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Usefulness of combined pseudo-continuous arterial spin labelling and spectroscopic analysis in schizophrenic Egyptian population sample

Aya Elsayed Ibrahim Allam[1], Alaa Mohamed Reda[2*], Mai Abd El Raouf Saed Ahmed Eissa[2] and Rania Essam Eldein Mohamed Ali Salem[2]

Abstract

Background Schizophrenia is a prevalent psychiatric disorder that affects 1% of the global population. Schizophrenia frequently begins in late adolescence or early adulthood, causing significant educational, social, and economic costs for people and society. Functional neuroimaging research on schizophrenia physiopathology has been beneficial. Arterial spin labelling (ASL) is one of functional magnetic resonance imaging (fMRI) technologies that assess brain function without radiation. ASL uses magnetic resonance (MR) imaging to quantify tissue-level brain perfusion non-invasively. Arterial spin labelling (ASL) is one of the functional magnetic resonance imaging (fMRI) technologies that assess the brain function without radiation hazards. ASL uses magnetic resonance (MR) imaging to quantify tissue-level brain perfusion non-invasively. Many publications were performed on role of different advanced MRI techniques in schizophrenia, but our study insisted on the added value of combined ASL and MRS over the conventional MRI in schizophrenic Egyptian population sample.

Aim of the work The purpose of this work was to evaluate the added value of combined ASL-perfusion MRI and MRS in schizophrenic patients.

Methods This prospective case–control study was carried out on two groups: First group was 30 patients who were diagnosed clinically as schizophrenic patients, and second group was 20 healthy volunteers as a control group for comparison in the period from August 2021 till July 2022.

Results The majority of newly diagnosed cases had significant higher positive symptoms than chronic cases. According to arterial spin labelling (ASL) data, rCBF was noticed to be reduced in anterior cingulate, frontal lobe, and parietal lobe of both patients' subgroups but more significant in chronically ill patients. Convergent results of decreased rCBF were also found in the parietal lobe and occipital lobe. Magnetic resonance spectroscopic analysis showed that NAA was decreased in the anterior cingulate cortex, thalami and basal ganglia of the newly diagnosed cases more than chronic cases. The ASL-MRI perfusion accurately detected the hypo-perfusion of different brain regions with sensitivity 100%, specificity 66.67%, positive predictive value 96.43%, negative predictive value 100%, and accuracy 96.67%, while MR spectroscopy showed sensitivity 100%, specificity 33.33%, positive predictive value 93.10%, negative predictive value 100%, and accuracy 93.33% in evaluation of changes of brain metabolites.

Conclusion ASL is a promising functional MRI technique that can produce together with MRS quantitative information about the metabolites of different brain regions. The ASL-MRI appears as a reliable non-invasive technique

*Correspondence:
Alaa Mohamed Reda
Alaa.khalil@med.tanta.edu.eg
Full list of author information is available at the end of the article

to measure cerebral blood flow and identify decreased rCBF without any contrast administration, and it could be repeatable which helps in early diagnosis as well as follow-up of the progression of the disease.

Keywords ASL-perfusion MRI, rCBF, Schizophrenia, MRS, Spectroscopy, Brain metabolites, NAA, Choline, Creatine

Background

Schizophrenia is a prevalent psychiatric disorder. It is one of the world's 10 leading causes of disability assessed by DALYs scale and, despite current progress in research and treatment, continues to be a major aetiologic, diagnostic, and therapeutic challenge. It has been estimated that around 1% of the world population suffers from this chronic and disabling disorder [1]. Functional neuroimaging studies have proved to be an invaluable source of information about the physiopathology of schizophrenia [2].

Regional cerebral blood flow (rCBF) is defined as the volume of blood delivered to a given mass of brain tissue over a given time [3]. Many previous studies have demonstrated that schizophrenia patients exhibited an increased or decreased resting-state CBF by SPECT in multiple brain regions, especially a decreased CBF in the prefrontal cortex. However, this technique is quite invasive, with a long acquisition time and low spatial resolution. In contrast, the arterial spin labelling (ASL) MRI is a non-invasive technique that can rapidly quantify CBF [4].

Arterial spin labelling (ASL), on the other hand, is based on the quantification of the water flow within brain regions, which is considered to provide an indirect measure of metabolism. Arterial spin labelling (ASL) is a magnetic resonance (MR) imaging technique that enables the measurement of brain perfusion non-invasively at the tissue level. Benefiting from the contrast of inflowing magnetically labelled blood, ASL obviates an exogenous contrast agent [5].

In arterial spin labelling, the tracer is a magnetic label (tag) applied to water molecules of flowing blood prior to their reaching the region under assessment [6]. Such labelling consists in an inversion of proton spins of arterial water. After an interval that allows water molecules to be exchanged within tissues, an image, referred to as 'labelled' or 'tagged', is acquired. In this image, blood water is in a different magnetization state from that of static tissue water. Also, a control image of the same slice is acquired in which inflowing blood is not labelled. By calculating the difference between control and tagged images, investigators can create an image that corresponds to the proportional rCBF in a given brain region [7]. This technique has been used to explore CBF alterations in schizophrenia and has demonstrated increased CBF in the striatum, decreased CBF in the prefrontal and anterior cingulate cortices, and increased or reduced CBF in the thalamus and temporal cortex. It is not known if these changes in rCBF are due to genetic influences, but findings of altered rCBF in first degree relatives of patients with schizophrenia indicate a familial and possibly genetic influence [8, 9].

Aim of the work

The aim of this study was to assess the capability of combined use of ASL and multi-voxel MRS in early detection and follow-up of schizophrenia by evaluation of the changes in ratios of neuronal metabolites by MRS and altered rCBF in different brain regions, compared to healthy individuals.

Methods

Study population

The current study design was prospective (case–control study), that was carried out on two groups: First group was 30 patients who were suspected clinically as schizophrenic patients, and the second group was 20 healthy volunteers as a control group for comparison. Those patients were referred to Diagnostic Radiology and Medical Imaging department from Neuropsychiatry department of our institute, over a period of 12 months, starting from August 2021 till July 2022. The study was performed after approval by the Research Ethical Committee.

The inclusion criteria of the studied cases were: patients who were clinically diagnosed as schizophrenic patient for the first time or stopped treatment more than 3 months. Their ages are between 20 and 60 years old. Both sexes were included in the study. Healthy volunteers of matched 20 patients (12 Males and 8 Females) were selected randomly as a control group to match the gender and aged of diseased group. While patients who are contraindicated to go under the magnet as patients with cardiac pacemakers, non-compatible intracranial clips of brain aneurysms and intraocular metallic foreign body were excluded from our study, as well as schizophrenic patients who were under medical medication (within 3 months from the study time). Also, claustrophobic people or patients with previous neurological disorders or traumatic brain injuries were excluded from the present study.

The present study was divided into 2 groups

Group 1 Thirty schizophrenic patients: 20 males and 10 females aged from 20 to 50 (12 of them were newly

diagnosed as schizophrenic who met the DSM 5 criteria [10], another 18 chronic schizophrenic patients and stopped their medical treatment for more than 3 months).

Group 2 Twenty age and sex matched healthy volunteers were enrolled in the current study as control group they were 12 males and 8 females with their ages ranged from 20 to 50 years they had no medical or neuropsychiatric illness.

All patients were subjected to the following:

Data collection

Full history

Proper history taking:—Personal history: as regard name, age and sex. Present history: as regard the presenting symptoms as positive symptoms, negative symptoms, and cognitive dysfunction. Past history: as regard any previous neurological diseases, trauma, intracranial haemorrhage, CNS infection, or previous operations, and family history of any CNS anomalies.

Check of all previous radiological studies

Clinical examination

Psychiatric Assessment using the psychiatric clinical sheet in Neuropsychiatry department.

Clinical diagnosis of schizophrenic cases was done using Mini International Neuropsychiatric Interview (MINI) [10], which is a short, comprehensive feasible, reliable, and valid systemic diagnostic psychiatric interview, with short administration time. Assessment of Schizophrenia severity using Positive and Negative Syndrome Scale (PANSS) [11]. The Positive and Negative Syndrome Scale was used for assessment of psychosis. The PANSS is a medical scale used for measuring symptom severity of patients with schizophrenia and was published in 1987. The scale is a 30-item, seven-point rating instrument. Of the 30 parameters assessed, seven were chosen to constitute a Positive Scale (score range 7–49), seven a Negative Scale (7–49) and the remaining 16 a General Psychopathology Scale (16–112).

All included individuals were subjected to MRI study

General Electric (GE) 1.5 Tesla (closed magnet) MR machine with a standard circularly polarized head coil was used to perform all MRI scans. Using a standard eight channels' head coil, head support pillow to minimize patient's movement.

