

The Role of Magnetic Resonance Spectroscopy in Evaluating the Rate of Brain Metabolic Variations in Chemical Veterans with Respiratory Problem In Comparison To Control Group

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Abstract

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BACKGROUND: During the eight years of the imposed war, Iraq used various chemical agents such as sulfur mustard and nerve agents (mainly tabun and sometimes soman) on Iran's soldiers. Using information obtained from specialist sequences and analysing information obtained from magnetic resonance imaging (MRI) in a susceptibility weighted imaging (SWI) sequence and magnetic resonance spectroscopy (MRS) provides valuable information on continuation of treatment and identifying functional disorders.

AIM: The objective of this research was to evaluate the rate of metabolic variations in chemically injured veterans based on chemical neuromarkers using the chemical sequence MRS, which would help patients and physicians in terms of time, economics, and selection of appropriate therapeutic methods, so if the can physician can get complete information about the metabolic properties of the brain through paraclinical (especially MRI) tools before treatment, he might change his treatment program to reduce the complications caused by it.

METHODOLOGY: In this research, 40 chemically injured veterans with brain dysfunction admitted to the screening centre for MRI with specialized MRS sequence participated. Accordingly, we examined the rate of brain metabolic variations about the level of neuromarkers and evaluated the relationship between the level of neuromarkers and brain damages.

RESULTS: The results of this research revealed that while the demographic characteristics such as age of the two groups of chemically injured veterans and control was similar, only the median of the NAA/Cr (N-acetylaspartate to creatine ratio) ratio in PONS of chemically injured patients was significantly lower than that of the control group, and this ratio was similar in other parts of the brain in two groups. The results also showed that the ratio of NAA to total choline and Cr was similar in all parts of the brain in two groups.

CONCLUSION: Based on the research results, using the MR (Magnetic Resonance) spectroscopy device and determination of the value and ratio of markers such as creatinine and N-acetylaspartate and choline, the brain injuries of chemically injured veterans can be examined. By conducting further studies and larger sample size, the brain damages in veterans can be diagnosed early, which would be a great contribution in their treatment.

Introduction

The First World War has been known as the birth of the modern chemical war. In this war, more than 480,000 tons of chemical agents were used and led to approximately one million injured people [1], [2]. After World War I, the largest and most extensive chemical attacks throughout history occurred in the Iran-Iraq war. During the eight years of this war, Iraq used various chemical agents against the Iranian forces on various fronts of the West and the South. Official reports (including the reports of UN experts) proved the using two important types of chemical agents, including sulfur mustard and the nerve agent

(mainly Tabun and sometimes Soman) by Iraq in the war. In addition to these two agents, there are other chemical agents, including blood agents (cyanide, etc.), and choking agents (phosgene) and some other rare agents, but the use of these agents in the Iran-Iraq war was not proven [1], [2], [3], [4]. Mustard gas usually does not cause significant symptoms in a few minutes or even a few hours of its releasing, while the onset of poisoning symptoms are seen at initial moments when nerve agents are used (2 and 5).

Inhalation of mustard gas usually causes inflammation in the respiratory tract, and it's entering to larynx also causes a violent sound. Gradually, inflammation of the respiratory tract and airways is intensified (depending on the severity of the infection

and the duration of exposure to the gas) and coughing, shortness of breath, wheezing, and symptoms similar to pneumonitis are created. The next infection might worsen the pulmonary lesions. The initial mortality caused by mustard gas is usually due to lung injury and respiratory failure [6], [7]. Medical examinations on those injured by mustard gas in the First World War and studies conducted on those injured by mustard gas in the Iran-Iraq war show that this chemical agent has long-term complications, which some of them are not very important and easily treatable, and some others are more important, and it is necessary to obtain more information on complications of each of them in order to find proper methods to treat it. At present, there are effective methods to treat the pulmonary and brain problems of chemically injured victims. The important point is that no research has been conducted on brain dysfunction in these patients, and chemists use magnetic resonance to examine the structure of molecules with very accurate measurement of peaks in the spectrum and the area of each peak is equal to the relative number of protons in that place. In the chemical environment of the human body, the number of water and fat proton is several thousand times greater than the number of protons in other molecules, so that we cannot make a distinction between these metabolites.

