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Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in Studies of Glaucoma: A Scoping Review

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ABSTRACT

Glaucoma is a disease that is chronic in nature and indistinctive at an early stage, leading to late detection. It has become a major public health problem and a leading cause of irreversible blindness worldwide. The proton magnetic resonance spectroscopy (^{1}H-MRS), of late, has been utilised to evaluate metabolites concentration in the brain, especially for detecting neurodegenerative disease. Considering that glaucoma is a neurodegenerative disease, studies on the concentration of metabolites using ^{1}H-MRS in glaucoma patients could be investigated. This scoping review aims to examine the extent, range, and nature of studies on the ^{1}H-MRS technique in glaucoma disease. The literature search was conducted using MEDLINE, CINAHL and SCOPUS, dated from 2000 until 2019. Studies on glaucoma using ^{1}H-MRS were considered. Any processing method used to characterise the metabolite concentration generated by the ^{1}H-MRS

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protocol has also been considered. Nine full-text studies were yielded after hundreds of literature identification, screening, and eligibility assessment. The included studies sought to determine the feasibility, change, and comparability of metabolite concentrations in glaucoma patients using ^{1}H-MRS with more studies utilising single-voxel spectroscopy (SVS) technique. The chosen anatomy of interest could have been influenced by several criteria, including the association between anatomy and the research query and the volume-sizing capabilities of the ^{1}H-MRS technique. Six (6) metabolites were significantly identified, and quantified, including N-acetyl aspartate, Creatine, Choline, Myo-Inositol, Lactate and Glutamate-Glutamine. This scoping review points out the ^{1}H-MRS application in different types and severity of glaucoma, highlighting the information on the type of patients, the detected metabolites in the anatomy involved, the ^{1}H-MRS technique used and the raw data decipherment processing software. Future studies are needed to validate existing glaucoma studies and to explore the potential of detecting incipient glaucoma.

Keywords: *Spectroscopy, Magnetic resonance spectroscopy, metabolites, glaucoma, neurodegeneration*

INTRODUCTION

Glaucoma is a group of chronic diseases often referred to as a "silent thief of sight" due to its unnoticed destruction of retinal ganglion cells (RGC). Difficult diagnosis of glaucoma leads to late detection and irreversible damage to the vision. Glaucoma is among the public's major health problems and a leading cause of irreversible blindness worldwide [1]. In 2040, the number of people with glaucoma is predicted to increase to 111.8 million worldwide [2].

The proton magnetic resonance spectroscopy (^{1}H-MRS) technique was used for metabolic studies in organisms [3] and the assessment of metabolites concentration in the brain, particularly for the detection of neurodegenerative disease [4]. The ^{1}H-MRS technique is capable of investigating the concentration of human metabolites without any invasive procedures. This technique is embedded in the Magnetic Resonance Imaging (MRI) scanner to produce metabolite spectrum data once it is employed.

Considering glaucoma being a neurodegenerative disease, and the capability of ^{1}H-MRS to investigate the extent of metabolites in the brain non-invasively, ^{1}H-MRS was then viewed as a potential method to investigate the metabolites in the brains and the eyes of patients with glaucoma. While published studies on metabolites evaluation in brain diseases using ^{1}H-MRS have been in existence for some time, published studies on the application of ^{1}H-MRS for metabolites evaluation in glaucoma are unconsolidated. Therefore, this review aims to examine the extent, range, and nature of studies on the ^{1}H-MRS technique in humans with glaucoma disease.

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EXPERIMENTAL

Stage 1: Identifying the Research Question

This scoping review was developed based on the question "what is the extent of published evidence on the application of ^{1}H-MRS in studies of glaucoma?".

Stage 2: Identifying Relevant Studies (Search Strategy)

Relevant articles in MEDLINE, CINALH and SCOPUS dated from 2000 until 2019 were screened by keywords, title, abstract or text words containing the search terms: "metabolites concentration" OR "metabolites", "magnetic resonance spectroscopy" OR "proton magnetic resonance spectroscopy" OR "1-HMRS" OR "spectroscopy" OR " MR spectroscopy", "ocular disease" OR "glaucoma" OR "chronic glaucoma" OR "open-angle glaucoma" OR "suspected glaucoma", "vitreous" OR "visual cortex" OR "optic nerve" OR "radiation optic". The boolean term "AND" and "OR" were used with the search term in the '*Advanced Search'* section in each database.