The patient lied supine during the exam in comfortable position during the MR exam to minimize his motion. The head was introduced first. The examination time ranged from 15 to 30 min. The slice thickness was 4 mm, the matrix was 256 × 256, and the field of view was 220–240 mm. No sedation was given.

Patient preparation

All metallic objects like pins and earrings were removed and emptied the bladder prior to examination (to prevent patient irritability during examination).

Magnetic resonance imaging protocol included

Axial T1WI (Repetition Time (TR)/Time to Echo (TE)= 400-600/10-20 ms).

Axial T2WI (TR/TE=2000–400/100–120 ms).

Coronal and/or axial Fluid—attenuated inversion recovery (FLAIR) images (TR/TE/Inversion time (TI) = 4000-6000/140/1200 ms).

Magnetic Resonance Spectroscopy (MRS) Magnetic Resonance Spectroscopy (MRS) was performed for all cases. Multivoxel localization proton MR spectroscopy was performed using a spin-echo mode sequence (SE) with long TE (TR/TE/average = 1600–2000/144 ms) and short TE (TR/TE = 1600–2000/35 ms). Proper shimming, saturation banding was performed, and water suppression was achieved with chemical shift selection (CHESS) technique. The metabolites were identified including: N-acetyl aspartate (NAA) at 2.0 ppm, creatine (Cr) at 3.03 ppm, choline (Cho) at 3.2 ppm, lipid containing compounds at the range of 0.9–1.3 ppm, lactate at 1.32 ppm and myoinositol at 3.56 ppm Glutamate (GLU), Glutamine (GLN) peaks at 2.1–2.5 ppm.

Post-processing of MRS was performed using MR workstation software (ADW 4.7 Vantage, GE Medical Systems), regions of interest (ROI) were placed in both frontal gyri (especially right medial frontal gyrus), medial cingulate gyri. Parieto-temporal cortices, hippocampi, thalami, basal ganglia, cerebellum). The peak ratios were calculated from the integration of the single peak including: NAA/Cr, NAA/Cho, and Cho/Cr. Interpretation of the spectral peaks was done quantitatively by three experienced radiologists (25, 11, and 3 years). This quantitative assessment represents a relative concentration of the metabolites using the integral ratios and comparing it to the control group.

Arterial spin labelling technique

The label was created by applying radiofrequency pulses to invert the bulk magnetization of the blood water protons. Images were acquired after the labelling and inflow period by using rapid acquisition techniques such as echo-planar imaging, gradient- and spin-echo imaging (GRASE), or three-dimensional fast spin-echo imaging using a stack-of-spirals approach.

During ASL image acquisition, repeated label and control images are typically interleaved. Perfusion contrast was obtained by pair-wise subtraction of the label and control acquisitions.

Post-processing of ASL was performed on GE workstation software, qualitative assessment of colour map, quantitative analysis was done, and absolute regional cerebral blood flow (rCBF) was estimated in the scanned hypo-perfused areas, by modelling expected signal changes in the brain.

The labelling is continuous through a thin slice at the neck level. The inversion of the magnetization is obtained by the joint application of continuous pulsed radiofrequency for 2–4 seconds and a magnetic field gradient in the direction of the flow.

Tagging efficiency of CASL may be affected by variations in flow velocity, which makes the average inversion efficiency of CASL (80–95%) lower than PASL (95%). However, the closer inversion to the imaging plane minimizes the loss of perfusion signal caused by T1 relaxation and somehow compensates for the lower inversion efficiency.

Statistical analysis

Data were analysed using the IBM® SPSS statistical software, version 21. We used the one-sample Kolmogorov—Smirnov test to check the normality of data and the data were parametric (normally distributed). Numerical data were presented as mean and standard deviation (SD), and categorical data were presented as number and percentage.

Chi-squared test was used for comparing the qualitative one-way of variance (ANOVA) test to compare the means in different groups. Post hoc test (LSD) was done to determine the significance between each two groups.

Linear correlation analysis was done by Spearman coefficient correlation and used for testing the positive or negative associations between different variables. The level of significant was adopted at $p<0.05$.

Sensitivity The capacity of the test to correctly identify diseased individuals in a population "true positive". The greater the sensitivity, the smaller the number of unidentified cases "false negative".

Specificity The capacity of the test to correctly exclude individuals who are free of the disease "true negative". The greater the specificity, the fewer "false positive" will be included.

Positive Predictive value (PPV) The probability of the disease being present, among those with positive diagnostic test results.

Negative Predictive value (NPV) The probability that the disease was absent, among those whose diagnostic test results were negative.

Accuracy Rate of Agreement

Intra-class Correlation coefficient It was used for the agreement between each two observers. The intra-class correlation coefficient (ICCs) were classified using a system suggested by Koo and Li (2016) as follows: less than 0.50 Z poor agreement; 0.50 to less than 0.75 Z moderate agreement; 0.75 to less than 0.90 Z good agreement; and 0.90 or greater Z high agreement. If a P value was less than 0.05 , this was considered a statistically significant data.

Results

This prospective case–control study was carried out in the period from 1st August 2021 till July 2022, the current study enrolled 30 schizophrenic patients and 20 age- and sex-matched healthy volunteers as a control group. The mean age in the patient group was 35.6 + 4.5 years, and in the control group, it was 31.1+ 3.8 years. Males constituted 66.7% of the patients' group (20 patients) and 60.0% of the control group (12 participants). Nineteen patients were educated and only 10 patients of the schizophrenic cases were employed representing (33.3% of cases). Concerning the marital status 18 patients are single. As for special habits 16 patients were smokers as described in table 1.

Regarding family history 16 out of 30 patients had positive family history of psychiatric illness who were 53.3% of cases, meanwhile the rest of the cases had no positive family history of any psychiatric disorder.

According to the patients' symptomatology data, majority of cases (93.3% and 90%) had delusions and hallucinations, respectively.

Regarding the clinical tests which were performed in the current study, as follows: correct response on Wisconsin Card Sorting Test (WCST) was significantly lower among both groups A and group B than control and group A had lower performance than group B with mean 31.47 ± 7.49 for group (A), 39.97 ± 8.17 for group (B), and 49.83 ± 5.36 for control patients. While both groups A and B had significantly higher errors than control, group A had significantly higher errors than group B with mean 31.27 ± 5.39 for group (A), 25.13 ± 7.57 for group (B), and 16.78 ± 6.76 for control patients. Patients of group A had significant higher score than both control and group B. Group B performed significantly better than group A and less than control group with mean 493.85 ± 36.74 for group (A), 370.25±58.67 for group (B), and 264.20 ± 96.23 for control patients.

Table 1 Sociodemographic data of the studied group

	Schizophrenia Cases ($n=30$)		Control ($n=20$)		Test of sig	*P*. value
	No	%	*No*	%		
Gender					*X2 = 0.231*	0.630
Male	20	66.7	12	60.0		
Female	10	33.3	8	40.0		
Age (Years)						
20–30	18	60.0	11	55.0	$X^2=2.8$	0.41
> 30–40	8	26.7	6	30.0		
> 40–50	4	13.3	3	15.0		
Range	20 -48			21–48	T = 2.6	0.41
Mean + SD	35.6 + 4.5			31.1 + 3.8		
Education					$X^2=0.24$	0.90
Illiterate	11	36.7	7	35.0		
Educated	19	63.3	13	65.0		
Occupation					$X^2=6.42$	0.011*
Employed	10	33.3	14	70		
Unemployed	20	66.7	6	30		
Marital status					$X^2=12.17$	0.04*
Single	18	60	5	25		
Married	4	23.3	12	60		
Divorced	7	13.3	2	10		
Widow	1	3.3	1	5		
Residence					$X^2=4.41$	0.43
Rural	10	33.3	8	40		
Urban	20	66.7	12	60		
Smoking Habits					$X^2=5.55$	0.01*
Yes	16	53.3	4	20		
No	14	46.7	16	80		

* Statistically significant at $p \leq 0.05$

SD: standard deviation

Conventional MRI findings reveal that there is atrophy of whole brain volume in 75% of the first episodic patients and 83.3% of the chronically ill patients. while, thalamic, temporal lobes atrophy occurred in 41.7% of 1st episodic cases, and in 72.2% of the chronic patients.

During analysis of arterial spin labelling (ASL) data, rCBF was noted to be reduced in anterior cingulate, frontal lobe and parietal with mean (47.58 + 4.95) (36.85 + 5.05)(42.93 + 4.55 in F.E and (44.05 + 6.45) (36.60+ 4.45) (38.31+ 5.19), respectively, at these areas in chronic schizophrenic patients. On the contrary to previous areas, putamen and hippocampus showed increased rCBF with mean (63.53 + 6.55) (63.25 + 5.95) in F.E and (54.34 + 5.64) (54.74 + 5.14), respectively, in chronic patients as mentioned in table 2.

