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Proton magnetic resonance spectroscopy of brain in obstructive sleep apnoea in north Indian Asian subjects

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Background & objectives: Repeated apnoeic/hypoapnoeic episodes during sleep may produce cerebral damage in patients with obstructive sleep apnoea (OSA). The aim of this study was to determine the absolute concentration of cerebral metabolites in apnoeic and non-apnoeic subjects from different regions of the brain to monitor the regional variation of cerebral metabolites.

Methods: Absolute concentration of cerebral metabolites was determined by using early morning proton magnetic resonance spectroscopy (¹H MRS) in 18 apnoeic patients with OSA (apnoeics) having apnoea/hypopnoea index (AHI) >5/h, while 32 were non-apnoeic subjects with AHI < 5/h.

Results: The absolute concentration of tNAA [(N-acetylaspartate (NAA)+N-acetylaspartylglutamate (NAAG)] was observed to be statistically significantly lower ($P<0.05$) in apnoeics in the left temporal and left frontal gray regions compared to non-apnoeics. The Glx (glutamine, Gln + glutamate, Glu) resonance showed higher concentration (but not statistically significant) in the left temporal and left frontal regions of the brain in apnoeics compared to non-apnoeics. The absolute concentration of myo-inositol (mI) was significantly high ($P<0.03$) in apnoeics in the occipital region compared to non-apnoeics.

Interpretation & conclusions: Reduction in the absolute concentration of tNAA in apnoeics is suggestive of neuronal damage, probably caused by repeated apnoeic episodes in these patients. NAA showed negative correlation with AHI in the left frontal region, while Cho and mI were positively correlated in the occipital region and Glx showed positive correlation in the left temporal region of the brain. Overall, our results demonstrate that the variation in metabolites concentrations is not uniform across various regions of the brain studied in patients with OSA. Further studies with a large cohort of patients to substantiate these observations are required.

Key words Brain metabolites - glutamate + glutamine (Glx) - magnetic resonance spectroscopy (MRS) - myo-inositol (mI) - N-acetyl aspartate (NAA) - obstructive sleep apnoea (OSA) - north Indian subjects

Obstructive sleep apnoea is a major public health problem worldwide¹⁻³. It is associated with several consequences including neurocognitive impairment⁴.

Magnetic resonance spectroscopy (MRS) is a non-invasive method, useful for evaluating local metabolic changes in various conditions affecting the

central nervous system, including tumours⁵, infarction and ischaemia⁵, multiple sclerosis, Alzheimer's disease, and epilepsy. Neuropsychological⁶ and electrophysiological tests have been used to evaluate central nervous system impairment in patients with obstructive sleep apnoea (OSA). In these patients, repeated apnoeic episodes during sleep may lead to changes in cerebral metabolism. Excessive daytime sleepiness and cognitive and emotional deficits are common daytime symptoms of OSA⁷⁻⁹. Hypoxic brain damage and fragmentation of sleep are generally thought to be causes of these deficits^{10,11}.

Several MRI studies in patients with OSA reported varying results. Regional gray matter loss in the frontal cortex, parietal cortex, temporal lobe, anterior cingulate, hippocampus, and cerebellum^{12,13} and hippocampal atrophy in patients with OSA have been reported¹⁴. However, O'Donoghue and colleagues¹⁵ found no evidence of gray matter change in hippocampus, temporal lobe, and whole brain in patients with OSA.

Previous MRS studies have documented cerebral metabolic changes in patients with OSA¹⁶⁻²². A decrease in n-acetyl aspartate/choline (NAA/Cho) in the periventricular white matter in patients with moderate to severe OSA¹⁶ and NAA/creatine (Cr) and Cho/Cr ratios in the frontal white matter of severe OSA patients compared with controls¹⁷ was reported. Recently, Tonon *et al*²⁰ reported lowered cortical NAA in OSA patients compared with controls. Reduced NAA/Cho in the left hippocampus and right frontal cortex was also reported in children with OSA²³.

Though several MRI and MRS studies on patients with OSA have been reported in literature; only two or three regions of the brain were investigated with varying findings. Further, only metabolite ratios were used instead of absolute concentration of metabolites, except in two studies¹⁸⁻²⁰. The aim of the present study was, therefore, to determine the absolute concentration of cerebral metabolites in apnoeic (OSA) and non-apnoeic subjects from five different regions of the brain with an objective to monitor the regional variation of cerebral metabolites in these patients.