Stage 3: Study Selection

All articles published in English were included. After removing duplicates, the screening of the titles, abstracts, and full texts was conducted. This scoping review also adopts the Arksey and O' Malley's method of eliminating the studies that did not address the central question [5]. For this, the inclusion and exclusion criteria were first developed.

Inclusion criteria:

- Sources of information include published primary research studies, reviews, systematic reviews, scoping review, and meta-analyses.
- Researches or review papers that are not limited to geographical location or setting.
- Participants of any age and sexes.
- Researches that include any types of glaucoma.
- Researches that consider the practice of in-vivo ^{1}H-MRS technique.
- Researches that use any types of software for processing the raw spectroscopy data.

Exclusion criteria:

- Case reports, magazine articles, opinions.

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Inclusion and exclusion criteria were then applied to all first stage relevant full articles. All full articles were read in deciding the eligibility to be considered in this review. Studies representing the best fit for the research question and inclusion criteria were selected for data extraction.

Stage 4: Charting the Data (Data Extraction)

Extracted data from the selected articles were organised into textual descriptions and data tables. Since each of the selected articles' critical evaluation is not a compulsory criterion for a scoping review [5], no method to assess study quality was used.

Stage 5: Collating, Summarizing, and Reporting the Result (Data Analysis)

The characteristics and findings of each selected literature were integrated through detailed descriptions, which were summarised into the following categories: (a) recruited participants, (b) the region of interest within the visual pathway, (c) the type of spectroscopy technique, (d) the processing software for generating raw data, and (e) the type of metabolites that were investigated. The overall picture of the detailed descriptions was reported in a narrative format.

RESULTS AND DISCUSSION

Descriptive Analysis

The systematic search of the electronic databases yielded 178 potential articles with 20 eligible articles to be assessed. After reading all 20 full articles, nine articles were selected for this review. An overview of the selection process using PRISMA scoping review technique is shown in Figure 1 [6]. From these nine selected studies, seven studies [7–13] were cross-sectional studies, while two [3,14] were review studies. One [11] of the seven studies was acquired from the result of the Google search engine. There were no published systematic review or meta-analyses articles that fulfilled the selection criteria. These studies were identified from six countries, and all were in English. Five of the included studies were from Europe (55.6 %), while three (33.3 %) were from Asia and one (11.1 %) was from the United States of America (USA).