Concerning MR spectroscopic analysis in the studied patients' group, it demonstrated NAA/Creatine ratios which had statistically significant difference between both groups at hippocampi and thalami (*P*. value = 0.06* and 0.08*), respectively. As well as, concerning NAA/Choline ratios, there was also significance at hippocampi and thalami, (*P* value = 0.04* and 0.06*) respectively. Moreover, regarding choline/creatine ratios, the lowest ratios were appreciated at cingulate gyrus region in both schizophrenic subgroups, with no statistically significant difference between healthy control group and patients in all scanned regions of interest as shown in Tables 3, 4, 5.

Assessment of the diagnostic performance of the two studied imaging modalities revealed that the highest area under the curve (AUC) was for ASL in inferior frontal gyrus (0.943) that showed sensitivity of 90.0% and specificity of 95.0% with a cut-of value of ≤ 44, medial frontal gyrus AUC was (0.913) with sensitivity of 93.33% and specificity of 85.0%, the parietal lobe AUC was (0.894) with sensitivity of 83.33% and specificity of 90.0%, while the anterior cingulate gyrus showed AUC of (0.876) with sensitivity and specificity of 76.67% and 90.0%, respectively, as mentioned in Fig. 1, Table 6.

Table 2 ASL diagnosis of the studied participants

	Schizophrenia Cases ($n=30$)		Control ($n=20$)	Test of sig (P. value)
	Newly diagnosed ($n=12$)	Chronic cases ($n=18$)		
Anterior cingulate				$F=27.6$ $P=0.001$* $P2, P3<0.05$
Min – Max	40–56	36–57	50–59	
Mean + SD	47.58 + 4.95	44.05 + 6.45	49.18 + 15.21	
Frontal lobe				$F=23.6$ $P=0.001$* $P2, P3<0.05$
Min – Max	32–48	32–47	35–60	
Mean + SD	36.85 + 5.05	36.60 + 4.45	45.35 + 4.59	
Medial frontal gyrus Lf				$F=2.1$ $P=0.32$
Min – Max	40 -62	39 -63	44 -69	
Mean + SD	58.98 + 6.85	57.51 + 6.13	62.52 + 6.75	
Medial frontal gyrus Rt				$F=2.6$ $P=0.25$
Min – Max	40–62	39–63	44–69	
Mean + SD	56.98 + 6.85	57.51 + 6.13	63.42 + 6.75	
Inferior frontal gyrus				$F=2.9$ $P=0.37$
Min – Max	31–50	34–52	36–59	
Mean + SD	48.13 + 5.35	47.31 + 4.45	53.44 + 4.94	
Parietal lobe				$F=17.7$ $P=0.001$* $P2, P3<0.05$
Min – Max	39–50	33–52	34–65	
Mean + SD	42.93 + 4.55	38.31 + 5.19	52.54 + 6.14	
Temporal lobe				$F=1.9$ $P=0.47$
Min – Max	32–47	30–47	30–48	
Mean + SD	39.35 + 5.51	39.61 + 5.19	43.24 + 4.35	
Occipital lobe				$F=2.4$ $P=0.47$
Min – Max	31–48	30–36	33–60	
Mean + SD	39.66 + 4.32	40.61 + 4.09	43.74 + 4.64	
Putamen				$F=21.7$ $P=0.001$* $P2, P3<0.05$
Min – Max	50–69	50–67	49–68	
Mean + SD	63.53 + 6.55	61.61 + 4.47	54.34 + 5.64	
Hippocampus				$F=19.4$ $P=0.001$* P2, P3 < 0.05
Min – Max	53–70	52–66	51–58	
Mean + SD	63.25 + 5.95	59.77 + 4.69	54.74 + 5.14	

F, *p*: *F* and *p* values for ANOVA test, Sig. bet. Groups were done using Post Hoc Test (LSD)

p1: *p* value for comparing between F.E and Chronic Schizophrenia patients

p2: *p* value for comparing between F.E Schizophrenia patients and Control group

p3: *p* value for comparing between Chronic Schizophrenia patients and Group Control group

*Statistically significant at $p \leq 0.05$

rCBF: Regional cerebral blood flow

Comparison between the three studied observers according to different parameters is shown in Table 7 (Figs. 2, 3, 4, 5).

Discussion

Schizophrenia spectrum and other psychotic disorders affect up to 3% of the population, are accompanied by prominent social and occupational functional impairments, and represent one of the leading causes of disability worldwide. Psychotic disorders have been suspected to be associated with disturbances in the cerebral blood flow (CBF) [12].

The two most common perfusion MRI methods are dynamic susceptibility contrast (DSC) and arterial spin labelling (ASL) [13].

Arterial spin labelling (ASL) imaging has been validated extensively against other perfusion methods by

Table 3 MRS finding regards to NAA/CR Ratios

	Schizophrenic patients group ($n=30$)		Control group ($n=20$)	*P*. Value
	1st attack ($n=12$)	Chronic patients ($n=18$)		
Right prefrontal cortex	2.15 ± 0.29	2.03 ± 0.21	2.21 ± 0.32	0.41
Left prefrontal cortex	2.13 ± 0.27	2.07 ± 0.22	2.23 ± 0.35	0.25
Temporal lobe	1.03 6 0.17	0.93 6 0.21	1.38 ± 6 0.14	0.09
Hippocampus	1.53 ± 0.13	1.20 ± 0.14	1.97 ± 0.22	0.06*
Thalamus	1.01 ± 0.21	1.1 ± 0.26	1.93 ± 0.20	0.08*
Dorsolateral prefrontal cortex	2.03 ± 0.23	2.02 ± 0.19	2.25 ± 0.31	0.27
parieto-occipital cortex	1.355 ± 0.1	1.341 ± 0.099	1.372 ± 0.109	0.35
Cingulate gyrus	1.185 ± 0.127	1.192 ± 0.13	1.176 ± 0.121	0.62

*Statistically significant at $p \leq 0.05$

Table 4 MRS finding regards to NAA ratio/ Choline

	Schizophrenia patients group ($n=30$)		Control group ($n=20$)	*P*. Value
	1st attack ($n=12$)	Chronic patients ($n=18$)		
Right prefrontal cortex	0.96 ± 0.27	0.91 ± 0.32	1.73 ± 0.27	0.52
Left prefrontal cortex	0.99 ± 0.28	0.90 ± 0.30	1.71 ± 0.25	0.53
Temporal lobe	0.983 ± 0.2	0.977 ± 0.23	1.52 ± 0.21	0.07
Hippocampus	0.783 ± 0.27	0.73 ± 0.25	1.61 ± 0.22	0.04*
Thalamus	0.72 ± 0.2	0.69 ± 0.23	1.91 ± 0.19	0.06*
Dorsolateral prefrontal cortex	0.95 ± 0.13	0.92 ± 0.14	1.73 ± 0.15	0.08
Parieto-occipital cortex	1.31 ± 0.13	1.29 ± 0.15	1.35 ± 0.13	0.36
Cingulate gyrus	0.76 ± 0.08	0.72 ± 0.1	1.98 ± 0.11	0.52

*Statistically significant at $p \leq 0.05$

Table 5 MRS finding regards to choline/creatine ratio

	Schizophrenia patients (30)		Control subjects (20)	*P*. Value
	F.E (12)	Chronic patients (18)		
Right prefrontal cortex	1.11 ± 0.15	1.11 ± 0.2	1.12 ± 0.11	0.87
Left prefrontal cortex	1.11 ± 0.18	1.12 ± 0.21	1.13 ± 0.22	0.88
Temporal lobe	0.73 ± 0.1	0.75 ± 0.1	0.69 ± 0.08	0.36
Hippocampus	1.03 ± 0.13	1.12 ± 0.12	0.98 ± 0.1	0.52
Thalamus	1.2 ± 0.23	1.14 ± 0.21	1.32 ± 0.27	0.32
dorsolateral prefrontal cortex	1.11 ± 0.15	1.11 ± 0.2	1.2 ± 0.13	0.68
parieto-occipital cortex	0.73 ± 0.08	0.75 ± 0.05	0.68 ± 0.05	0.77
Cingulate gyrus	0.63 ± 0.06	0.65 ± 0.03	0.59 ± 0.04	0.58

*Statistically significant at $p \leq 0.05$

NAA: N-acetyl apartate, Cho: choline, Cr: creatine

demonstrating accurate and reproducible quantification of CBF. Since it is completely non-invasive, ASL imaging has become useful for many clinical and research applications [12].

The aim of this work was to evaluate the combined added value of p-CASL-perfusion MRI and MR spectroscopy (MRS) in schizophrenia.