Spectroscopy is a compound in a living environment, using gradients for selective stimulation of a small volume of tissue, then recording the FID (Free induction decay) and creating a spectrum of that voxel instead of creating an image. This technique has been problematic technically and yielded uncertain results for many years, but it has become very accurate and sensitive, and many people consider it an essential part of the brain MR test. Phosphor spectroscopy is another developed application of spectroscopy in the living environment, used mainly for muscle metabolism [8]. Magnetic resonance imaging is a method developing rapidly. High sensitivity and contrast in soft tissues and its inherent safety for patients due to the lack of using ionising radiation are the main advantages of this method over other methods of imaging. A powerful non-invasive tool for characterising spatial variations in metabolic profiles for patients with brain disorders is magnetic resonance spectroscopy (MRS). Metabolic parameters obtained using this technique has shown to predict treatment response, disease progression, and transformation to a more malignant phenotype. The availability of ultra-high-field MR systems has the potential to improve the characterisation of metabolites. In reality, magnetic resonance spectroscopy (MRS) is a magnetic resonance-based imaging modality that allows non-invasive sampling of metabolic changes in normal and abnormal brain parenchyma. MRS is particularly useful in the differentiation of developmental or non-neoplastic disorders from neoplastic processes. MRS is also useful during routine imaging follow-up after radiation

treatment or during antiangiogenic treatment and for predicting outcomes and treatment response [9].

The objective of this research was to evaluate the rate of metabolic variations in chemically injured veterans based on chemical neuromarkers using the chemical sequence MRS, which would help patients and physicians in terms of time, economics, and selection of appropriate therapeutic methods, so if the can physician can get complete information about the metabolic properties of the brain through paraclinical (especially MRI) tools before treatment, he might change his treatment program to reduce the complications caused by it. No research has been conducted so far on the brain damages of chemically injured veterans and the evaluation of neuromarkers function, such as N-acetylaspartate, choline, creatinine, lactate, and the role of these markers in examining the cause of seizure, weakness, brain impairment, and other injuries. In fact, by using specialised MRI sequences, we aim to establish a significant relationship between the brain damage of chemically injured patients and the rate of variations in neuromarkers and contribute to the treatment of these patients.

Material and Methods

This research is a fundamental-analytical study conducted on chemically injured veterans whose respiratory problems were confirmed as well as asthmatic patients who are candidates for the continuation of the treatment with coordination of a specialist physician for MRI imaging using the MRS protocol. A total of 40 chemically injured veterans admitted to imaging centre of Arian Hospital in Tehran due to respiratory problems were selected as research sample. Research inclusion criteria included lack of contraindication for MRI and having the willingness to participate in a research project after study and signing the written consent. Moreover, 40 patients with asthma were selected as the control group, and they were matched with case group regarding age and gender. Patients' data including age, gender, and other demographic information were recorded before entering the study. Patients were controlled regarding lack of contraindications for MRI tests.

Contraindications for MRI tests were as follows:

1. Patients with a heart pacemaker.
2. Patients who have metallic objects in their eyes or have a cerebral aneurysm clipping, as the magnetic field may interfere with the metal.
3. Patients with severe claustrophobia which may not be able to tolerate an MRI scan.

4. Patients with metal devices in their spinal cord (such as bolts and nuts) can have an MRI scan, but the scan resolution is often impaired by the metal object, and the spinal cord is not well displayed.

The imaging was performed with the 1.5 GHz Tesla Device (Magnetom Model). The patient was placed in a supine position on the bed and the patient's head was placed in a 16-channel coil special for brain imaging and some of the routine sequences of the brain imaging, including TSE-T2w axial images with parameters of TR/T: 5600/98 msec, slice thickness: 5mm and FLAIR Axial with parameters of TR/T: 8500/103 msec, slice thickness: 5 mm. Then, the MRS images with single & multi-voxel sequence were performed according to five parameters: 230 * 182 * 107 TR/TE: 2000/144 msec, NSA: 128. The images were evaluated by one radiologist and one MRI imaging physicians. Then, the severity of the markers obtained through the software was analysed after plotting the charts. Then, the results were given to the clinician to determine the proper treatment for the patients.