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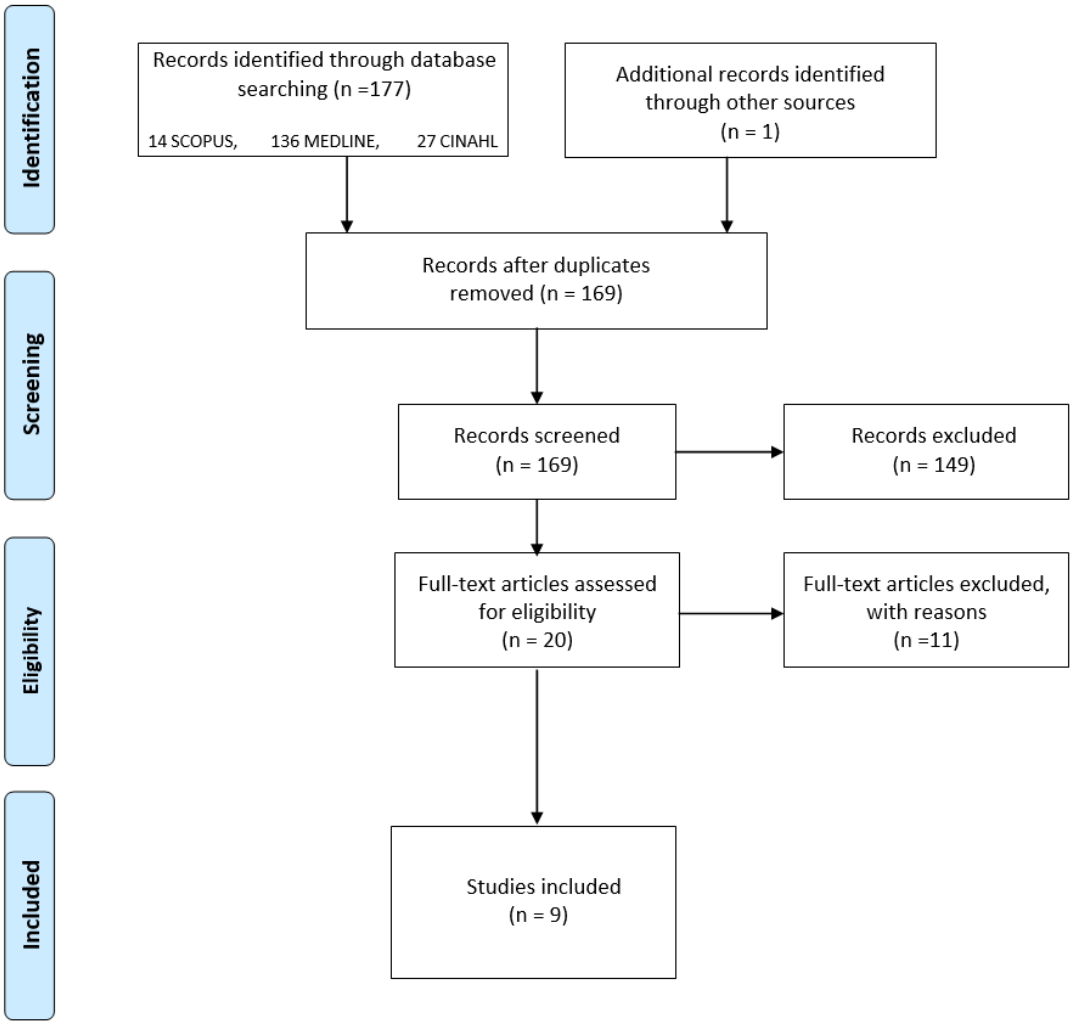

Figure 1: PRISMA Flow Diagram for the scoping review process [6].

The bibliometric trends in this research area show an increasing trend. Figure 2 highlights a substantial chronological increase in the number of published studies relating to glaucoma and ^{1}H-MRS. The primary focus of the selected papers fitted broadly into several themes. The themes were: (a) participants' background; (b) anatomy of interest; (c) spectroscopy technique; (d) processing software; and (e) metabolite of interest. Table 1 summarises the nine published studies that were included in this scoping review.

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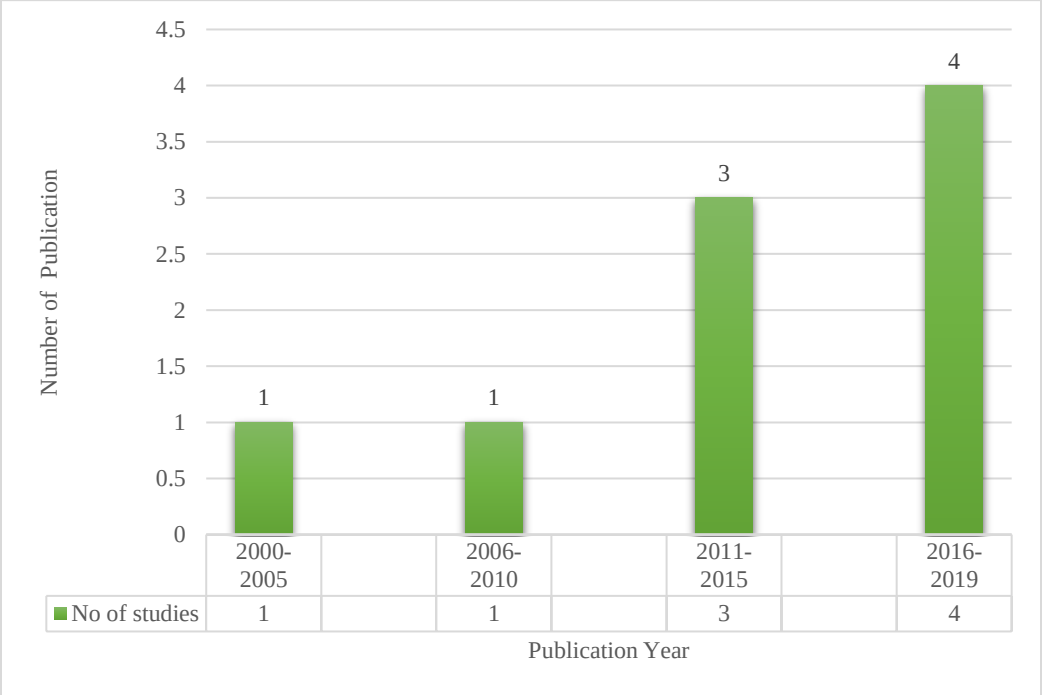