Material & Methods

Patient selection: The investigations were conducted at the All India Institute of Medical Sciences Hospital, New Delhi, a tertiary level referral center. The study protocol was reviewed and approved by the research review committee and the Institute ethical committee. A written informed consent was obtained from all

patients. Subjects attending medical inpatient and outpatient departments from April 2004 to October 2006 were eligible to participate in this study. Subjects were taken up for study using the following criteria: subjects of either gender in the age group of 18-60 yr, residents of New Delhi and nearby areas. Patients with history of alcoholism, chronic anxiolytic/sedative drug use, associated respiratory, renal, hepatic or cardiovascular disease or upper respiratory tract infection within the past one week as well as those who were pregnant or critically ill were excluded.

A detailed physical examination was carried out in all subjects. Blood pressure was measured in recumbent position in all subjects after at least five minutes of rest. Subjects were advised to refrain from smoking or ingesting caffeine during the 30 min preceding the blood pressure measurement. A variety of large cuff sizes were used where necessary to ensure that bladder length was at least 80 per cent of the arm circumference. A history of antihypertensive medication intake was also obtained in subjects with hypertension.

A specialist otorhinolaryngologist blinded to polysomnography (PSG) findings carried out examination of upper airway in all subjects included in the study. A specific note of the following abnormalities was made; macroglossia, pharyngeal crowding, bulky uvula, retrognathia, tonsillar enlargement and deviated nasal septum. All subjects underwent complete blood count (CBC), liver and renal function tests, thyroid functions, lipid profile, chest radiograph and an electrocardiogram.

Anthropometric profile: Body weight was recorded (to nearest 0.5 kg) in all patients, in erect position without shoes and wearing only light indoor clothes, with an electronic scale. Total body fat, excess body fat and total body water were estimated by bipedal bioelectric impedance technique using Tanita body composition analyzer-TBF 300 G.S., Japan. Height was measured to the nearest 1 cm and body mass index (BMI) was calculated as body weight/height² (kg/m²). Neck circumference (NC) was measured at the level of cricothyroid membrane using a non-elastic measuring tape. Neck length (NL) was measured from occipital tubercle to the vertebra prominens. A height-corrected measure for NC, percentage predicted neck circumference (PPNC) was computed using the formula, PPNC = (1000 x NC)/(0.55 H+310)^{20,21}. Waist circumference was measured midway between the lower rib cage margin and the anterior superior iliac spine. Hip circumference was measured at the

maximum circumference of the buttocks, the subject standing with feet placed together and waist-hip ratio (W-HR) was calculated.

Skinfold thickness was measured using Lange skinfold calipers (Beta Technology Inc., Santa Cruz, CA, USA) to the nearest 1 mm. Mid-arm circumference (cm) was measured at the level of mid-arm taking acromion process and olecranon as reference points. Triceps and biceps skinfold thicknesses were measured midway between the acromion process of scapula and the olecranon process. Subscapular skinfold thickness (SSFT) was measured at the inferior angle of scapula in mid-axillary line and suprailiac skinfold thickness was measured just above the highest point of iliac crest. All measurements were carried out at least 3 times and the mean value was recorded.

Polysomnography (PSG): The PSG was performed as described previously²⁵. All subjects were asked to complete a questionnaire before the test. Subjects were asked not to sleep in the afternoon on the day of study and not to take alcohol anxiolytic/sedative drugs. They were called to the Sleep Laboratory at 0800 h and each patient was hooked to Alice 3 infant and adult computerized PSG machine (Healthdyne Technologies, USA) by standard gold cups after cleansing the area of attachment by spirit followed by Omni prep[®]. Subjects were requested to go to sleep around 0900 h. The recording of sleep was started after ensuring that the impedance of recording electrodes was set to zero. Various parameters monitored included electroencephalogram (EEG), electro-oculogram (EOG), electrocardiogram (ECG), chin and leg electromyogram (EMG), nasal airflow, tracheal breath sounds, thoracic wall and abdominal movements, transcutaneous oxygen saturation, body position and continuous positive airway pressure (CPAP) titration, where required. All subjects underwent PSG for at least 6 h. The sleep data recorded by the computer were manually scored for sleep stages, apnoeas, and hypopnoeas. An experienced laboratory technician blinded to any clinical data scored all polysomnograms. Apnoea was defined as cessation of oro-nasal airflow for ≥ 10 sec. Obstructive apnoeas were scored when airflow was absent but respiratory efforts were present. Hypopnoea was defined as a discernible reduction in respiratory airflow during a preceding period of normal breathing for ≥ 10 sec accompanied by a decrease of 4 per cent or more in oxyhaemoglobin saturation during sleep. Apnoea-hypopnoea index (AHI) was calculated based on the following formula: $AHI = (\text{total no.}$

of obstructive apnoeas + total no. of hypopnoeas)/total sleep time (h). Subjects with polysomnographic evidence of $AHI \geq 5/h$ were categorized as 'apnoeics' and the subjects with $AHI < 5/h$ as 'non-apnoeics'. The severity of OSA was graded as mild (5-14.9), moderate (15-29.9) and severe (>30 events/h).