After collecting data, information was extracted from the files and analysed using SPSS 25 software. Mean, median, standard deviation, minimum and maximum, and range was calculated. In this research, T-test was used to analyse the data and to examine the normal distribution of data, KS (Kolmogorov–Smirnov) test was used. For rank data or data with non-normal distribution, non-parametric tests such as Kruskal-Wallis were used. Charts were plotted by using GraphPad Prism software. In all cases, $p < 0.05$ was considered significant. The data are presented as mean \pm SD.

Results

In this research, 40 chemically injured veterans with respiratory problems and 40 patients with asthma as control were evaluated. The mean age of them was 54.6 years, with a standard deviation of 8.6. Examining and comparing the mean age of the patient group and the control group showed that the mean age was not significantly different in the two groups ($p > 0.05$). Injury percentage of the case group had a mean of 51.3% with a standard deviation of 16.88%. These patients were injured between the years 1983 and 1987, and the highest prevalence of injury was as follows: lung (66.7%), lung with eyes and skin (20%) and lung and gastrointestinal tract (6.7%). The harmful chemical agents were unknown in these patients. They were mustard gas in 20%, mustard gas and nerve gas in 20%, and blood and choking agents in 6.7%. The data distribution about the NAA/Cr ratio in patients studied by KGV test was examined. Results show that the NAA/Cr ratio in the studied population does not follow the normal curve.

Thus, to compare this ratio in two groups, non-parametric tests were used. Based on the results obtained, the median of NAA/Cr ratio in Pons in the two groups is significantly different. As shown in Fig. 1, the mean of this ratio in chemically injured patients is significantly lower than that in the control group (p -value = 0.03) (Fig. 1).

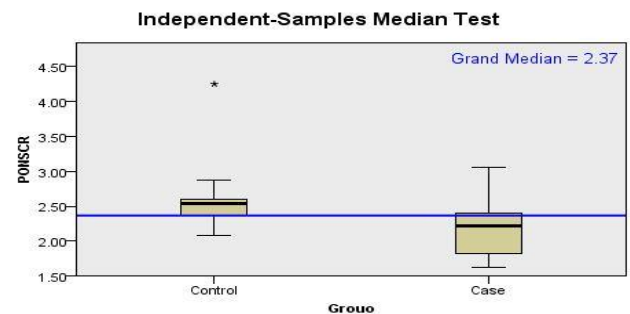


Figure 1: The median of Pons NAA/CR ratio in two groups

Examining the distribution of NAA/CR ratio in basal ganglia of the left and right sides of brain as well as their mean of normal curve showed that this ratio does not follow the normal curve in the basal ganglia of the right side, but the curve function is normal in the left side of the brain and the mean of the two sides of the curve. Non-parametric test on the comparison of the median of NAA/CR ratio in the basal ganglia of the right side of the brain in both patient and control groups showed that the mean of this ratio was not significantly different in the two groups (p -value > 0.99).

Also, the comparison of the mean NAA/CR ratio in basal ganglia in the two groups of patients and control by Students T-test showed that the mean of this ratio was not significantly different in two groups (p -value = 0.142). Finally, the comparison of the mean NAA/CR ratio of right and left brain ganglia in both groups of patients and control by Students T-test showed that the mean of this ratio was not significantly different in two groups (p -value = 0.336) (Fig. 2).