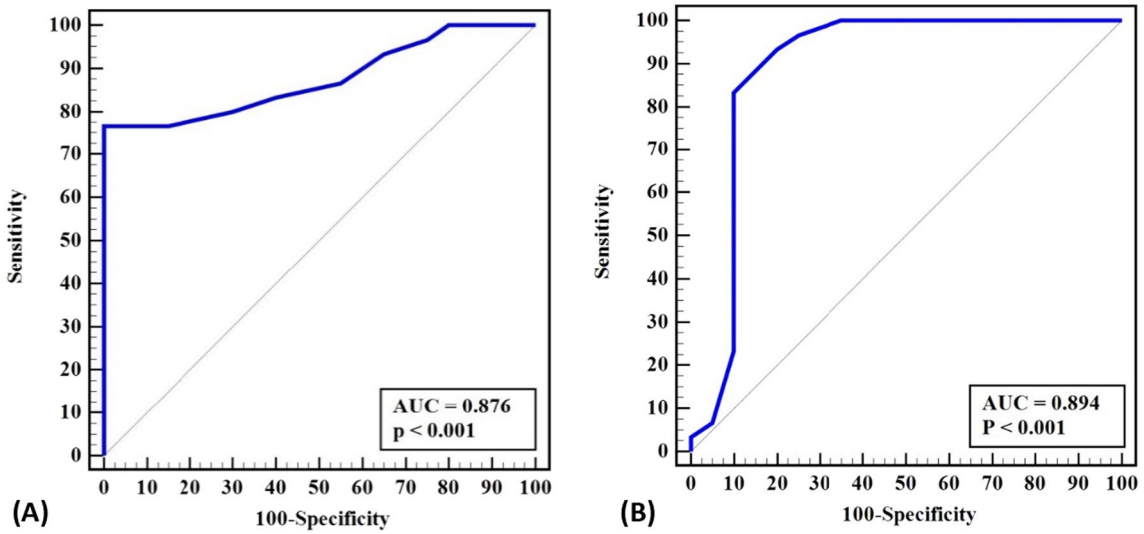


Fig. 1 ROC analysis for the diagnostic performance of ASL, **A** ROC curve for ASL performance at the anterior cingulate cortex, **B** ROC curve for ASL performance at the parietal lobe

Table 6 The differences of paired sample area under the ROC curve for the diagnostic performance of ASL

ASL	AUC	*p*	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Anterior cingulate	0.876	< 0.001*	0.780–0.972	≤ 50	76.67	90.0	92.0	72.0
Frontal lobe	0.874	< 0.001*	0.774–0.975	≤ 38#	83.33	95.0	96.2	79.2
Medial frontal gyrus Lt	0.906	< 0.001*	0.805–1.000	≤ 61	93.33	80.0	87.5	88.9
Medial frontal gyrus Rt	0.913	< 0.001*	0.813–1.000	≤ 61#	93.33	85.0	90.3	89.5
Inferior frontal gyrus	0.943	< 0.001*	0.860–1.000	≤ 44#	90.0	95.0	96.4	86.4
Parietal lobe	0.894	< 0.001*	0.775–1.000	≤ 46#	83.33	90.0	92.6	78.3
Temporal lobe	0.758	0.002*	0.616–0.899	≤ 36#	70.0	90.0	91.3	66.7
Occipital lobe	0.830	< 0.001*	0.717–0.943	≤ 36#	76.67	85.0	88.5	70.8
Putamen	0.803	< 0.001*	0.664–0.941	> 55#	86.67	85.0	89.7	81.0
Hippocampus	0.805	< 0.001*	0.687–0.923	> 57	60.0	85.0	85.7	58.6

AUC: Area Under a Curve, p value: Probability value, CI: Confidence Intervals, NPV: Negative predictive value, PPV: Positive predictive value

*Statistically significant at $p \leq 0.05$ #Cut off was choose according to Youden index

In the present study, 30 patients (20 males and 10 females aged from 20 to 50 years old) who were known to be schizophrenic underwent conventional brain MRI, together with ASL-MRI and MRS. Also, a matched group of 20 healthy volunteers (12 males and 8 females aged from 20 to 50 years old) were subjected to the same MRI examinations.

In this study, more than 66.7% of patients were males. This matches with the study of Nawka et al. [14] who found by clinical observation that males are more affected than females with earlier onset by (3–5) years.

A wide range of age groups were included in this study, ranging from 20 to 48 years, with most patients in the schizophrenic group (60%) were at the age ranged from 20 to 30, and the mean age was 35.6 ± 4.5 years. This agrees with Nawka et al. [14] who provided robust, global epidemiological estimates of age at onset for mental disorders, including schizophrenia, through a systematic review with meta-analysis, and found that the peak age of patients with schizophrenia was 33 years. In addition, according to the Global Burden of Disease Study 2016 that was published by Charlson et al. [15] and demonstrated the global epidemiology and burden of schizophrenia, as with prevalence, the peak disease burden was observed at around 30–40 years of age, where individuals are most likely to be economically productive.

The current study showed that about half of the patients (46.7%) had a positive family history of schizophrenia. This is in line with what has been reported that schizophrenia is a highly heritable

Table 7 Inter-observer agreement of rCBF values between observers in different brain regions

	1st Observer	2nd Observer	3rd Observer	1st vs. 2nd		1st vs. 3rd		2nd vs. 3rd	
	Mean ± SD	Mean ± SD	Mean ± SD	*p*	ICC (95% C.I)	p	ICC (95% C.I)	p	ICC (95% C.I)
Anterior cingulate	45.27 ± 5.16	44.27 ± 4.51	43.33 ± 4.58	< 0.001*	0.946 (0.747–0.984)	< 0.001*	0.845 (0.330–0.955)	< 0.001*	0.930 (0.753–0.978)
Frontal lobe	41.13 ± 4.27	40.27 ± 4.32	40.0 ± 3.89	< 0.001*	0.963 (0.668–0.991)	< 0.001*	0.913 (0.583–0.975)	< 0.001*	0.964 (0.901–0.988)
Medial frontal gyrus Lf	50.47 ± 7.37	49.73 ± 7.40	49.27 ± 7.56	< 0.001*	0.990 (0.918–0.997)	< 0.001*	0.978 (0.739–0.995)	< 0.001*	0.996 (0.967–0.999)
Medial frontal gyrus Rt	50.47 ± 7.37	49.73 ± 7.40	49.27 ± 7.56	< 0.001*	0.990 (0.918–0.997)	< 0.001*	0.978 (0.739–0.995)	< 0.001*	0.996 (0.967–0.999)
Inferior frontal gyrus	46.40 ± 7.34	46.07 ± 7.38	45.80 ± 7.35	< 0.001*	0.997 (0.986–0.999)	< 0.001*	0.992 (0.951–0.998)	< 0.001*	0.998 (0.991–0.999)
Parietal lobe	45.93 ± 6.71	45.53 ± 6.51	45.20 ± 6.75	< 0.001*	0.994 (0.975–0.998)	< 0.001*	0.982 (0.923–0.994)	< 0.001*	0.992 (0.975–0.997)
Temporal lobe	38.47 ± 5.58	38.40 ± 5.62	38.07 ± 5.47	< 0.001*	0.990 (0.972–0.997)	< 0.001*	0.985 (0.953–0.995)	< 0.001*	0.988 (0.964–0.996)
Occipital lobe	40.53 ± 5.44	40.27 ± 5.12	40.07 ± 4.89	< 0.001*	0.993 (0.978–0.998)	< 0.001*	0.986 (0.946–0.996)	< 0.001*	0.996 (0.987–0.999)
Putamen	59.60 ± 6.59	60.0 ± 6.60	60.33 ± 6.48	< 0.001*	0.992 (0.973–0.998)	< 0.001*	0.985 (0.909–0.996)	< 0.001*	0.993 (0.978–0.998)
Hippocampus	59.67 ± 5.46	60.07 ± 5.39	60.27 ± 5.36	< 0.001*	0.991 (0.963–0.997)	< 0.001*	0.985 (0.912–0.996)	< 0.001*	0.997 (0.989–0.999)

Value of ICC	Strength of agreement
Below 0.50	Poor
0.50 and < 0.75	Moderate
0.75 and 0.90	Good
Above 0.90	Excellent

ICC: Intra-class Correlation coefficient

Data were expressed using Mean ± SD

SD: Standard deviation

ICC: Intra-class Correlation coefficient

CI: Confidence interval

LL: Lower limit

UL: Upper Limit

P: p value for comparing between the two studied Observers

*Statistically significant at $p \leq 0.05$

(See figure on next page.)

