Original Article

Rationalization of Using the MR Diffusion Imaging in B12 Deficiency

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Abstract

Context: The structural imaging of brain does not demonstrate any changes in the vast majority of patients with vitamin B12 deficiency, even in the advanced stages. **Aims:** We investigated the microstructural changes in the brain with diffusion imaging among patients with biochemical evidence of B12 deficiency. **Patients and Methods:** We retrospectively analyzed all diffusion-weighted MRI images between the periods 2014–2016 who had biochemical evidence of B12. The age-sex matched controls were chosen from the group with normal B12 levels. Patients with pathological findings in conventional MRI images were excluded from the study. **Results:** About 37 patients were recruited (22 women, 15 men; mean age, 34.1 ± 9.9 years; age range). They were about thirty-four age-and sex-matched controls (with normal B12 levels), which were also included in the study. The mean apparent diffusion coefficient (ADC) value of amygdala (773.8 ± 49.9 vs. 742.2 ± 24.2, $P = 0.01$), hypothalamus (721.3 ± 39.2 vs. 700.2 ± 38.2, $P = 0.02$), striate cortex (737.6 ± 77.6 vs. 704.3 ± 58.2, $P = 0.04$), suprafrontal gyrus (740.7 ± 46.9 vs. 711.6 ± 40.7, $P = 0.007$) and medulla oblongata-olivary nucleus (787.3 ± 56.4 vs. 759.7 ± 46.2, $P = 0.02$) were significantly higher in B12 deficiency group compared to controls, whereas ADC values were similar at hippocampus, thalamus, insula, corpus striatum, cingulate gyrus, occipital gyrus, dentate nucleus, cerebral pedicle, tegmentum, pons, and posterior medulla oblongata. **Conclusions:** Our study indicates that a significant increase in ADC values occurs in multiple brain regions in patients with vitamin B12.

Keywords: B12 deficiency, diffusion imaging, MRI

Introduction

The importance of observational studies lies in the identification of important disorders and providing impetus to future research.[1] Vitamin B12 is an essential vitamin nervous system because it is necessary for myelinization. Its deficiency has been associated with important neurological disease, especially peripheral neuropathy. However, most patients may be asymptomatic in severe deficiency. Currently, the medical practice has gone through moments of great renewal.[2,3] There has been rapid development in novel MR technologies, which is a useful tool for non-invasively assessing not only brain structure, but also provides measures of cerebral volume, and brain water content. Today, it is different from the past.[4] While knowledge and technical skills are crucial in medicine, science, the manner in which they are used is also equally important.[5] The increased use of technology in neurological and medical practices,[6] and the advent of magnetic resonance imaging (MRI) and the progressive increase in definition of this modality of imaging in the last decades[7] have considerably contributed to the knowledge of the changes of brain structures with vitamin B12 deficiency. Vitamin B12 deficiency may produce some changes in MRI images. However, conventional MR imaging of the brain may not always demonstrate any changes in the vast majority of patient's deficiency, even in its advanced stages. Altered B12 levels may be associated with microstructural alterations in diffusion MR imaging. With the advent of advanced imaging, diffusion-weighted imaging (DWI) is a powerful technique for analyzing microstructure changes caused by myelin damage, axonal damage, cellular atrophy, loss of tissue integrity, and gliosis.[8] DWI may detect changes in water diffusion associated with cellular dysfunction secondary to B12 deficiency and have been used in numerous studies in neurological science. The aim of this present trial is to investigate the microstructural changes in the brain with diffusion imaging among patients with biochemical evidence of B12 deficiency.

Patients and Methods

We retrospectively analyzed all diffusion-weighted MRI images of patients between the periods 2014–2016 who had biochemical evidence of B12 deficiency according to the hospital database. The patients who have vitamin B12 deficiency more than two years were included in this study. The age-sex matched controls were chosen from the group with normal B12 levels. Patients with pathological findings

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in conventional MRI images were excluded from the study. Local Ethics Committee approved the study, and participants provided written informed consent.