Figure 2: Published studies by year

Participants Background

Each study incorporated a unique set of participant group categories. Rucker et al. [11] enrolled only one patient with optic neuropathy and four healthy controls in their study, as they were primarily interested in determining the feasibility of using ^{1}H-MRS in glaucoma studies. Meanwhile, most of the studies [9,12,13] focused on how metabolite concentration differed from the standard concentration, thus they enlisted a larger number of participants, both healthy and those with glaucoma.

Another observation was the increasing intricacy in the later studies where investigation of the metabolite concentration involved groups of different glaucoma severities [7] and glaucoma types [10]. It was also interesting to note that the very recent study by Guo et al. [8] had recruited participants with early open-angle glaucoma, aimed to associate detected metabolites changes with cortical plasticity. The two review studies, Barbosa-Breda et al. [3] and Fiedorowicz et al. [14], did not discuss the participant's background specifically. These two review studies, however, reported on the possibility of heterogeneity of the recruited glaucoma [3], small sample size [3], and different neurodegenerative mechanism [14] as the factor of insignificant findings.

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Table 1: Publication included in this scoping review

Authors	Participants	Aim	Anatomy of interest, (voxel size)	MRS Method, MRI strength	Processing Software	Metabolites studied
[3]	N/A	Review	N/A	N/A	N/A	N/A
[7]	15 controls 15 mild POAG, 15 severe POAG	comparison	optic radiation (20x20x20mm^3)	SVS, 3.0 T	LCMODEL VERSION 6.2	NAA, NAAG, Cr, PCr, GPC, PCh, Gln, Glu
[8]	23 POAG, 21 controls	changes	visual cortex (20x30x20mm^3)	SVS, 3.0 T	Siemens Syngo MR software	NAA, Cho, Cr, Glx, mI,
[9]	7 POAG, 7 AMD	changes	occipital region, (N/A)	SVS, 3.0 T	Philips Intera software	NAA, Cho, Cr
[10]	30 controls, 25 OAG, 16 GS 16 OHT	changes	corpus geniculatum laterale & visual cortex (20x20x20mm^3)	SVS, 1.5 T	Siemens Syngo MR version B17 software	NAA, Cho, Cr, mI
[11]	4 healthy, 1 optic neuropathy	feasibility	Vitreous, (10x10x10 mm^3)	SVS, 1.5 T	Philips NT software	Lactate
[12]	29 Glaucoma, 13 controls	changes	Vitreous (13x13x4 mm^3) & laterale geniculate body (12x12x2 mm^3)	SVS, 1.5 T	Philips Gyroscan software	NAA, Cho, Cr, Glx,
[13]	20 controls 20 primary Glaucoma	changes	geniculocalcarine & striate area of the occipital lobe (18x18x10mm^3)	MVS, 3.0 T	GE SIGNAL EXCITE software	NAA, Cho, Cr, Glx
[14]	N/A	Review	N/A	N/A	N/A	N/A

Abbreviations: AMD = age-related macular degeneration; POAG = primary open angle glaucoma; OAG = open angle glaucoma; OHT = ocular hypertension; GS = glaucoma suspect; SVS = single voxel spectroscopy; MVS = multi-voxel spectroscopy; NAA = N-Acetyle Aspartate; Cho = Choline; Cr = Creatine, Glx = Glutamate-Glutamine; mI = Myo-Inositol; NAAG = N-acetyle aspartylglutamate; PCr = Phosphocreatine; GPC = Glycerophosphocholine; PCh = Phosphocholine; Gln = Glutamine; Glu = Glutamate; N/A = not applicable.

