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Iron Quantification in Deep Subcortical Nuclei and its Correlation with Extracranial Venous System in Multiple Sclerosis and Controls

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ABSTRACT

Background: Multiple sclerosis (MS) is an acquired demyelinating disease of the central nervous system presumed to be of autoimmune nature. This MRI based study was done to see iron deposition in deep subcortical nuclei and look for any abnormalities in extracranial venous drainage system and to look for any correlation with clinical parameters .

Material and Methods: This case control study was done in a single large north Indian institute and had two groups of 25 cases each. One group being of consecutive MS patients and another of other neurological disease (OND). The two groups were age and sex matched. Both the groups underwent Magnetic Resonance Venography (MRV) of neck vessels and azygous system and cross sectional areas of internal jugular veins, azygous veins were measured. Iron stores in deep grey matter nuclei were quantified with Susceptibility weighted Imaging (SWI) studies.

Results: The predefined cross sectional areas of internal jugular veins (IJV) and azygous veins were comparable. On comparing the flows of right and left IJVs, there was a clear dominance of right side in both MS and OND groups. We found more iron in all the nuclei in MS group compared to OND group. But statistically significant difference between the two groups was seen in bilateral pulvinar thalami and red nuclei, right putamen, right caudate nucleus and left substantia nigra. The absence of any difference in anatomical parameters in two groups goes against any vascular hypothesis of iron deposition in deep subcortical nuclei. The iron deposition may be an epiphenomenon of underlying disease rather than having to do something with etiopathogenesis.

Key Words: Iron quantification, Deep subcortical nuclei, Venous abnormalities

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS) of unknown pathogenesis; it is considered to be of autoimmune nature.^[1,2] and continues to challenge investigators trying to understand the pathogenesis of the disease and prevent its progression.^[3]

Importance of iron in multiple sclerosis has been recently studied and its significance in pathogenesis of disease has generated a lot of interest. Many opposing theories have been put forth and this is shown by the fact that on one side chelating therapy was tried in one study to reduce iron deposition

in brain by Levine et al; the hypothesis being to decrease free radical damage induced by iron via Fenton reaction.^[4] On the converse side iron deficiency has been postulated to increase relapse rate and even put forth as a reason for increased incidence of MS in females.^[5,6] Having said that, increased iron deposition has been demonstrated in multiple sclerosis brain in many studies mostly in deep subcortical nuclei and around demyelinating plaques. Exact process of iron deposition is not known. The presence of iron was thought to be secondary to immune response causing local accumulation of iron by disrupting the blood brain barrier and by attracting iron-rich macrophages.^[7] Impaired axonal clearance of iron has also been postulated.^[8]

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In the past decade a vascular theory for MS was proposed; with some similar insinuations made in 19th century.^[9,10] The theory put forth is that iron deposits in MS are a consequence of altered cerebral venous return and chronic insufficient venous drainage.^[9,11] It is shown diagrammatically **Figure 1**.

Moreover it was postulated that venous hypertension and local reflux in medial venous drainage system of brain (internal cerebral veins and vein of Galen) leads to deposition of iron in thalamus and basal ganglia.^[12] Furthermore several studies have demonstrated a greater correlation between grey matter lesions with fatigue and deficits in cognitive than with white matter lesions, this has suggested that measuring iron in grey matter might be of clinical significance.^[13]

The vascular theory put forth proposes structural venous abnormalities in extracranial draining veins viz; internal jugular and azygous veins in the form of stenosis and abnormal valves. This would lead to decreased flow from primary draining veins and reflux of venous blood getting transmitted to medial venous system in brain and opening of collateral channels in vertebral veins and lumbar veins. In fact pathological changes have been shown in jugular veins and absence of endothelium on defective valves shown by scanning electron microscope.^[14] The venous abnormalities were looked via doppler studies initially and later on via percutaneous selective venography. MRI studies being non-invasive and with more interoperative reliability than Doppler were used to investigate this theory subsequently. However conflicting results were shown regarding both anatomical and flow dynamics in draining veins and furthermore correlation with clinical parameters of MS was not done in all. The lack of a clear explanation for iron deposition in deep subcortical nuclei has kept the vascular hypothesis in debate even though a randomised study was published to demolish the vascular hypothesis.^[15]

In this context our study was conceptualised to look into iron deposition in brain in context of vascular hypothesis.