Fig. 2 A twenty-one-year-old male patient complained of visual Hallucinations and Delusions, on clinical psychiatric examination: correct response on Wisconsin Card Sorting Test (WCST) was 28, errors response on Wisconsin Card Sorting Test (WCST) was 30 and that on Stroop test was 470. A Conventional MRI (**A**): axial FLAIR MRI brain image shows no significant abnormalities B- ASL (**B**): Axial ASL colour map shows mildly reduced frontal blood flow, (rCBF = 39.38 ml/min/100g) C- ASL (**C**) Axial ASL colour map shows mildly reduced parietal lobe blood flow, (rCBF = 39.61ml/min/100g) D- ASL (**D**) Axial ASL colour map shows mildly increased putamen blood flow, (rCBF = 63.03ml/min/100g) E- ASL (**E**) Axial ASL colour map shows moderately increased hippocampus blood flow, (rCBF = 70.55 ml/min/100g) F- MRS (**F**) MRS (long TE144) of Anterior Cingulate Cortex shows decreased NAA and reduced glutamate and increased glutamine, with NAA/Choline ratio 0.768 and NAA/Creatine ratio 1.19 G- MRS (**G**) MRS (long TE144) of Thalamus shows reduced NAA and increased both glutamate and glutamine with NAA/choline ratio 0.71 and NAA/creatine ratio 1.11. Final Radiological diagnosis: a case of acute schizophrenia, matching with clinical tests

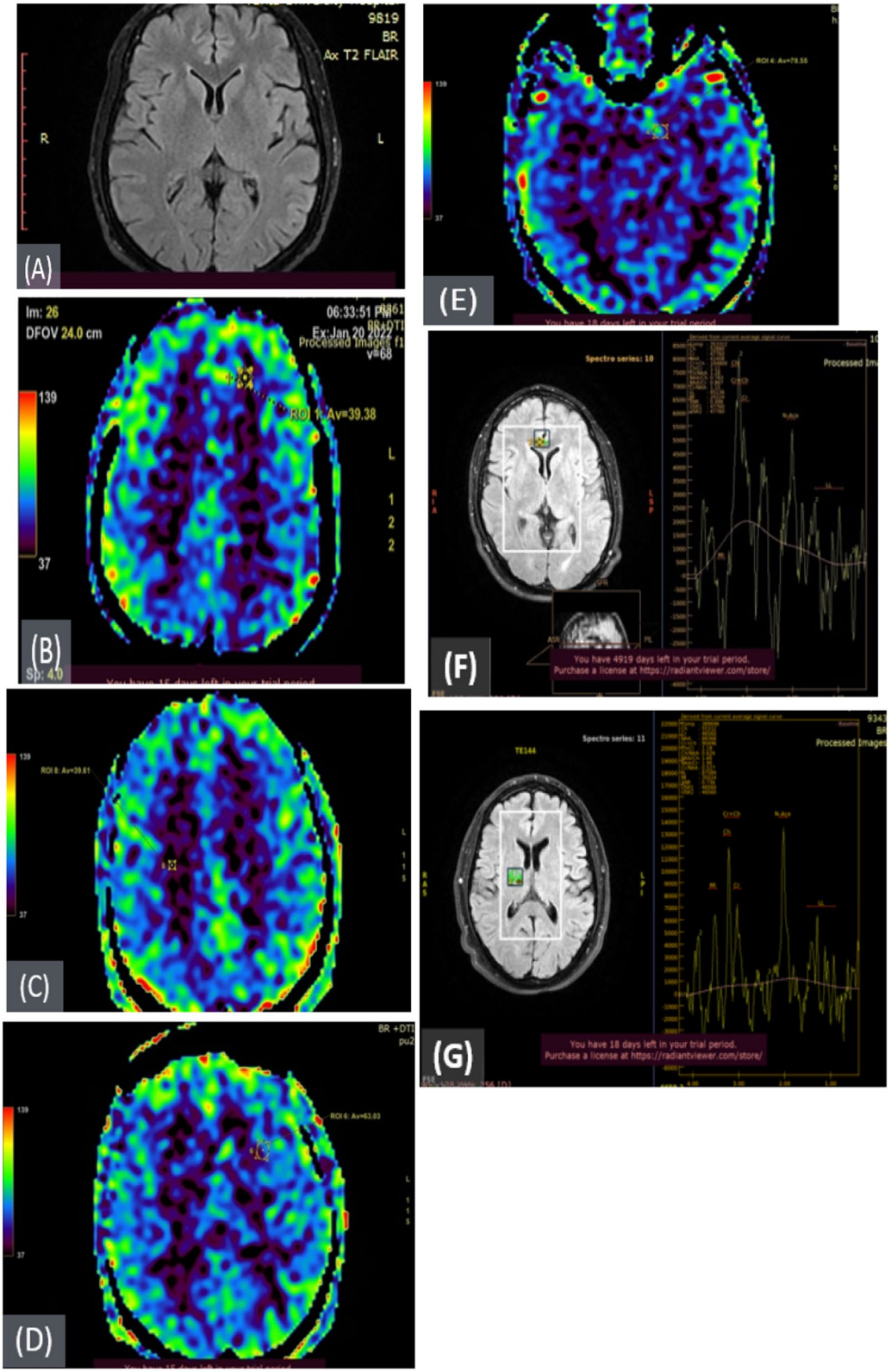


Fig. 2 (See legend on previous page.)

disorder according to the study made by Hilker et al. [16] who showed in their study that a substantial genetic component in liability to schizophrenia, with a heritability estimate of 79%.

All patients underwent conventional brain MRI to evaluate brain volume and any structural abnormalities, and it was noted that there is significant difference in the whole brain, thalamus, frontal, and temporal lobe volumes between the schizophrenia patients and the control group. These results are congruent with previous study of brain MRI by Guo et al. [17] who investigated schizophrenia individuals and controls with MRI brain and found that schizophrenia cases exhibited greater progressive brain reductions than controls, mainly in the frontal and temporal lobes.

In the current study, significant rCBF decrease in ACC, parietal and frontal lobes and increase putamen and hippocampus were found. In agreement with the current findings, Guimarães et al. [18] reported decreased rCBF in the frontal lobe of patients with schizophrenia. Convergent results of decreased rCBF in the ACC are consistent with clinical observations and other imaging studies. These results were in line with Zhu et al. [19] who identified decreased CBF in the left medial frontal gyrus, a cortical region of the frontal lobe and identified decreased CBF in the anterior cingulate cortex as well.

The striatum is studied in schizophrenia by Howes and McCutcheon [20] who reported higher flow in schizophrenia patients.

The present work implemented the use of MRS for assessment of patients with schizophrenia and the control healthy subjects. We found that NAA concentration in anterior cingulate cortex, thalami, basal Ganglia and hippocampus is significantly lower than in healthy controls, this was in agreement with the findings of Szulc et al. [21] study.

Glutamate was decreased in ACC and left superior temporal gyrus in both chronic cases and first attacks by same ratio. In basal ganglia and thalami, it was increased in 1st diagnosed cases rather than chronically ill patients. This goes with the study done by Poels et al. [22], who reported higher levels of glutamine in the ACC in 21 antipsychotic-naïve schizophrenia subjects than levels in 21 chronic, medicated schizophrenia subjects.

In the present study, there was significant reduction in creatine peaks in the prefrontal cortex and cerebellum. In similarity, previous studies have found reduced creatine in the anterior cingulate cortex in schizophrenia [23].

Concerning choline peak, it was significantly increased in visual cortex and dorsolateral prefrontal cortex in new cases of schizophrenia. This was also found by Plitman et al. [24].

Choline-containing compounds act as markers of membrane metabolism [24, 25]. Choline is present in higher concentrations within glial cells than in neurons and has been investigated in patients with schizophrenia. Elevated levels of these neuro-metabolites have been proposed to reflect glial activation and have been observed in several neuroinflammatory disorders [25].

Limitations of the study

The present study was limited by the relative small sample size, a large number of uncooperative patients they had been excluded from our study, non-long term follow-up of the patients after medications and assessment of the potential changes in MRS, ASL and their relation to the patients' clinical condition this will open new perspective about the role of advanced MRI techniques in diagnosis and follow-up of mental disorders, and in particular, schizophrenia.