Magnetic resonance imaging

MRI examinations were performed using a twelve-channel head coil on a 1.5 Tesla MR scanner (Siemens Magnetom Aera, Erlangen, Germany). T2 weighted axial images; TR; 5480 ms, TE; 100 ms, FoV; 230 mm, FoV phase; 81.3, Slice thickness; 5 mm, Averages; 1, slices; 24.

T1 weighted axial images; TR; 417 ms, TE; 8.9 ms, FoV; 230 mm, FoV phase; 81.3, Slice thickness; 5 mm, Averages; 1, slices; 24.

FLAIR axial image; TR; 8000 ms, TE; 86 ms, FoV; 240 mm, FoV phase; 78.1 mm, Slice thickness; 5 mm, Averages; 1, slices; 24.

T2 sagittal image; TR; 5480 ms, TE; 100 ms, FoV; 250 mm, FoV phase; 93.8 mm, Slice thickness; 5 mm, Averages; 1, slices; 23.

The DWI (Diffusion-weighted imaging) protocol consisted of a single-shot SE echo-planar sequence with fat suppression technique (TR; 5100 ms, TE; 75 ms, FoV; 230 mm, FoV phase; 100 mm, Slice thickness; 5 mm, Averages; 1, slices; 24, b = 0 s/mm^2 and b = 1000 s/mm^2).

The post-processing of DWI datasets was carried out on a workstation (Syngo.Via VA20 software, Siemens Healthcare, Forchheim, Germany). ADC (apparent diffusion coefficient) maps were reconstructed.

The diffusion-weighted magnetic resonance imaging allows for the quantification of water molecular motion with the ADC.[9] An important step of many MRI imaging studies consists of selecting regions of interest (ROIs), from which signal intensity time courses will be extracted.[10] ROIs were manually drawn on the images acquired using a b value of 0 s/mm^2 based on ROI_{LS} and ROI_{WT} methods as used Ren *et al*.[9] The region of interest (ROI) dimensions were determined parallel to the size of the measured brain area. All ROI's were obtained from the left cerebral hemisphere; left amygdala, hypothalamus, hippocampus, thalamus, insula, corpus striatum, cingulate gyrus, striate cortex, occipital gyrus, dentate nucleus, superior frontal gyrus, cerebral pedicle, tegmentum, pons, medulla oblongata posterior, and medulla oblongata-oliver nucleus. The areas of ROIs were 52 mm^2 in the left corpus striatum and dentate nucleus, 6 mm^2 in striat korteks, occipital gyrus, pons, medulla oblongata posterior, oliver nucleus, and 17 mm^2 in other fields.

Statistical analysis

Statistical analysis were performed using SPSS 21.0 for Windows (IBM Corporation, Armonk, NY, USA). Continuous variables are presented as mean ± SD, and qualitative values were given as percentages. One sample Kolmogorov Smirnov test was performed to determine the distribution of continuous variables. Normally distributed continuous variables were compared by paired sample T-tests whereas non-normally distributed continuous variables were compared with that of a Wilcoxon Signed Ranks test. A P value <0.05 was taken to indicate statistical significance.

Results

There were about 37 patients who met the above criteria were recruited (22 women, 15 men; mean age, 34.1 ± 9.9 years; age range). About thirty-four age-and sex-matched controls (with normal B12 levels) were also included in the study (25 women and 9 men; mean age, 30.7 ± 5.4 years). No significant differences in age and sex were observed between the patient group and controls.

The mean hemoglobin level in patients with vitamin B12 deficiency was 10.7 ± 3.1 g/dL, the mean serum vitamin B12 concentration was 134.3 ± 31.1 pg/mL. The mean hemoglobin level in controls was 12.5 ± 2.4 g/dl, serum vitamin B12 concentration was 310.4 ± 101.2 pg/mL.