MATERIAL AND METHODS

It is a case control observational study. It consisted of two groups one being multiple sclerosis patient group (diagnosed as per McDonald Criteria) and another was a control group consisting of patients having other neurological disease OND. First 25 sequential patients of those who gave consent were enrolled as a case in the MS group. The study was cleared by our hospital's ethics committee.

Other neurological disease OND (Control Group): These included those patients who were suffering from other neurological disease OND and needed MRI plain and contrast for their basic disease; e.g. Headache, leucodystrophy, Dementias. Twenty five patients were enrolled as controls in

this Other Neurological disease (OND) group.

Exclusion criteria

- History of surgery in head, neck or mediastinum .
- Radiation to neck or chest.
- Past history of cerebral venous thrombosis,
- History of Transient global amnesia.
- Thrombosis in veins of neck or any central venous catheter in the Internal Jugular Vein.
- Chronic Heart or lung disease.
- Budd–Chiari syndrome.

An informed consent was taken from all patients .A detailed history was taken and thorough physical examination was done. Type of MS, disease duration, relapse rate, Expanded Disability Scoring Scale (EDSS) score was noted.

Non invasive evaluation for extracranial venous drainage in MS patient group as well as OND controls was done by MRI studies and this included, CEMRI Brain and spine, Contrast MRV of head, neck and azygous venous system (**figure 2**), Susceptibility weighted imaging of brain. The MRI studies were done on a 3T SEIMES VERIO machine and it is a left sided system.

Susceptibility weighted imaging (SWI): For doing iron quantification we acquired the susceptibility weighted images SWI images. The SWI study was done as per the protocol keeping TR= 27, TE= 20, FOV 240, Slice thickness= 4mm, Distance factor= 20, FOV phase 81.3, FA flip angle= 150° and bandwidth BW=287hz/px.

In the post processing we quantified iron in sub cortical deep grey matter nuclei viz., Caudate nucleus, Globus pallidus, putamen, red nucleus, pulvinar thalamus, red nucleus, substantia nigra. On the phase images of the MS and OND cases regions of interests (ROIs) were hand-drawn following the contour of nucleus. We used SPIN software for processing SWI phase images and it generated the number of pixels, mean, SD, maximum intensity and minimum intensity within the ROI.

For converting phase values to microgram iron we took 180 units (0.276 radians) of phase to be equivalent to 480µg Fe/g/cc tissue. And for calculating total iron content following formula was used.¹⁶

Iron content = - $\{(\phi_{SPU} - 2048) \times 3.14 \times 480 \times \text{pixel \#} \times 3.23\} / (2048 \times 0.276 \times 1000) \mu\text{g/cc.}$

ϕ_{SPU} = mean phase

pixel # = number of pixels

Anatomical details of draining veins: Measurement of the maximum and minimum cross sectional area (CSA) in both the internal jugular veins IJVs (right and left) in both the groups was done. Cross sectional area CSA at the bulb of

internal jugular vein IJV i.e. just below its formation from the jugular sinus was also measured. This acts like a reference point as it is taken at a predetermined level in all the cases of MS or OND group. Stenosis in any IJV was defined as a CSA of less than 0.125cm^2 at or above 3rd cervical vertebral level or a CSA of less than 25cm^2 below 3rd cervical vertebral level.^[16,17] Morphological measurements in azygous vein included measuring maximum CSA of the horizontal part of the azygous vein as it enters superior vena cava. We also measured the minimum CSA of the vertical portion of the azygous vein.

RESULTS

Cohort demographics: The two groups were similar in age and sex distribution (**Table 1**). The mean of relapses in previous 2 years was $2.76(\text{SD}=1.535)$. The relapses in previous 2 years in the multiple sclerosis cohort were; minimum 1 and maximum 8. Of the 25 patients, 22 were having Relapsing Remitting MS (RRMS) type of multiple sclerosis; while 3 patients were having Secondary progressive MS (SPMS).

On comparing the total iron; which was calculated from mean phase, number of pixels i.e. volume and as per equation discussed in material and methods, in various deep subcortical nuclei between the two groups (**figure 3**) we found more iron in all the nuclei in MS group compared to OND group (**Table 2**). Statistically significant difference was seen in bilateral pulvinar thalami and red nuclei, right putamen, right caudate nucleus and left substantia nigra.

