



Research Article

Reduction in Forebrain Parenchymal and Cortical Grey Matter Swelling across Treatment Groups in Patients with Inflammatory Illness Acquired Following Exposure to Water-Damaged Buildings

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Abstract

Exposure to the complex mixture of inflammagens and toxigenic microbes growing in water-damaged buildings (WDB) can lead to a chronic inflammatory response syndrome (CIRS). Many CIRS patients exhibit a neurological component of illness that includes structural brain changes. This study shows some of those structural brain changes are potentially reversible when patients are removed from the WDB environment and follow sequential steps of a published treatment protocol. We evaluated MRIs from 91 subjects classified into four groups: controls, untreated, partially treated and fully treated/recovered CIRS-WDB patients using the MRI volumetric software NeuroQuant®. The current study reinforced previous findings of increased forebrain parenchymal, cortical gray matter and pallidum volumes, as well as decreased caudate nucleus volumes in untreated CIRS patients compared to controls. All changes were found bilaterally. When an ANOVA was performed on brain structures across all patient classes, statistically significant decreases were seen in forebrain and cortical gray matter between untreated and fully treated/recovered patients as these structures trended towards control levels after sequential treatment. Both the caudate and pallidum volumes also trended towards control values but were not significant by ANOVA. These data are consistent with clinical improvement of executive functioning seen in patients as they progressed through the treatment steps, suggesting that volumetric brain imaging is a useful tool for monitoring therapy longitudinally.

Keywords

NeuroQuant; Caudate nucleus; Inflammation; Volumetric MRI; Neuropsychiatry; CIRS; Chronic inflammatory response syndrome; Blood brain barrier

Introduction

Chronic inflammatory response syndrome (CIRS) is a chronic, progressive, multi-system, multi-symptom syndrome characterized

by HLA genetic predisposition, exposure to biotoxins, altered innate and adaptive immunity, peripheral hypoperfusion at multiple sites and multiple hypothalamic-pituitary-end organ dysregulations. Several objective lab abnormalities in patient plasma are commonly seen, including key regulators of inflammation such as elevated TGF β -1 (transforming growth factor beta 1) and depressed VIP (vasoactive intestinal peptide) [1] and MSH (melanocyte stimulating hormone) [2,3]. This inflammatory dysregulation can affect virtually any organ system of the body and if left untreated can become debilitating.

There are several known triggers for CIRS; more are likely to be discovered. Ciguatera fish poisoning results from eating fish contaminated with the marine toxin ciguatera. A single exposure to this toxin may result in CIRS-Ciguatera [4]. Many who suffer chronic illness seen after antibiotic therapy of acute Lyme disease, Post Lyme disease syndrome, present with CIRS-Lyme [5]. CIRS-WDB is CIRS developed after chronic exposure to the interior of water-damaged buildings typified by resident microbial growth, including bacteria, filamentous fungi (molds), mycobacteria and actinomycetes; together with resultant biologically produced toxins and inflammagens, including mannans, beta glucans, hemolysins, and proteinases [6].

Regardless of initial trigger, all CIRS show similar characteristics in their final manifestations. Although the IRB for this study does not allow reporting of patient treatments, the protocol used at the clinics presented here is based on the protocol published by Shoemaker et al. [2,6]. The first phase of therapy includes eliminating offending toxin exposures, short term use of a bile acid sequestrant such as cholestyramine (CSM); and eradicating nasal MARCoNS (multiply antibiotic resistant coagulase negative staphylococci) if present. The second phase of therapy includes the correction of anti-gliadin antibodies, dysregulated androgens, dysregulated ADH (antidiuretic hormone) and osmolality; elevated MMP-9 (matrix metalloproteinase 9), VEGF (vascular endothelial growth factor; bimodal dysregulation), complement components C3a, C4a and TGF β -1. All fully treated patients have proceeded through these two phases. A third phase is used in some but not all patients (those with specific persisting deficits).