The ADC values at 16 different brain locations in the study group are given in Table 1. The mean ADC value of amygdala (773.8 ± 49.9 vs. 742.2 ± 24.2, P = 0.01), hypothalamus (721.3 ± 39.2 vs. 700.2 ± 38.2, P = 0.02), striate cortex (737.6 ± 77.6 vs. 704.3 ± 58.2, P = 0.04), suprafrontal gyrus (740.7 ± 46.9 vs. 711.6 ± 40.7, P = 0.007), and medulla oblongata, olivary nucleus (787.3 ± 56.4 vs. 759.7 ± 46.2, P = 0.02) were significantly higher in B12 deficiency group compared to controls. ADC values were

Table 1: Comparison of demographic characteristics and ADC values between patients with B12 deficiency and control group. ADC values were similar at the hippocampus, thalamus, insula, corpus striatum, cingulate gyrus, occipital gyrus, dentate nucleus, cerebral pedicle, tegmentum, pons, and posterior medulla oblongata

	Patients with B12 Deficiency (n=37)	Control group (n=34)	P
Age	34.1±9.9	30.7±5.4	0.08
Sex (Female %)	46.8% (22)	53.2% (25)	0.78
Amygdala	773.8±49.9	742.2±24.2	0.01
Hippocampus	789.0±50.3	780.7±40.2	0.44
Hypothalamus	721.3±39.2	700.2±38.2	0.02
Thalamus	738.0±39.2	724.2±38.1	0.13
Insula	754.1±41.0	751.2±32.8	0.75
Corpus striatum	668.2±32.2	659.0±39.8	0.28
Cingulate gyrus	780.6±37.8	765.9±41.3	0.12
Striat cortex	737.6±77.6	704.3±58.2	0.04
Occipital gyrus	716.1±145.3	679.1±133.0	0.26
Dentat nucleus	675.6±26.3	680.9±31.1	0.44
Supfrontal Gyrus	740.7±46.9	711.6±40.7	0.007
Cerebral pedicul	748.2±40.9	734.9±45.0	0.19
Tegmentum	732.9±34.3	727.3±37.6	0.51
Pons	709.8±55.3	698.2±68.0	0.43
Medulla oblongata posterior	770.7±68.4	769.0±59.4	0.91
Medulla oblongata oliverni	787.3±56.4	759.7±46.2	0.02

similar at hippocampus, thalamus, insula, corpus striatum, cingulate gyrus, occipital gyrus, dentate nucleus, cerebral pedicle, tegmentum, pons and posterior medulla oblongata. Our findings were shown in Figures 1-8 and Table 1.

Discussion

Key message

We investigated the microstructural brain changes in 37 patients with biochemical evidence of Vitamin B12 deficiency using DWI by placing the ROIs from different regions of the brain. The ADC values from these different brain regions were compared with age/sex-matched controls, and observed significantly increased mean ADC value of amygdala, hypothalamus striate cortex, supra frontal gyrus and medulla oblongata olivary nucleus in B12 deficiency group compared to controls. There were no specific changes that were observed.

The nervous system is affected by vitamin B12 deficiency. The reason for this effect may be explained by two main biological mechanisms in the available literature. First, Vitamin B12 is a cofactor for methionine synthase. The second mechanism is the low B12 levels may lead to reduced availability of methyl groups which is associated with impaired myelin formation, cell membrane phospholipids and different neurotransmitters.[11] Neuroimaging studies often aim to investigate the progressive changes of brain structure in various diseases. In hospitals in the tropics, the availability of magnetic resonance imaging (MRI) facilities in urban areas and especially in teaching institutions have resulted in white matter diseases being frequently reported in a variety of clinical settings.[12] For example, Tangney *et al.* investigated the relationship between B12 deficiency and brain volumes and cerebral infarcts which determined by MRI.[13] They analyzed the brain MRI scans, biochemical analyses of B12 markers and metabolites and cognitive performance test of 121 patients, and found that vitamin B12–related markers, but not serum vitamin B12 itself, were associated with global cognitive function and total brain volume.[13] Gupta *et al.* also performed a study to investigate the relationship between biochemically proven B12 deficiency and conventional MRI findings in the mean of DTI.[14] They found significant reductions in fractional anisotropy whereas an increase in ADC and radial diffusivity in multiple brain regions in patients with B12 deficiency. Similar with our study microstructural changes were not present in all brain regions.[14] The present study shows that a significant increase in ADC values occurs in multiple brain regions in patients with vitamin B12 deficiency.