Various cross sectional areas (CSA) of IJV at maximum, minimum and at IJV bulb were comparable in cases and controls on both sides as described in (**Table 3**) P value was insignificant between two groups. Similarly maximum and minimum cross sectional area (CSA) of the horizontal segment of azygous vein in MS cases and OND group were again comparable (**Table 3**)

The number of cases having stenosis (by definition adopted by as discussed above) in Right IJV was 2 and 5 in MS and OND groups respectively. Similarly on left side 2 and 3 cases had stenosis in MS and OND group respectively (**figure 4**).

DISCUSSION

The anatomical parameters of draining veins in MS cases and OND controls were comparable. Similar results were shown in other studies as well. The presence of stenosis in IJV looks as normal variation in caliber of draining veins as they were present in comparable numbers in OND group as well (**figure 3**); we have used a definition for defining stenosis similar to most studies so as to make our results comparable. As we know well that veins have a considerable

variation in their size, shape as compared to other anatomical structures in body and may depend on position of body respiratory cycle and hydration status.^[18] Thus these so called "stenosis" seem to be normal anatomical variations rather than any pathological entities (**figure 5**). These observations go against the theory of CCSVI which proposes stenotic lesions as the basis of the etiopathogenesis of multiple sclerosis. As per CCSVI hypothesis we should have had lesser cross sectional areas and more stenosis in the MS group which we did not observe in our study.

Similar findings refuting the CCSVI hypothesis and showing no evidence of more IJV stenoses/narrowing in MS patients were observed by other authors.^[19,20,21,22]

Iron quantification: we documented increased iron deposition in multiple sclerosis and we have quantified it as well. As has been demonstrated in other studies in multiple sclerosis, we found more iron in all the nuclei in MS group compared to OND group. Statistically significant difference was seen in bilateral pulvinar thalami and red nuclei, right putamen, right caudate nucleus and left substantia nigra. Similarly iron deposition has been shown in other studies.^[23,24]

As regarding Clinically isolated syndrome (CIS) we did not have CIS patients in our study. But there have been contradicting results in previous studies; while a study by Khalil et al revealed no iron deposition; other studies showed increased iron in CIS patients.^[25,26,27] It has been shown in a previous study that basal ganglia iron accumulation in MS occurs with advancing disease and is related to the extent of morphologic brain damage.^[25] We could not find any correlation between total iron in the six deep grey matter nuclei on both sides with the various clinical parameters as EDSS, Disease duration, Relapses in previous 2 years and whether stenosis was present or not. Lebel et al found significant correlations of disability with pulvinar iron deposition; marginally significant correlations were also observed in the thalamus and red nucleus. No significant correlations were observed with duration since index relapse.^[28]

The apparent lack of any correlation is not clear. The MS patients in which we could get iron quantification was only 19 which is very small sample for such a correlation. Second we had only 3 MS cases having stenosis (one having bilateral and two having unilateral stenosis), hence getting any sort of correlation was not possible. Third our cohort of patients were having relatively less disease duration with median age being 4 years only and we had no CIS patients in our study. Though we need longitudinal studies ideally to look into such a correlation, but there might be a suggestion here that iron deposition in subcortical nuclei is more of a bystander phenomenon rather than something involved in pathogenesis of disease. As regarding reason for iron deposition in these deep subcortical nuclei we feel this could be because of impaired

axonal transport of iron because of axonal injury secondary to multiple sclerosis lesions.

Thus it is difficult to accept vascular theory for iron deposition. Its promoters argue that the pattern of iron deposition seen in the basal ganglia, thalamus and midbrain of most MS patients represents a backward iron accumulation and is consistent with the hypothesis of venous hypertension i.e. MS is a perivenular disease first and an inflammatory demyelinating disease second. But it is very difficult to accept that iron deposition is because of venous hypertension without first proving the basic step i.e. anatomical and physiological abnormalities of draining veins. The comparison between CIS and RRMS in another study discussed above showed significantly increased iron in RRMS and thus this iron deposition could very well be epiphenomenon of the underlying disease. [25]

Strength and limitations of our study

The strength of our study is that unlike other studies it is a case control study and has attempted to verify the CCS-VI hypothesis critically. We have used a noninvasive MRI techniques to study the two groups and thus involved no risk whatsoever to the cases and controls unlike studies done involving conventional venography.

