PRELIMINARY REPORT



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Opto-magnetic imaging spectroscopy in characterization of the tissues during hyperbaric oxygen therapy

Karakterizacija tkiva tokom hiperbarične oksigenacije primenom optomagnetne imidžing spektroskopije

Mariana Sedlar*, Gorana V. Nikolić[†], Aleksandra Dragičević[†], Djuro Koruga[†]

*Centre for Hyperbaric Medicine, Belgrade, Serbia; [†]Department of Biomedical Engineering, Faculty of Mechanical Engineering, University of Belgrade, Belgrade, Serbia

Abstract

Background/Aim. Opto-magnetic imaging spectroscopy (OMIS) was used as a novel method to determine tissue molecular conformation changes during hyperbaric oxygen (HBO) therapy. The aim of this study was to examine the usefulness of OMIS for the assessment of HBO therapy effectiveness on the diseased tissue. Methods. OMIS is concerned with obtaining paramagnetic/diamagnetic properties of materials, related to the presence of unpaired/paired electrons based on their interaction with visible light. The basic tool is light of wavelength in the range between 400 nm and 700 nm and its interaction with tissue. The study included 22 subjects: 16 angiopathy patients and 6 healthy subjects as the control group. OMIS was used with patients on the 1st, 10th and 20th session and with the control group on the 1st, 10th and 20th day without HBO therapy in between. Results. The obtained results showed that healthy skin of all the control group subjects had the same shape curve. In the angiopathy patient group, before the first session OMIS showed tissue disorder and after the last session results resembled more closely the results in healthy tissue. The differences in the tissue state in the angiopathy group before each session were noticeable, showing normalized tissue under the influence of HBO. Conclusion. The results show that OMIS could be used as a diagnostic tool for detection of the tissue state before and after the HBO therapy.

Key words:

hyperbaric oxygenation; tissues; spectrum analysis: biomedical engineering; methods; optical imaging.

Apstrakt

Uvod/Cilj. Optomagnetna imidžing spektroskopija (OMIS) je nova metoda primenjena za određivanje molekularnih promena u tkivu tokom hiperbarične oksigenoterapije (HBO). Cilj rada bio je da se ispita efikasnost OMIS u proceni dejstva HBO na obolela tkiva. Metode. OMIS se bavi određivanjem paramagnetnih/dijamagnetnih karakteristika materijala, koje su posledica prisustva sparenih i nesparenih elektrona usled interakcije materijala sa vidljivom svetlošću. Osnovni alat je bela svetlost talasne dužine 400-700 nm i njena interakcija sa tkivom. Studija je obuhvatila 22 ispitanika: 16 bolesnika sa angiopatijama različitog porekla i 6 zdravih osoba kao kontrolnu grupu. OMIS je primenjena kod bolesnika tokom prvog, desetog i dvadesetog tretmana, a kod kontrolne grupe prvog, desetog i dvadesetog dana bez HBO terapije, u međuvremenu. Rezultati. Dobijeni rezultati pokazuju da je rezultantna kriva kod svih članova kontrolne grupe bila istog oblika. Kod bolesnika sa angiopatijom krive dobijene merenjem pre prvog tretmana prikazivale su neuređenost tkiva, dok je nakon poslednjeg tretmana kriva bila istog oblika kao kod zdravog tkiva. Postojala je uočljiva razlika u stanju tkiva kod bolesnika pre i nakon HBO tretmana. Zaključak. Dobijeni rezultati pokazuju da bi OMIS mogla da se primenjuje kao dijagnostičko sredstvo za utvrđivanje stanja tkiva pre i posle HBO terapije.

Ključne reči:

hiperbarička oksigenacija; tkiva; spektar, analize; biomedicinski inženjering; metodi; optičko snimanje.