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Anatomy of Interest

It could be seen that most of the studies chose the occipital region [8–10,13] as the region of investigation, while the rest reasonably chose vitreous [11,12], laterale geniculate nucleus [10,12] and geniculocalcarine related area [7,13]. Meanwhile, Barbosa-Breda et al. [3] published a review paper that discussed the results of other ^{1}H-MRS studies in vitreous and lateral genicular, as well as their relevance to glaucoma pathogenesis. Additionally, the review paper by Fiedorowicz et al. [14] highlighted the ^{1}H-MRS studies done in the vitreous and visual cortex. They remarked that spectroscopy studies in vitreous could help track the pathological changes in glaucoma.

It was understood that the anatomy of interest in those studies was chosen in accordance with the researchers' research interests. However, given the variation in voxel size and MRI strength demonstrated in Table 1, the volume-sizing capability of the ^{1}H-MRS technology might also play a significant role in the anatomy selection criteria. Presumably, the more capable the volume-sizing capability in a ^{1}H-MRS technology is at covering smaller sizes of the anatomy of interest, the more anatomy associated with the eye may be studied. Thus, perhaps the more precise spectroscopy data can be gathered.

Spectroscopy Technique

Researchers can use a series of added methods to the MRI scan to analyse the molecules of the human tissue in 1H-MRS. The common methods used are the single-voxel spectroscopy (SVS) technique or the multivoxel spectroscopy (MVS) technique. Only one of the included studies [13] used the MVS technique, while the others [7–12] used the SVS technique. Meanwhile, the two review studies [3,14] reported the SVS and MVS with little explanation.

As anticipated, many studies preferred to use the SVS method due to its short acquisition time and higher signal to noise ratio, which would provide good spectral quality [15]. It was argued that MVS offered more accurate information through its capability to yield data from multiple adjacent voxels in a single measurement, thus avoiding errors caused by multiple acquisitions times [16]. The particularly small voxels within the multivoxel also enabled the localisation of the region of interest individually and independently; thus, the metabolites' concentration was relatively unaffected by different adjacent tissue [16]. Nonetheless, each technique has advantages and disadvantages that could vary depending on the scanned region.

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Processing Software

MRS acquisition generates raw data in the form of the scanned region's total signal strength. To distribute the signal strength to each individual metabolite, the total signal strength would be subjected to some algorithm. Eventually, the signal strength of each metabolite would be converted to its absolute concentration [17]. The absolute concentration is the arbitrary unit that researchers prefer to use when conducting more complex analyses.

Advanced processing software like the linear combination of model (LCModel) and the Java-based magnetic resonance user interface (jMRUI) are widely used to convert raw spectroscopic data into a quantifiable or understandable form for non-expert users [18]. All studies in this review [8–13] used embedded scanner MRI manufacturer software except one study by Sidek et al. [7] which used an external processing software, LCModel version 6.2. It was shown in Table 1 that there were more metabolites [7] that could be studied by using external software compared to using embedded manufacturer software. The variation of versions and vendors of manufacturer scanner software was also noted in Table 1, implying that these studies' findings must be interpreted with caution as they might not be directly transferable or extrapolated to other studies.

Metabolite of Interest

In the published studies included in Table 1, at least six metabolites were sought or addressed in depth. The metabolites were N-acetyl aspartate, Creatine, Choline, Myo-Inositol, Lactate and Glutamate-Glutamine [7–13] that were detected and measured by ^{1}H-MRS from within the eyeball and from several brain locations involved in visual function. However, another original investigation by Rucker et al. [11] pinpointed only lactate concentration as the metabolite of concern in their publication. Perhaps this was because the primary objective of this pioneering study of metabolites in glaucoma was to determine the viability of using ^{1}H-MRS in glaucoma studies.

The concentration of a metabolite is directly proportional to the total area under the metabolite resonance in the ^{1}H-MRS spectrum. Commonly, the metabolites' concentration is presented in the form of ratio, using Cr as the reference metabolite because it is the fastest and most employed method in clinical practices. Additionally, Cr is used as a reference because it is the most stable metabolite in normal and pathological tissue.

For this reason, four studies [7,8,12,13] used the relative quantification method by taking Cr as a reference for the metabolites ratio value. This metabolites ratio value was essential in interpreting the spectroscopy data. However, Boucard et al. and Aksoy et al. [9,10] did not use this

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relative quantification because their studies' reported metabolite concentration was in absolute concentration value. The rationale for using the absolute concentration value was not addressed in Boucard et al. and Aksoy et al. findings. It was presumed that these two studies used the other opinion that abnormal tissue has different amounts of Cr than normal tissue [19].