The limitations include that; we have not taken a healthy control group as our ethics committee did not allow for contrast MRI studies in healthy controls. The sample size in our study was not large and we could do iron quantification studies in 19 out of 25 patients only due to technical reasons. We have not taken any clinically isolated syndromes (CIS).

Conflict of interest: Authors declare no conflict of interest.

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Conclusion: There was increased iron deposition in most of the deep subcortical grey matter nuclei in MS patients as compared to controls. This may be an epiphenomenon of underlying disease rather than having to do something with etiopathogenesis. The significance of deposition in subcortical deep grey matter needs to be studied further. We could not find evidence in support of vascular theory on doing non-invasive evaluation of MS patients and comparing with control group. There was no significant correlation between clinical parameters of MS patients viz. Age, EDSS, Disease duration, Relapses in preceding two years and anatomical measurements and stenosis.

Thus even though CCSVI is an interesting hypothesis but we could not find any evidence in its favour in our study. But on a positive site CCSVI theory has generated a lot of interest

and research into studying venous hemodynamics, venous abnormalities and iron deposition as possible mechanisms of various disease processes in CNS.

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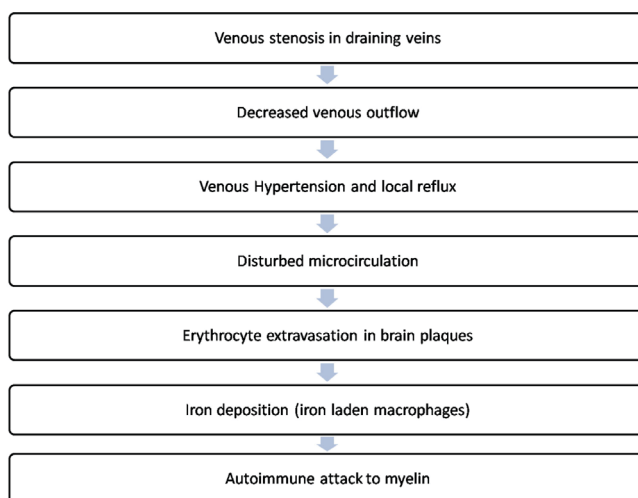


Figure 1: Showing the basic concept behind vascular theory of multiple sclerosis and iron deposition.

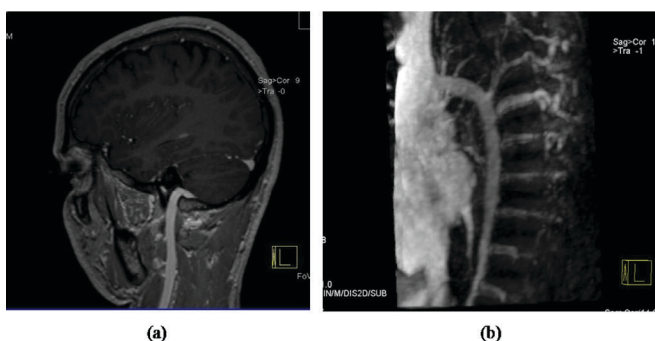


Figure 2: Showing internal jugular vein(a) and azygous vein(b) in a multiple sclerosis patient

