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Assessment of functional and structural damage in brain parenchyma in patients with vitamin B12 deficiency: A longitudinal perfusion and diffusion tensor imaging study

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ABSTRACT

Introduction: Vitamin B12 deficiency may cause neural tissue damage. Even in advanced stages, conventional imaging of brain usually appears normal in vitamin B12 deficient patients. The aim of this study was to assess the structural and functional changes in brain of patients with vitamin B12 deficiency before and after six weeks of vitamin B12 supplementation using diffusion tensor imaging and pseudo-continuous arterial spin labelling (PCASL).

Methods: MR imaging including DTI and PCASL and neuropsychological tests (NPT) were performed in 16 patients with vitamin B12 deficiency and 16 controls before and after 6 weeks of therapy. Cerebral blood flow (CBF) derived from PCASL and DTI indices was calculated in brain of patients with vitamin B12 deficiency and controls.

Results: Patient with vitamin B12 deficiency showed altered neuropsychological scores and altered CBF as well as fractional anisotropy (FA) values in various brain regions as compared with controls. Both CBF values and neuropsychological scores showed complete reversibility at 6 weeks post therapy. Though FA values showed significant recovery, it failed to show complete recovery.

Conclusion: Our results suggest that micro-structural recovery lags behind functional recovery in patients with vitamin B12 deficiency following therapy and CBF change may be used as an early predictor of complete recovery in patients with B12 deficiency.

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1. Introduction

Vitamin B12, an essential water soluble vitamin, is involved in different epigenomic regulatory mechanisms and especially in brain development [1]. It contributes significantly to myelin synthesis, hematopoiesis and synthesis of epithelial tissue. Inadequate dietary intake and malabsorption of the vitamin from food are two main causes of vitamin B12 deficiency. Commonly vitamin B12 deficiency manifests in humans as: 1) hematopoietic disorders, 2) neurological/psychiatric disorders and 3) epithelial changes in the mucosa of the digestive tract [1]. Among these manifestations, neurological disorders are often the earliest. According to the Institute of Medicine (IOM, Washington, DC, USA), 75%–90% of persons with a clinically relevant B12 deficiency have neurological disorders, and in about 25% of cases these are the only clinical manifestations of the B12 deficiency [2]. In combination with folic acid, vitamin B12 maintains low homocysteine (Hcy) levels in plasma. Plasma concentrations of methylmalonic acid and Hcy are known indicators of vitamin B12 status [3].

Vitamin B12 deficiency leads to several central and peripheral nervous system dysfunctions. The spinal cord manifestation, called subacute combined degeneration (SACD) is characterized by T2-hyperintensity of spinal cord columns with restricted water diffusion on MRI [4]. The involvement of brain has also been reported in vitamin B12 deficiency. Both Fluid attenuated inversion recovery (FLAIR) and T2-weighted images demonstrate hyper-intense areas in the peri-ventricular white matter regions [4]. Diffusion tensor imaging (DTI), has shown significant changes in DTI matrices in various brain regions in respect to vitamin B12 deficiency [5,6]. Few studies have also reported disturbance in cerebral blood flow (CBF) and explained an association between the mental symptoms observed in vitamin B12 deficient patients and decrease in cerebral metabolism [7,8]. Arterial spin labeling (ASL), has emerged as a completely non-invasive MRI technique for quantification of CBF that uses magnetic labeling of protons in blood to provide an

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endogenous tracer of flow. It has shown its potential to investigate various brain pathologies [9,10]. In the vitamin B12 deficient population, brain perfusion with ASL has not been previously studied.

Functional and structural changes in a number of brain pathologies have been studied by combining DTI and ASL techniques [11,12]. Based on the facts (i.e. altered myelin structure and CBF disturbances in vitamin B12 deficient population) available in literature, we hypothesize that both DTI and ASL would be useful in assessing and complementing the structural and functional changes in patients with vitamin B12 deficiency. To verify the aforementioned hypothesis both healthy controls and patients with vitamin B12 deficiency were studied. Both longitudinal DTI and ASL data were acquired in these groups just before initiation (baseline study) and after six weeks (follow-up study) of vitamin B12 supplementation for the first time in the literature.

2. Materials and methods

Patients with clinical features of SACD and biochemical evidence of Vitamin B12 deficiency were included in this study. Sixteen patients who met the above criteria were recruited (males = 11, females = 5, mean age = 32.06 ± 10.07 years). Sixteen age-and sex-matched healthy controls were also included in the study (males = 12, females = 4, mean age = 34.50 ± 7.59 years).

Detailed neurologic examination was performed by a neurologist to assess the severity of impairment in patients with vitamin B12 deficiency. The vitamin B12 deficiency diagnosis was based on low serum vitamin B12 levels (<200 pg/mL) [13]. Routine hematologic examination including red cell mean corpuscular volume was also performed. Serum vitamin B12 level and assessment of cognitive and neurological status in healthy controls were also evaluated. All subjects underwent brain MRI.

Neuropsychological test (NPT) battery was also performed in all groups including controls, baseline and follow-up. NPT battery included the Trail-Making test [number connection tests A and B (NCT A & B), and figure connection tests A and B (FCT A & B)]. In addition the modified Wechsler Adult Intelligence Scale (modified for Indian population), which included picture completion (PC), digit symbol (DS), block design (BD), picture arrangement (PA), and object assembly (OA), was also performed [14].

