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Original Work

High-density lipoprotein functionality in patients with hyperbaric oxygen therapy

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ABSTRACT: High-density lipoprotein (HDL) cholesterol levels are associated with decreased risk of atherosclerotic disease, but also not all HDL are functionally equivalent. The functional status of HDL is closely linked to its primary protein component, apolipoprotein A-1 (ApoA-I) levels and paraoxonase 1 (PON1) enzyme. Functional changes of HDL may arise from hyperbaric oxygen therapy (HBO) induced posttranslational modification of ApoA-1 and PON1 levels. A total of 41 patients who met the research criteria were included in the study. On average, 30 sessions of HBO therapy were performed (range: 20-39). Laboratory measurements were performed at the beginning and at the end of HBO treatment in two groups of the same patients. We measured serum levels of Apo A-1, PON1, oxidized LDL (OxLDL) and routine lipid laboratory parameters to determine possible changes in HDL function with HBO therapy. As unexpected, long term HBO treatment have no effect on OxLDL and also on PON1 enzyme. However, the mean ApoA-1 values in the second group were statistically significantly increased than their pre-treatment values ($P < 0.003$). This preliminary study showed that HBO therapy increased the amount of serum ApoA-1. Actually, it can be assumed that the treatment of HBO does not have a negative effect on HDL functionality. The increase in ApoA-1 with HBO therapy is probably aimed at protecting against oxidative stress in patients. As a result, there is a need for larger clinical trials to determine the possible effects of HBO therapy on HDL functionality.

KEY WORDS: ***ApoA-1; Paraoxonase; Hyperbaric oxygen therapy; OxLDL; Inflammation; Oxidative stress; Reactive oxygen species***

INTRODUCTION

Hyperbaric oxygen (HBO) therapy serves as the primary or secondary treatment for various medical conditions. The use of HBO therapy has increased significantly over the past decade in various diseases[1]. So how does HBO therapy have a common effect in these very different diseases?

It has been understood that the combined effect of hyperoxia and hyperbaric pressure results in improved mitochondrial metabolism, which targets both oxygen and anti-oxidative stress genes, leads to a significant improvement in tissue oxygenation and has anti-apoptotic and anti-atherosclerotic effects[2]. In fact, the use of O_2 in basic supra-physiological pressures has the potential to support systemic overproduction of O_2 radicals[2]. HBO therapy is also associated with changes in lipid metabolism. It is known that the imbalance of free radical formation or intensive consumption of antioxidant molecules leads to deleterious

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modification of biological macromolecules such as lipids, lipoproteins and enzymes[3].

The effect of HBO therapy on HDL functionality is unknown. HDL functionality hypothesis suggests that measurement of serum HDL levels has no major relationship with how the HDL concentrations are being dynamically remodeled or the state of HDL measurement[4]. The tremendous functional heterogeneity inherent to HDL is driven mostly by its compositional heterogeneity. However, the majority of HBO patients may have HDL dysfunction because diabetes and the metabolic syndrome are common in these patients. HDL is known to undergo dramatic modification in structure and composition as a result of the concerted actions of the acute-phase response and inflammation. This 'dysfunctional' HDL is characterized by decreased levels and activities of anti-inflammatory and anti-oxidant factors, such as ApoA-I and PON1[5].

Furthermore, transgenic over-expression of ApoA-1 gene or direct infusion of plasma derived or recombinant wild type or mutant ApoA-1 and gene transfer of other HDL associated proteins have been shown to be athero-protective in limited clinical settings, thereby justifying current focus on HDL based therapies[6]. So, HDL inhibits LDL oxidation, scavenges toxic phospholipids from OxLDL and protects the vascular wall from the damaging effects of OxLDL[5].

