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Assessment of Ozone Autohemotherapy on Patients with Multiple Sclerosis by Time-frequency Analysis of Near-infrared Spectroscopy Signals

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Abstract. During the last several decades, the availability of ozone autohemotherapy is gradually being understood through the comprehensive study and clinical experiment. Ozone autohemotherapy is advocated as a form of alternative or combination with orthodox medicine in treating vascular and neurological diseases. In order to study the therapeutic effect of ozone autohemotherapy on multiple sclerosis (MS), we used a near-infrared spectroscopy system to monitor the oxygenhemoglobin (O_2Hb) and dexoygenhemoglobin (CO_2Hb) concentration changes of 6 MS suffer subjects under ozone autohemothearpy for about 150 minutes each.The NIRS-Ozone signals were performed a time-frequency analysis in three time intervals:1)blood removal; 2)blood reinfusion and 3) at the end of the monitoring (a few minutes before the end of the monitoring). By computing and comparing the relative power of O_2Hb and CO_2Hb signals in the very low frequency (VLF) and low frequency (LF) bands during three different recording periods, it can be observed that the VLF power decreases and the LF power increases, which is possible an indication of a clear vascular effect of ozone. From a technical point of view, it is a quantitative assessment of the therapeutic effect of ozone autohemotherapy by means of time-frequency analysis.

Keywords: Ozone autohemotherapy, near-infrared spectroscopy, time-frequency analysis

1. Introduction

Ozone is always considered as a harmful gas present in the photochemical smog. Continuous ozone inhalation eventually causes leisions in the respiratory system and extrapulmonary organ because of the releasing of some inflammatory substances [1-2]. However, its toxicity is related to its dosage. In medicine, many studies and clinical application demonstrated that an appropriate ozone dosage with a precise concentration can be used as a treatment [1-2]. Ozone autohemotherapy (OA) achieves the therapeutic result by the reinfusion of the ozonated blood to the patient. When a medical controllable ozone dose reacts with biomolecules present in plasma, there is a biochemistry process that can trigger a precisely calculated minimal oxidative stress which is able to upregulate the antioxidant defenses [1-2]. Thus, the medical ozone therapy has a different result from the oxidative stress induced by continuous inhalation. OA can improve blood circulation, activate antioxidant enzymes and scavenge free radicals [3]. Thanks to the development of dose-adjustable ozone generator, OA as a treatment has been applied in an extensive range of pathologies. Recent studies showed that OA has been already used to treat vascular disease, advanced ischemic diseases and neurological disease [4-8].

This paper introduced the research on the therapeutic effect of OA in multiple sclerosis (MS). NIRS was applied to monitor the changes of cerebral oxygenation level affected by OA on MS patients. The NIRS signals were processed in the time-frequency domain with the method of Choi-Williams Distribution (C-W Distribution). This study is an in-vivo case of investigating the long-term effect of OA in neurological disease.

2. Material and Methods

2.1. Subjects and Experiment protocol

We enrolled 6 MS patients. All the subjects were instructed about the system and the experimental protocol and signed a written informed consent.

The experiment protocol consisted of three steps: 1) the subjects were drawn 240 grams of blood from the antecubital vein; 2) the blood was mixed with 180ml of a O_2/O_3 gas mixture, which was composed by O_2 at 50%, with an O_3 concentration equal to 40μg/ml (M95, Multioxygen, Gorle, Italy); 3) the blood was slowly reinfused into the subject via the antecubital vein, after being passed through a sterile filter [5]. The ozone therapy experiment were taken under a precise medical observation to see whether they were in comfort and relaxation situation or not during the long-term recording.

2.2. NIRS recording and NIRS signal processing

Near-infrared spectroscopy (NIRS) is a spectroscopic method to non-invasively monitor the cerebral oxygenation level by detecting changes in hemoglobin concentration associated with neuron activity. The chromophores, oxyhemoglobin (O_2Hb) and deoxyhemoglobin (CO_2Hb), are considered as the main tissue oxygenation parameters [9]. The specific periods of the NIRS monitoring during the OA treatment were applied to assess its therapeutic effect.