¹H MRS of brain: Volume-localized ¹H MR spectroscopy was carried at 1.5 Tesla (MAGNETOM SONATA/AVANTO, Siemens, Germany) using a phased array head coil. Prior to performing ¹H MR spectroscopy, multislice T1-weighted images in the coronal and sagittal planes of the whole brain were acquired using a standard spin-echo pulse sequence [time to echo (TE)= 15 msec; time for repetition (TR)= 520 msec; 3-5 mm slice thickness; 256x256 matrix]. Then T2-weighted axial images were acquired using the parameters TE= 90 ms; TR= 2500 ms; 3-5 mm slice thickness; 256 x 256 matrix. These images were then used to select the region of interest for performing the MR spectroscopy.

Single voxel proton MR spectra were recorded using the PRESS pulse sequence with the following parameters: TR = 2000 ms; TE = 30 ms; NS = 128. Care was taken to optimize the magnetic field homogeneity by carrying out both global and voxel shim. The line-width after voxel shim ranged from 5 to 9 Hz depending on the voxel size and the region studied. Spectra were acquired from five different areas of the brain: namely from left temporal, left frontal white and gray matter, hippocampus and occipital regions. However, spectra from all these brain regions could not be recorded from all subjects due to long acquisition time and patient non co-operation. The voxel size used in various brain regions studied varied; 2 to 2.5 ml for the left temporal region; 3 to 3.4 ml for the frontal lobe; 1 to 1.2 ml for the hippocampal region and 3.4 ml for the occipital region. Absolute concentration of metabolites from 5 different regions of the brain was estimated by LC Model which allows deconvolution of spectra by using a basis set of reference spectra. The errors in metabolite concentration determined are expressed in per cent standard deviation (%SD) of the concentration and represent the 95 per cent confidence interval (95% CI) of the estimated concentration. Further, the concentrations of metabolites were included only if their %SD was within 20 per cent. The concentration of metabolites was expressed as millimoles per liter (mmol/l).

Statistical analysis: Data were recorded on a pre-designed data sheet and managed on an 'Excel'

spreadsheet. All entries were double checked for any possible feeding error. Anthropometric measurements and PSG findings in apnoeics and non-apnoeics were compared using Student's t-test or Wilcoxon's Rank sum test as appropriate. The concentrations of each metabolites in different regions of the brain were compared using one way analysis of variance (ANOVA). Pearson's correlation coefficient of AHI with various brain metabolites was calculated. The significance of these correlations was assessed by t-test. Statistical analysis was performed using statistical software package STATA version 10.2 (Stata Corporation, Houston, Texas, USA).

Results

A total of 50 subjects underwent overnight polysomnography and had early morning ^1H MRS of the brain. Single spectrum from each volume of interest (VOI) was acquired from various brain regions and none was excluded because of poor quality. Of these, apnoeic group consisted of 18 patients who had $\text{AHI} > 5/\text{h}$ (female 6, male 12) while the remaining 32 were non-apnoeic with $\text{AHI} < 5/\text{h}$ (female 11, male 21). The mean age of apnoeics and non-apnoeics was 48.0 ± 8.8 and 39.9 ± 10.2 yr, respectively. The apnoeic patients had a mean BMI of $30.0 \pm 5.5 \text{ kg/m}^2$ while non-apnoeic subjects had a mean BMI of $27.2 \pm 5.9 \text{ kg/}$

m^2 . The waist-hip ratio and neck circumference were significantly higher ($P < 0.05$) in apnoeic patients as compared to non apnoeics (Table I).