Introduction

Hyperbaric oxygen (HBO) therapy is breathing 100% oxygen at a pressure higher than atmospheric pressure while in the hyperbaric chamber. It is used to treat patients with different

vascular problems, microangiopathy, diabetes mellitus complications, embolism, gangrene, burns, etc^{1,2}.

Various optical methods, such as pulse oximetry, near infrared spectroscopy, and fluorescence spectroscopy have been used for measuring hemoglobin oxygen saturation, tissue

Correspondence to: Mariana Sedlar, Department of Biomedical Engineering, Faculty of Mechanical Engineering, University of Belgrade, Belgrade, Serbia. E-mail: <u>mariana.sedlar@gmail.com</u>

oxygenation as well as the glucose level in blood in some diseases $^{3-5}$, but due to different limitations in methods and specificities of HBO therapy, their evaluation value is limited 6,7 .

In this paper, opto-magnetic imaging spectroscopy (OMIS) is presented as a method used for skin characterization during hyperbaric oxygen therapy. Until now OMIS was used on different types of matter, starting with non-organic compounds such as water⁸, biological tissue such as healthy skin⁹, contact lenses¹⁰ and live microorganisms such as viruses¹¹.

The aim of this study was to identify the expected positive effects of HBO therapy on the diseased tissue and to characterize skin properties during the therapy. The results of applied OMIS in the control group are also reported.

Methods

The OMIS method is concerned with obtaining paramagnetic/diamagnetic properties of materials (unpaired/paired electrons) based on their interaction with visible light. The basic tool is the light of wavelength in the range between 400 nm and 700 zed by the sample itself (Figure 1)¹². The longitudinal wave of the reflected light will dominantly have an electrical component whose properties will depend on the electrical state of the surface. However, the magnetic component of the reflected light will be perpendicular to the electrical one, but as a transverse wave. Since the transverse wave has a small intensity in the longitudinal direction its influence on the sensor will be negligible. Therefore, the electrical properties of a material can be obtained by measuring the reflected light. The difference between reflected non-polarized and polarized lights will give us magnetic properties of the sample.

The digital image is recorded with a standard digital camera, which uses the specially self-constructed extra component placed in front of the objective (Figure 2) ¹². This extra component contains the set of the diodes for illuminating the sample. The NL-B53 device with the Canon digital camera, model IXUS 105, 12.1 Mpix was used. The illumination of the sample was achieved by using white diffused light from the diodes (three light diodes aligned under the angle of 53° to vertical axis, and with the mutual angle distance of 120° in the hori-



Fig. 1 – The experimental arrangement sketch showing relative positions of light sources for white (a) and reflected polarized light (b). The degree of light polarization is 95.4%, while angular diffusion of the light source (six white lightemittind diodes arranged in a circle) is $\pm 1:6^{\circ}$ (difference between angles θ and θ_{1})¹².



Fig. 2 – Basic operational setup (prototype) for opto-magnetic imaging spectroscopy (NanoLab, Faculty of Mechanical Engineering, University of Belgrade)¹².

nm. Since light is composed of coupled electrical and magnetic field perpendicular to each other in propagation wave, if sample is exposed to the white light under the angle of 90°, reflected unpolarized light will have information about electromagnetic properties of the sample. On the other hand, if the light beam is incident under a particular angle (the Brewster angle) to a sample of the material of interest, the reflected light will be polari-

zontal plane). The recording can be conducted over the circle like shaped area with 25 mm in diameter.

This method uses the color system analogous to human eye perception. It consists of red, green, and blue colors (RGB). The algorithm for image processing and chromatic information determination is based on a chromatic diagram called the Maxwell triangle and on an operation of the spectral convolution referring to the blue (B) and the red (R) channel. It is marked as (R-B)&(W-P) which indicates the convolution operation of the blue and the red channels (R-B) of "diffusive" (white light-W) and "polarization" (polarized light-P) response (W-P). The result of this operation is the convolution spectrum. The difference of the convolution spectra of the reflected white light and the polarized light of blue and red channels yields the opto-magnetic convolution spectra ¹².

