ORIGINAL ARTICLE

Serum oxidized low-density lipoprotein level as a marker of oxidative stress in patients undergoing hyperbaric oxygen therapy

Hiperbarik oksijen tedavisi gören hastalarda oksidatif stresin bir göstergesi olarak serum okside düşük yoğunluklu lipoprotein seviyesi

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ABSTRACT

Objective: Oxidative stress (OS) is involved in the pathogenesis of atherosclerosis. Hyperbaric oxygen therapy (HBOT), in which 100% oxygen is inhaled under hyperbaric pressure, may create OS. Therefore, the aim of this research was to measure the serum oxidized low-density lipoprotein (oxLDL) level in patients undergoing HBOT.

Methods: Twenty-nine patients who underwent HBOT to treat various diseases were enrolled in this study. The serum oxLDL level was measured at the beginning of the first and after the 10th therapy session.

Results: There was no significant difference between the oxLDL level of patients before and after HBOT (4.96±0.1 *vs.* 4.94±0.1 U/mL; p=0.36).

Conclusion: HBOT seems to be safe in terms of oxLDL production up to 10 sessions. However, further large-scale studies investigating longer duration of HBOT treatment are required to understand the role of OS.

ÖZET

Amaç: Aterosklerozun patogenezinde oksidatif stres yer almaktadır. Hiperbarik oksijen tedavisinde yüksek basınç altında %100 oksijen verilmesinden dolayı oksidatif stres için bir yatkınlık oluşabilir. Bu nedenle çalışmamızda hiperbarik oksijen tedavisine giren hastalarda oksidatif stresin bir göstergesi olan okside düşük yoğunluklu lipoprotein (oxLDL) seviyelerini ölçtük.

Yöntemler: Çalışmaya, çeşitli hastalıklar sebebiyle hiperbarik oksijen tedavisine giren 29 hasta alındı. İlk seasın başlangıcında ve 10. seansın sonunda kan örnekleri alınarak oxLDL seviyeleri ölçüldü.

Bulgular: Hiperbarik oksijen tedavisi uygulanan hastalarda oxLDL seviylerinde anlamlı bir değişiklik gözlenmemiştir (4.96±0.1 ve 4.94±0.16 U/ml, p=0.36).

Sonuç: Onuncu seansa kadar olan hiperbarik oksijen tedavisi oxLDL oluşumu açısından güvenli gözükmektedir. Oksidatif stresin aydınlatılması için daha uzun süreli tedavilerin incelendiği büyük çaplı çalışmalara ihtiyaç vardır.

There is a significant amount of data showing that oxidative stress (OS) plays a crucial role in the pathogenesis of atherosclerosis.[1] The excessive reactive oxygen species (ROS) produced by OS interact with lipids, proteins, and DNA, causing structural changes, inflammation, and cellular damage.[2,3] Chemicals, ultraviolet and ionizing radiation, environmental toxins, and mutagens are well-recognized external sources of OS.[4] Free radicals, particularly superoxide, also interfere with nitric oxide, leading to the production of a number of molecules, such as peroxinitrate, all of which are collectively called reactive nitrogen species (RNS). RNS act with ROS to damage

Abbreviations:

ATA	*Atmospheres absolute*
HBOT	*Hyperbaric oxygen treatment*
LDL	*Low-density lipoprotein*
MDA	*Malondialdehyde*
OS	*Oxidative stress*
oxLDL	*Qxidized low-density lipoprotein*
RNS	*Reactive nitrogen species*
ROS	*Reactive oxygen species*

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cells, causing nitrosative stress. Qxidized low-density lipoprotein (oxLDL) is a reliable marker of oxidative status, and is one of the products of the exposure of low-density lipoprotein (LDL) cholesterol to ROS/RNS.[5] Since oxLDL promotes endothelial dysfunction, and contributes to atherosclerotic plaque formation and progression, it is believed to be more atherogenic than its native form.[6]

Hyperbaric oxygen treatment (HBOT) involves the intermittent inhalation of 100% oxygen under 2.5 atmospheres absolute (ATA), which is pressure greater than that of the ambient air (1 ATA), 1 standard atmosphere of pressure at sea level. It has been used successfully in the treatment of various clinical conditions, including decompression sickness, soft tissue infections, wound healing, and carbon monoxide intoxication.[7] Although there are some experimental studies speculating that HBOT increases antioxidant activity by providing more oxygen to the body, and thus decreases the production of ROS and lipid peroxidation, these results have not been validated.[8] Therefore, the goal of our study was to evaluate the oxidative status of patients by measuring their serum oxLDL level pre and post HBOT.