Polysomnography (Tables I, II): The mean Epworth sleepiness scale (ESS) value in apnoeics (12.0 ± 5.6) was significantly higher ($P < 0.001$) as compared to non-apnoeics (5.5 ± 4.1). The AHI was significantly higher ($P < 0.001$) in apnoeics (46.7 ± 29.8 events/h) as compared to non-apnoeics (0.89 ± 1.3 events/h). Both the groups showed no significant difference between total sleep time and sleep efficiency. The minimum SaO_2 , $\text{SaO}_2 < 90$ per cent, stage I% and slow wave sleep showed significant differences between the two groups ($P < 0.001$). In addition, stage II% and REM% were also significantly different between the groups ($P < 0.05$).

Brain metabolites on ^1H MRS (Tables III, IV and Figs 1-4): The most prominent signal in the proton spectra of brain is from NAA that appears at 2.01 ppm (Fig. 1). The singlet at 2.04 ppm which appeared as a shoulder to the NAA CH_3 peak (2.01 ppm) arose from the dipeptide N-acetylaspartylglutamate (NAAG). At low fields, NAA and NAAG are not easily distinguishable^{26,27} and hence the peak around 2.01 ppm in the proton MR spectrum is usually referred to tNAA. Other major resonances were from creatine (Cr) plus phosphocreatine (PCr) at 3.02 ppm and Cho [also has contributions from phosphocholine (PC) and glycerophosphocholine (GPC)] at 3.2 ppm. The multiplet resonance pattern around 2.2 to 2.5 ppm is due to Glx [glutamate (Glu) and glutamine (Gln)] and the peak at 3.54 ppm is due to myo-inositol (mI).

Table I. Baseline characteristics of the study groups

Variables	Non-apnoeics (n=32)	Apnoeics (n=18)
Age (yr)	39.9 ± 10.2	48 ± 8.8
Males (%)	21 (65.6%)	12 (66.7%)
BMI (kg/m^2)	27.2 ± 5.9	30 ± 5.5
Fat (%)	29 ± 11.1	34.7 ± 10.3
TSFT (mm)	20.8 ± 8.8	21 ± 7.8
BSFT (mm)	12.8 ± 6	14 ± 5
SSFT (mm)	26.3 ± 8.2	28.6 ± 9
SIFT (mm)	31.8 ± 7.5	31.5 ± 9
Neck circumference (cm)	33.6 ± 5	$37.8 \pm 5.6^*$
Waist-hip ratio	0.97 ± 0.06	$1.03 \pm 0.1^*$
Systolic blood pressure (mmHg)	129.7 ± 12.4	$141.4 \pm 18.9^*$
Diastolic blood pressure (mmHg)	87.9 ± 11.2	$93.4 \pm 11.6^*$
Epworth sleepiness scale	5.5 ± 4.1	$12 \pm 5.6^{**}$
AHI (events/h)	0.89 ± 1.3	$46.7 \pm 29.8^{**}$

Data are expressed as mean \pm SD or No. (%), unless otherwise indicated; * $P < 0.05$, ** $P < 0.001$ compared to non-apnoeics BMI, body mass index; AHI, apnoea hypoapnoea index; TSFT, triceps skin fold thickness; BSFT, biceps skin fold thickness; SSFT, subscapular skin fold thickness; SIFT, subiliac fold thickness

Table II. Comparison of polysomnography parameters between the study groups

Parameters	Non-apnoeics (n=32)	Apnoeics (n=18)
Baseline SaO_2 (%)	97.3 ± 1.2	95.4 ± 5
Minimum SaO_2 (%)	88 ± 7.7	$65.9 \pm 14.8^{**}$
$\text{SaO}_2 < 90\%$, (%TST)	1.6 ± 4.9	$32.3 \pm 35.6^{**}$
Total sleep time (min)	361.6 ± 75.6	368.8 ± 64.3
Stage I (%)	14.3 ± 8	$23.5 \pm 6.7^{**}$
Stage II (%)	42.3 ± 16.2	$56.7 \pm 8.6^*$
Stage III & IV (SWS %)	24.9 ± 16.7	$8.2 \pm 4^{**}$
REM %	10.7 ± 5.4	$7.1 \pm 3.5^*$
Sleep efficiency (%)	83.7 ± 13.2	83 ± 11.9

Values are given as the mean \pm SD, unless otherwise indicated; * $P < 0.05$, ** $P < 0.001$ compared with non-apnoeics SaO_2 , arterial oxygen saturation; TST, total sleep time; REM, rapid eye movement; SWS, slow wave sleep

Table III. Concentration of brain metabolites (mmol/l) measured by ¹H MRS in different regions of brain in apnoeics and non-apnoeics