The NIRS signals were recorded using a commercially oximeter (NIRO300, Hamamatsu Photonics K.K., Japan) with the sampling rate of 2Hz. Four different wavelength (775, 810, 830 and 910nm) of near- infrared source and a photo-detector were applied to monitor the concentration changes of chromphores. The detecting area is on the forehead 2 cm away from midline and 1cm above the supraorbital ridge [5]. The whole monitoring lasted for about 150 minutes. It was divided into the following stages: 1) baseline condition; 2) blood removal; 3) blood reinfusion; 4) post reinfusion. The NIRS monitoring recorded the concentration changes of O_2Hb and CO_2Hb in the entire process, which reflect the cerebral vasomotor reactivities of the subjects. In this study we limited the observation to 150 minutes, due to the subjects' physiological situation (for example, feeling tired or hungry) and the physiological constraints of the NIRS system.

The nonstationary NIRS-ozone signals were processed by a time-frequency analysis through Choi-Williams Distribution (with σ=0.5) in three time intervals:1) blood removal; 2) blood reinfusion and 3) at the end of the monitoring (a few minutes before the end of the monitoring).

Many studies present the oscillations of cerebral hemodynamics and metabolism in adult human head can be detected by using NIRS, which also provide the possibility of frequency-derived parameters used to assess cerebral autoregulation [10]. These oscillation have been classified within the power spectrum essentially consisting of two different bands: in very low frequency (VLF) (20-40 mHz) and low frequency (LF) band (40-140 mHz), VLFs are thought to be generated by brain stem nuclei, which modulated the lumen of the small intracerebral vessels. LFs reflect the systemic oscillations of the blood pressure and are modulated by the sympathetic system activity [11].

We also computed the time-frequency Squared Coherence Function (SCF) between the O_2Hb and the CO_2Hb concentration signals. Being x(t) the O_2Hb concentration signal and y(t) the CO_2Hb, the SCF between the two signals was defined as

$$SCF_{xy} = \frac{\left|D_{xy}(t,f)\right|^2}{D_{xx}(t,f)\cdot D_{yy}(t,f)} \quad (1)$$

Where,

$D_{xy}(t,f)$ = cross time-frequency C-W representation of the O_2Hb and the CO_2Hb concentration signals

$D_{xx}(t,f)$ = time-frequency C-W representation of the O_2Hb signal

$D_{yy}(t,f)$ = time-frequency C-W representation of the CO_2Hb signal

All the auto and cross time-frequency distributions were computed on a suitable time window according to the length of event, with the event centered in the middle part of the window. This value of time window is chosen to keep the experimental protocol sufficiently short.

3. Results

From Figure 1 to Figure 3 respectively, shows the O_2Hb (left) and CO_2Hb (right) concentration changes of one MS subject during the three specific periods.

