/GD-TRACKER/ A SOFTWARE FOR BLOOD-BRAIN BARRIER PERMEABILITY ASSESSMENT

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Abstract

Epilepsy is chronic neurological disease characterized by occurrence of spontaneous recurrent seizures affecting approximately 1% of people. One of the most common causes of epilepsy in adults is a stroke. The precise mechanism of development of vascular epilepsy is not known, and thus no reliable biomarker of postischemic epileptogenesis currently exists. Blood-brain barrier (BBB) impairment is phenomenon observed in several pathologies including stroke after which epilepsy often develops. Blood components such as albumin or thrombin have been experimentally shown to possess the capacity to increase excitability which results in seizures and epileptogenesis. We hypothesize that severity of the BBB breakdown during first weeks after the stroke can be used as a biomarker of postischemic epileptogenesis. This paper describes free MATLAB based software /Gd-Tracker/ and methodology developed for assessment of BBB permeability from magnetic resonance (MR) scans based on statistical voxel to voxel comparison of sequences with and without Gd-DTPA contrast. This software allows to evaluate extent and magnitude of impaired BBB region and thus serve as a tool for visualization and quantification of BBB breakdown.

Keywords

blood-brain barrier, MRI, Gd-DTPA, permeability, stroke, epileptogenesis, MATLAB, freeware, Gd-Tracker

Introduction

Epilepsy is a chronic disorder of the brain that affects people of all ages. According to WHO approximately 50 million people worldwide suffer from epilepsy, which makes it one of the most common neurological disease globally [1].

The most common cause of epilepsy in adults older than 35 years (more than 50% of all new cases) is cerebral ischemia (stroke). The risk of developing epilepsy in patients after the stroke rises up to 9%. Patients who develop vascular epilepsy after the stroke have a significantly higher disability and are less likely to be independent in activities of daily living [2]. Search for new treatments to preventing development of epilepsy represents the main direction of contemporary epilepsy research.

Although the precise mechanism of postischemic epileptogenesis is not known, several proposed cascades are currently in the focus of the neuroscience teams world-wide. It seems reasonable to therapeutically intervene only in those patients where epileptogenesis occurs. Currently, we lack a reliable marker to identify patients at increased risk of developing postischemic epilepsy.

Blood-brain barrier (BBB) dysfunction is one of the candidate mechanisms which plays an important role in the development of epilepsy after stroke. The BBB is an anatomical structure separating vascular and neural brain compartments. Its proper function is essential for normal brain activity by maintaining the homeostasis

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of the brain microenvironment [3]. Loss of BBB integrity leads to extravasation of plasma proteins into the neuronal tissue and vasogenic brain edema [4]. Defective BBB can persist for long periods after the stroke and may cause secondary inflammation and neuronal dysfunction [5–6]. Several plasma proteins including albumin and thrombin [7–8] as well as inflammatory cytokines have been shown to trigger both acute epileptiform activity (acute symptomatic seizures) and epileptogenesis [9–10]. Conversely, experimentally induced epileptic seizures in animal models transiently compromise BBB [11–12].

We hypothesize that severity of BBB dysfunction and extent and time profile of inflammation induced by stroke correlate with epileptogenesis. To address this hypothesis a reliable method for detection and quantification of BBB is needed. Routine diagnostic protocol usually includes MRI with different sequences allowing sensitive identification of ischemic area only. Therefore, gadolinium (Gd) based contrast MR imaging must be implemented to provide information about the BBB properties. Because of its paramagnetic pro-perties, intravenously administered chelated organic gadolinium complexes enhance nuclear relaxation rates and increase contrast. Standard perfusion imaging protocol is based on dynamic acquisition during fast bolus perfusion of chelated Gd solution. However, it is demanding method which prevent its use as a routine diagnostic protocol. In this paper, we present a soft-ware /Gd-Tracker/ based on statistical methods [13] which allows semi-automatic BBB permeability quan-tification from two sets of T1-weighted MR images acquired before and after Gd-DTPA application. The software is freely accessible on http://bbb.biomed.cas.cz.