Deliberation and recommendation

Current overviews from this scoping review provide essential insights of ^{1}H-MRS application in glaucoma disease. However, several considerations of the existing application of ^{1}H-MRS in glaucoma studies should be addressed.

The different current studies employed a variety of methods, MRI technologies, and data processing techniques. While the arrangements for these studies may still serve as a guide for future researchers, the replication of the study arrangements and the extrapolation of the findings from those studies are still challenging to other researchers and should be done with caution. This is due to the fact that no validation studies for the existing research have been published to date. Thus, a pilot study validating these glaucoma studies in a research setting, as well as additional glaucoma studies using ^{1}H-MRS, should be conducted to further ones understanding of glaucoma pathophysiology, particularly when ^{1}H-MRS is used.

Besides, current studies have a variety of preferences in terms of the anatomy of interest, the technique used for spectroscopy acquisition, or the software used for raw data decipherment. It is also worth noting that due to the volume-sizing capability of the ^{1}H-MRS technology, the selection of anatomy of interest may be limited. Furthermore, the bulk of recent research used the SVS approach rather than the MVS methodology, and they depended on embedded processing software that could detect fewer metabolites rather than advanced software like the LCModel programme that could detect more metabolites. To address this, researchers may benefit from collaborating with and integrating their research with that of other countries or a highly experienced international organisation, allowing them to leverage a variety of resources in order to achieve the best possible study results.

CONCLUSION

In this scoping review, the existing MRS application in glaucoma studies has been revealed and synthesised, pointing out the application of ^{1}H-MRS in different types and different severity of glaucoma disease. This synthesis highlights information on the type of patients, the detected metabolites in the anatomy involved, the MRI technology used and the raw data decoding processing method. More studies are needed to comprehend and validate existing glaucoma studies

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that employ ^{1}H-MRS. Additionally, future research is also needed to explore the potential of detecting incipient glaucoma, which will eventually contribute to better glaucoma management and a reduction in the prevalence of glaucoma-related blindness.

CONFLICT OF INTEREST STATEMENT

The authors declare absence of any self-benefits, commercial or financial conflicts.

REFERENCES

[1] Abdull, M. M., Chandler, C., & Gilbert, C. (2016). Glaucoma, "the silent thief of sight": Patients' perspectives and health seeking behaviour in Bauchi, northern Nigeria. *BMC Ophthalmology*, *16*(1), 1–10. https://doi.org/10.1186/s12886-016-0220-6

[2] Tham, Y. C., Li, X., Wong, T. Y., Quigley, H. A., Aung, T., & Cheng, C. Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology*, *121*(11), 2081–2090. https://doi.org/10.1016/j.ophtha.2014.05.013

[3] Barbosa-Breda, J., Himmelreich, U., Ghesquière, B., Rocha-Sousa, A., & Stalmans, I. (2018). Clinical Metabolomics and Glaucoma. *Ophthalmic Research*, *59*(1), 1–6. https://doi.org/10.1159/000479158

[4] Jones, R. S., & Waldman, A. D. (2004). ^{1} H-MRS evaluation of metabolism in Alzheimer's disease and vascular dementia. *Neurological Research*, *26*(5), 488–495. https://doi.org/10.1179/016164104225017640

[5] Arksey, H., & O'Malley, L. (2005). Scoping studies: Towards a methodological framework. *International Journal of Social Research Methodology: Theory and Practice*, *8*(1), 19–32. https://doi.org/10.1080/1364557032000119616

[6] Moher D, Liberati A, Tetzlaff J, Altman DG, T. P. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med*, *6*(7), 1000097. https://doi.org/10.1371/journal.pmed1000097

[7] Sidek, S., Ramli, N., Rahmat, K., Ramli, N. M., Abdulrahman, F., & Kuo, T. L. (2016). In vivo proton magnetic resonance spectroscopy (1H-MRS) evaluation of the metabolite concentration of optic radiation in primary open angle glaucoma. *European Radiology*, *26*(12), 4404–4412. https://doi.org/10.1007/s00330-016-4279-5