Metabolite	Group	Left hippocampus absolute value	Left temporal	Left frontal white	Left frontal gray	Occipital gray	P
Cr+PCr	Non-apnoeic	5.21 ± 0.84 (N = 13)	4.42 ± 0.48 (N = 25)	4.33 ± 0.57 (N = 3)	4.59 ± 0.36 (N = 7)	4.91 ± 0.57 (N = 8)	< 0.01
	Apnoeic	4.78 ± 1.00 (N = 9)	4.17 ± 0.83 (N = 15)	5.12 ± 0.59 (N = 6)	4.68 ± 0.70 (N = 5)	5.18 ± 0.62 (N = 4)	0.08
	P	0.29	0.22	0.10	0.76	0.48	---
tNAA	Non-apnoeic	6.13 ± 0.93 (N = 13)	8.53 ± 0.78 (N = 25)	7.57 ± 2.07 (N = 3)	6.80 ± 0.23 (N = 7)	7.60 ± 0.97 (N = 8)	< 0.001
	Apnoeic	6.19 ± 1.22 (N = 9)	7.29 ± 2.14 (N = 15)	7.50 ± 0.69 (N = 7)	6.46 ± 0.27 (N = 5)	7.42 ± 0.76 (N = 4)	0.33
	P	0.90	0.01	0.94	0.04	0.76	---
mI	Non-apnoeic	5.87 ± 0.91 (N = 12)	4.23 ± 0.82 (N = 21)	*	3.64 ± 0.38 (N = 5)	3.46 ± 0.41 (N = 8)	< 0.001
	Apnoeic	5.92 ± 1.09 (N = 9)	4.26 ± 1.02 (N = 12)	*	4.52 ± 0.95 (N = 5)	4.10 ± 0.36 (N = 4)	0.001
	P	0.92	0.93	---	0.09	0.03	---
Cho	Non-apnoeic	1.84 ± 0.39 (N = 12)	1.62 ± 0.23 (N = 25)	1.87 ± 0.21 (N = 3)	1.13 ± 0.15 (N = 6)	0.92 ± 0.12 (N = 8)	< 0.001
	Apnoeic	1.60 ± 0.32 (N = 8)	1.65 ± 0.26 (N = 15)	2.22 ± 0.25 (N = 5)	0.90 ± 0.56 (N = 4)	1.10 ± 0.22 (N = 4)	< 0.001
	P	0.16	0.70	0.09	0.35	0.09	---
Glx	Non-apnoeic	11.46 ± 2.02 (N = 9)	6.93 ± 1.15 (N = 13)	*	9.47 ± 1.62 (N = 7)	9.48 ± 1.58 (N = 8)	< 0.001
	Apnoeic	11.23 ± 0.94 (N = 6)	7.96 ± 1.21 (N = 7)	*	7.92 ± 0.86 (N = 5)	10.30 ± 1.89 (N = 4)	< 0.001
	P	0.81	0.08	*	0.08	0.44	

*Metabolite concentration was not calculated in these two regions. One-way ANOVA was used to compare the P values among different regions

tNAA; N-acetyl aspartate + NAAG; dipeptide N-acetylaspartylglutamate, Cr; Creatine, PCr; phosphocreatine Cho; Choline containing compound, Glx; glutamate (Glu) and glutamine (Gln); myo-inositol (mI)

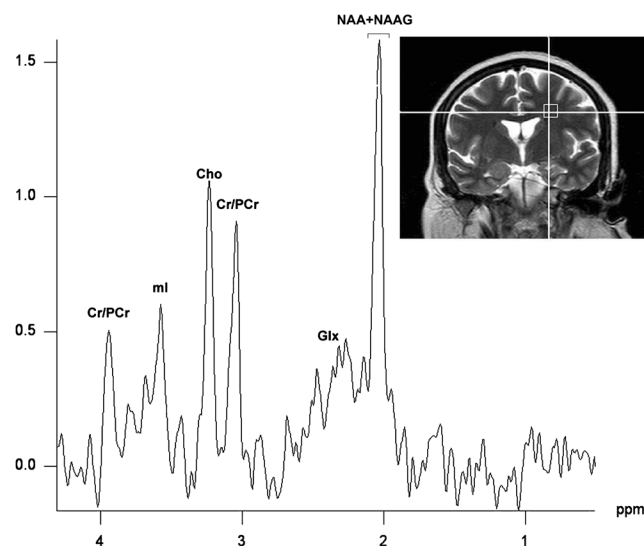


Fig. 1. Proton MR spectrum from the frontal gray matter of an apnoeic patient. The insert shows the voxel position in the coronal MR image of the brain. The abbreviations shown in figure correspond to: NAA (N-acetyl aspartate); Cr (Creatine); PCr (phosphocreatine); Cho (Choline); NAAG (N-acetylaspartylglutamate); Glx [glutamate (Glu) and glutamine (Gln)]; mI(myo-inositol).