Methods and materials

In total 30 patients (17 men, age 65 ±11 years and 13 women, age 69 ± 10 years) were included in this study to verify sensitivity and selectivity of algorithms. The study was approved by the institutional ethical committee of Motol University Hospital and all subjects provided their informed consent. Only patients with acute stroke in supratentorial region were selected. Patients with previous cortical ischemic lesions, allergy to contrast agent and established epilepsy were excluded.

A modified clinical MRI protocol with Gadovist (Gd-DTPA, Bayer Pharma AG, Germany) contrast agent was performed 7–12 days after the stroke. A solution of contrast agent (10 ml) was injected approximately 3 minutes before the acquisition of contrast sequences start. MRI was performed using 1.5T Phillips Ingenia scanner.

Algorithms used in this study are adopted from [13] and the entire procedure is summarized and graphically presented in Fig. 1. The method is based on comparison of two consecutively recorded T1-weighted MR images of head: native MRI and MRI with contrast agent Gd-DTPA (Gadovist). Gadovist does not cross BBB in healthy tissue while in regions with compromised barrier it leaks to the brain tissue and increases signal intensity. Thus regions with significantly higher signal after Gadovist injection are considered to have BBB impairment.

Acquired data were processed in our software /Gd-Tracker/ written in Matlab environment (version R2014b, The Mathworks, Nattick, USA). The software processes datasets in several steps: construction of

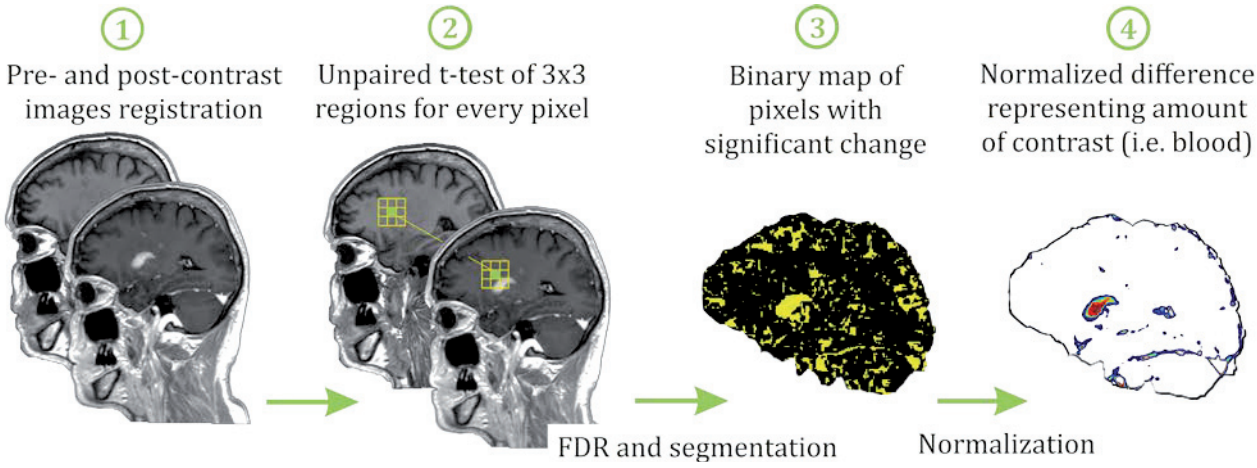

Fig. 1: Method summarization. (1) Pre and post contrast images are registered. (2) T-test of corresponding regions is performed (3) resulting in binary significance map where areas with significant changes are highlighted. (4) Difference of intensities in these areas is calculated and eye-sinus normalized.

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binary map of voxels with significant difference (after Gd contrast), quantification of this difference, normalization of the data by eye(0)/blood(1) scale and visualization in brain tissue containing voxels which were identified by segmentation.