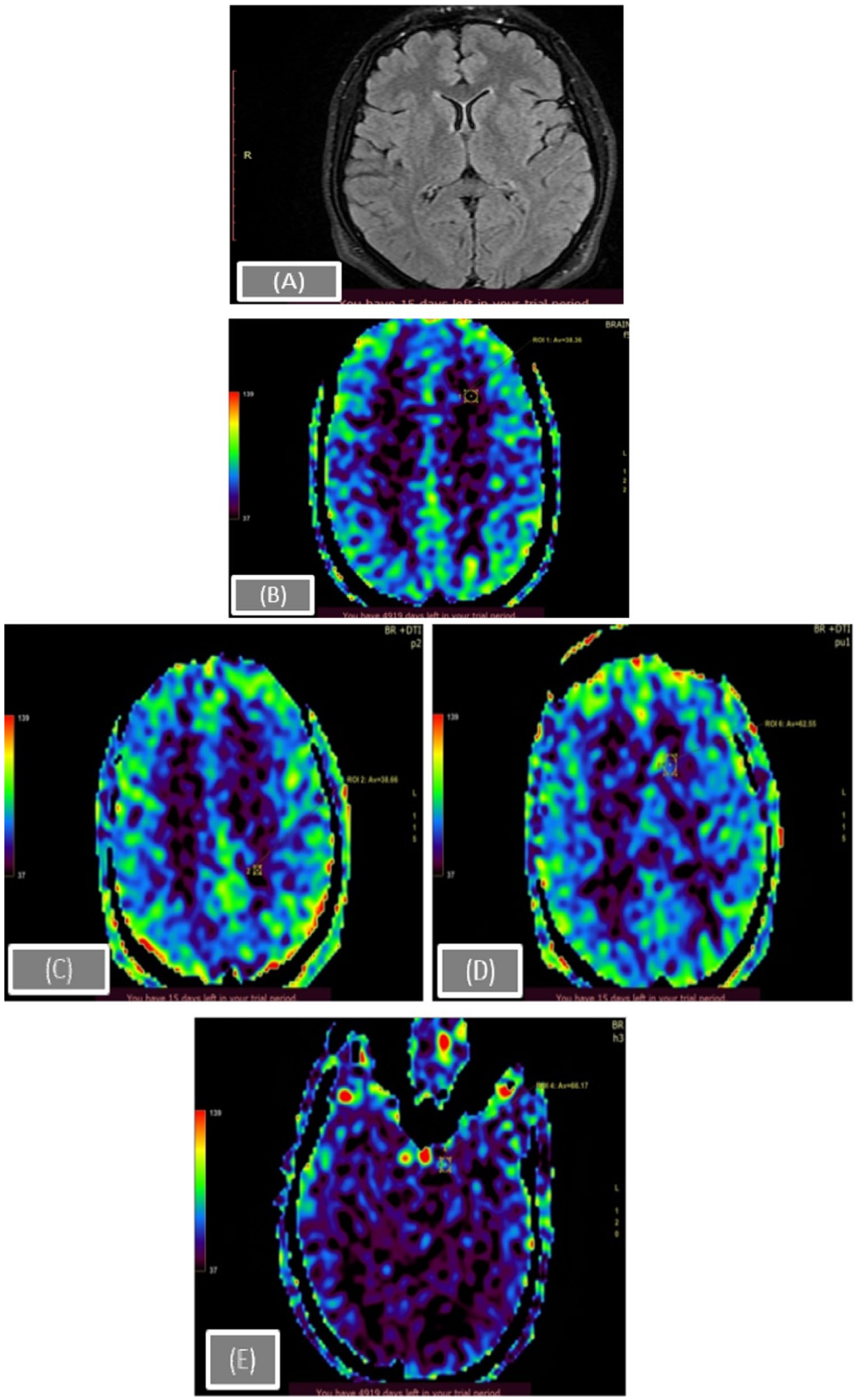
Conclusions

Combination of both ASL and MR spectroscopy can provide better diagnosis and differentiation between cases either it is acute or chronic.

As early detection of schizophrenia is an important tool to improve the outcome of the disease and adjust the

(See figure on next page.)

Fig. 3 A twenty-two-year-old male patient complained of Obsessions, Paranoia and hallucinations (visual and auditory), on clinical psychiatric examination: correct response on Wisconsin Card Sorting Test (WCST) was 27, errors response on Wisconsin Card Sorting Test (WCST) was 33 and that on Stroop test was 440. A Conventional MRI (**A**): axial FLAIR MRI brain image shows no significant abnormalities B -ASL (**B**): Axial ASL colour map shows mildly reduced frontal blood flow, (rCBF= 38.36ml/min/100g) C -ASL (**C**) Axial ASL colour map shows mildly reduced parietal lobe blood flow, (rCBF=39.66ml/min/100g) D -ASL (**D**) Axial ASL colour map shows moderately increased putamen blood flow, (rCBF = 62.55ml/min/100g) E -ASL (**E**) Axial ASL colour map shows moderately increased hippocampus blood flow, (rCBF=66.17 ml/min/100g) F—MRS (**F**) MRS (long TE144) of Frontal lobe shows decreased NAA and reduced glutamate and increased glutamine, with NAA/Choline ratio 1.29 and NAA/Creatine ratio 2.32 G -MRS (**G**) MRS (long TE144) of Thalamus shows reduced NAA and increased both glutamate and glutamine with NAA/choline ratio 0.92 and NAA/creatine ratio1.21 Final Radiological diagnosis: a case of acute schizophrenia, matching with clinical tests



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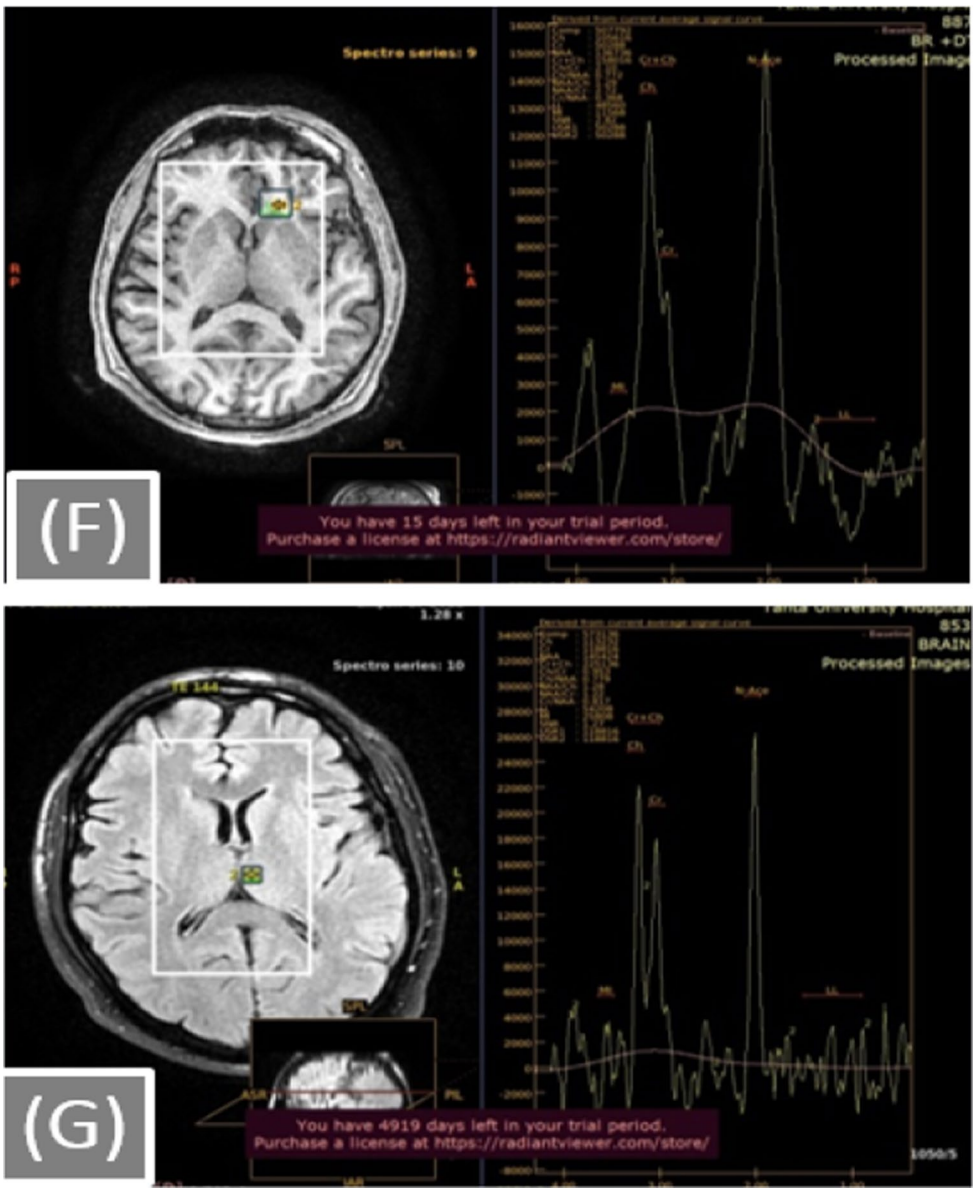