In patients, conventional MR Imaging, DTI, 3D PCASL and NPT battery were performed at the time of recruitment and after six weeks of appropriate therapy. The study was approved by the local Institutional ethics committee. Informed and written consent was obtained from each subject.

2.1. MRI imaging

Conventional MRI, DTI and 3D PCASL were performed using an eight channel (phased array) head coil on a 3 T MR scanner (Signa Hdxt, General Electric, Milwaukee, WI, USA). DTI data were acquired using a dual spin-echo single-short echo-planar sequence with 30 uniformly distributed directions and ramp sampling on. The acquisition parameters were: repetition time (TR) = 17 s/echo time (TE) = 88.7 ms/number of slices = 62/slice thickness = 3 mm/interslice gap = 0/field of view = 240 × 240 mm/image matrix = 256 × 256/number of excitation (NEX) = 1/b-factor = 1000s mm^{-2} in addition to the reference measurement with b = 0 s mm^{-2}. T2-weighted, FLAIR axial images and 3D T1-weighted inversion recovery prepared fast-spoiled gradient-echo imaging were also performed.

The 3D PCASL was performed using 3D FSE spiral acquisition with eight in-plane spiral interleaves using the following parameters: NEX = 3/slices = 46/FOV = 240 mm/slice thickness = 3 mm/band width = 62.5Hz/post-label-delay = 1025 ms/tagging duration = 1450 ms/TR = 4511 ms/TE = 10.44 ms/acquisition matrix = 512 × 8/reconstructed matrix = 128 × 128 along with 3D proton density weighted FSE. The equation of the model used was based on the previously published work [15]. The CBF maps were generated by the scanner with background suppression.

2.2. Voxel wise analysis

The CBF maps were spatially pre-processed using SPM8. These maps were co-registered to the corresponding T1-weighted images and were spatially normalized to a 2 × 2 × 2-mm^3 "T1-template" Montreal Neurologic Institute template hence permitting voxel-wise analysis of the CBF maps in a common stereotactic space and smoothed in space with a three-dimensional, 8-mm full width-at-half-maximum Gaussian kernel.

2.3. DTI processing

The Diffusion Toolbox software tool in the Functional MRI of the Brain (FMRIB) Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/fdt/index.html) was used for calculating the DTI indices including fractional anisotropy (FA), mean diffusivity (MD), axial (AD) and radial (RD) diffusivity. The DWI was corrected for eddy current induced distortions and minor head movements by using affine registration to the reference B0 images. The Brain Extraction Tool was used for extracting the brain [16].

2.4. Tract-based spatial statistics (TBSS) analysis

Voxelwise analysis of FA was performed by using tract-based spatial statistics (TBSS) [17], part of the FMRIB Software Library (FSL) package [18]. Individual skeletonized FA maps were aligned to the MNI 152 template by using the nonlinear registration tool in FMRIB. Each subject's aligned FA map was then projected onto this skeleton, and the voxelwise general linear model (GLM) was applied by using permutation based nonparametric testing, corrected for multiple comparisons. Using the similar registration parameters from the FA maps, we also spatially transformed MD, RD, and AD maps to the MNI space.

2.5. Region of interest (ROI) analysis

The voxel wise analysis of CBF and TBSS analysis of DTI metrics were done to define the regions which showed significant changes. ROIs were placed on regions defined on the basis of voxel wise analysis and TBSS analysis to quantify the changes in different brain region of CBF and DTI maps. For ROI analysis, elliptic ROIs of sizes varying from 25 to 50 mm^2 were placed on the defined regions.

2.6. B12 supplementation

All vitamin B12 deficient subjects were administered 1000 μg of vitamin B12 intramuscularly, daily for ten days after sensitivity testing followed by once a week for four weeks and then once a month.

2.7. Statistical analysis

For whole brain analysis the threshold for minimum number of voxel present in a cluster was set to 10 and $p = 0.001$ was considered to be significant after family-wise error correction. Independent t-test was performed for comparing ROI based CBF value and DTI indices between controls and baseline as well as follow-up study. P values of ≤ 0.05 were considered to be significant. All statistical analyses for ROI based values

were performed using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) software.

3. Result

The mean hemoglobin, the mean cell volume level, serum vitamin B12 concentration, serum homocysteine levels in B12 deficient patients were 11.3 ± 1.9 g/dL, 98.9 ± 11.9 fL, 157.4 ± 31.6 pg/mL, and 21.6 ± 5.9 μmol/L respectively. The mean serum vitamin B12 concentration in healthy controls was 270.7 ± 55.7 pg/mL. All patients had gait disturbance, sensory disturbance, mental impairment, and neuropathy. Only one patient presented clinically with pyramidal tract signs.

After 6 weeks of appropriate therapy, both the mean serum vitamin B12 (1058.1 ± 666.4 pg/ml) and serum homocysteine levels (13.7 ± 4.4 μmol/L) were improved.

Conventional MR Imaging of the brain showed no visible abnormality in vitamin B12 deficiency subjects as well as in controls.

3.1. Neuropsychological test (NPT) scores

All the NPT scores except OA and NCT A and B showed a significant difference between controls and baseline study of the patients (Table 1). At the time of follow-up study, entire test battery showed recovery when compared with controls.