Localization of significant changes

First for each pixel (all algorithms are 2D - each slice of 3D volume is computed individually, thus we use term pixel instead of voxel) of pre- and post- contrast images surrounding 3×3 area is selected for comparison. The reason why 3×3 neighborhood is used instead of comparing single pixels is an effort to suppress possible inaccurate registration. During the image registration, one image is spatially transformed to exactly match other image. Some pixels do not have perfect alignment and false positive difference between such pixels can be observed. This effect is decreased by using a matrix of surrounding pixels and its statistical comparison.

Each pair of the two corresponding neighborhoods (from pre and post contrast images) is compared using unpaired t-test to determine changes between pre and post contrast images. Pixels (represented by its neighborhood) with P-values smaller than 0.05 are considered to be statistically significant and are used for the creation of binary map, where significant pixels are represented by 1 and nonsignificant by 0. FDR (false discovery rate) statistical correction is used to suppress correct the impact of multiple comparisons.

Calculation of difference in intensity

The difference between pre and post contrast images in pixels defined by significance map is calculated by equation 1 in following step. The difference corresponds to amount of contrast agent in the tissue.

$$I_{diff}(x,y) = I_{post}(x,y) - I_{pre}(x,y) \qquad (1)$$

Data normalization

To enable comparison between subjects and sessions calculated values of difference are normalized by two reference values [13]. Value in eye ball is considered as minimal (0), because Gd contrast does not leak to a vitreous body of healthy eye ball. On the other hand, intensity in dural venous sinuses (sinus sagittalis superior, transversus or rectus) is considered as maximal (1), because the highest concentration of contrast agent is in blood.

Larger area (9×9 pixels) is repeatedly selected (two or three different places from one tissue) instead of representing tissue intensity by one value from a single pixel.