CIRS patients routinely are observed to suffer from a decline in executive cognitive functioning, as well as other neurological deficits. NeuroQuant® (NQ) is an FDA approved software that takes data from a standardized brain MRI and calculates the volume of 11 paired brain structures [7]. What previously took 100 hours of skilled radiologist's time to assess can now be done in roughly 10 minutes. Additionally, the NQ software can quantify subtle changes in volume for discrete brain structures that a radiologist's eye may not be able to appreciate. A prior study by our group using MRI brain structure quantization of CIRS-WDB patients showed statistical enlargement of the left and right pallidum, right forebrain, left amygdala and bilateral diminution of the caudate nucleus [8]. This current study used similar, standard statistical methods to determine if volumetric abnormalities in discrete brain structures of untreated CIRS-WDB patients persist across stages of therapy.

Methods

Institutional Review Board approval for this study was granted by Copernicus Group IRB LLC, Research Triangle Park, NC. Patients

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seeking or undergoing treatment at 3 separate specialized clinics for a CIRS, potentially acquired following exposure to the interior environment of a WDB, signed HIPAA releases to participate in a study to assess the hypothesis that their illness parameters included volumetric abnormalities in the CNS. Each of the patients demonstrated exposure to a building with a history of water intrusion followed by evidence of microbial growth as shown by either presence of visible mold or presence of elevated levels of speciated fungi as shown by qPCR using ERMI (Environmental Relative Mold Index) testing. Patients were interviewed by a physician to determine the presence of symptoms consistent with CIRS. Patients then underwent extensive blood labs to verify CIRS illness (for a detailed discussion of CIRS diagnosis and blood labs see [8]). Briefly, positive CIRS diagnosis requires (i) exposure criteria; (ii) presence of a multi-system, multi-symptom illness; and (iii) laboratory abnormalities in 5 of the following 8 blood protein CIRS bio-markers: VIP, MSH, complement component C4a, MMP9, VEGF, TGF β -1, osmolality/ADH balance and cortisol/ACTH balance. Patient groups consisted of new patients, untreated (n=28; 12 male and 16 female, mean age 50.4, std 6.7, range 34-61 years of age, 100% Caucasian), as well as 40 patients currently undergoing treatment. The first treatment group, partially treated (n=20, 9 male and 11 female, mean age 50.1, std 8.9, range 33-63 years of age, 100% Caucasian) were those treated through the first phase of the CIRS treatment protocol but still recorded abnormal blood labs. The second treatment group, fully treated (n=20, 9 male and 11 female, mean age 51.0, std 7.0, range 36-61 years, 100% Caucasian) had completed the CIRS treatment protocol prior to receiving their MRI. Patients categorized as untreated had not been treated by a CIRS specialist. Unfortunately, restrictions of the IRB prohibit discussion of treatments for these patients and limit reporting to structural brain changes.

A group of 23 medical controls (n=23, 11 male and 12 female, mean age 51.3, std 8.4, range 29-57 years of age, 96% Caucasian (n=22) and 4% Asian (n=1)) was recruited from a patient group coming to the clinic for well evaluation or non-CIRS related care (i) who had no symptoms or lab abnormalities suggesting CIRS; (ii) nor history of brain injury or executive cognitive impairment; (iii) and who had no significant uncontrolled medical illnesses. No statistical differences were seen in average length of education for groups (controls mean = 17.0, std 2.91; untreated mean = 16.2, std 2.29; partially treated mean = 16.4, std 2.69; fully treated mean = 15.7, std 2.75).

Each subject in this study had a 3D magnetic resonance image (MRI) scan of the brain using a 3.0 Tesla (minimum) MRI scanner followed by volumetric analysis of the image using NQ. The protocol for scanning can be found on the NQ website (www.cortechlabs.net). Each brain MRI was uploaded to the NQ server and processed, resulting in a full-volume, spatially corrected and anatomically labeled dataset containing the absolute and relative volumes on 11 brain regions, with left and right hemispheres reported separately. All mapping outputs were visually inspected for segmentation irregularities.

All volumetric data analyzed were in the normalized format of intracranial volume percentage. Comparison of the control group vs. the untreated group for each structure was assessed using a two-sided sample t-test and a Holm-Bonferroni stepwise multiple test correction. For each structure that showed a significant difference by t test, we applied an ANOVA followed by a Tukey-Kramer post hoc analysis to determine if significant differences persisted across different stages of treatment. The null hypotheses for comparison

between the different treatment stages was that there would be no difference (H_0 = treatment would not correct volumes).