3.2. Voxel wise analysis

It showed a significant difference in CBF in several gray and white matter regions between patients and control groups.

3.2.1. Quantitative imaging

When controls were compared with baseline in gray matter, the occipital and parietal regions showed significantly decreased CBF (Fig. 1A & B), whereas in frontal, temporal and temporal–parietal regions, the CBF was found to be significantly increased (Fig. 1C). In white matter, frontal and occipital regions showed significantly decreased CBF (Fig. 1D & E), while it increased significantly in temporal, frontal and parietal regions (Fig. 1F & G).

After B12 supplementation, significant improvement was observed in CBF in gray matter in follow up patient group as compared to controls. CBF was significantly decreased in occipital region (Fig. 2 A and B) and significantly increased in occipital and temporal regions (Fig. 2 C). Also in white matter significant improvement was observed. The CBFs in frontal and occipital region were both significantly decreased (Fig. 2 D and E) and increased (Fig. 2 F and G).

Table 1
Summary of neuropsychological tests scores in controls, patients' baseline as well as follow-up study.

NPT	Controls (A) (Mean ± SD)	Baseline (B) (Mean ± SD)	Follow-up (C) (Mean ± SD)	(p value <0.05)	
				A vs B	A vs C
PC	15.1 ± 1.1	12.0 ± 1.9	14.7 ± 1.9	<0.001	*NS*
DS	11.6 ± 1.7	8.2 ± 2.0	9.8 ± 2.2	<0.001	*NS*
BD	13.0 ± 0.8	9.1 ± 2.0	11.31 ± 2.5	<0.001	*NS*
PA	13.4 ± 1.0	11.1 ± 1.7	12.1 ± 2.1	0.001	*NS*
OA	11.0 ± 1.2	9.7 ± 1.5	10.5 ± 1.8	*NS*	*NS*
NCT-A	40.7 ± 8.6	47.1 ± 10.0	46.1 ± 15.0	*NS*	*NS*
NCT-B	61 ± 11.6	73.0 ± 18.8	62.5 ± 15.0	*NS*	*NS*
FCT-A	50.2 ± 16.6	68.4 ± 21.3	53.4 ± 18.0	0.026	*NS*
FCT-B	70.4 ± 13.1	92.1 ± 16.8	81.1 ± 13.4	0.000	*NS*

NPT: Neuropsychological tests, PC: Picture completion, DS: Digit symbol, BD: Block designing, PA: Picture arrangement, OA: Object assembling, NCT-A: Number connection test A, NCT-B: Number connection test B, FCT-A: Figure connection test A, FCT-B: Figure connection test B.

3.2.2. TBSS analysis

The FA changes in some regions were found to be significantly reduced in pre- and post-treated patients as compared to controls (Fig. 3), AD, RD and MD maps showed no significant changes among patient groups and controls.

3.3. ROI analysis

ROI analysis confirmed the voxel wise analysis results of CBF, which are summarized in Table 2.

When compared with controls, patients with vitamin B12 deficiency showed significant alteration in CBF values in both gray and white matter regions. The CBF values in bilateral temporal and left frontal white matter regions were found to be significantly increased, while in gray matter the values were significantly decreased in left occipital and right parietal, and increased in right frontal, temporal and temporal–parietal regions. When the control and follow-up groups were compared, no significant change was observed in CBF values in gray and white matter.

The results of TBSS were confirmed by ROI analysis and are summarized in Table 3.

When compared with controls significantly decreased FA values were observed in both frontal and occipital white matter, genu, splenium, right anterior and posterior limb of internal capsule in baseline study group. Nevertheless, when follow up was compared to controls the FA values in some regions do not come back to normal. Both the bilateral frontal and occipital white matter and genu showed significantly decreased FA values while splenium and right anterior and posterior limb of internal capsule showed recovery.

4. Discussion

In the present study, PCASL MRI and DTI have been used to measure CBF and diffusion indices, respectively, in the brain parenchyma of patients with vitamin B12 deficiency in comparison to age matched healthy controls. The VBM analysis provided morphological differences in gray and white matter region in brain of patients group as compared to control, however ROI analysis imparted regional quantification with anatomical validation. The result of VBM analysis depicted altered CBF in different gray and white matter regions of the brain when comparing between control, baseline and follow up study. However, on ROI based analysis no significant change in CBF values between controls and follow up study group was observed. The region depicting significant change in CBF values obtained after ROI analysis has been further discussed. Though, both ASL and DTI techniques showed altered perfusion and diffusion indices in patient group compared with controls at baseline and follow-up study, only CBF values were able to demonstrate complete recovery in B12 deficient individuals after 6 weeks of therapy. Our study is the first longitudinal study that showed the potential of CBF values in monitoring therapeutic response in patients with vitamin B12 deficiency and also reported the functional/microstructural damage in both gray and white matter regions in these patients.