METHODS

Study population

From May 2015 to December 2015, a total of 29 patients (17 males; 58%) aged between 21and 85 years (mean: 55±17 years) who underwent HBOT were enrolled in this study prospectively. The inclusion criteria consisted of patients older than 18 years with no contraindications to HBOT. The exclusion criteria included smoking, chronic inflammatory disease, and current antioxidant therapy. The demographic, clinical, and historical data were recorded for all of the patients. The pathologies treated with HBOT were primarily vasculopathies (n=20; 69%). Each consecutive session was conducted 6 days a week, and consisted of 120 minutes of 100% oxygen inhalation at 2.5 ATA, with 5 minute intervals every 30 minutes, in a multiplace hyperbaric chamber. Each HBOT session also included 15-minute compression and decompression periods.

Blood sampling and laboratory assays

Blood samples were drawn from the peripheral vein immediately before the first and immediately after the 10th session, which represented the minimum duration of HBOT. The samples were then centrifuged at 3000 rpm for 15 minutes, and the serum was stored at -80°C for later biochemical analysis. The samples were tested for oxLDL using a sandwich enzyme-linked immunosorbent assay, according to the manufacturer's instructions (Catalog no: CSB-E07931h; Cusabio Biotech Co., Ltd, Wuhan, China). The results were expressed as U/mL. Our study plan was reviewed and approved by the institutional review committee (approval no: 782), and written, informed consent was obtained from all of the patients.

Statistical analysis

Continuous variables were expressed as mean±SD, and categorical variables were presented as numbers and percentages. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables, and a paired samples t-test was used to compare measurements. A p value of <0.05 was considered statistically significant.

RESULTS

This study included 29 patients requiring HBOT for various reasons (17 males; mean age: 55±17 years). The demographic characteristics and clinical and laboratory findings of the patients are presented in Table 1. The mean body mass index was 27±4.9 kg/m^2, and the estimated glomerular filtration rate was 76±25 mL/minute. The frequency of diabetes mellitus, hypertension, peripheral artery disease, and ischemic heart disease was 44% (n=13), 27% (n=8), 69% (n=20), and 10% (n=3), respectively. Ten patients were on antihypertensive therapy and 15 were on statins. The mean serum oxLDL level for both pre and post HBOT are provided in Table 2. Post-HBOT serum oxLDL values were slightly lower than pre-HBOT values (4.96±0.1 vs 4.94±0.1 U/mL, p=0.36). There was no statistically significant difference in lipid parameters after the treatment (Table 2). Statin treatment had no effect on serum oxLDL level (pre HBOT: 4.92±0.1 vs 4.98±0.1 U/mL; p=0.53 and post HBOT: 4.93±0.1 vs 4.97±0.1 U/mL; p=0.45, on statin treatment vs no statin treatment, respectively).

Of the 29 patients who underwent HBOT, 20 had peripheral artery disease. Three patients had sudden

Table 1. Baseline characteristics of the patients

	n	%	Mean±SD
Age (years)			55±17
Gender(male)	17	58	
Body mass index (kg/m^2)			27±4.9
eGFR (mL/min), (mL/dk)			76±25
Diabetes mellitus	13	44	
Hypertension	8	27	
Peripheral artery disease	20	69	
Ischemic heart disease	3	10	
Glucose, mean (mg/dL)			138±50
Total cholesterol, mean (mg/dL)			235±33
LDL cholesterol, mean (mg/dL)			147±28
HDL cholesterol (mg/dL)			42±12
Triglyceride (mg/dL)			200±20
Antihypertensive medication	10	34	
Statin	15	51	