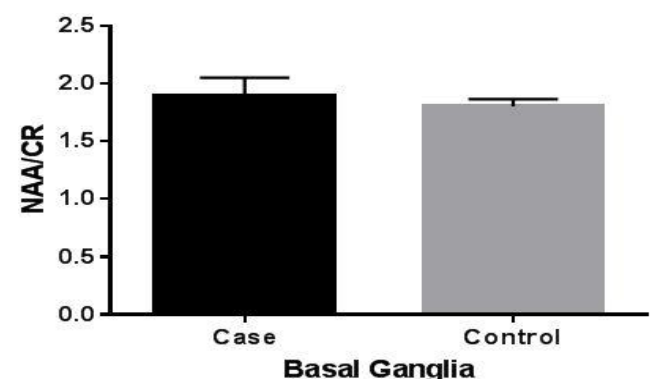


Figure 2: Mean and deviation from of NAA/CR ratio of basal ganglia of right and left sides of the brain in case and control groups

Comparison of mean NAA ratio to total Choline and Cr in the Pons region of the chemically injured patients and the control group by Students T-test showed that the mean of this ratio was not significantly different in the two groups (p-value = 0.117) (Fig. 3).

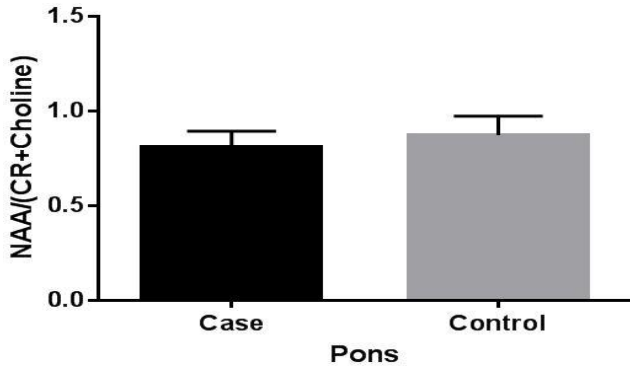


Figure 3: Mean and SD of the ratio of NAA to Total Choline and Cr in the Pons region of the case and control groups

The analysis of data related to ratio of NAA to Choline and Cr in the basal ganglia of left and right sides of brain and their mean in chemically injured and control groups showed that this ratio has a normal distribution in each of the two halves, but the ratio of mean of two halves is not normally distributed. Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of the left half of brain in the chemically injured and control groups by Students T-test showed that the mean of this ratio was not significantly different in the two groups (p-value = 0.702).

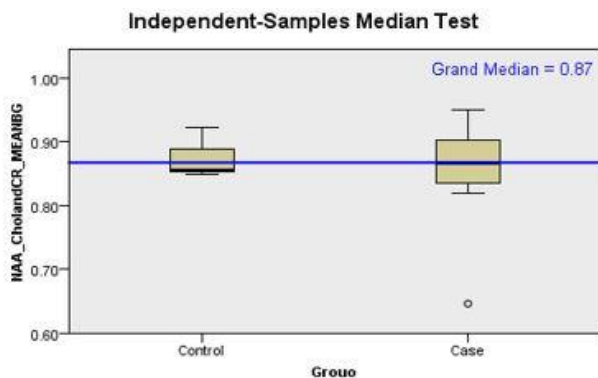


Figure 4: Median of mean ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in the chemically injured group and control group

Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of the right side of the brain in the chemically injured patients and control group by Students T-test showed that the mean of this ratio was not significantly different in two groups (p-value = 0.753). Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in chemically injured patients

group and control group by non-parametric test showed that the mean of this ratio did not differ significantly between two groups (p-value > 0.99). The results obtained from the comparison of the median of the ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in chemically injured patients group and the control group by the non-parametric test are shown in (Fig. 4).

Discussion

Disorders are occurring in the human neurological system cause changes in the concentration of some of the metabolites, depending on their type and severity [10]. Studies have shown that brain cells, based on their type, have unique metabolites at least in vitro, which these patterns can be distinguished by using the MRS [11]. The MRS method is used to measure the level of various metabolites in tissues such as choline, creatinine, citrate and lactate. This method measures the level of various chemical agents in the tissues by showing waves with different peaks which each peak is related to a specific chemical agent [12]. By quantitative measuring of these chemicals in tissues, the level of cellular activity and the presence of malignant cells in the tissues can be detected. MRS has been used so far in diagnosing and assessing the diseases such as brain tumours, stroke, oxygen deficiency, epilepsy, and multiple sclerosis. However, there is little accurate and comprehensive research in this regard [13]. Previous studies, indicating the application of ratio of these metabolites in diagnosing of neurological diseases, have shown that masses of high-grade malignancy (Grade 3 anaplastic glioma and Grade 4 multiform glioblastoma), compared to low-grade masses, have higher choline and lower NAA. Increased Cho is related to proliferation and cell density. Sarin and mustard gases were used by the Iraqi during their war against Iran and even its Kurdish regions [14]. To examine these effects on the nerve system, new imaging techniques can be used. Effective methods have been identified in the treatment of pulmonary and brain problems of chemically injured veterans, and complementary research would reveal the outcome of these treatments.