There are several factors which may contribute to the development of cognitive impairment and dementia, e.g. thyroid disorders, electrolyte disturbances, nutritional factors, toxins [19]. Among these, two intimately linked B-vitamins B12 and folate with an accompanying Hcy elevation are important nutritional factors [20]. Literature suggests that the effects of B12 are mediated by the altered methylation reaction as these are required for the synthesis of methyl group donor of the brain i.e. *S*-adenosyl methionine [21]. B12/folate deficiency decreases the access of *S*-adenosyl methionine, thereby, changing cellular methylation reactions and resulting in the

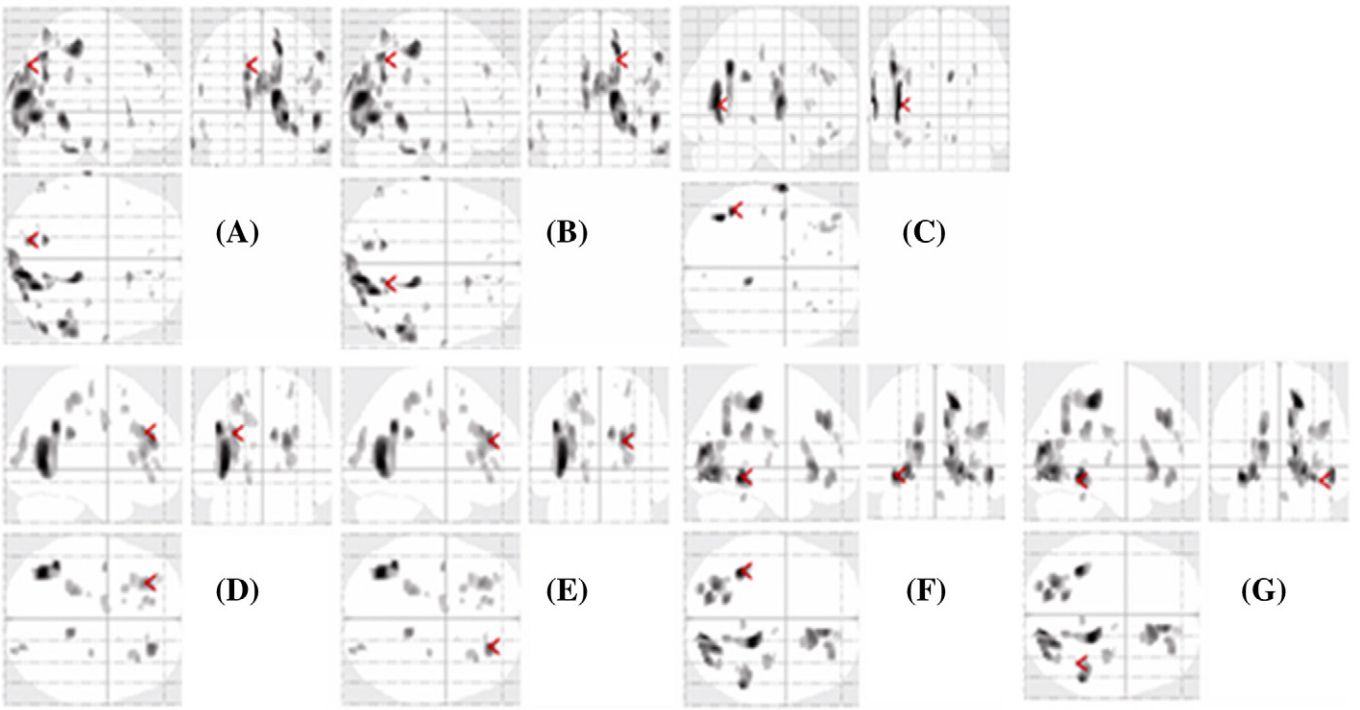

Fig. 1. Figure showing gray matter regions where CBF has decreased at (A) left (−15, −81, 42) and (B) right (17, −59, 42) and has increased at (C) left (−47, −53, 2) in patients with vitamin B12 deficiency as compared to control. White matter region shows decreased CBF at (D) left (−29, 36, 30) and (E) right (23, 42, 23) and increased CBF at (F) left (−38, −45, −7) and (G) right (39, −48, −12) in patients with vitamin B12 deficiency as compared to control.

impaired myelin formation [1]. On serial volumetric MRI study, it has been shown that accelerated rate of brain atrophy in elderly with mild cognitive impairment can be slowed by homocysteine-lowering B vitamins therapy [22]. A recent diffusion tensor tractography study has shown decreased FA values in white matter tracts of vitamin B12 deficient individuals which were well correlated with