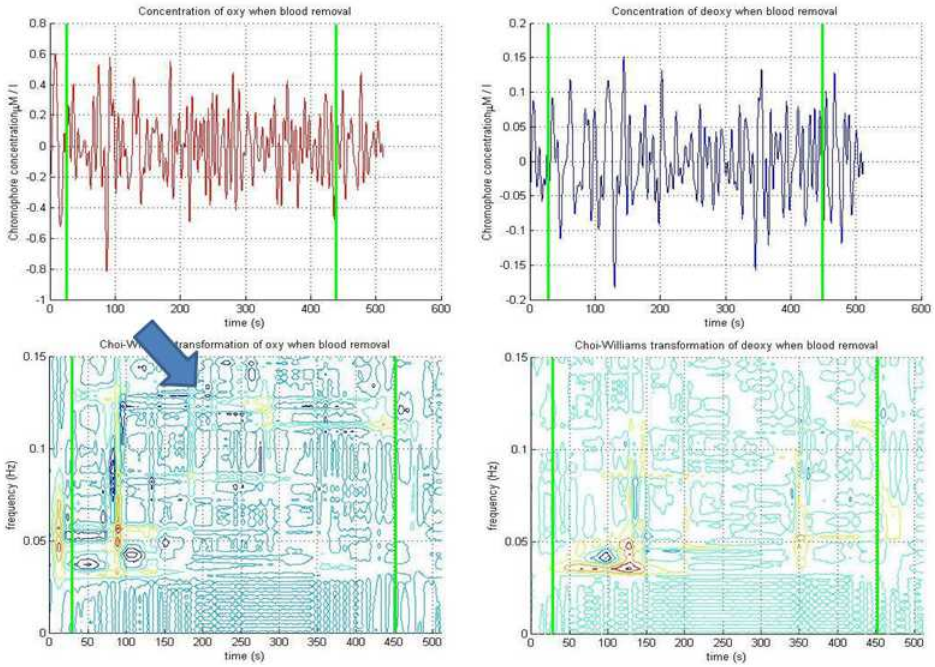

Figure 1. *C-W distribution of O_2Hb (left) and CO_2Hb (right) concentration signals during blood removal. The upper panel shows the time course of the O_2Hb and CO_2Hb concentration changes, the lower the C-W distribution (σ=0.5). The vertical green lines mark the onset and offset of blood removal.*

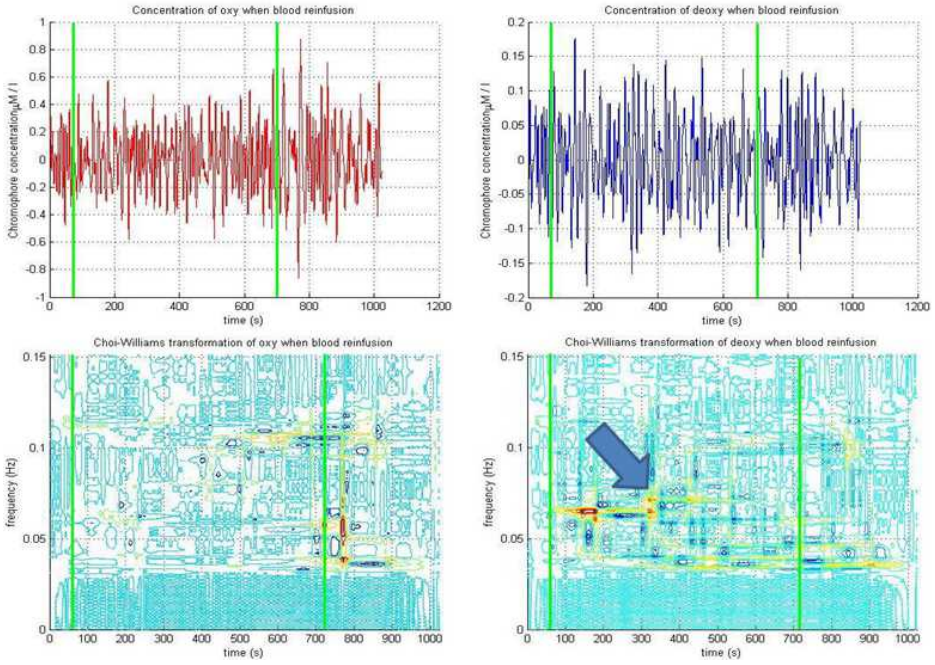

Figure 2. *C-W distribution of O_2Hb (left) and CO_2Hb (right) concentration signals during blood reinfusion. The upper panel shows the time course of the O_2Hb and CO_2Hb concentration changes, the lower the C-W distribution (σ=0.5). The vertical green lines mark the onset and offset of blood reinfusion.*