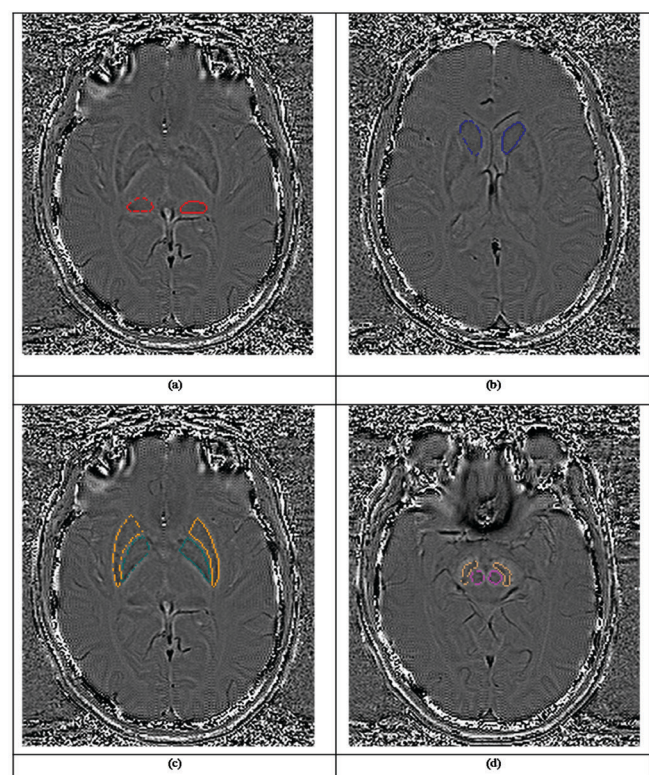


Figure 3: Iron quantification in susceptibility weighted images SWI images and showing ROIs for various deep subcortical nuclei. a) Bilateral pulvinar thalamus in red, b) Bilateral caudate nucleus in blue, c) Bilateral globus pallidus in green, putamen in yellow, d). Bilateral substantia nigra in yellow and red nuclei in purple.

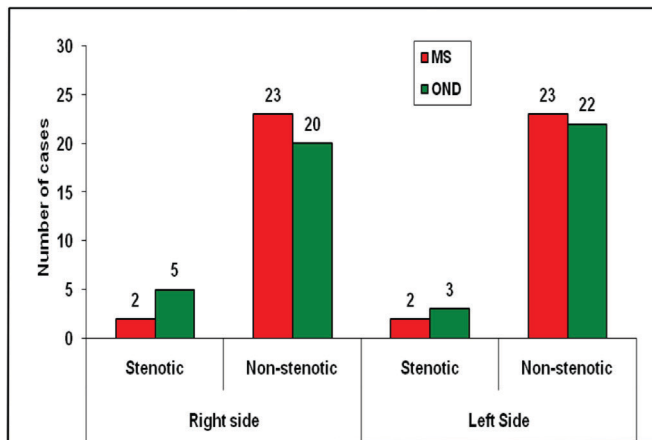


Figure 4: Graph showing comparison of number of cases in MS and OND groups having stenosis.



Figure 5: MRV of neck showing narrowing of bilateral IJVs (arrows).

Table 1: Demographics of MS cases and OND controls

	MS cases	OND controls
Number	25	25
age	32.24 years	30.12 years
females	19	17
EDSS	2.84 (0-8.5)	-
Disease duration	4 (0.25-11)	-

Table 2: Comparison of total phase iron ($\mu\text{g/ml}$ brain tissue) in MS and OND group in the subcortical grey matter nuclei.

Group	MS (N=19)	OND(N=25)	p-value
Right Caudate nucleus	26.25 (\pm 8.18)	20.01(\pm 7.16)	0.011*
Left Caudate nucleus	23.00(\pm 7.61)	20.52(\pm 9.35)	0.35
Right Globus pallidus	41.47(\pm 14.06)	39.92(\pm 14.26)	0.726
Left Globus pallidus	50.81(\pm 11.30)	45.13(\pm 13.64)	0.159
Right pulvinar thalamus	9.03(\pm 4.63)	6.09(\pm 3.09)	0.017*
Left Pulvinar thalamus	8.78(\pm 5.97)	5.23(\pm 2.26)	0.031*
Right putamen	31.96(\pm 15.71)	22.71(\pm 12.07)	0.035*
Left Putamen	23.52(\pm 12.86)	18.47(\pm 14.38)	0.242
Right red nucleus	8.73(\pm 5.90)	5.31(\pm 2.87)	0.037*
Left red nucleus	7.29(\pm 4.6)	4.44(\pm 1.91)	0.013*
Right substantia nigra	22.99(\pm 10.63)	20.53(\pm 8.17)	0.391
Left Substantia nigra	27.12(\pm 8.03)	21.48(\pm 8.83)	0.035*

* p value is significant

Table 3: Comparison of measurements of internal jugular and azygous veins in cases and controls.

Groups	MS #	OND#	P value
Right maximum CSA	1.44 ±0.53	1.32±0.64	0.463
Right minimum CSA	0.45±0.23	0.44±0.24	0.908
Left maximum CSA	1.18±0.58	0.94±0.56	0.138
Left minimum CSA	0.35±0.18	0.30±0.26	0.419
Right Bulb CSA	0.55±0.26	0.62±0.26	0.383
Left Bulb CSA	0.37±0.18	0.34±0.122	0.436