The importance of the present study

Our results are functionally important because vitamin B12 deficiency is associated with central and peripheral nervous system abnormalities. Nutritional myelopathies resulting from the deficiency of vitamin B12, vitamin E and folic acid.[15] In humans, Vitamin B12 deficiency leads to two main pathologic conditions[16] First it is megaloblastic anemia. The second pathology is demyelination of the corticospinal tracts of the spinal cord,[17] wherein it is known as subacute combined degeneration (SCD), the dorsal and lateral columns of the spinal cord are affected by vitamin B12 deficiency. This kind of deficiency is also a cause of myelopathy. Our clinical practice is being guided by scientific principles.[18,19] It is important to distinguish B12 deficiency from other causes of myelopathy, as it is treatable and early detection and management is important for good clinical recovery. The subacute combined degeneration (SACD) is the initial neurological finding of B12 deficiency,[20] which is manifested by T2-hyperintensity of spinal cord columns with restricted water diffusion on MRI.[21] Although the involvement of the brain in B12 deficiency is not well-defined as the peripheral nervous system, fluid-attenuated inversion recovery (FLAIR) and T2-weighted images demonstrate hyper-intense areas in the periventricular white matter regions. Diffusion tensor imaging (DTI), has shown significant changes in DTI matrices in various brain regions in respect to vitamin B12 deficiency.[14] In this study, we only used the diffusion MR imaging and obtained significant finding. MRI scans are an excellent, noninvasive means of imaging the whole brain structures. It is very sensitive and specific to tissue disruptions.[22,23] In Medline research with keyword "MR diffusion imaging and B12 deficiency, there are only three studies,[14,24,25] all of these studies used the tensor diffusion imaging,[14,24,25] so our study is different from them." In addition, the present study adds some new valuable MR diffusion imaging information about B12 deficiency and neurological findings; firstly, we confirmed the association between B12 deficiency and microstructural changes in brain, secondly; these changes were present even without clinical findings of B12 deficiency, thirdly; we could demonstrate changes by DWI study which is a readily available protocol in many radiology centers. This study indicates that the rationale of utilizing DWI in patients with B 12 vitamin deficiency. Our findings were shown in the table and Figures 1-6. We copiously stress the fact that we are the first ones to report this rationalization. If, indeed one is the first to report something, that something is of much value.[26,27] In addition, our study is an observational study. Observational studies are important to provide the impetus for future studies.

Limitations of the study

Firstly; the relatively small number of patients may be the major limitation, secondly; only one measurement of serum B12 level was used to define B12 deficiency and we did not measure B12 markers and metabolites, thirdly; control MRI scans after B12 therapy would add much value. Roy *et al.* studied this subject previously. They investigated the structural and functional changes in the 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 labeling,[25] and found that micro-structural recovery lags behind the functional recovery in patients with vitamin B12 deficiency following therapy.[25] In addition, despite its great potential for studying brain anatomy and structure in the patient with B12 deficiency, DWI is marred by artifacts more than any other commonly used MRI technique.[28] Determining the optimal acquisition protocol in DWI is governed by a series of