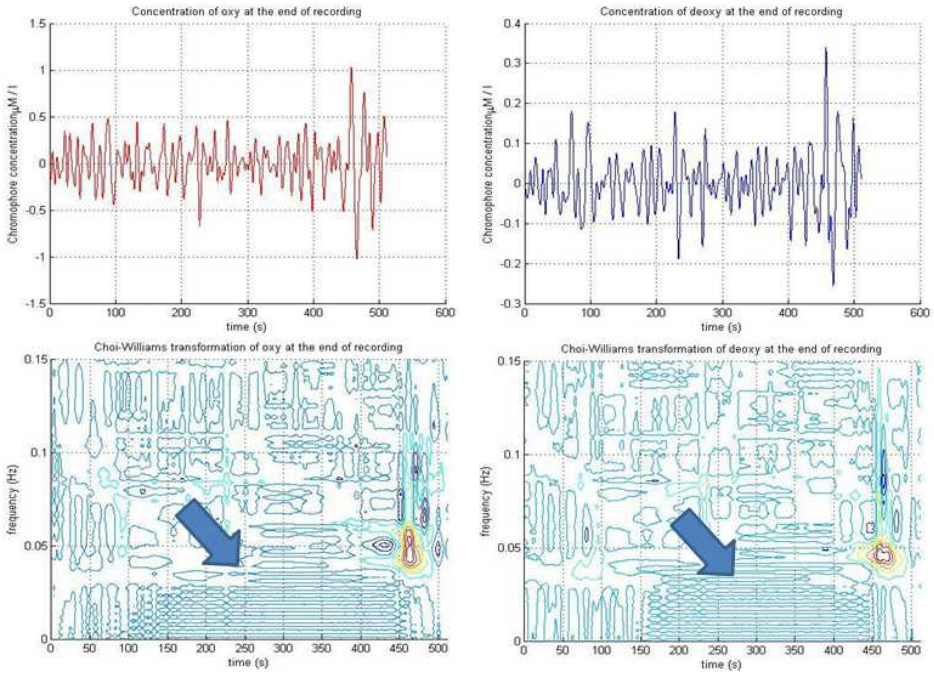

Figure 3. *C-W distribution of O_2Hb (left) and CO_2Hb (right) concentration signals during at the end of the monitoring. The upper panel shows the time course of the O_2Hb and CO_2Hb concentration changes, the lower the C-W distribution (σ=0.5).*

The results in Figure 1-3 were shown both in time domain (upper) and time-frequency domain(lower).The axes of the upper figures are: horizontal axis is time(s) and the vertical axis is chromphore concentrations (μmol/L). The axes of the lower figures are: horizontal axis is time(s) and the vertical axis is frequency (Hz).

For each subject, we computed the following variables derived from the Choi-Williams Distribution:
1) The O_2Hb and CO_2Hb power in the VLF and LF bands (P_{VLF} and P_{LF}), during blood removal, blood reinfusion and at the end of the monitoring, for a total of 12 variables.
2) The total power of O_2Hb and CO_2Hb (P_{TOT}), during the same three time intervals, for a total of 6 variables.
3) The O_2Hb and CO_2Hb SCF value in the two bands (SCF_{VLF} and SCF_{LF}) and total (SCF_{TOT}), during the same three time intervals, for a total of 9 variables.
Thus, we organized the data in a matrix containing the 6 subjects as row and 27 measured variables as columns, the total number of variables was 162.

The signal power in the VLF band, LF band and total frequency band were computed by integration of the corresponding time-frequency representation. Since there are three analytical stages, the VLF band and LF band power values were converted into percentage by comparing to the total signal power, see Table 1-3.