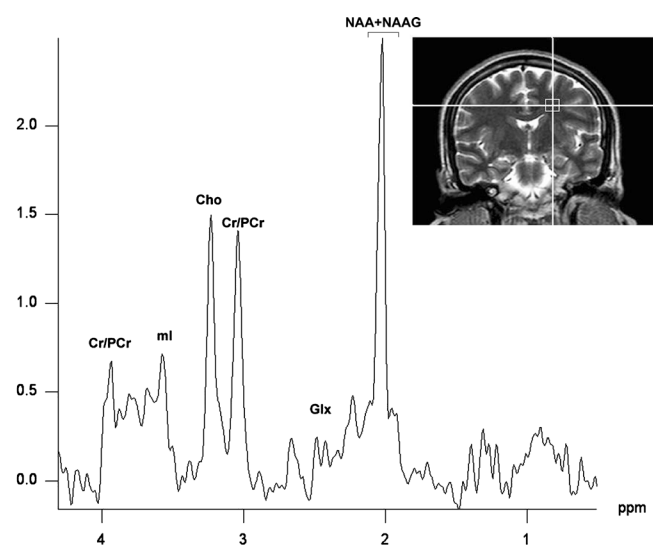


Fig. 2. Proton MR spectrum from the frontal gray matter of a non-apnoeic subject. The insert shows the voxel position in the coronal MR image of the brain. The abbreviations shown in figure correspond to: NAA (N-acetyl aspartate); Cr (Creatine); PCr (phosphocreatine); Cho (Choline); NAAG (N-acetylaspartylglutamate); Glx [glutamate (Glu) and glutamine (Gln)]; mI(myo-inositol).

Table IV. Correlation of various metabolites with AHI in apnoeics and non-apnoeics from different regions of the brain

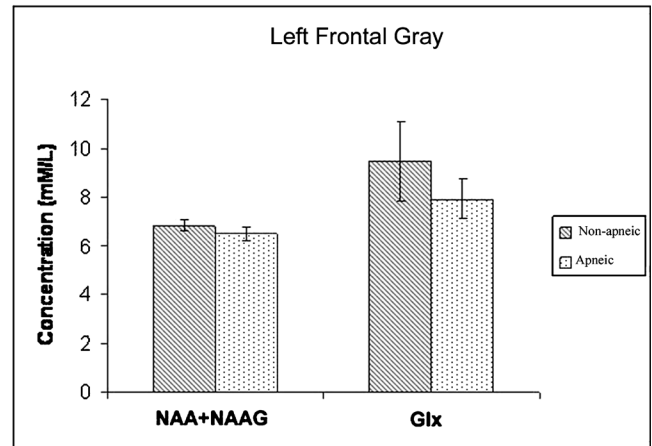
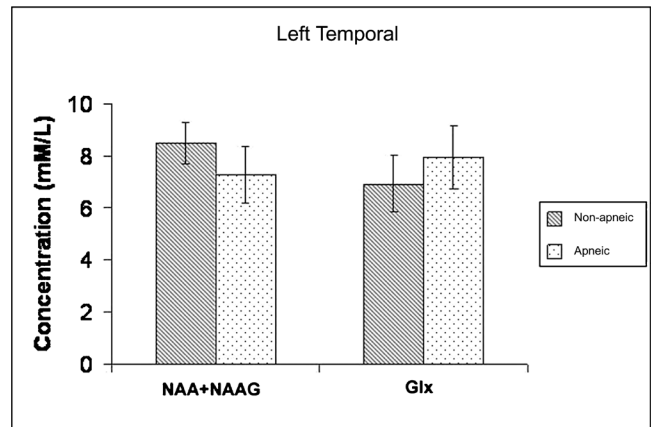
Area	Metabolite	N	<i>r</i> (<i>P</i>)
Left hippocampus absolute value	Cr+PCr	22	- 0.0743 (0.74)
	tNAA	22	- 0.0058 (0.98)
	mI	21	0.0773 (0.74)
	Cho	20	- 0.0366 (0.88)
	Glx	15	0.0175 (0.95)
Left temporal	Cr+PCr	40	-0.1767 (0.28)
	tNAA	40	- 0.2745 (0.09)
	MI	33	0.1472 (0.41)
	Cho	40	0.0375 (0.82)
	Glx	20	0.5392* (0.01)
Left frontal white	Cr+PCr	9	0.5471 (0.13)
	tNAA	10	0.0091 (0.98)
	mI	4	0.4991 (0.50)
	Cho	8	0.4852 (0.22)
	Glx	3	0.2870 (0.81)
Left frontal gray	Cr+PCr	12	- 0.0126 (0.97)
	tNAA	12	- 0.6908* (0.01)
	MI	10	0.5188 (0.12)
	Cho	10	- 0.2416 (0.50)
	Glx	12	- 0.4554 (0.14)
Occipital gray	Cr+PCr	12	0.2454 (0.44)
	tNAA	12	- 0.0691 (0.83)
	MI	12	0.5972* (0.04)
	Cho	12	0.6414* (0.02)
	Glx	12	0.2814 (0.38)