SD: Standard deviation; EGFR: Estimated glomerular filtration rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Table 2. Mean oxidized low-density lipoprotein and other lipid levels pre and post hyperbaric oxygen treatment

	Pre HBO T	Post HBOT	*p*
	Mean±SD	Mean±SD	
Ox-LDL (U/mL)	4.96±0.1	4.94±0.1	0.36
Total cholesterol (mg/dL)	235±33	233±32	0.86
LDL cholesterol (mg/dL)	147±28	143±27	0.73
HDL cholesterol (mg/dL)	42±12	41±11	0.92
Triglyceride (mg/dL)	200±20	188±19	0.11

HBOT: Hyperbaric oxygen therapy; SD: Standard deviation; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; OxLDL: Qxidized low-density lipoprotein.

Table 3. Hyperbaric oxygen treatment indications

Indications	n	%
Sudden hearing loss	3	10
Sudden vision loss	3	10
Peripheral artery disease	20	69
Venous ulcers	3	10

hearing loss, sudden vision loss, or venous ulcers, respectively (Table 3). Figure 1 shows each patient's oxLDL level before and after HBOT. The serum oxLDL level of 12 patients increased, while the level decreased in 14 patients, and remained the same in 3 patients.

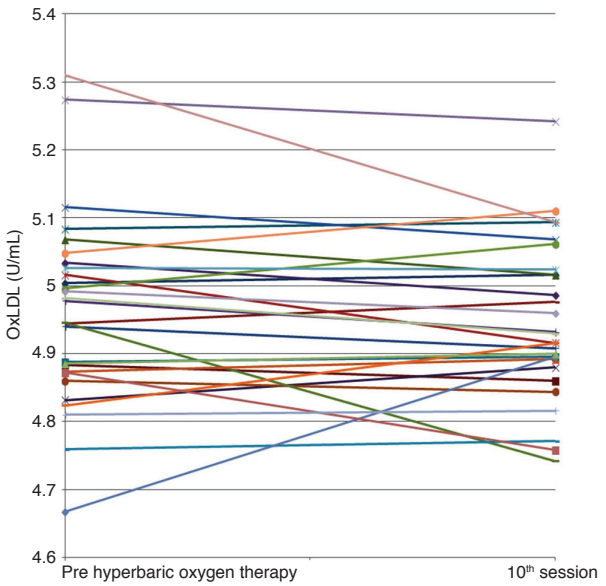

Figure 1. The change in the oxidized low-density lipoprotein level of each patient. OxLDL: Qxidized low-density lipoprotein.

DISCUSSION

In our study, there was no statistically significant difference in serum oxLDL level after HBOT. Furthermore, other lipid parameters did not seem to be affected. Although there are some studies reporting that statin treatment may decrease oxLDL level, in our study group, baseline level was similar and statin use had no major impact in terms of oxidative stress prevention.[9] In fact, to date, there is no evidence that statin administration might attenuate the OS created by HBOT.

In the search for the presence and quantification of OS that results during HBOT, several biomarkers have been studied, each of which showed several advantages and disadvantages. Lipoproteins are highly susceptible to oxidative damage due to their complex lipid-protein composition and large number of polyunsaturated fatty acid chains, which makes them attractive for OS investigation.[10] Although the exact mechanism for the modification of LDL has not yet been fully established, the extensive fragmentation of fatty acid chains along with the generation of hydroperoxides and aldehydes are believed to be the main factors.[11] There is much evidence showing that

oxLDL activates endothelial cells and induces endothelial dysfunction, smooth muscle cell proliferation, and apoptosis. Moreover, it is involved in the transformation of macrophages into foam cells, and the induction of platelet activation, all of which promote atherosclerotic plaque formation and destabilization.[12] Thus, oxLDL plays a pivotal role in the pathogenesis of atherosclerosis.[13] Its close association with atherosclerosis and the susceptibility of lipoproteins to oxidative damage makes oxLDL a good target to measure the possible deleterious effects of HBOT.