Fig. 3 continued

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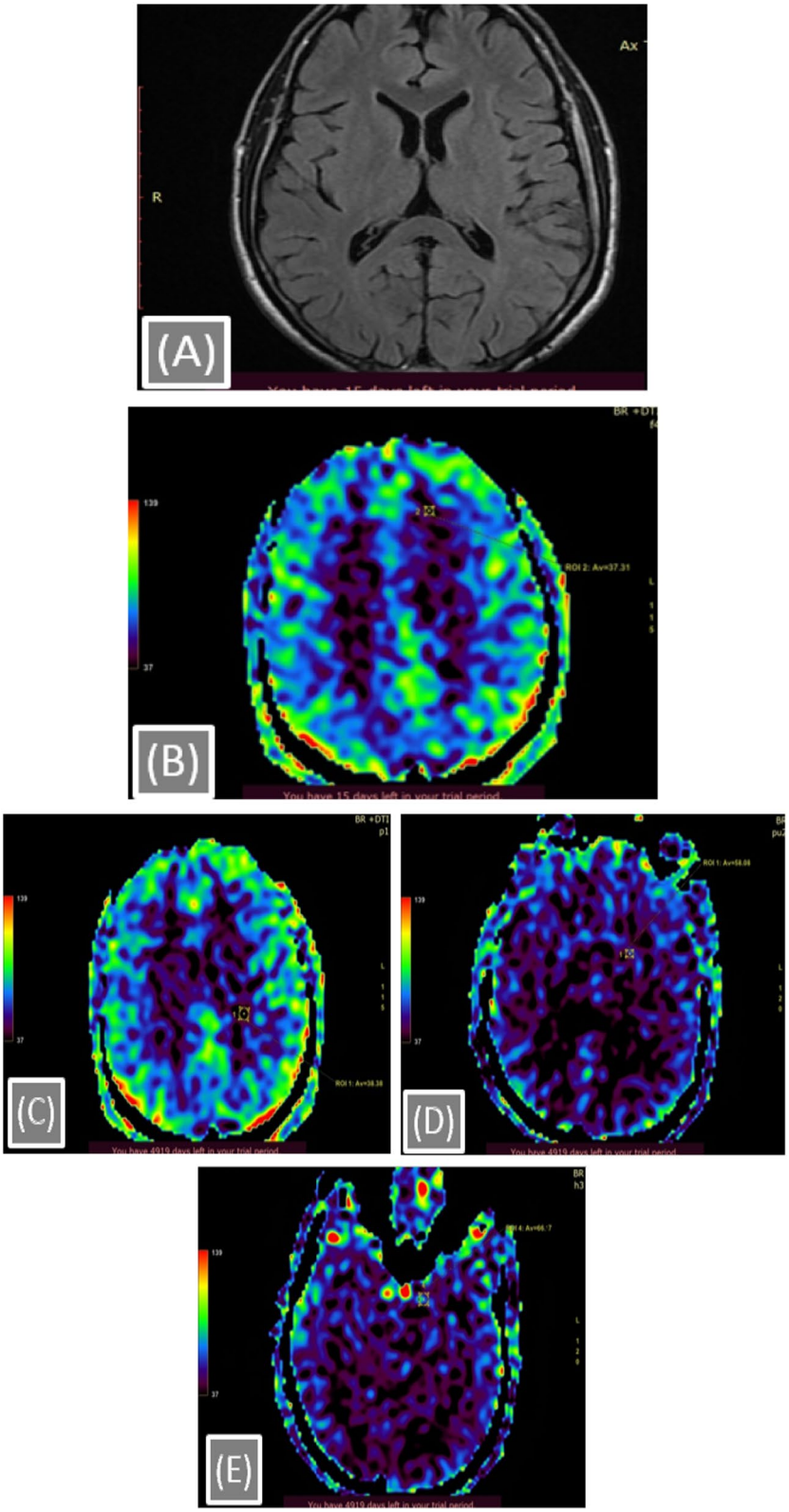
Fig. 4 A twenty-nine-year-old male patient complained of Hallucinations, Delusions, Obsessions and Paranoia, on clinical psychiatric examination: correct response on Wisconsin Card Sorting Test (WCST) was 38, errors response on Wisconsin Card Sorting Test (WCST) was 25 and that on Stroop test was 330. A—Conventional MRI (**A**): axial FLAIR MRI brain image shows slightly reduced whole brain volume B -ASL (**B**): Axial ASL colour map shows mildly reduced frontal blood flow, (rCBF= 37.31ml/min/100g) C -ASL (**C**) Axial ASL colour map shows mildly reduced parietal lobe blood flow, (rCBF=38.38ml/min/100g) D -ASL (**D**) Axial ASL colour map shows mildly increased putamen blood flow, (rCBF = 58.08ml/min/100g) E -ASL (**E**) Axial ASL colour map shows moderately increased hippocampus blood flow, (rCBF=66.17 ml/min/100g) F—MRS (**F**) MRS (long TE144) of Frontal lobe shows decreased NAA and reduced glutamate and increased glutamine, with NAA/Choline ratio 0.98 and NAA/Creatine ratio 2.23 G -MRS (**G**) MRS (long TE144) of Thalamus shows reduced NAA and also reduced both glutamate and glutamine with NAA/choline ratio 0.714 and NAA/creatine ratio1.36 Final Radiological diagnosis: a case of chronic schizophrenia, matching with clinical tests



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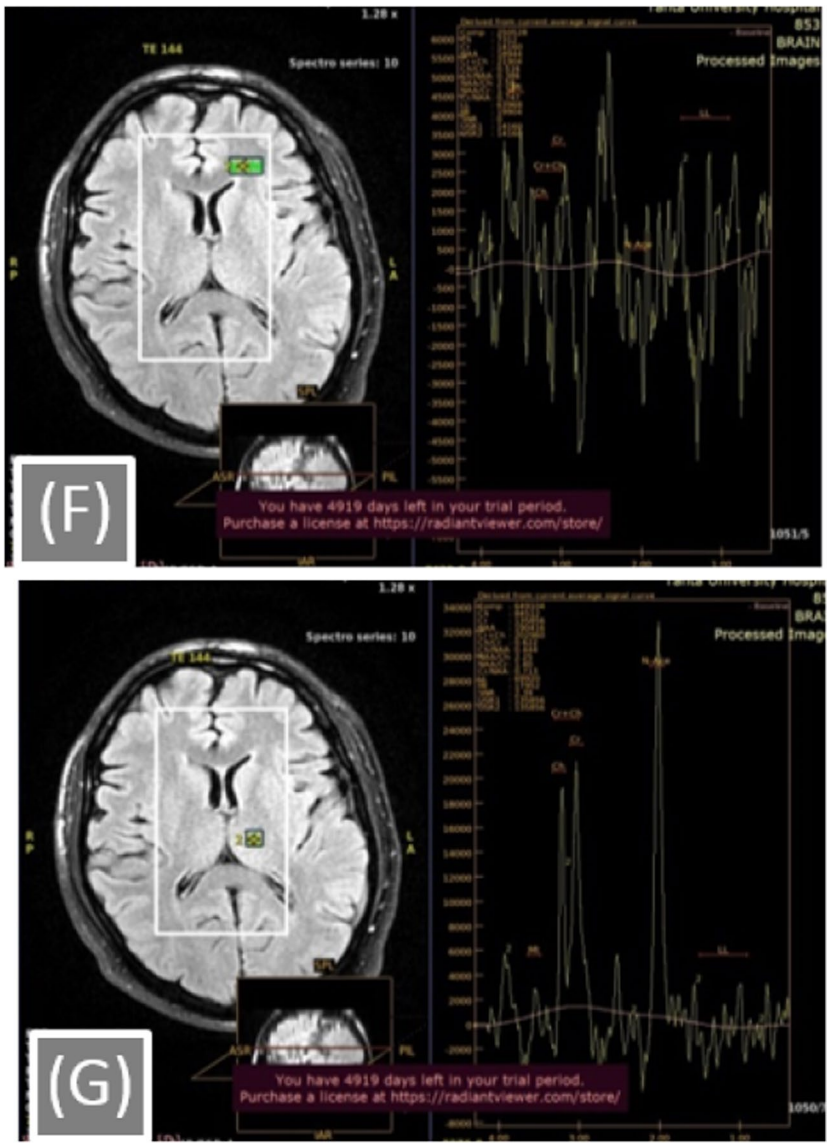