tNAA; N-acetyl aspartate + NAAG; dipeptide N-acetylaspartylglutamate, Cr, Creatine; PCr, phosphocreatine; Cho, Choline containing compound; Glx, glutamate (Glu) and glutamine (Gln); myo-inositol (mI)

The concentration of tNAA [(N-acetylaspartate (NAA)+NAA glutamate (NAAG)] was observed to be significantly lower ($P<0.05$) in apnoeics in the left temporal (7.3 ± 2.1 vs 8.5 ± 0.8 mmol/l) and left frontal regions (6.4 ± 0.30 vs 6.8 ± 0.23 mmol/l) compared to non-apnoeics (Figs 3, 4). The absolute concentration of myo-inositol (mI) was significantly higher ($P<0.03$) in apnoeics in the occipital region compared to non-apnoeics.

The minimum oxygen saturation significantly correlated with tNAA in left temporal region ($r=0.39$ & $P=0.01$) and left frontal gray region ($r=0.61$ & $P=0.04$). The ESS also significantly correlated with Glx in left temporal region ($r=0.46$ & $P=0.04$) and with tNAA in left frontal gray region ($r=0.72$ & $P=0.01$).

The Glx (glutamine, Gln + glutamate, Glu) resonance showed higher concentration (but not

**Fig. 3.** Metabolite concentration of tNAA (NAA+NAAG) and Glx (Glu+Gln) metabolites for the left frontal gray matter of the brain in apnoeics and non-apnoeics.**Fig. 4.** Metabolite concentration of tNAA (NAA+NAAG) and Glx (Glu+Gln) metabolites for the left temporal region of the brain in apnoeics and non-apnoeics.

statistically significant) in the left temporal and occipital regions of the brain in apnoeics compared to non-apnoeics. Other metabolites like Cho and Cr did not show any difference between the two groups in various regions of the brain studied. Further, the concentrations of brain metabolites were similar in the left hippocampal region between apnoeics and non-apnoeics. The correlation of various brain metabolites with AHI for the different regions of the brain was also calculated and the results are presented in Table IV.

Discussion

Our results indicated that the variation of brain metabolites was not uniform across various regions of the brain in apnoeics and non-apnoeics. The absolute concentrations of metabolites (tNAA, Cho, Cr/PCr, mI, and Glx) from left hippocampus, temporal, frontal white and gray matter and occipital gray matter in both

the group of subjects were determined. In general, the concentrations of most metabolites were similar in the five brain regions studied between the two groups except for lower tNAA in left temporal and left frontal regions in apnoeics compared to non-apnoeics. Further, our data showed that apnoeic patients had higher concentration of Glx in the left temporal and occipital regions although values were not statistically significant compared to non-apnoeics. No lesion or signs of atrophy was observed in any of the OSA patients on MR images.

The prominent peak in proton MRS of brain is the acetyl resonance at 2.01 ppm that is predominantly from NAA. This peak also has contribution from protons of the dipeptide NAAG²⁷. NAA is an acetyl donor and possibly as an osmoregulator of the nerve cell²⁸ and is believed to be a marker of neuronal density, whereas NAAG is the most abundant peptide neurotransmitter in the human brain²⁷. It is difficult to differentiate NAAG from NAA by *in vivo* MRS especially at low fields (e.g. 1.5T) due to the similarity of the structure and the spectral profile of these compounds and thus normally designated as total NAA (tNAA). NAAG is reported to be unevenly distributed in the brain²⁷. NAAG has been reported to be abnormal in schizophrenia patients²⁹ and reduced in amyotrophic lateral sclerosis (ALS)³⁰. Thus, the reduction in the concentration of tNAA in apnoeics is suggestive of neuronal damage, probably caused by repeated apnoeic episodes.