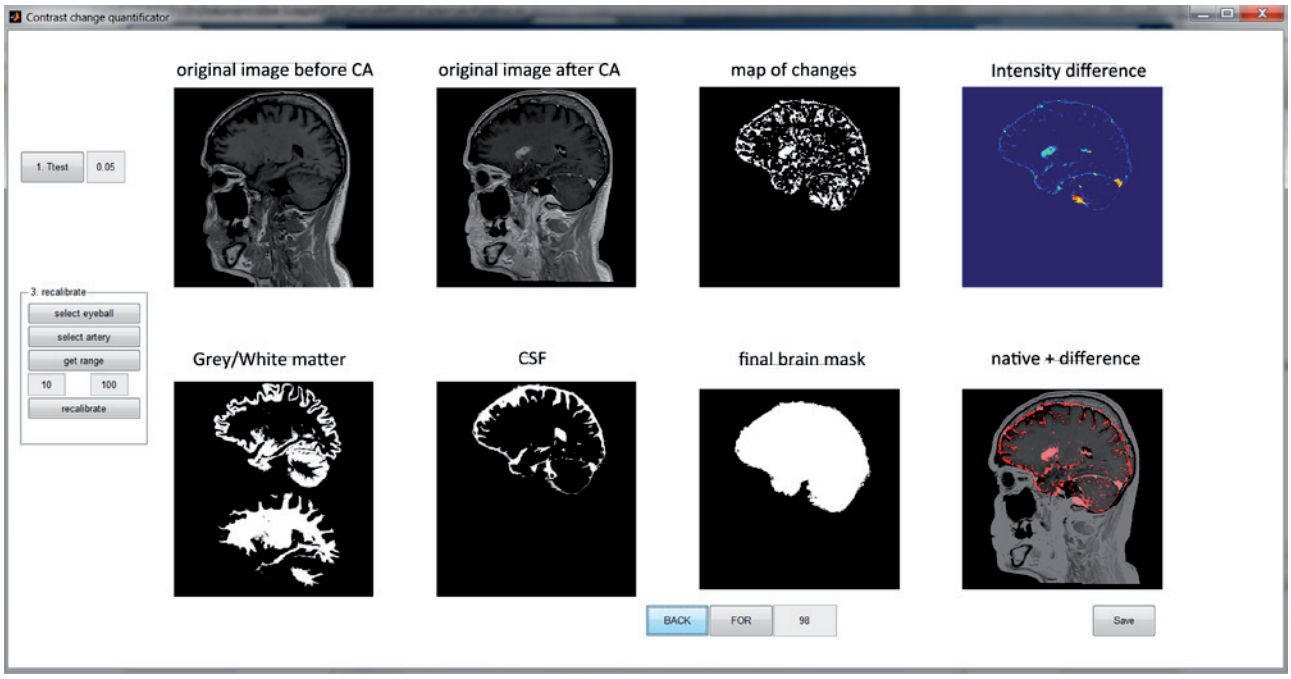

Fig. 2: Screen shot of the /Gd-Tracker/ software user interface. Partial-results of all steps of the described algorithm are shown in subplots: pre-contrast, post-contrast image, binary map of the significant changes and the normalized intensity difference respectively in upper row, brain maps with grey/white matter and CSF, all maps merged to final brain mask and the native image merged with the intensity difference in lower row. Buttons for program control on the left side of the window and buttons for listing though slices (mediolateral plane) are located below these subplots together with the button for saving results of analysis to NIfTI.

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Significant outliers due to various artifacts or other disturbances coming from method of data acquisition were often present in these sets. These outliers are excluded to obtain homogenous data sets. Gaussian fit is consequently applied on outliers-free data and quartiles of resulting distributions are considered as exact values for normalization.

The result of the normalization is a dataset of values between 0 and 1 corresponding to individual minimal (tissue with no Gd) and maximal value (blood containing a maximal amount of Gd). This transformation allows direct comparison between two different sessions and between individuals.

Image segmentation

To separate brain tissue from other tissues, a set of image segmentation techniques provided by SPM12 toolbox for Matlab (Wellcome Trust Centre for Neuroimaging, UCL, UK) were used. New Segmentation tool (function of SPM12 toolbox) performs an image registration, tissue classification (based on a mixture of Gaussians and nonlinear registration with tissue probability maps) and bias correction. The process successfully identifies grey matter, white matter, cerebrospinal fluid (CSF), bone, air, and background. Final segmentation mask, which is used in our algorithm is obtained as the unification of grey, white matter and CSF – i.e. brain tissue [14].

Results

The result of this study is a semi-automatic software which enables to quantitatively assess permeability of the BBB in MRI scans acquired without and with gadolinium contrast. User interface of the software is introduced in Fig. 2 where each steps of the entire procedure are shown.

The implemented pre-post comparison (with-without contrast) detects areas with significantly different intensities which correspond to regions with impaired BBB. Detected regions precisely overlap contrast-enhanced areas in T1 images as shown in Fig. 3. Images were further eye-sinus normalized, thus the change of BBB impairment for individual patient and session can be quantified.

Regions with impaired BBB were successfully detected in all 30 studied patients by /Gd-Tracker/ software and confirmed by conventional visual analysis. The quantification was successful either in cases of strong and obvious impairment but also in cases where the leakage was barely visible by human eye.

Datasets obtained by our processing can be further used for either visualization or analysis in other software. For example, 3DSlicer, an open source software package [15] can be used to calculate leakage size from permeability change map or to construct 3D model (Fig. 4.).