The important point is that no research has been conducted on brain function disorders in these patients so far. The objective of this study was to evaluate the brain damages in chemically injured veterans using magnetic resonance imaging (MRI) and to evaluate the concentrations of choline, creatinine (Cr) and N-acetylaspartate (NAA). While estimating the pure concentration of choline is possible, it is sensitive to many of the errors, which their assuming is necessary. The ratios of NAA/cho

and cr/cho are used for very accurate examination. Hence, the ratios of N-acetylaspartate to creatinine and the ratio of N-acetylaspartate to total choline and creatinine were examined [15]. Measurement of brain biomarkers by MR spectroscopy shows that neuronal activity significantly decreased in the brain stem of war veterans compared to that of healthy control subjects. In the investigation conducted, the highest prevalence of injury was observed in the lung alone, lung along with eyes and skin, and lung along with the gastrointestinal tract, respectively. Measurement of NAA/Cr in MR spectroscopy showed a significant reduction in the veteran group compared to the control group in the brain stem.

In the measurement of NAA/Cr in the right and left basal ganglia with MR spectroscopy, results showed no significant reduction in this marker in the right and left basal ganglia. The other marker measured in this study was the ratio of NAA to total choline and Cr in Pons. Results showed no significant difference between the veterans group and the healthy control group. No significant reduction was seen in the ratio of NAA to total choline and Cr in the left and right basal ganglia in the control and case groups. In a study conducted on 34 thousand Iranians injured by mustard (13 to 20 years after exposure), 57.7% of them had pulmonary dysfunction, and 34%, 4.5% and 1% had weak, moderate and severe complications, respectively [16]. Studies conducted by Hollingworth et al., in 2006 showed that MRS can accurately distinguish between high glioma and low-grade glioma. However, the results of glioma grading using MRS are very extensive. This diversity can be due to different methods and metabolites. Among the different grades of the tumour, the Cr/Cho and NAA/Cho overlap is significant [11], [17].

In a study conducted by Robert et al., in 2000, Gulf war veterans were examined. In this study, 22 veterans of the Persian Gulf War were selected as case group, and 18 veterans who were matched with case group subjects regarding age and education level were selected as a control group. In this study, the Gulf War syndromes with the level of brain metabolites were examined. The Gulf War Syndrome was introduced in 1997 by Haley. It consists of three levels. In this study, the ratio of NAA to creatinine was examined. It showed neuronal mass activity. In this study, the level of N-acetylaspartate to creatinine, in contrast to our results, decreased significantly both in basal ganglia and brain stem in all three Gulf War syndromes [18]. However, in this study, the level of NAA / Cr decreased significantly only in the brainstem (and not in the basal ganglia) in chemically injured veterans with respiratory problems. However, this level in the right and left basal ganglia showed no significant difference with that of the control group. Physiological studies show that the respiratory centre located in the medulla spine plays a major role in the endogenous regulation of respiratory rhythm, chemo-regulation and mechano-regulation in an integrated

way [19]. As a result, a significant reduction in the ratio of NAA/Cr in the brain stem of veterans with respiratory disorders suggests the important role of this region in central respiration regulation.

Based on the research results, the brain damages of chemically injured veterans can be examined by using MR Stereoscopy device and determining the value and ratio of markers such as creatinine and N-acetylaspartate and choline. Also, conducting further studies with larger sample size can be helpful in early diagnosis of brain damages in veterans, and it would be a great contribution in the treatment of these patients.

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