In the literature, few studies have looked for serum oxLDL level changes during HBOT. For example, Kudchodkar et al. speculated that chronic and intermittent treatment with HBOT might induce the components of the antioxidant defense response, and even lead to decreased oxLDL level, which may be atheroprotective.[14,15] It has also been proposed that depletion of the antioxidants at the target sites due to OS activates several pathways utilizing antioxidant mobilization from the body's antioxidant stores.[16] In a recent study, Matzi et al. measured oxLDL level, along with other OS parameters, and found no differences, although malondialdehyde (MDA) and glutathione peroxidase levels increased significantly.[17] Likewise, Eken et al. found no significant difference in the erythrocyte antioxidant capacity or lipid peroxidation, although they claimed that it could induce genotoxicity through different mechanisms.[18] One of the reasons these studies have conflicting results may be related to the duration of HBOT. In a study conducted by Ma et al., MDA level increased slightly at session 10, and significantly at session 20, together with increased expression of proteins and genes (superoxide dismutase-1 and catalase) after 14 days of treatment. Based on these findings, the authors suggested that the accumulation of ROS and OS was parallel to the number of HBOT sessions.[19] In a similar study performed by Dennog et al., antioxidant response to a single HBOT exposure (3 x 20-minute periods) did not reveal any significant difference in antioxidant levels and antioxidant enzymes in healthy subjects.[20] Similar findings have also been seen in animal studies. For instance, Simsek et al. measured rats' plasma levels of MDA, protein carbonyl content, and superoxide dismutase, which are all markers of OS. In their study, there was no change for the first 5 to 15 HBOT sessions; however, all of these levels were found to be significantly higher in sessions 20 to 40.[21] In accordance with the above-mentioned studies, we did not find statistically significant difference in the serum oxLDL level, and this may be related to the duration of the HBOT.

Another explanation for our results may be related to the marker we studied. The mechanism by which OS markers are affected is related to the complex interplay between ROS, various cellular structures, and the antioxidant capacity of the body. The susceptibility of lipoproteins to OS, the stability of oxLDL when stored at -80°C, and its good reproducibility from frozen samples using ELISA are advantages.[22] In addition, when compared to the other OS markers available, oxLDL is the one that is most closely associated with atherosclerosis. Another alternative would be the "total antioxidant capacity" method, which measures the combined antioxidant effect of nonenzymatic pathways in biological fluids and does not take into account the enzymatic antioxidant systems, like superoxide dismutase and catalase. It measures both water and lipid soluble antioxidants, such as carotenoids and vitamin C. However, there are some technical concerns regarding the specificity and sensitivity of these assays, and as such, it is not recommended for decisions affecting population health.[23] Studies incorporating the multimarker approach have shown inconsistent results. The ideal marker for the quantification of OS depends on the type of investigation and the investigator; therefore, we can speculate that HBOT is safe, at least in terms of oxLDL production. However, further research is needed in this area.

The small sample size and measuring serum oxLDL as the sole marker for OS may be considered limitations of our study. In addition, our study population mostly consisted of patients with uncontrolled diabetes mellitus and advanced atherosclerosis, in whom OS should be expected to be substantial and therefore leaving no room for further increase. HBOT treatment of more than 10 sessions might also yield different results.

Conclusion

HBOT seems to be safe in terms of oxLDL production up to the 10th session. However, further large-scale studies are required to explain the role of OS and its relationship to HBOT.