Results

Controls vs. untreated CIRS-WDB patients

Comparison of medical controls (n=23) against untreated patients (n=28) revealed a number of statistically significant differences by t test (Table 1). Specifically, untreated CIRS-WDB patients had significant enlargement in both hemispheres, or bilaterally, of forebrain parenchyma, cortical gray and pallidum, as well as significant bilateral diminishment of the caudate nucleus, compared to controls (Figure 1). The change in forebrain was small but as it comprises 31% of the volume inside the skull as measured in controls, even a small change in that structure can impact intracranial pressure. The pallidum exhibited the largest degree of change at a 20% increase in size, followed closely by the lateral ventricle with a 19% reduction. The caudate was also reduced 13%, which is interesting since the caudate and pallidum are elements of the basal ganglia, and lay along the lateral ventricle. Although these are smaller structures and do not contribute much to the overall volume inside the skull (raw data found in Supplementary Table 1), their change is dramatic and may point to the basal ganglia as having increased sensitivity in CIRS-WDB.

Comparison of patients during different phases of treatment

To determine if treatment had any effect on the four significantly changed structures identified in CIRS-WDB patients (Table 1), an ANOVA was performed using controls, untreated patients, partially treated patients and fully treated patients. The ANOVA was run using both hemispheric distinction, e.g., left caudate and right caudate were separate tests, as well as combined, where the measurements were

Table 1: T test p values for brain structures of controls vs untreated subjects. Shading indicates significance after multiple test correction.

Test	P value
Caudate RH	3.07E-07
Forebrain LH	3.19E-06
Cortical Gray LH	6.02E-06
Forebrain RH	7.51E-06
Cortical Gray RH	1.34E-05
Pallidum RH	6.83E-04
Caudate LH	1.10E-03
Pallidum LH	2.69E-03
Inf Lat vent RH	3.05E-02
Thalamus RH	3.90E-02
Age	9.15E-02
Lat Ventricle RH	1.23E-01
Amygdala LH	1.88E-01
Cerebellum LH	2.36E-01
Putamen RH	2.41E-01
Thalamus LH	2.69E-01
Amygdala RH	2.79E-01
Inf Lat ventr LH	3.94E-01
Cerebellum RH	4.09E-01
Hippocampus RH	4.85E-01
Lat Ventricle LH	5.78E-01
Yrs of Education	6.94E-01
Hippocampus LH	7.53E-01
Putamen LH	9.81E-01

binned only by structure and not distinguished by hemisphere. When the structures are not distinguished by hemisphere, it effectively doubles the number of data points (using L+R, instead of segregating L and R as different) for the test. The results of the ANOVA (Table 2) show a significant diminution of forebrain and cortical gray swelling in fully treated patients compared to untreated. The caudate was resistant to treatment and although the pallidum decreased in size, the improvements were not statistically significant between untreated and fully treated patients. It is noteworthy to add that when fully treated patients were compared to controls in the ANOVA using L and R distinctions, no significant differences were noted for all the structures that showed significant changes between controls and untreated. When removing the L and R distinction on structures,

only the caudate remained significantly different between controls and fully treated patients.

Discussion

Our first study of volumetric changes in brain structures of CIRS-WDB showed bilateral changes in both the caudate and pallidum, as well as single hemisphere changes in forebrain and amygdala [8]. This current follow up study with a larger sample size also showed these same bilateral changes in the pallidum and caudate, but also bilateral enlargement of the forebrain parenchyma and cortical gray. NeuroQuant® extends the reach of a traditional MRI's ability to analyze and track the effect of disease upon the brain. This process is extremely