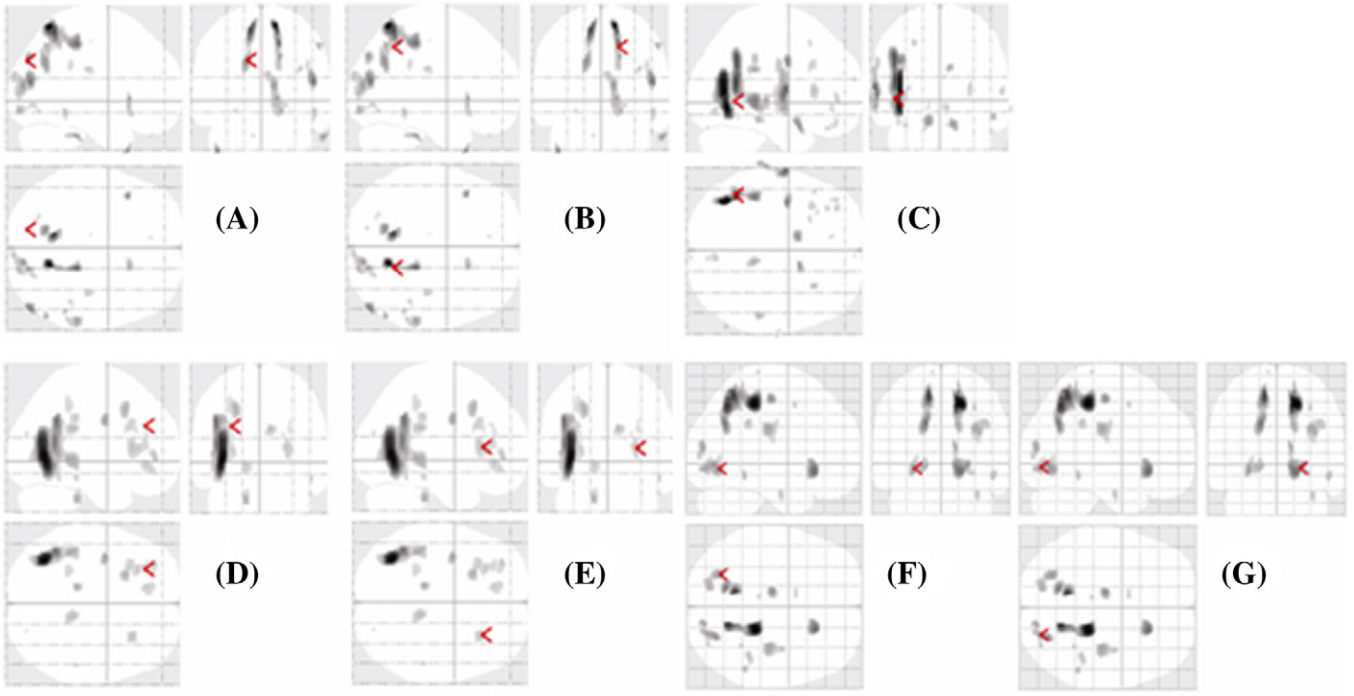

Fig. 2. Figure showing decreased CBF in (A) left (−15, −81, 35) and (B) right (17, −54, 45) gray matter region in patients after treatment with vitamin B12 as compared to control. Figure (C) showing gray matter region (−45, −54, 2) where CBF remained increased in patients after treatment with vitamin B12 as compared to control. White matter region showing decreased CBF in (D) left (−29, 36, 30) and (E) right (27, 25, 11) and increased CBF in (F) left (−27, −68, −4) and (G) right (23, −80, −3)in patients after treatment with vitamin B12 as compared to control (however there are no significant changes in temporal region).

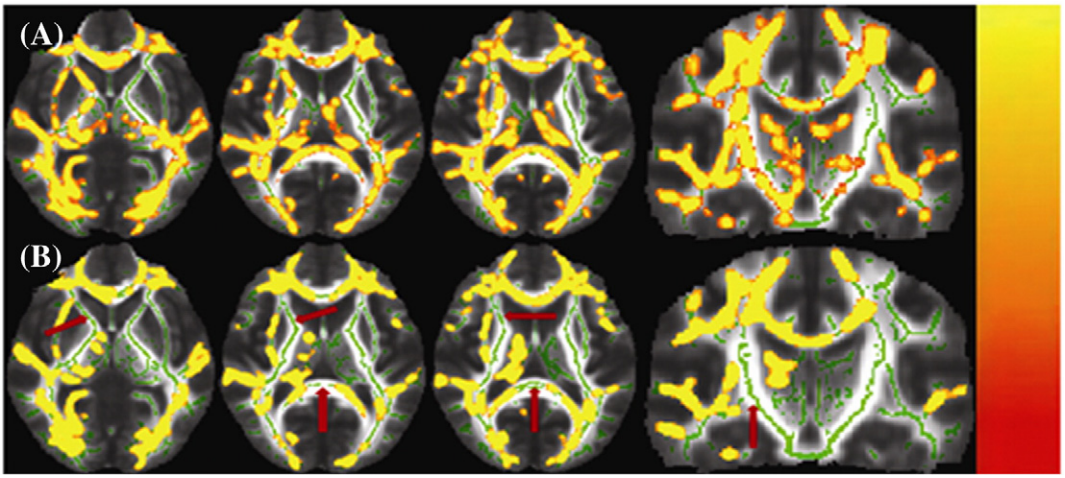

Fig. 3. Statistical maps corrected for age and gender show lower FA in patients with vitamin B12 deficiency (A) pre-therapy and (B) post-therapy as compared to controls. Changes (marked in dark red arrows) are observed in post therapy patients with control when compared with pre-therapy & control (TBSS analysis, two-sample, $p < 0.05$, tfce corrected).

the neuropsychological scores [5]. The decreased FA values in vitamin B12 population in our study also reflect a net loss or disorganization of the structural barriers to molecular diffusion of water in white matter tracts.