PON1 is a HDL-associated enzyme with potent anti-oxidative and atheroprotective effects, having the potential to hydrolyze OxLDL-cholesterol. One of the anti-oxidative components of HDL is PON1, which is synthesized in the liver. The various functions of PON1 are: Inhibition of LDL oxidation, inhibition of HDL peroxidation, and detoxification of homocysteine thiolactone, a pro-atherogenic compound. Also, serum PON1 activity directly correlates with the amount of stearic acid and dihomo-gamma- linolenic acid present in Apoprotein A-1 and phospholipids in HDL[4,7].

Lipoproteins and their alterations are wholly linked with increased risk of developing an atherosclerotic disease. Loss of ApoA-1 content of HDL is the mainstay in the development of atherosclerosis with reference to HDL. Hence our attention has to shift towards determining the qualitative aspects of HDL in order to reduce the risk of atherosclerosis[8]. Furthermore, as far as we know, there have been no previous studies showing the functionality of HDL in patients receiving HBO therapy. However, a number of lipid metabolism changes have been observed in many in vitro and animal studies after the administration of HBO therapy.

Lipid changes associated with HBO therapy, especially focusing on HDL functionality, have not yet been identified in humans. In the case of HBO therapy, levels of ApoA-1 and PON1 enzyme linked to HDL have not been measured yet. The measurement of ApoA-1 and PON1 enzyme activity along with the routine lipid profile provides a simple way to determine the quality of the HDL particle. Our aim in this study is to assess the possible effect of HBO therapy on HDL associated PON1 and ApoA-1 levels in HBO patients. For this purpose, we measured serum levels of PON1, ApoA-1 and routine laboratory parameters of patients before and after HBO treatment.

METHODOLOGY

Study population and clinical examinations

In 2017, 41 patients (male, 66.3%) between 20-87 years of age (mean: 50.7 ± 13.2) who received HBO treatment were prospectively included in this study for a period of 6 months. All patients had a comprehensive physical examination, completed a generalized questionnaire and gave informed consent before being included in the study. In the questionnaire: age, socio-economic status, roots of ancestors, physical activity status, history of smoking and alcohol consumption and detailed medical history were recorded.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Hypertension was defined as systolic blood pressure of at least 140 mmHg, diastolic blood pressure of at least 90 mmHg according to the American Heart Association/American College of Cardiology Guidelines. Consequently, blood pressure was measured manually with a sphygmomanometer in all patients. Initially, the inclusion criteria consisted of patients older than 18 years who had no contraindication to HBO therapy. Exclusion criteria included smoking, alcohol consumption, current anti-lipid and antioxidant treatments. Clinical and laboratory data were recorded for all patients. The protocol was approved by the Institutional Review Board and performed in accordance with the Declaration of Helsinki as revised in 2001. Informed consent was obtained from all participants.

HBO treatment

Every sequential session was conducted five days a week with 120 minutes each session. Each participant breathed 100% oxygen at 2.5 atmospheres absolute, with five-minute air break periods every 30 minutes, in a multi place hyperbaric chamber (Baroks MUL 35®). Each HBO treatment session also included 15-minute

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compression and 10-minute decompression periods.

Blood sampling and laboratory measurements
Blood sample collection

Blood samples were taken from all patients before starting HBO therapy, after 12 hours fasting. Blood samples were drawn from the peripheral vein immediately before the first and after the 20th sessions, which represented the minimum duration of HBO treatment. Blood samples were obtained after an overnight fasting state in the morning. The samples were then centrifuged at 3,000 rpm for 15 minutes, and the serum was stored at -80°C for later analysis. Lipid parameters were measured immediately.

Routine Laboratory parameters

The levels of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and serum creatinine were determined by using commercially available assay kits (Abbott) with an auto analyzer (Architect® c16000, Abbott Diagnostics). LDL-cholesterol (LDL-C) levels were calculated from Friedewald formula: LDL-C = Total cholesterol – HDL – TG/2.2.

Measurement of PON1 and ApoA-1 levels in serum

The samples were tested for Human ApoA-1 (Biont®, Catalog no: YLC0115HU), Human PON1 (Biont®, Catalog no: YLA0984HU) and Human OxLDL (Biont®, Catalog no: YLA0257HU) using a sandwich enzyme linked immunosorbent assay, according to the manufacturer's instructions. The results were expressed as ng/ml, ng/ml and ng/L, respectively.