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REFERENCES

1. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003;91:7A–11A. [CrossRef]
2. Speit G, Dennog C, Radermacher P, Rothfuss A. Genotoxicity of hyperbaric oxygen. Mutat Res 2002;512:111–9. [CrossRef]
3. Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F, Canestrari F. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. Clin Biochem 2004;37:312–7. [CrossRef]
4. Limón-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res 2009;674:137–47. [CrossRef]
6. Maiolino G, Pedon L, Cesari M, Frigo AC, Barisa M, Rossitto G, et al. Antibodies to malondialdehyde oxidized low-density lipoproteins predict long term cardiovascular mortality in high risk patients. Int J Cardiol 2013;168:484–9. [CrossRef]
7. Leach RM, Rees PJ, Wilmshurst P. Hyperbaric oxygen therapy. BMJ 1998;317:1140–3. [CrossRef]
8. Cuzzocrea S, Imperatore F, Costantino G, Luongo C, Mazzon E, Scafuro MA, et al. Role of hyperbaric oxygen exposure in reduction of lipid peroxidation and in multiple organ failure induced by zymosan administration in the rat. Shock 2000;13:197–203. [CrossRef]
9. Aydin MU, Aygul N, Altunkeser BB, Unlu A, Taner A. Comparative effects of high-dose atorvastatin versus moderate-dose rosuvastatin on lipid parameters, oxidized-LDL and inflammatory markers in ST elevation myocardial infarction. Atherosclerosis 2015;239:439–43. [CrossRef]
10. Choi SH, Sviridov D, Miller YI. Oxidized cholesteryl esters and inflammation. Biochim Biophys Acta 2017;1862:393–7.
11. Uchida K. Aldehyde adducts generated during lipid peroxidation modification of proteins. Free Radic Res 2015;49:896–904. [CrossRef]
12. Usman A, Ribatti D, Sadat U, Gillard JH. From Lipid Retention to Immune-Mediate Inflammation and Associated Angiogenesis in the Pathogenesis of Atherosclerosis. J Atheroscler Thromb 2015;22:739–49. [CrossRef]
13. Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and atherosclerosis. Mediators Inflamm 2013;2013:152786. [CrossRef]
14. Kudchodkar B, Jones H, Simecka J, Dory L. Hyperbaric oxygen treatment attenuates the pro-inflammatory and immune responses in apolipoprotein E knockout mice. Clin Immunol 2008;128:435–41. [CrossRef]
15. Kudchodkar BJ, Pierce A, Dory L. Chronic hyperbaric oxygen treatment elicits an anti-oxidant response and attenuates atherosclerosis in apoE knockout mice. Atherosclerosis 2007;193:28–35. [CrossRef]
16. Elsayed NM. Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. Nutrition 2001;17:828–34. [CrossRef]
17. Matzi V, Greilberger JF, Lindenmann J, Neuboeck N, Nuhsbaumer S, Zelzer S, et al. Application of Hyperbaric Oxygen Reduce Oxidative Damage of Plasmatic Carbonyl Proteins and 8-OHdG by Activating Glutathion Peroxidase. Clin Lab 2015;61:587–93. [CrossRef]
18. Eken A, Aydin A, Sayal A, Ustündağ A, Duydu Y, Dündar K. The effects of hyperbaric oxygen treatment on oxidative stress and SCE frequencies in humans. Clin Biochem 2005;38:1133–7. [CrossRef]
19. Ma L, Li P, Shi Z, Hou T, Chen X, Du J. A prospective, randomized, controlled study of hyperbaric oxygen therapy: effects on healing and oxidative stress of ulcer tissue in patients with a diabetic foot ulcer. Ostomy Wound Manage 2013;59:18–24.
20. Dennog C, Radermacher P, Barnett YA, Speit G. Antioxidant status in humans after exposure to hyperbaric oxygen. Mutat Res 1999;428:83–9. [CrossRef]
21. Simsek K, Ay H, Topal T, Ozler M, Uysal B, Ucar E, et al. Long-term exposure to repetitive hyperbaric oxygen results in cumulative oxidative stress in rat lung tissue. Inhal Toxicol 2011;23:166–72. [CrossRef]
22. Pai JK, Curhan GC, Cannuscio CC, Rifai N, Ridker PM, Rimm EB. Stability of novel plasma markers associated with cardiovascular disease: processing within 36 hours of specimen collection. Clin Chem 2002;48:1781–4.
23. Griendling KK, Touyz RM, Zweier JL, Dikalov S, Chilian W, Chen YR, et al; American Heart Association Council on Basic Cardiovascular Sciences. Measurement of Reactive Oxygen Species, Reactive Nitrogen Species, and Redox-Dependent Signaling in the Cardiovascular System: A Scientific Statement From the American Heart Association. Circ Res 2016;119:e39–75. [CrossRef]

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