Skin represents the largest organ of the human body. It consists of three main parts: epidermis, dermis and hypodermis. The boundary between epidermis and dermis is the basement membrane which presents a barrier for adverse influences from the external environment.

Patients with compromised circulation are candidates for HBO therapy because it is well known that, eventually, angiopathy disarranges the structure and function of the skin with certain consequences^{1, 2, 13}. HBO therapy improves regeneration of wounded tissue and promotes healing.

This investigation was performed at the Centre for Hyperbaric Medicine, Belgrade, Serbia in the period from February to March 2012 in a multiplace hyperbaric chamber and included 16 patients with angiopathy of diverse origin and 6 healthy subjects as the control group. All the subjects involved gave written consent to participate in this research. While sitting in the chamber before the regular HBO therapy, OMIS was used on the skin of the leg or arm, depending on the patient diagnosis and on the skin of the arm of the control group subjects. Pictures were taken both with white and polarized light twice each. The same procedure was repeated after 30 min on 222.915 kPa [2.2 absolute atmosphere (ATA)], and the third time immediately at the end of the therapy on 101.325 kPa (1 ATA). Measurements were repeated in the same way on the 10th and 20th session. OMIS was applied with the control group in the same way as angiopathy group but without HBO therapy in between.

Results

The results obtained using the OMIS method of a few randomly chosen subjects from both groups (control and angiopathy) were presented. The differences between these groups were presented as the characteristics of peaks, wavelength differences and intensity given in the tables below each figure.

The diagrams in the Figure 3 show the results in the control group, i.e. healthy people. By taking the pictures with OMIS and processing them ¹², the results obtained show that healthy skin of all the control group subjects had the same curve shape.

These values represent dynamic state of healthy tissue. Small variations are expected because each subject is unique (Figure 3). It has to be stressed that curves of all the subjects in the control group had the same shape on the first, 10th and 20th day. Two examples of healthy subjects of the control group are shown in Figure 4.







Fig. 4 – Healthy skin opto-magnetic imaging spectrometry spectra comparison on the first, 10th and 20th day: a) of the control group subjects JL (female) and b) DR (male). The same persistency in the curve shape is present in all the control group subjects.

(for abbreviations see under Figure 3).

The values of characteristic peaks are given in Table 1. The negative values represent diamagnetic and positive paramagnetic characteristics. It has to be stressed that diamagnetism indicates stable state of the tissue.

The diagrams in Figure 5 represent the patients with angiopathy. The results in the Figure 5 a (the left figure) show the state of the patients' skin before the first and Figure 5 b (the figure right) after 20th session. After the last session the results obtained show similarity with the control group subjects.

All curves in Figure 5a, (left) start as paramagnetic with one exception indicating the presence of unpaired electrons, which generate tissue disorder. After the last HBO session (Figure 5b, right), the curves within some of the patients again starts as paramagnetic, but all the curves are of narrower shape and higher amplitude.

Wavelength differences and the intensity of the characteristic peaks before the first and after the last therapy are shown in Tables 2 and 3, respectively.

Table 1

Intensities and wavelengths corresponding to characteristic peaks for all the control group subjects

Peaks -	DR		JT		AL		JL		MN		JP	
	w [nm]	I [a.u.]*	w [nm]	I [a.u.]								
Peak 1	114.76	-27.169	117.993	30.131	115.341	-26.576	115.341	-38.47	114.76	-14.283	115.142	-37.246
Peak 2	116.637	21.32	121.224	-90.057	119.22	40.768	117.079	28.63	116.87	23.351	117.079	26.493
Peak 3	120.968	-59.897	123.832	26.49	122.505	-11.402	119.48	-17.528	120.708	-34.856	119.991	-39.842
Peak 4	124.161	51.257	/	/	125.497	0.604	122.249	-11.005	123.343	31.906	122.973	36.665
Peak 5	/	/	/	/	/	/	126.558	29.176	/	/	/	/

*a.u.-arbitrary units ¹²; i – intensity; w – wavelength.