Statistical analysis of data

Continuous variables were expressed as mean ± SD, and categorical variables were presented as numbers and percentages. The statistical analysis was performed using MedCalc© Statistical Software version 15.8 (MedCalc Software® bvba, Ostend, Belgium; https://www.medcalc.org; 2018). The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables, and paired samples t-test, chi-squared test, and Wilcoxon signed ranks test were used to compare measurements. A P-value of <0.05 was considered statistically significant.

RESULT

The demographic and clinical characteristics of the subjects are shown in **Table 1**. In addition, 29% of the patients included in the study were using different antihypertensive drugs. The mean BMI was 25.3 ± 3.6 kg/m^2, and number of sessions received ranged from 20-40.

Table 1. Baseline characteristics of patients receiving hyperbaric oxygen treatment

Parameter (n=41)	Mean ± SD/ Median	Range/ IQR
Age	50.7 ± 13.2	20-87
Male gender, n (%)	27 (66)	p*=0.01
Weight (kg)	76.7 ± 14.0	50-102
BMI (kg/m^2)	25.3 ± 3.6 kg/m^2	20-37
Number of sessions received	30	20-40
Antihypertensive	29%	

**Chi-squared test*

Percentage distribution of patients entering HBO treatment according to diseases is presented in **Table 2** and **Figure 1**. Frequency of distribution of the disease; Idiopathic sudden sensorineural hearing loss 32%, diabetic foot 25%, avascular necrosis 12%, chronic osteomyelitis 11%, peripheral vascular disease 9%, Buerger disease 5% and others 6%.

Table 2. Percent distribution of patients with hyperbaric oxygen treatment according to diseases

Disease	(n=41)
Idiopathic sudden sensorineural hearing loss	13
Diabetic foot	10
Avascular necrosis	5
Chronic osteomyelitis	4
Peripheral vascular disease	3
Buerger's disease	3
Others (venous ulcer, graft-related complication, prosthetic joint infection)	3

Post-treatment status of HBO therapy patients is given in **Table 3**. There were no adverse signs observed in HBO-treated patients. At baseline prior to therapy, serum ApoA-1 levels was mean ± SD 1.684 (range; 0.791-2.156). Interestingly, there was a statistically significant rise in ApoA-1 levels during HBO treatment (**Table 4**). Indeed, second group HBO patients serum ApoA-1 values were statistically significantly higher than pre-treatment ApoA-1 values; 1.913 µg/ml mean ± SD (range; 1.717-2.109), P<0.038, **Figure 1**.

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Table 3. Post-treatment status of hyperbaric oxygen treatment patients

Status	(n=41)
Cured	9
Improved	28
No Change	4

Table 4: Comparison of the biomarkers of patients receiving hyperbaric oxygen therapy at baseline and at the end of Session 20

Parameter (n=45)	Baseline	Session 20	p value
ApoA-1* (µg/ml)	1.684 (0.791-2.156)	1.913 (1.717-2.109)	0.038
PON1** (ng/ml)	17.2 ± 5.3	15.9 ± 4.6	0.17
OxLDL** (ng/L)	545 ± 29	549 ± 33	0.45
LDL cholesterol (mg/dL)	137 ± 28	140 ± 25	0.37
HDL cholesterol (mg/dL)	39 ± 13	41 ± 12	0.52
Triglyceride (mg/dL)	213 ± 28	197 ± 15	0.12
ApoA-1/ HDL***	0.0431	0.0466	0.05