[8] Guo, L., Wang, R., Tang, Z., Sun, X., Wu, L., Wang, J., Zhong, Y., Xiao, Z., & Zhang, Z. (2018). Metabolic Alterations Within the Primary Visual Cortex in Early Open-angle Glaucoma Patients: A Proton Magnetic Resonance Spectroscopy Study. *Journal Of Glaucoma*, *27*(12), 1046–1051. https://doi.org/10.1097/IJG.0000000000001098

[9] Boucard, C. C., Hoogduin, J. M., van der Grond, J., & Cornelissen, F. W. (2007). Occipital

https://scilett-fsg.uitm.edu.my/
Vol. 15(2) JUNE 2021

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v15i2.13835

proton magnetic resonance spectroscopy (1H-MRS) reveals normal metabolite concentrations in retinal visual field defects. *PLoS ONE*, *2*(2), e222–e222. https://doi.org/10.1371/journal.pone.0000222

[10] Aksoy, D. Ö., Umurhan Akkan, J. C., Alkan, A., Aralaşmak, A., Otçu Temur, H., & Yurtsever, İ. (2018). Magnetic Resonance Spectroscopy Features of the Visual Pathways in Patients with Glaucoma. *Clinical Neuroradiology*, 9–11. https://doi.org/10.1007/s00062-018-0728-7

[11] Rucker, J. C., Biousse, V. V., Mao, H., Sandbach, J., Constantinidis, I., Newman, N. J., & Anlanta. (2003). Detection of Lactate in the Human Vitreous Body using Proton Magnetic Resonance Spectroscopy. *Archives of Ophthalmology*, *121*(6), 909–911. http://dx.doi.org/10.1001/archopht.121.6.909

[12] Doganay, S., Cankaya, C., & Alkan, a. (2012). Evaluation of corpus geniculatum laterale and vitreous fluid by magnetic resonance spectroscopy in patients with glaucoma; a preliminary study. *Eye (London, England)*, *26*(8), 1044–1051. https://doi.org/10.1038/eye.2012.84

[13] Zhang, Y., Chen, X., Wen, G., Wu, G., & Zhang, X. (2013). Proton Magnetic Resonance Spectroscopy (1H-MRS) Reveals Geniculocalcarine and Striate Area Degeneration in Primary Glaucoma. *PLoS ONE*, *8*(8), 8–13. https://doi.org/10.1371/journal.pone.0073197

[14] Fiedorowicz, M., Dyda, W., Rejdak, R., & Grieb, P. (2011). Magnetic resonance in studies of glaucoma. *Med Sci Monit*, *17*(10), RA227-32. https://doi.org/881973 [pii]

[15] Anith Alfred J, Abubacker Sulaiman F, Divya Shree, Ashraf Ahmed, Saravanan K, V. P. (2018). Comparison of Single Voxel and Multi Voxel Magnetic Resonance Spectroscopy in Evaluation of Brain Tumors. *International Journal of Contemporary Medicine, Surgery and Radiology*, *3*(2), 36–40. https://doi.org/10.21276/ijcmsr.2018.3.2.9

[16] Yang, Z.-X., Huo, S.-S., Cheng, X.-F., Xu, Z.-F., Cao, Z., Zeng, J.-X., Xiao, Y.-Y., You, K.-Z., Chen, W., Liu, Y.-Y., & Wu, R.-H. (2011). Quantitative multivoxel proton MR spectroscopy study of brain metabolites in patients with amnestic mild cognitive impairment: a pilot study. *Neuroradiology*, *54*(5), 451–458. https://doi.org/10.1007/s00234-011-0900-0

[17] Ernst, T., & Chang, L. (2006). Data Processing and Interpretation. In *ISMRM*.

[18] Blüml, S. (2013). Magnetic Resonance Spectroscopy: Basic. In S. Blüml & A. Panigraphy (Eds.), *MR Spectroscopy of Pediatric Brain Disorders* (pp. 11–23). Springer New York. https://doi.org/10.1007/978-1-4419-5864-8

[19] Jansen, J. F. A., Backes, W. H., Nicolay, K., & Kooi, M. E. (2006). 1H MR spectroscopy of the brain: Absolute quantification of metabolites. *Radiology*, *240*(2), 318–332. https://doi.org/10.1148/radiol.2402050314