Table 1*. analysis results of power changes of O_2Hb and CO_2Hb during the period of blood removal*

Variables	***P_{VLF}_O_2Hb(%)***	***P_{LF}_O_2Hb(%)***	***P_{VLF}_CO_2Hb(%)***	***P_{LF}_CO_2Hb(%)***
Subject1	30.68867551	61.5442055	11.61998647	81.00513545
Subject2	16.64811763	71.52079894	17.84358881	75.33780148
Subject3	27.44047107	63.47086868	24.58152207	65.72830057
Subject4	16.87925651	73.92453294	13.47963792	83.0026557
Subject5	11.73696386	80.61834618	15.84533642	78.30320422
Subject6	9.260498848	81.82446692	22.74394493	70.90363871
Mean	**18.776**	**72.151**	**17.686**	**75.713**
SD	**8.546**	**8.446**	**5.119**	**6.493**

Table 2. *analysis results of power changes of O_2Hb and CO_2Hb during the period of blood reinfusion.*

Variables	P_{VLF}_O_2Hb(%)	P_{LF}_O_2Hb(%)	P_{VLF}_CO_2Hb(%)	P_{LF}_CO_2Hb(%)
Subject1	25.29527784	69.09768801	11.5624517	82.28847226
Subject2	18.82648998	70.45358962	28.20568403	67.01128425
Subject3	18.03080133	68.17864408	17.59350509	73.14711004
Subject4	18.71469784	76.28820607	17.91248429	77.81918129
Subject5	0.607001994	65.68945505	0.916270821	73.64317435
Subject6	12.86079412	75.04595931	14.84359756	77.66561136
Mean	**15.723**	**70.792**	**15.172**	**75.262**
SD	**8.393**	**4.103**	**8.940**	**5.232**

Table 3. *analysis results of power changes of O_2Hb and CO_2Hb at the end of the recording*

Variables	P_{VLF}_O_2Hb(%)	P_{LF}_O_2Hb(%)	P_{VLF}_CO_2Hb(%)	P_{LF}_CO_2Hb(%)
Subject1	7.355220366	85.45522974	11.02800894	84.10531039
Subject2	15.10873463	77.38068006	13.69045667	78.94990268
Subject3	10.6233097	70.80230521	15.30362044	78.93009618
Subject4	22.58885968	70.63325517	26.86932401	65.87562301
Subject5	0.220733154	76.01119815	0.223373446	79.46801936
Subject6	10.08532773	79.88689619	16.44253697	77.04052917
Mean	**10.997**	**76.695**	**13.926**	**77.395**
SD	**7.505**	**5.645**	**8.625**	**6.114**

4. Discussion and Conclusion

Figure 1 showed that power in the LF band, denoting a vagal response due to blood flow perturbation, which is more evident on O_2Hb than on CO_2Hb. Figure 2 showed that there was an increased LF power of CO_2Hb due to cerebral autoregulation because the increased oxygen triggered vasoconstriction. Figure 3 showed that there is no power in the VLF band. Oscillations are all in the LF band because it is a long period vascular response.

According to Table 1-3, it can be observed that the power of O_2Hb and CO_2Hb in the VLF band were progressively reduced during the monitoring. Conversely, the LF power neatly increased for O_2Hb (from 72.151% to 76.695%) and weakly for CO_2Hb (from 75.713% to 77.395%).

The combination of VLF power decrease and LF power increase indicated a clear vascular effect of ozone. In fact, The LF component originates from fluctuations in sympathetic vasomotor control by the central nervous system [12]. It means that we observed a vasomotor activity following OA that lasted about 150 minutes. This numerical result is in accordance to the patients' judgment. Many patients reported that the subjective sensation of overall physical improvement and wellness given by ozone lasted for days after treatment.

In conclusion, we applied the Choi-Williams distribution to the NIRS signals to assess the cerebral oxygenation of patients with multiple sclerosis under the ozone autohemotherapy. By computing and comparing the relative power of O_2Hb and CO_2Hb signals in the VLF and LF bands during three different recording periods, it is obvious that the VLF power decreases and the LF power increases. This indicated the endothelial reactivity and showed to be a good result. The time-frequency analysis is an useful tool for characteristics analysis of ozone signals.

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