**Median (IQR) / Wilcoxon signed ranks test*
***Mean ± SD / Paired samples t-test*
**** Paired samples t-test*

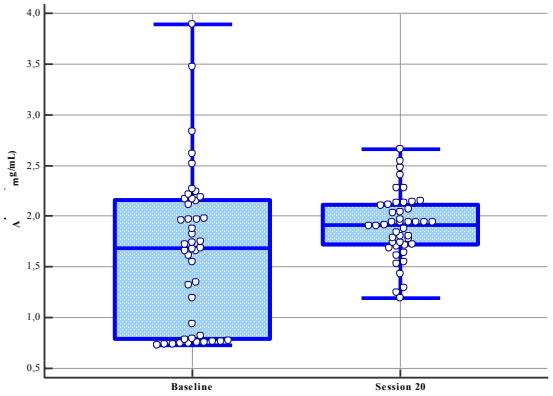

Figure 1: Serum Apolipoprotein A-1 concentration distributions of the patients at baseline and at the end of Session 20

Whereas, compared to pretreatment values (mean ± SD; ng/L), PON1 levels did not change significantly after the HBO therapy, P=0.17 **Figure 2**. Also, compared to pretreatment values (mean ±S D; 545 ± 29 ng/L), OxLDL levels did not change significantly after the 20th session (mean ± SD; 549 ± 33 ng/L), P=0.45, **Table 4** and **Figure 3**. In addition, the mean serum lipid levels for both pre- and post-HBO treatment are provided in **Table 4**. There were no statistically significant differences in all routine lipid parameters after the HBO treatment

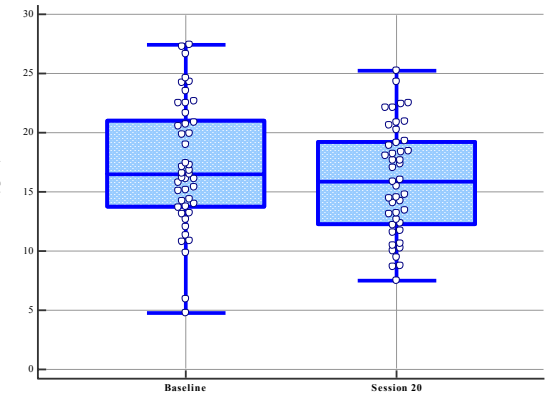

Figure 2: Serum Paraoxonase-1 concentration distributions of the patients at baseline and at the end of Session 20

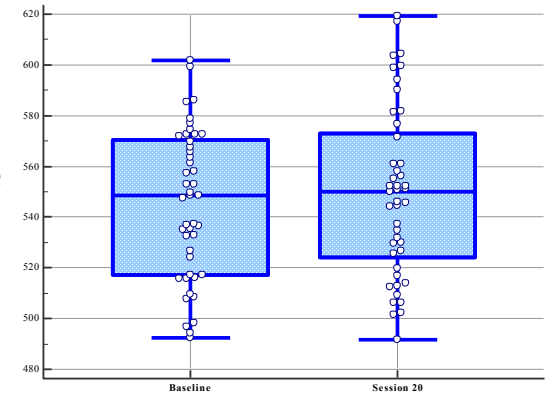

Figure 3: Serum Oxidized LDL concentration distributions of the patients at baseline and at the end of Session 20

DISCUSSION

The first result we obtained is that long-term HBO therapy may have a positive effect on patients' HDL functionality. After HBO therapy, the level of ApoA-1 required for normal functionality of HDL was significantly increased in all patients. The most important finding of this study is, of course, the increase in the ApoA-1 level in the serum of patients receiving HBO therapy. It should be kept in mind that HDL is made up of many proteins, lipids and ApoA-1 (for over 70% of the total protein mass). So, HDL serves as a prominent anti-atherogenic function by mediating reverse cholesterol transport[5,9]. It is the first clinical study to show this relationship between HBO therapy and ApoA-1.

Pharmacological treatment of dyslipidemia and therapeutic lifestyle changes has been shown to

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prolong the survival of the patient and reduce the incidence of cardiovascular events. However, some non-pharmacological cholesterol lowering interventions such as HBO therapy has not been well studied. However, this study shows that HBO treatment carries the potential to cause lipid metabolic changes in a variety of patients, which may reduce atherosclerotic diseases.