Fig. 4 continued

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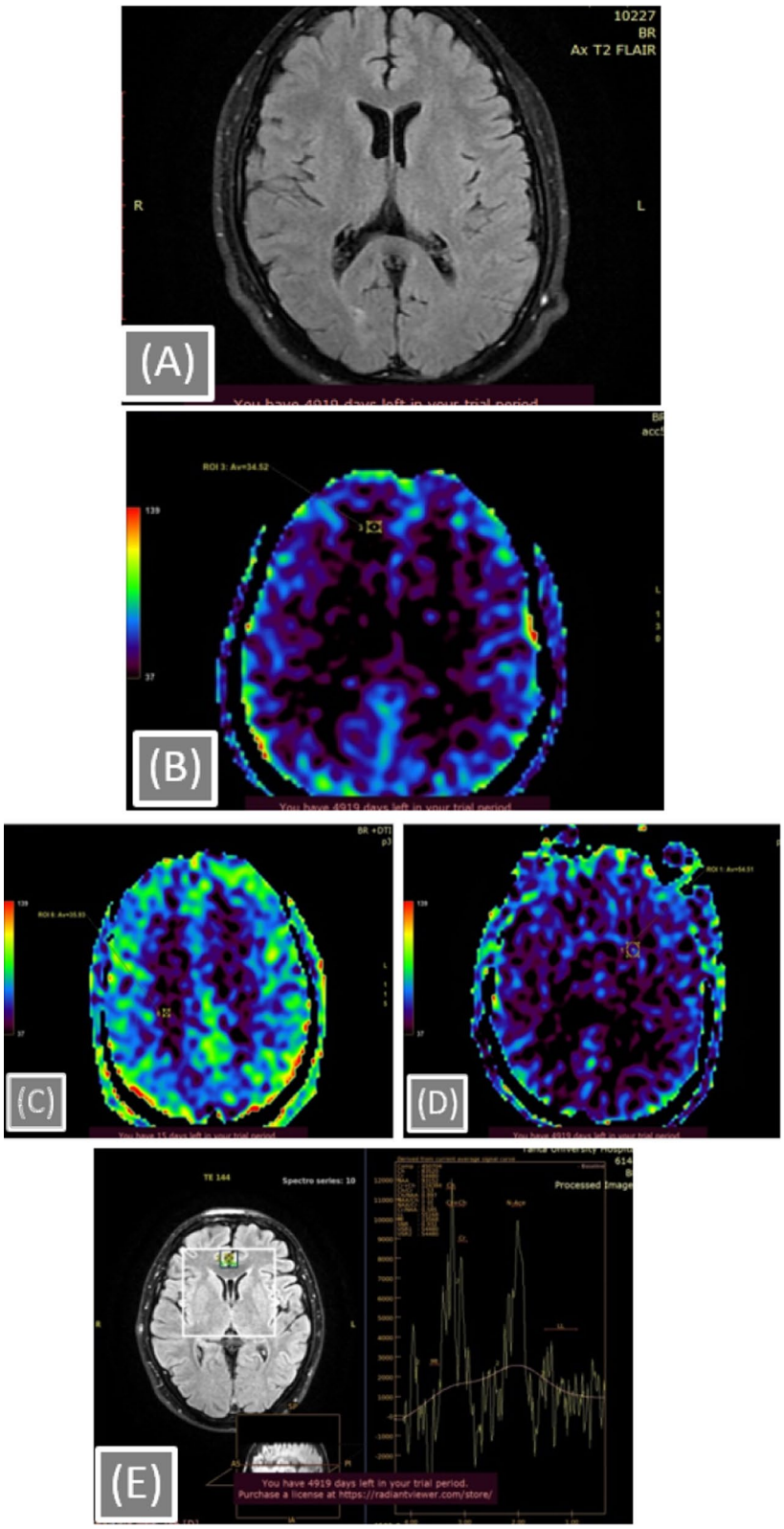
Fig. 5 A thirty-two-year-old male patient complained of Aggression, Delusions and Hallucinations (visual and auditory), on clinical psychiatric examination: correct response on Wisconsin Card Sorting Test (WCST) was 40, errors response on Wisconsin Card Sorting Test (WCST) was 22 and that on Stroop test was 370. A—Conventional MRI (**A**): axial FLAIR MRI brain image slightly reduced brain volume. B -ASL (**B**): Axial ASL colour map shows mildly reduced frontal blood flow, (rCBF= 34.52ml/min/100g) C -ASL (**C**) Axial ASL colour map shows mildly reduced parietal lobe blood flow, (rCBF= 35.93ml/min/100g) D -ASL (**D**) Axial ASL colour map shows moderately increased putamen blood flow, (rCBF = 54.51ml/min/100g) **E**- MRS (**F**) MRS (long TE144) of Anterior Cingulate Cortex shows decreased NAA and reduced glutamate and increased glutamine, with NAA/Choline 0.80 ratio and NAA/Creatine ratio 1.26 G -MRS (**G**) MRS (long TE144) of Thalamus shows reduced NAA and also reduced both glutamate and glutamine with NAA/choline ratio 0.705 and NAA/creatine ratio 1.25 Final Radiological diagnosis: a case of chronic schizophrenia, matching with clinicaltests



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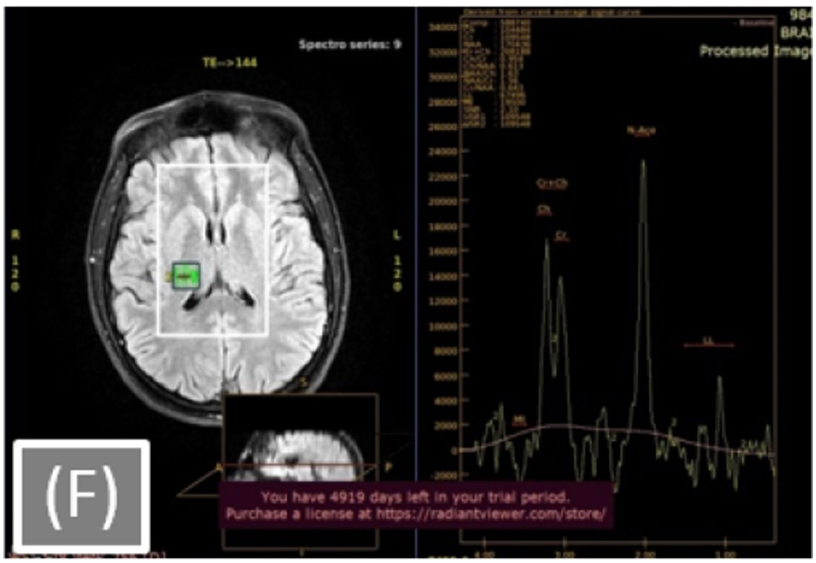

Fig. 5 continued

plan of treatment, it is recommended to use quantitative ASL-MRI for early detection of rCBF changes.

Recommendations

Finally, from the current study, we recommend ASL and MR spectroscopy techniques in screening for psychiatric disease among the high-risk individuals, especially those with positive family history, which will help in early detection of schizophrenic patients and their first-degree relatives even with no obvious clinical symptomatology.

Continued data collection in a larger sample, longer duration with the use of powerful MRI 3.0-Tesla scanners will be helpful in further studies to develop the best practice for ASL and MR spectroscopy.

Follow-up studies for longer period up to 6–12 months will be valuable in treatment assessment and remodelling treatment plan according to course of the disease for every patient.

Abbreviations

ANOVA	One-way of variance
ASL	Arterial spin labelling
BOLD	Blood-oxygen level dependency
CASL	Continuous arterial spin labelling
CBF	Cerebral blood flow
CHESS	Chemical shift selection
CHO	Choline
Cr	Creatine
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
GLN	Glutamine
GLU	Glutamate
GRASE	Gradient- and spin-echo imaging
MINI	Mini International Neuropsychiatric Interview
MRS	Magnetic resonance spectroscopy
NAA	N-acetyl aspartate
PANSS	Positive and negative syndrome scale
rCBF	Regional cerebral blood flow
ROI	Regions of interest
SD	Standard deviation
SE	Spin-echo mode sequence
SNR	Signal-to-noise ratio
TE	Time to echo
TI	Inversion time
TR	Repetition time
WCST	Wisconsin card sorting test

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Author contributions

RE suggested the research idea, ensured the original figures and data in the work, minimized the obstacles to the team of work, correlated the study concept and design and had the major role in analysis, ME supervised the study with significant contribution to design the methodology, manuscript revision and preparation, AM correlated the clinical data of patient and matched it with the findings, drafted and revised the work. AE collected data in all stages of manuscript, performed data analysis.

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Availability of data and materials

The authors confirm that all data supporting the finding of the study are available within the article and the raw data supporting the findings were generated and available at the corresponding author on request.

Declarations

Ethics approval and consent to participate

Informed written consents were taken from the patients and healthy volunteers; the study was approved by ethical committee of Tanta University hospital, faculty of medicine.

Consent for publication

All participants included in the research gave written consent to publish the data included in the study. Authors accepted to publish the paper.

Competing interests
The authors declare that they have no competing of interests.

Author details
[1]Tanta, Gharbia Governorate, Egypt. [2]Faculty of Medicine, Tanta University, El-geish Street, Tanta, Gharbia Governorate, Egypt.

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