Potential pathogenic processes related to direct toxic effect of Hcy other than altered myelin synthesis are damage to blood–brain barrier (BBB). Dysfunction of the BBB is the most recent theory on the cause of cerebral small vessel dementia related white matter lesions [23]. Although structural BBB derangement has never been proven in vitamin B12 deficiency, it is possible that BBB permeability is increased at a functional level; while structural integrity is retained. Increased TNF-alpha and decreased IL-6 levels in the central nervous system have been found in vitamin B12 deficient rats [24]. The CSF/serum albumin ratio which is considered to reflect the permeability of the BBB was found to be decreased in patients treated with vitamin B12–folate combination [25]. Though, the pathophysiology of functional/structural damage of gray matter still needs to be illuminated, available literature suggests a relationship between vitamin B12 and gray matter damage. Erickson et al. in their voxel based morphometry study found that people with greater B12 intake had greater volume in the left and right superior parietal sulcus [26]. Both folic acid and vitamin B12 play an important role in maintaining low Hcy levels in plasma. Studies have demonstrated that B-vitamin treatment markedly reduces gray matter atrophy in regions specifically vulnerable to the AD process, including the medial temporal lobe [27]. Altered CBF values (in both gray and white matter) in patients with vitamin B12 deficiency in our study further strengthen the fact that the damage caused by vitamin B12 deficiency is beyond the white matter tracts.

A very limited literature is available on the neuro-perfusion in vitamin B12 deficient population. An early study in patients with pernicious anemia has reported altered pattern of CBF and metabolism measured by the nitrous oxide method [28]. They observed increased CBF with decreased cerebral vascular resistance in patients with severe anemia; and patients with moderate or no anemia showed decreased CBF with increased vascular resistance [28]. In recent case reports, researches have reported hypoperfusion in frontal [7] and fronto-temporo-parietal region [29]. In this study, both increased as well as decreased CBF values were observed in patients with vitamin B12 deficiency. Our CBF results are in line with the available literature. In Alzheimer's disease, both decreased and increased CBF values in different brain regions have been reported by

Table 2
Summary of the comparison of CBF values as obtained using region of interest analysis (ROI) from the controls and patients' baseline.

Regions	Controls	Patients' Baseline	p value
RTWM	26.05 ± 5.94	31.29 ± 4.51	0.009
LTWM	27.22 ± 4.69	32.49 ± 4.45	0.003
ROWM	23.32 ± 5.87	25.00 ± 5.26	*NS*
LOWM	26.06 ± 3.83	28.24 ± 5.04	*NS*
RFWM	29.26 ± 5.88	28.37 ± 3.88	*NS*
LFWM	27.33 ± 5.33	32.62 ± 4.15	0.004
RPWM	28.88 ± 4.47	28.31 ± 4.24	*NS*
LPWM	28.11 ± 6.26	28.93 ± 3.55	*NS*
LFPVPCWM	23.77 ± 6.48	24.37 ± 7.21	*NS*
RPOWM	27.41 ± 6.21	26.44 ± 4.74	*NS*
RFGM	55.35 ± 6.95	62.65 ± 7.43	0.007
RTGM	56.99 ± 6.12	64.43 ± 8.53	0.009
LTGM	61.06 ± 7.38	57.24 ± 6.89	NS
RTPGM	61.01 ± 8.77	70.55 ± 8.87	0.005
RPGM	60.76 ± 8.36	54.19 ± 7.39	0.026
LPGM	59.80 ± 8.01	63.63 ± 8.59	NS
ROGM	54.85 ± 7.31	50.93 ± 7.29	NS
LOGM	63.65 ± 7.26	57.46 ± 6.73	0.018

RTWM, Right (Rt) temporal white matter (WM); LTWM, left (Lt) temporal WM; ROWM, Rt occipital WM; LOWM, Lt occipital WM; RFWM, Rt frontal WM; LFWM, Lt frontal WM; RPWM, Rt parietal WM; LPWM, Lt parietal WM; LFPVPCWM, Lt frontal periventricular pericaudate WM; RPOWM, Rt parieto-occipital WM; RFGM, Rt frontal gray matter (GM); RTGM, Rt temporal GM; LTGM, Lt temporal GM; RTPGM Rt temporo-parietal GM; RPGM, Rt parietal GM; LPGM, Lt, parietal GM; ROGM, Rt occipital GM; LOGM, Lt occipital GM; NS, non-significant.

Table 3
Summary of the comparison of FA values as obtained using region of interest analysis (ROI) from the controls, patients' baseline as well as follow-up study.

Fractional Anisotropy	Controls (A) (Mean ± SD)	Baseline (B) (Mean ± SD)	Follow-up (C) (Mean ± SD)	(P value <0.05)	
				A vs B	A vs C
RFWM	0.57 ± 0.05	0.51 ± 0.04	0.53 ± 0.04	0.002	0.017
LFWM	0.55 ± 0.05	0.50 ± 0.06	0.52 ± 0.05	0.011	0.039
ROWM	0.66 ± 0.09	0.57 ± 0.07	0.59 ± 0.06	0.002	0.013
LOWM	0.66 ± 0.09	0.57 ± 0.05	0.60 ± 0.05	0.002	0.023
GENU	0.79 ± 0.04	0.66 ± 0.05	0.72 ± 0.05	<0.001	0.001
SPLENIUM	0.78 ± 0.06	0.65 ± 0.06	0.77 ± 0.06	<0.001	NS
RALIC	0.57 ± 0.07	0.51 ± 0.06	0.56 ± 0.06	0.011	NS
RPLIC	0.69 ± 0.05	0.56 ± 0.05	0.68 ± 0.04	<0.001	NS

RFWM: right frontal white matter, LFWM: left frontal white matter, RALIC: right anterior limb of internal capsule, LPLIC: right posterior limb of internal capsule.