In the literature, there is very little research showing the effect of HBO therapy on lipoproteins. For example, in one study, changed lipoprotein subfractionation was demonstrated in diabetic patients with HBO therapy. As a result, it has been reported that the VLDL and IDL subfractions decrease after HBO therapy[10].

In addition, several other studies have shown that HBO therapy does not cause an increase in OxLDL levels[11]. Similar to these studies, the HBO therapy did not make any statistically significant change at the level of OxLDL in our study. In the past decades, OxLDL has attracted attention as a blood marker that is associated with atherosclerosis. The oxidative modification of LDL in the artery wall has been implicated as one of the major physiologically relevant mechanisms for the pathogenesis of atherosclerosis[11].

Unfortunately, we have not found any studies in the literature to evaluate serum ApoA-1 levels and PON1 activity with HBO therapy in humans but an increase in PON1 level was demonstrated in preclinical animal experiments. Evidently, the HDL-associated serum PON1 enzyme activity did not show an analytical change in our patients as a result of HBO therapy. As a result, this study suggests that long-term high-pressure oxygen therapy may not affect serum PON1 activity.

Although the release of reactive oxygen species was recognized as a critical step in combating invaders, excessive production of reactive oxygen species can trigger oxidative stress that causes significant harm to cells. It is well known that oxidative stress can lower PON1 activity and increase the OxLDL[12]. At least in our patients the oxidative stress on PON1 and OxLDL during HBO treatment was not adversely affected. In addition, HBO therapy did not alter routine blood lipid parameters.

Many studies indicate that the HDL proteome can change in a variety of disease states and these changes are often related to in vitro measures of HDL function. Normal functional HDL has high levels of active antioxidant proteins, enzymes with high antioxidant potential and has anti-inflammatory activity. So, HDL can directly inhibit oxidation of LDL. PON1 may be interacting with ApoA-1 to inhibit LDL oxidation. Anti-atherogenic properties of HDL have been largely ascribed to ApoA-I, the major protein component of the lipoprotein particle. Apo-A1 also manifests anti-inflammatory and anti-thrombotic activities that contribute to HDL's key role in preventing atherosclerosis development [4,13].

Recent data show that ApoA-1 concentrations are superior to HDL in predicting cardiovascular risk, but this approach has not yet been incorporated into national guidelines.

Furthermore, novel therapeutic interventions that increase ApoA-1 concentrations may be superior to those that primarily increase HDL concentrations, but this superiority remains largely theoretical at present. These results suggest that ApoA-1 overexpression may reduce oxidative stress by decreasing ROS levels in patients[8,13].

ApoA-1 mimetics have been shown in animal models and humans to have therapeutic potential for reversing atherosclerosis. However, still nothing is known about the ApoA-1 gene expression under oxidative stress in commonly used treatments like HBO therapy. This is a critical step for the molecular basis of a number of therapeutic mechanisms. Because, Apo-A1-deficient mice exhibit significant progression of atherosclerosis, an approximately 5-fold increase compared to LDL receptor-deficient mice[6,14,15]. Substantially, HBO therapy though has many disadvantages, which require further investigation. First, HBO treatment requires special equipment, which limits its daily practice for atherosclerotic patients. Second, long-term effects of HBO treatment, for example harmful oxidative stress on lipid function, are still unclear and need further study.

CONCLUSION

In this study, a limited sample set was investigated and measurements were recorded over a relatively short-term period. Further investigation should be carried out with long-term prognostic follow-up of the patients. Therefore, the relationship between HDL function and HBO therapy should be demonstrated by larger clinical trials. This preliminary study of patients with HBO therapy gives promising results for HDL functionality. In the future there will be need for expanded clinical trials. In this study, a limited patient group was investigated and laboratory measurements were recorded over a relatively short-term period. Future investigation should be achieved with long-term prognostic follow-up of the patients.

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