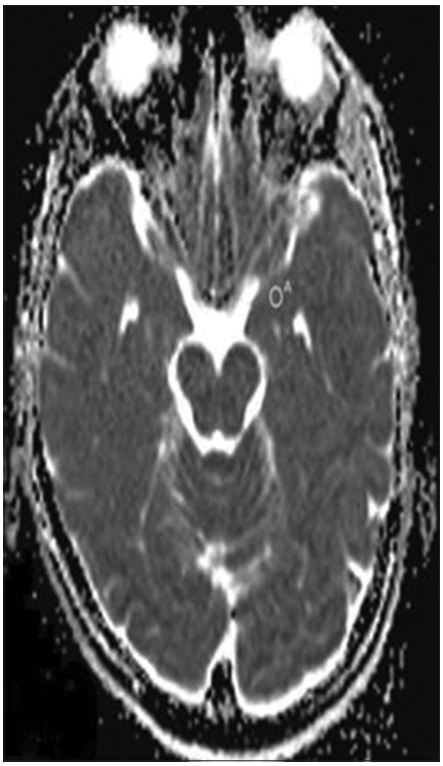

Figure 1: A: Amygdala, B: Hippocampus

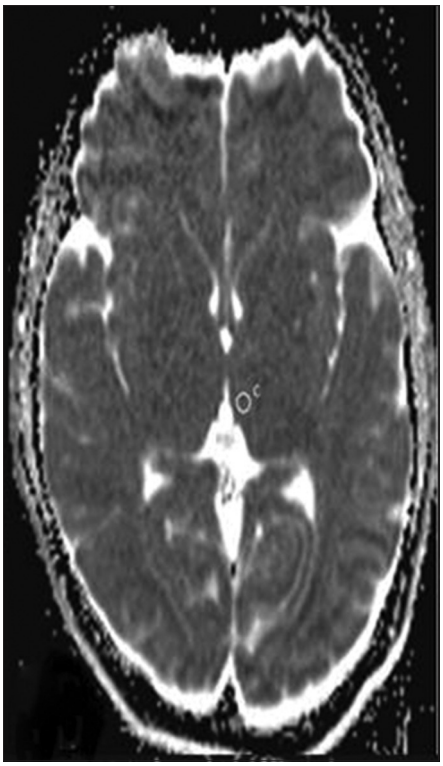

Figure 2: C: Hypothalamus

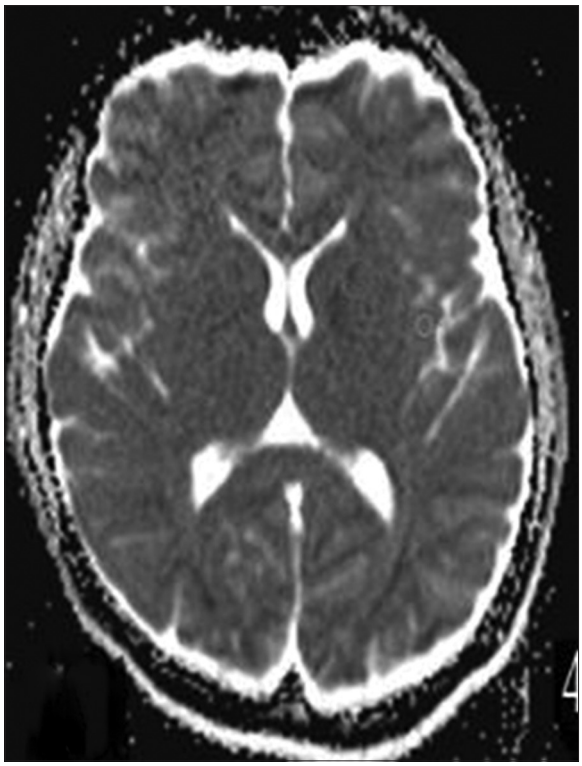

Figure 3: D: singular gyrus, E: corpus stratum, F: thalamus, G. Insula

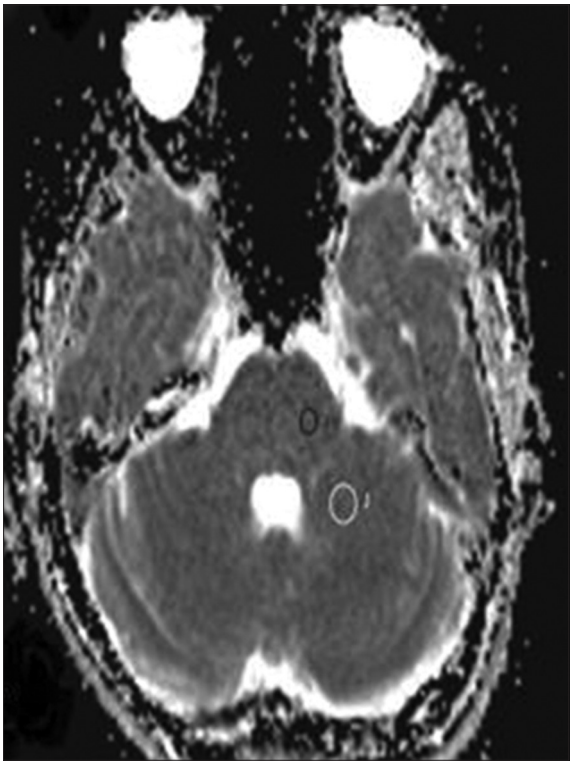

Figure 4: I: Pons, J: dentate nucleus

trade-offs. An increase in the spatial resolution of the acquisition yields lower signal to noise ratio.[29] In addition, we used a manual selection of ROIs. Ideally, the automatic approach for comparison like voxel-based morphometry (VBM) to define the abnormal regions with voxel size to avoid the voxel selection bias might be used. This is a standard methodology to be used today. Another limitation, we know that currently all clinicians ask for Vitamin B12 levels in their subjects and