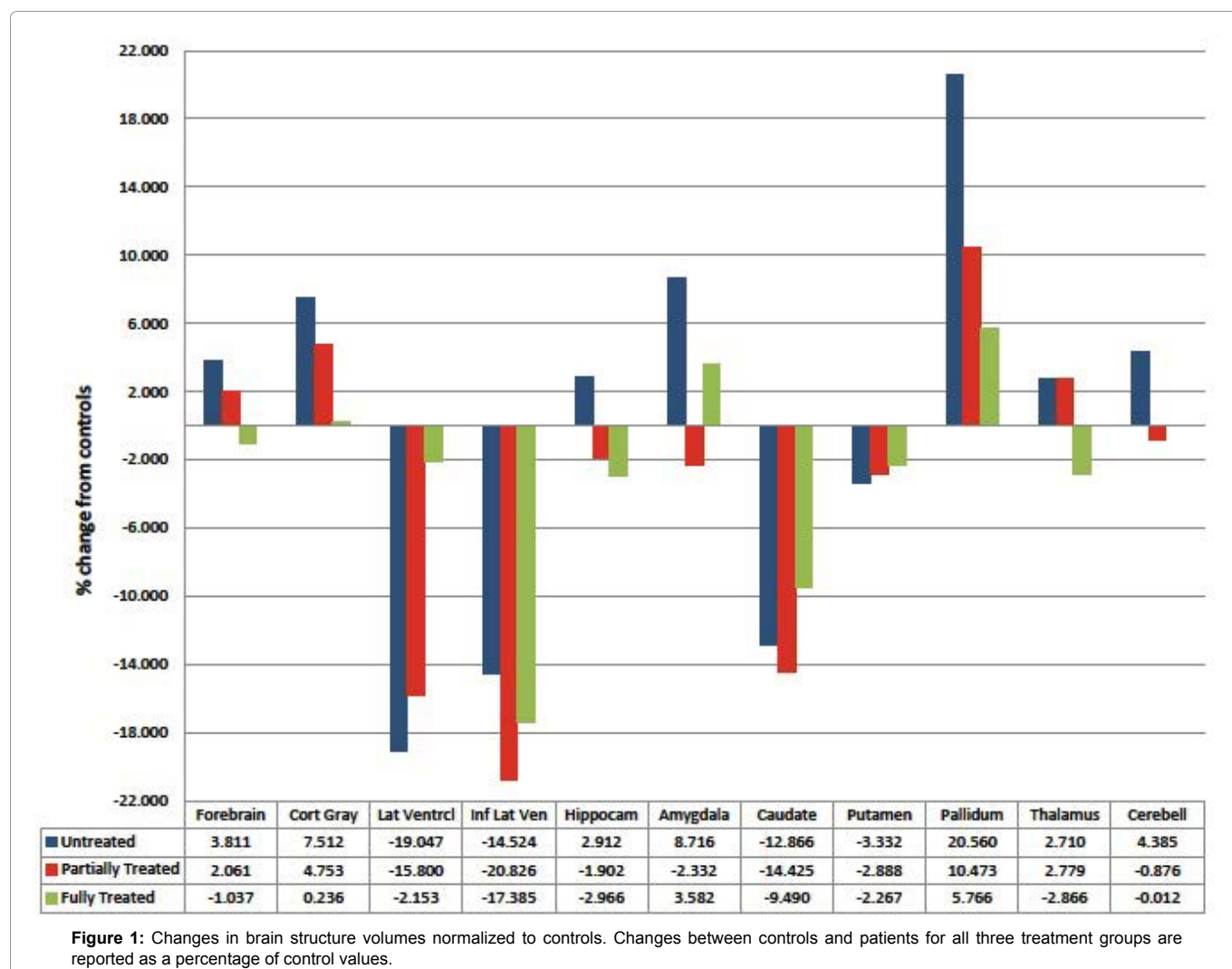


Table 2: ANOVA Results. P values from ANOVA run on forebrain (FB), cortical gray (CG) caudate nucleus (Caud) and pallidum (Pal). Values are reported for each hemisphere individually (left or right, L&R respectively) and with both hemispheres combined (C). Shaded cells indicate significant changes

Group 1	Group 2	L FB	R FB	C FB	L CG	R CG	C CG	LCaud	RCaud	CCaud	L Pal	R Pal	CPal
Untreated	Control	-1.3E-05	-5.3E-06	-2.0E-05	3.7E-05	3.9E-05	-2.0E-05	7.3E-03	6.0E-05	-2.0E-05	6.9E-03	2.0E-03	-1.4E-05
Untreated	Treated	1.7E-05	2.8E-05	-2.0E-05	1.1E-04	2.5E-04	-2.0E-05	NA	1.0E-01	1.0E-01	2.5E-01	3.0E-01	5.4E-02
Untreated	Part Treated	1.2E-01	7.2E-02	4.8E-03	1.2E-01	1.2E-01	7.2E-03	NA	9.7E-01	7.6E-01	NA	NA	NA
Part Treated	Control	2.9E-02	1.0E-01	1.4E-03	8.1E-02	7.9E-02	3.0E-03	9.0E-04	4.9E-05	-2.0E-05	2.5E-01	1.0E-01	2.1E-02
Part Treated	Treated	5.9E-02	NA	3.7E-03	NA	NA	3.8E-02	2.0E-01	3.9E-02	6.7E-03	NA	NA	NA
Treated	Control	9.8E-01	NA	9.8E-01	NA	NA	9.1E-01	1.9E-01	1.9E-01	2.6E-02	NA	NA	1.7E-01