Fig. 5 – Comparison of the opto-magnetic imaging spectrometry spectra of the angiopathy patients: a) before the first, and b) after the 20th therapy. (for abbreviations see under Figure 3)

Table 2

Intensities and wavelengths corresponding to characteristic peaks for randomly selected patients with angiopathy before the first therapy

	~		~		17					
Peaks	MS		BS		DD		BP		DS	
	w [nm]	I [a.u.]								
Peak 1	117.33	7.865	116.87	21.999	113.421	21.738	112.461	-8.725	117.737	18.524
Peak 2	119.991	-51.528	119.22	-54.887	117.33	-36.931	116.423	29.326	120.708	-39.044
Peak 3	121.992	45.67	121.224	11.213	120.968	10.09	119.22	-31.797	/	/
Peak 4	125.26	-13.746	/	/	/	/	/	/	/	/

For abbreviations see under Table 1.

Table 3

Intensities and wavelengths corresponding to characteristic peaks for randomly selected patients with angionathy after the last therapy

angiopatity after the last therapy										
Peaks	MŠ		ВŠ		DD		В	Р	DS	
	w [nm]	I [a.u.]								
Peak 1	115.725	29.000	115.725	35.433	114.189	-9.923	114.571	-13.49	114.571	-1.181
Peak 2	119.48	-73.687	118.967	-54.288	116.423	34.668	116.241	16.241	117.079	26.005
Peak 3	122.973	37.139	122.764	8.257	119.48	-22.107	120.498	-47.428	120.245	-46.19
Peak 4	/	/	128.373	4.086	/	/	123.343	36.673	112.764	4.114

For abbreviations see under Table 1.

After the last therapy, the state of the patients showed an improvement because the differences compared to the control group were diminished.

The diagrams in Figure 6 indicate that OMIS spectra in the angiopathy patients significantly changes during the period of HBO therapy.

the same magnetic state as before the first therapy and in some of them it was changed (Table 3) that needs our further investigation. The result was that all the subjects with the first peak in diamagnetic field belonged to the peripheral neuroangiopathy patients.

Opposite to the results showed in Figure 4, in the



Fig. 6 – Comparison of the opto-magnetic imaging spectrometry spectra of the diseased tissue of two patients with angiopathy on the first, 10th and 20th session. (for abbreviations see under Figure 3).

Discussion

Small variations of the results of the control group are present which was expected as the skin of the female and male genders differ ^{9, 14}. The OMIS spectra of healthy skin start as diamagnetic which indicates biophysical stable state of the tissue. After the repeated measurements the shape of the curves for all the control group subjects was not significantly changed (Figure 4) which confirms the stability of healthy tissue. Irregularity in one healthy subject (JT) was noticed as the curve in each measurement started as paramagnetic without proper biophysical explanation. However, the shape of the curve, in general, matched the rest of the curves of other healthy subjects. Further investigation is needed.

The results in Figure 5 within the angiopathy group show shape disorder before the first therapy and after the last therapy. The shape of the curves became more orderly with significantly more resemblance to the healthy tissue results.

The values given in Table 2 show that the first peak of the curves was paramagnetic with one exception (BP) without biophysical explanation according to our knowledge. After the last therapy, some of the subjects had first peaks in

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angiopathy patients the curve shape before the first, 10th and 20th session was significantly different showing effects of HBO therapy on the tissue normalization (Figure 6).

Conclusion

The obtained results show that opto-magnetic imaging spectrometry can be used as diagnostic tool for detection of the skin state before and after the hyperbaric oxygen therapy. There are the differences between the diagrams and additional biophysical explanation is needed. The study needs to be continued with a larger number of subjects for better understanding of the correlation between opto-magnetic imaging spectrometry, increased level of oxygen and tissue metabolism.

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