ASL studies and have [30,31] suggested that hyper-perfusion serves as a compensatory mechanism against cognitive decline in early stages of AD, and mild cognitive impairment [32,33].

Patients with vitamin B12 deficiency at all age groups shows cognitive decline. Neuropsychological studies have shown an association between cognitive decline and vitamin deficiency [34]. Our study is in agreement with the earlier study where we observed a decline in visuospatial and performance skills in patients with vitamin B12 deficiency at baseline study. An earlier study has reported cognitive decline in patients (age ranging from 35 to 50 years) with vitamin B12 deficiency, which subsequently recovered following supplementation of vitamin [35].

Though, both FA and CBF maps showed altered values at baseline and follow-up (after weeks of appropriate therapy) studies, only CBF values showed complete recovery. Voxel wise analysis showed altered CBF values in gray and white matter region including frontal, parietal, temporal and occipital regions in pre-treatment patient compared to controls. After appropriate treatments partial improvement was seen in gray (frontal, temporal, parietal and occipital regions) and white matter (temporal, parietal and occipital regions). Also, ROI based analysis showed no significant changes in CBF values between control and follow-up. TBSS analysis showed no significant difference in FA values between baseline and follow-up study. However, ROI based analysis showed significant difference in FA values among all groups. In an earlier study changes in DTI metrics have been observed after 8 weeks of therapy [7]. In our study, after 6 weeks of therapy DTI indices failed to show the process of complete recovery. The reason behind this discrepancy might be the duration between baseline and follow-up study. It appears that ASL based CBF quantification is probably an early imaging biomarker for therapeutic response assessment in patients with vitamin B12 deficiency.

We conclude that both DTI metrics and CBF are altered in patients with Vitamin B12 deficiency; however CBF shows measures of early response to therapy compared to DTI metrics. This may be used as an early imaging biomarker in early assessment of therapeutic response in patients who suffer from Vitamin B12 deficiency.

Conflict of interest

There is no conflict of interest.