tedious by hand but performed in a few minutes by computer. Small differences in one dimension may not be appreciated by the eye of even a skilled radiologist but these changes can correspond to significant volume changes. An increase of 5% in any dimension may not be noticed by a radiologist but as volume is a cubic function, this small change amplified throughout the three dimensions can result in a 16% difference in volume. A recent study showed that NQ software is significantly better at detecting atrophy and abnormal asymmetry (98.5%) vs the traditional radiologist's approach (12.5%) [9]. The statistically different volumes between controls and CIRS-WDB patients found here represented a 3.8% (forebrain), 7.5% (cortical gray), 12.9% (caudate) and 20.6% (pallidum) change from controls. Although we do not know the biological significance of a 4% change in structure volume, we do know that cognitive and other neurological symptoms in these patients heal with therapy, as do forebrain and cortical gray structures return to control levels. As mentioned in the results, the larger changes, by proportion, seem to point to the basal ganglia as particularly sensitive. As well, the basal ganglia are known targets of Parkinson's and Huntington's Disease [10], however, unlike CIRS, damage in these diseases is currently intractable. CIRS-WDB patients often suffer from memory loss, inattention, easy confusion, difficulty assimilating new information, frequent difficulty finding words while speaking, tremors, mood swings, sleep disturbances, even true disorientation to person or place [8]. In fact, most CIRS patients have some degree of "brain fog" ranging from mild to debilitating. NQ provides objective evidence that areas of the brain are impacted in untreated CIRS patients. The fact that these structures can now be identified as statistically different from normal provides a basis to understand the illness symptoms of patients.

CIRS-WDB is a chronic inflammatory disorder. While it has been documented that CIRS patients are in a chronic peripheral inflammatory state, the enlargement of brain parenchyma would suggest inflammation in the brain as well. As discussed in earlier work on volumetric abnormalities in the brains of CIRS-WDB patients [8] many of the immune mediators found dysregulated peripherally contribute to blood brain barrier (BBB) maintenance such as TGF β -1 [11], VEGF [12] and MMP9 [13]. Once the BBB becomes "leaky" or is breached, peripheral molecules are allowed to enter the parenchyma, which will evoke an immune response from resident microglia.

Several mechanisms could be proposed to account for the volumetric increases in structures of patients with CIRS-WDB. The most likely scenario is that microscopic interstitial edema, but not vasogenic edema, is causing swelling. The larger volumes of brain matter juxtaposed against the fixed volume of the adult skull would require a decrease in blood volume and/or CSF. This most likely explains the reduced volumes of the lateral and inferior lateral ventricles. Evidence of reduced blood flow in CIRS-WDB is shown by the magnetic resonance spectroscopy findings of increased lactate in the brains of patients (unpublished data). As increasing cranial pressure is relieved by forcing CSF from the ventricles, the walls of the ventricles will deform. This could introduce sheering pressures upon the tissues fixed against these moving walls, adding mechanical stress to the nearby, inflamed tissue, such as the caudate.

When peripheral inflammatory cytokines are controlled and the BBB has time to heal, it is likely parenchymal volumes return to normal and blood flow to these structures also improves. Although the caudate in these patients was resistant to change throughout treatment, despite control of peripheral inflammation and therefore

a less permeable BBB, the observation of atrophy compared to microscopic interstitial edema suggests a different mechanism of injury. Further research will focus on time course studies through therapy as well as long-term follow up MRIs/NQ on patients to monitor the caudate for healing. Although easily missed by the naked eye, the sensitivity for identifying these small changes in brain structure volumes is easily obtained through volumetric imaging software such as NQ.

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