occipital cortex, and fractions of thalamic grey and white matter. They found that quantitative $^1\text{H-MRS}$ and VBM provide further information for the involvement of the thalamus in the generation of seizures in IGE patients. The mean thalamic and cerebral volume was reduced in patients compared with controls ($p=0.0001$, $p=0.0003$, respectively) [106].

3.5. The Relationship between SPECT and PET results and $^1\text{H-MRS}$ Alterations

As mentioned above, currently, MRI, SPECT, and PET are the most important non-invasive methods for the detection of structural and functional abnormalities in TLE and ETLE patients. Decreased blood flow in the epileptogenic zone is detected by inter-racial SPECT and can aid in the lateralization of TLE patients with normal MRI, whereas it is less sensitive than PET [49, 97, 107, 108]. D. Lu *et al.* assessed the relationship between $^1\text{H-MRS}$ alterations (NAA reduction) and glucose metabolism in 12 patients with unilateral TLE ($n=7$ normal MRI and $n=5$ positive MRI). They found a positive correlation between the NAA/(Cho+Cr) ratio and glucose metabolism ($r=0.54$, $p<0.01$) [107]. Another study demonstrated the sensitivity of PET and $^1\text{H-MRS}$ (NAA/Cr and NAA/Cho ratios), which was 85% where the results were compared with MRI findings on only the ipsilateral side [108].

3.6. The Relationship between Clinical Characteristics of Epilepsy and $^1\text{H-MRS}$ Alterations

Some studies have examined the relationship between $^1\text{H-MRS}$ results and seizure frequency [4, 109]. In a study conducted by P. A. Garcia *et al.* there was a negative correlation between NAA concentration and seizure frequency in FLE and TLE patients ($r=-0.72$, $p<0.02$ and $r=-0.60$, $p<0.06$, respectively). However, the correlation between NAA/Cr ratio, FLE, TLE and the duration of epilepsy was poor ($r=0.10$, $p<0.41$ and $r=0.18$, $p<0.34$, respectively) [5].

4. Conclusion

Taking everything into consideration, there is methodological heterogeneity in applied methods (including CSI vs. SVS, MRS sequences, and their parameters) and the quantification methods. Nevertheless, increasing the field strength, improving RF pulse design methods, localization sequences, and post-processing and quantification procedures, especially at short TEs, can improve the overall diagnostic sensitivity and provide a valuable implication for epilepsy diagnosis in clinics. Mohammadi *et al.* suggested an interesting approach for the quantification of MRS data. They employed a dimension reduction technique for the spectral fitting of the MR spectroscopy data with short TE (=30 ms) and showed that the sensitivity and specificity of this modality in TLE patients are 60% and 82%, respectively. Overall, H. Aydin *et al.* suggested that proton MR spectroscopy imaging can be used for the lateralization of an epileptic focus in mTLE patients with 96% sensitivity (sensitivity in the right hippocampi was 92%) and 50% specificity.

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