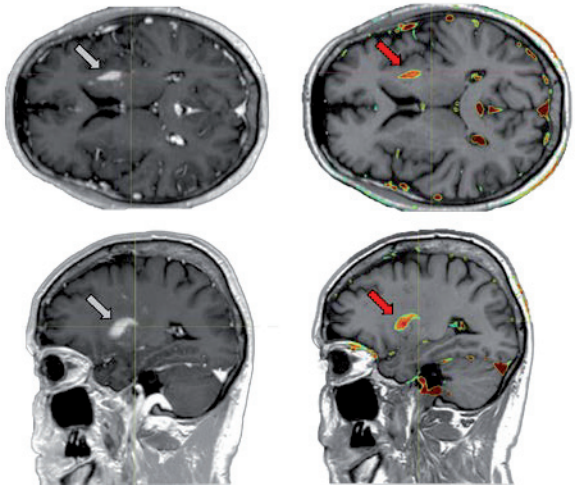

Fig. 3: Comparison of Gd contrast MRI images (left) and images enhanced by described method (right). Stroke lesion visible on contrast images (marked by an arrow) was precisely identified and highlighted by our software.

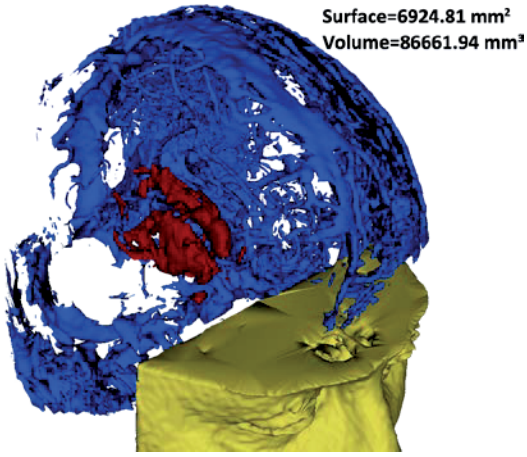

Fig. 4: 3D model of the brain tissue. Brain vessels are blue, region with impaired BBB detected by our software is depicted in red color. Surface and volume of lesion can be calculated for comparison with other prospective measurements.

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Discussion

We have shown that software /Gd-Tracker/ presented in this paper is a useful tool for quantification of changes in BBB permeability in vicinity of ischemic lesion. To detect and quantify discrete changes in the tissue intensity after the Gd contrast injection, high sensitivity of the algorithm is set. Automatic lesion detection would require to adopt a different approach because high algorithm sensitivity frequently leads to detection of larger area then ischemic region due to leak of contrast in periischemic tissue and to false positive results located mostly around vessels and other well-perfused tissues. These false positive pixels can be avoided by precise segmentation of the brain while it is mostly located outside of the brain tissue (retrobulbar structures). High sensitivity settings were chosen to avoid false negative results of pixels with mild increase in signal intensity due to enhanced BBB permeability which happens especially on lesion surroundings where intensity differences are usually low. The problem with hypersensitivity is minor in our study design because exact location of lesion is known from other MRI series as assessed by conventional visual analysis prior to BBB permeability detection.

We have elaborated eye-sinus normalization to enable interindividual and intersession comparison. While vitreous body of the eye ball represents stable level and can be efficiently used for setting the lower limit range, setting of the maximal value used for upper limit seems to be more challenging. We used intensity as measured in the region of interest corresponding to dural venous sinuses which yielded stable results. The normalization to the level of the muscle tissue is other commonly used method. However, with this approach we have observed high variability in the intensity and in some cases the muscle intensity was lower than that observed in the ischemic area (data not shown). Altogether the eye-sinus normalization approach seems to be more appropriate for the purpose of this study. Current version of the /Gd-Tracker/ software was tested on datasets and is reliable tool to visualize and quantify poststroke BBB impairment.

Conclusion

The result of this study is MATLAB based software /Gd-Tracker/ allowing quantitative assessment of blood-brain barrier (BBB) permeability from MRI with and without gadolinium contrast agent. This software is able to detect significant intensity changes corresponding to pathological areas of the brain and following normalisation to estimate size and severity of the BBB impairment. /Gd-Tracker/ can by freely downloaded at http://bbb.biomed.cas.cz/.

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