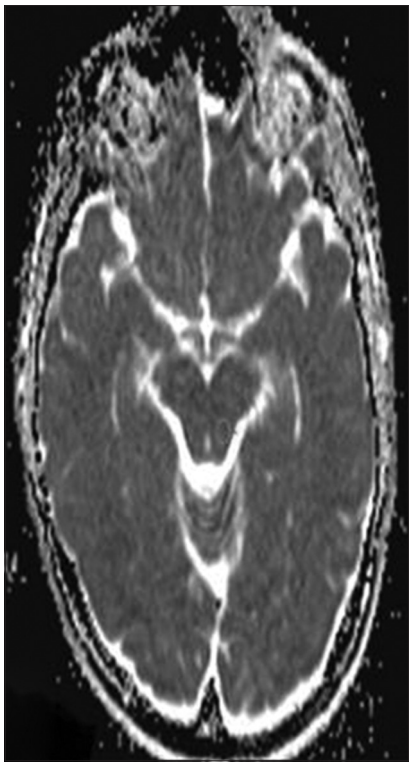

Figure 5: K: Cerebral pedicle, L: Tegmentum

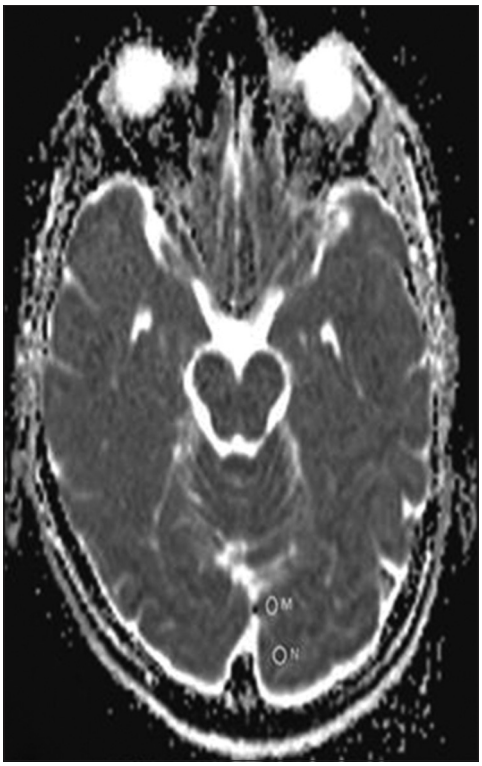

Figure 6: M: Striat cortex, N: Occipital gyrus

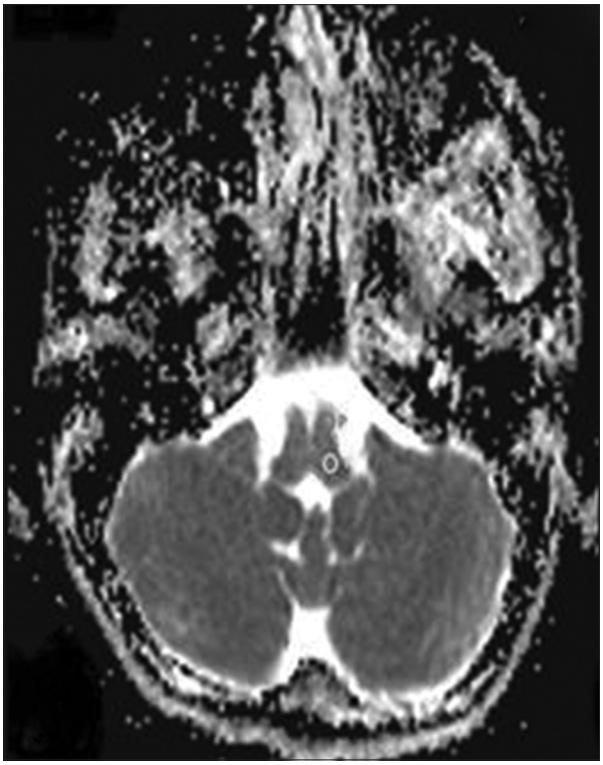

Figure 7: P: Medulla oblongata olivary nucleus, R: Medulla oblongata inferior cerebellar pedicle

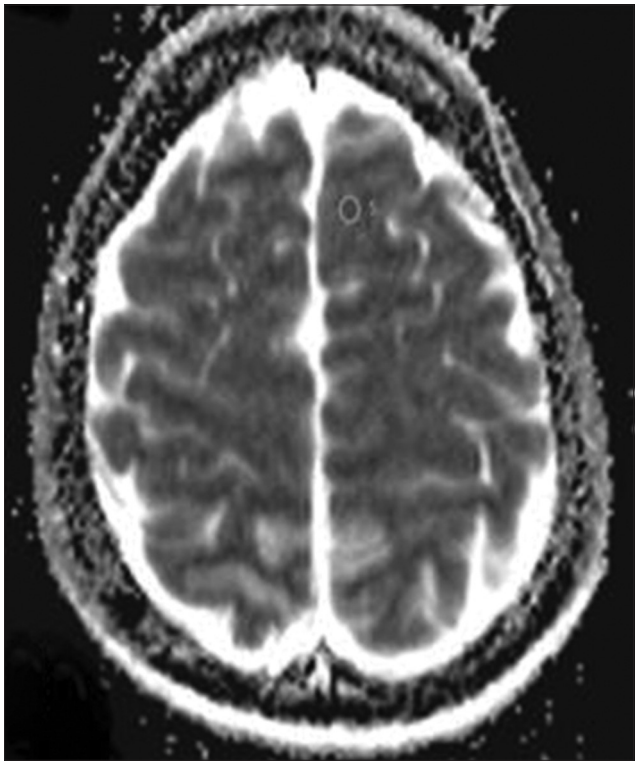

Figure 8: S: Superior frontal gyrus

are found to have the biochemical B12 deficiency. Do all these patients have clinical manifestations? We don't know. If they have some neurological abnormalities, we have not assessed it to know the relationship of the neurological abnormalities with ADC values in different brain regions. In other words, there are no biological correlates of the Vitamin B12 deficiency and its relationship with high ADC in different brain regions, the

neurological symptoms were not studied in this study, because the aim of our present study to evaluate the value of DWI in the patient with B12 vitamin deficiency.

Conclusion

This study indicates that a significant increase in ADC values occurs in multiple brain regions in patients with vitamin B12 deficiency. Neurodegeneration due to B12 deficiency can progress over time before clinical symptoms become apparent. A readily available MRI technique may have a value in defining B12 deficiency in the pre-clinical phase. This study shows that MRI may play a key role in the clinical care and scientific investigation of B12 deficiency.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in this study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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