Kamba *et al* have documented a decrease in NAA/Cho in the periventricular white matter in patients with moderate to severe OSA¹⁶. They also reported cerebral hypoperfusion with a significant decrease in the NAA/Cho ratio in cerebral white matter in moderate to severe OSA compared with mild OSA and healthy control individuals¹⁶. We also analyzed our MRS data in apnoeics by categorizing them as mild, moderate and severe form and observed no change in the concentration of tNAA. This probably may be due to less number of patients in these three categories. A study involving more number of patients is required. Alchanatis *et al*¹⁸ reported both NAA/Cr and Cho/Cr ratios to be lower in the frontal white matter of severe OSA patients compared with controls. The absolute concentrations of NAA and Cho were also reported to be reduced in the frontal white matter of patients with OSA. NAA/Cho was found to be correlated with the severity of OSA in periventricular white matter but not in cerebral cortex¹⁷. Reduced NAA/Cho was also found in children with OSA compared with control

children in the left hippocampus and right frontal cortex²³.

Increase in NAA/Cr has also been reported in patients with OSA compared with controls from the left hippocampal area²⁶. The increase was attributed to a decrease in hippocampal creatine-containing compounds rather than an increase in NAA. Authors also reported that lower levels of Cr containing compounds correlated with OSA severity and neurocognitive performance. Tonon *et al*²⁰ recently reported lowered cortical NAA in OSA patients compared with controls and a positive correlation between cortical NAA and minimum nocturnal oxygen saturation.

Myo-inositol (mI) was significantly increased in the occipital gray matter in apnoeics in our study. The function of mI is not clearly understood, although it is believed to be an essential requirement for cell growth, an osmolyte, and storage for glucose²⁸. A significant increase of mI/Cr ratio was reported from the temporal and frontal regions of brain in OSA patients by Sarchielli *et al*²¹. Altered levels have been associated with Alzheimer's disease, hepatic encephalopathy and brain injury.

Our results also showed that apnoeic patients had higher concentration of Glx in the left temporal and left frontal regions of the brain compared to non-apnoeics, even though the values were not statistically significant. Glx resonance is composed of Glu and Gln resonances. Studies with more number of patients in the both the groups are required. Changes in other brain metabolites were non significant in different areas of the brain studied. Localized gray matter volumetric changes have been reported from various regions of the brain in patients with OSA including the frontal region and the magnitude approximates with age¹³. A unilateral loss in well-perfused structures was also observed in OSA patients suggestive of the onset of neural deficits¹³. The knowledge of the distribution of tNAA in brain thus may give insight on the role of cell neurochemistry.

It has been reported that elevated CO₂ increases the oxygen uptake by its influence on the regulation of alveolar ventilation and ventilation-perfusion matching and facilitates oxygen delivery to the tissues and increases cerebral blood flow. These biological effects of hypercapnia may be especially important in patients with severe OSA³². However, studies have documented alterations in the NAA and Cho concentration primarily in the frontal lobe white matter^{16,22}.

NAA was found to be negatively correlated with AHI in the left frontal region, indicating that higher the AHI, lower is the concentration of NAA, indicating possible neuronal damage of the brain. Similar negative correlation was reported by Sarchielli *et al*²¹ between AHI and NAA/Cr ratio in the frontal regions of the brain of OSA patients. Cho and mI were positively correlated with AHI in occipital region of the brain while only Glx shows positive correlation in the left temporal region. The correlation of tNAA and Glx from different regions of the brain was also calculated but no significant correlation was observed.

In conclusion, our results demonstrate that the variation of brain metabolites is not uniform across various regions of the brain studied in apnoeics and non-apnoeics. Reduction in the concentration of NAA in the left temporal and left frontal regions of the brain in apnoeics may be due to neuronal damage, caused probably by repeated apnoeic episodes. In addition, there is an increase of mI in the occipital region of the brain in apnoeics compared to non-apnoeics. Other metabolites did not show any significant changes in both the groups. Our results indicate the need for further systematic study on a large cohort of patients with varying severity (mild to severe) to understand the metabolic abnormalities and adaptive mechanisms associated with obstructive sleep apnoea.

Conflict of interest: None.

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