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References

[1] Gröber U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12—underestimated neurological significance. Nutrients 2013;5:5031–45. http://dx.doi.org/10.3390/nu5125031.
[2] Vitamin B12. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. A report of the standing committee on the scientific evaluation of dietary reference intakes and its panel on folate, other B vitamins, and choline and subcommittee on upper reference levels of nutrientsWashington DC, Md: National Academy Press; 1998. p. 306–56.
[3] Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med 1994;96:239–46. http://dx.doi.org/10.1016/0002-9343(94)90149-X.
[4] Briani C, Dalla Torre C, Citton V, Manara R, Pompanin S, Binotto G, et al. Cobalamin deficiency: clinical picture and radiological findings. Nutrients 2013;5:4521–39. http://dx.doi.org/10.3390/nu5114521.
[5] Gupta PK, Garg RK, Gupta RK, Malhotra HS, Paliwal VK, Rathore RK, et al. Diffusion tensor tractography and neuropsychological assessment in patients with vitamin B12 deficiency. Neuroradiology 2014;56:97–106. http://dx.doi.org/10.1007/s00234-013-1306-y.
[6] Gupta PK, Gupta RK, Garg RK, Rai Y, Roy B, Pandey CM, et al. DTI correlates of cognition in conventional MRI of normal-appearing brain in patients with clinical features of subacute combined degeneration and biochemically proven vitamin B (12) deficiency. Am J Neuroradiol 2014;35:872–7. http://dx.doi.org/10.3174/ajnr.A3785.
[7] Blundo C, Marin D, Ricci M. Vitamin B12 deficiency associated with symptoms of frontotemporal dementia. Neurol Sci 2011;32:101–5. http://dx.doi.org/10.1007/s10072-010-0419-x.
[8] Nilsson K, Warkentin S, Hultberg B, Fäldt R, Gustafson L. Treatment of cobalamin deficiency in dementia, evaluated clinically and with cerebral blood flow measurements. Aging 2000;12:199–207.
[9] Petcharunpaisan S, Ramalho J, Castillo M. Arterial spin labeling in neuroimaging. World J Radiol 2010;2:384–98. http://dx.doi.org/10.4329/wjr.v2.i10.384.
[10] Pollock JM, Tan H, Kraft RA, Whitlow CT, Burdette JH, Maldjian JA. Arterial spin-labeled MR perfusion imaging: clinical applications. Magn Reson Imaging Clin N Am 2009;17:315–38. http://dx.doi.org/10.1016/j.mric.2009.01.008.
[11] Fudaba H, Shimomura T, Abe T, Matsuta H, Momii Y, Sugita K, et al. Comparison of multiple parameters obtained on 3T pulsed arterial spin-labeling, diffusion tensor imaging, and MRS and the Ki-67 labeling index in evaluating glioma grading. Am J Neuroradiol 2014;35:2091–8. http://dx.doi.org/10.3174/ajnr.A4018.
[12] Zhang Y, Schuff N, Ching C, Tosun D, Zhan W, Nezamzadeh M, et al. Joint assessment of structural, perfusion, and diffusion MRI in Alzheimer's disease and frontotemporal dementia. Int J Alzheimers Dis 2011:546871. http://dx.doi.org/10.4061/2011/546871.
[13] Remacha AF, Souto JC, Piñana JL, Sardà MP, Queraltó JM, Martí-Fabregas J, et al. Vitamin B12 deficiency, hyperhomocysteinemia and thrombosis: a case and control study. Int J Hematol 2011;93:458–64. http://dx.doi.org/10.1007/s12185-011-0825-8.
[14] Dhiman RK, Saraswat VA, Verma M, Naik SR. Figure connection test: a universal test for assessment of mental state. J Gastroenterol Hepatol 1995;10:14–23. http://dx.doi.org/10.1111/j.1440-1746.1995.tb01041.x.
[15] Roy B, Awasthi R, Bindal A, Sahoo P, Kumar R, Behari S, et al. Comparative evaluation of 3-dimensional pseudocontinuous arterial spin labeling with dynamic contrast-enhanced perfusion magnetic resonance imaging in grading of human glioma. J Comput Assist Tomogr 2013;37:321–6. http://dx.doi.org/10.1097/RCT.0b013e318282d7e2.
[16] Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17:143–55. http://dx.doi.org/10.1002/hbm.10062.
[17] Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. NeuroImage 2006;31:1487–505. http://dx.doi.org/10.1016/j.neuroimage.2006.02.024.
[18] Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 2004;23:S208–19. http://dx.doi.org/10.1016/j.neuroimage.2004.07.051.
[19] Bachman DL, Wolf PA, Linn R, Knoefel JE, Cobb J, Belanger A, et al. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. Neurology 1992;42:115–9.
[20] Quadri P, Fragiacomo C, Pezzati R, Zanda E, Tettamanti M, Lucca U. Homocysteine and B vitamins in mild cognitive impairment and dementia. Clin Chem Lab Med 2005;43:1096–100. http://dx.doi.org/10.1515/CCLM.2005.191.
[21] Werder SF. Cobalamin deficiency, hyperhomocysteinemia, and dementia. Neuropsychiatr Dis Treat 2010;6:159–95.
[22] Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, Agacinski G, et al. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One 2010;5:e12244. http://dx.doi.org/10.1371/journal.pone.0012244.
[23] Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease—systematic review and meta-analysis. Neurobiol Aging 2009;30:337–52. http://dx.doi.org/10.1016/j.neurobiolaging.2007.07.015.
[24] Scalabrino G. The multi-faceted basis of vitamin B12 (cobalamin) neurotrophism in adult central nervous system: lessons learned from its deficiency. Prog Neurobiol 2009;88:203–20. http://dx.doi.org/10.1016/j.pneurobio.2009.04.004.
[25] Lehmann M, Regland B, Blennow K, Gottfries CG. Vitamin B12–B6–folate treatment improves blood–brain barrier function in patients with hyperhomocysteinaemia and mild cognitive impairment. Dement Geriatr Cogn Disord 2003;16:145–50. http://dx.doi.org/10.1159/000071002.
[26] Erickson KI, Suever BL, Prakash RS, Colcombe SJ, McAuley E, Kramer AF. Greater intake of vitamins B6 and B12 spares gray matter in healthy elderly: a voxel-based morphometry study. Brain Res 2008;1199:20–6. http://dx.doi.org/10.1016/j.brainres.2008.01.030.
[27] Douaud G, Refsum H, de Jager CA, Jacoby R, Nichols TE, Smith SM, et al. Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. Proc Natl Acad Sci U S A 2013;110:9523–8. http://dx.doi.org/10.1073/pnas.1301816110.
[28] Scheinerg P. Cerebral blood flow and metabolism in pernicious anemia. Blood 1951;6:213–27.
[29] Akdal G, Yener GG, Kurt P. Treatment responsive executive and behavioral dysfunction associated with vitamin B12 deficiency. Neurocase 2008;14:147–50. http://dx.doi.org/10.1080/13554790802032242.
[30] Alsop DC, Casement M, de Bazelaire C, Fong T, Press DZ. Hippocampal hyperperfusion in Alzheimer's disease. NeuroImage 2008;42:1267–74. http://dx.doi.org/10.1016/j. neuroimage.2008.06.006.
[31] Ding B, Ling HW, Zhang Y, Huang J, Zhang H, Wang T, et al. Pattern of cerebral hyperperfusion in Alzheimer's disease and amnestic mild cognitive impairment using voxel-based analysis of 3D arterial spin-labeling imaging: initial experience. Clin Interv Aging 2014;9:493–500. http://dx.doi.org/10.2147/CIA.S58879.

[32] Clément F, Belleville S. Compensation and disease severity on the memory-related activations in mild cognitive impairment. Biol Psychiatry 2010;68: 894–902. http://dx.doi.org/10.1016/j.biopsych.2010.02.004.
[33] Mozolic JL, Hayasaka S, Laurienti PJ. A cognitive training intervention increases resting cerebral blood flow in healthy older adults. Front Hum Neurosci 2010;4: 16. http://dx.doi.org/10.3389/neuro.09.016.2010.
[34] Morris MS. The role of B vitamins in preventing and treating cognitive impairment and decline. Adv Nutr 2012;3:801–12. http://dx.doi.org/10.3945/an.112.002535.
[35] Aaron S, Kumar S, Vijayan J, Jacob J, Alexander M, Gnanamuthu C. Clinical and laboratory features and response to treatment in patients presenting with vitamin B12 deficiency-related neurological syndromes. Neurol India 2005;53: 55–8. http://dx.doi.org/10.4103/0028-3886.15057.