SUMMARY

OBJECTIVE: Reactive oxygen species and oxygen free radicals cause oxidative damage to lipids, proteins, and cell DNA in the cell membrane. Although many DNA products are produced during oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most common one, since it can be produced in vivo environment. In recent years, diving has been done quite frequently for business and sports purposes all over the world. Increased environmental pressure in diving leads to hyperoxia and causes oxidative stress.

METHODS: The acute effects of diving on DNA damage were evaluated by comparing 8-hydroxy-2'-deoxyguanosine values of 15 professional diver groups before and after diving. In addition to the demographic characteristics, the serum 8-hydroxy-2'-deoxyguanosine levels of these 15 divers were compared with the control group consisting of nondiving medical students to examine the chronic effect of diving on DNA damage.

RESULTS: After deep dive, the amount of 8-hydroxy-2'-deoxyguanosine increased significantly in the diver group and acute DNA damage was observed (T1: 38.86±4.7; T2: 51.77±4.53; p<0.05). In the control group, the amount of 8-hydroxy-2'-deoxyguanosine was insignificant (C1: 47.48±3.73; T1: 38.86±4.7; p>0.05).

CONCLUSIONS: It was found that air dives caused an increase in serum 8-hydroxy-2'-deoxyguanosine levels, leading to acute oxidative stress and aging. However, there is no chronic side effect, according to the study of samples taken from the control group. This was thought to be due to the relative sedentary life of the control group. The duration of the effect or the ability to return to normal values should be investigated with further studies planned with large populations.

KEYWORDS: 8-Hydroxy-2'-deoxyguanosine. Air pressure. Naval medicine. DNA damage. Diving.

INTRODUCTION

Exposure of some workers and healthcare workers to hyperbaric environment means breathing air at high pressure of more than 10% of 1 atm. Operations in hyperbaric environments can take place in wet environments such as tunnels, tunnel scraping and pressure chambers, and search-rescue and military purposes.1

According to Henry’s gas law, limited depth and time of the dive is suggested to avoid oxygen toxicity during diving.2 Witte et al. reported DNA damage increases with depth and bottom time during diving.3 These partial pressure changes of oxygen and nitrogen in body tissues are responsible for many biochemical reactions that cause the formation of reactive oxygen and reactive nitrogen species.4 These radicals cause a decrease in the activities of antioxidant enzymes, leading to lipid peroxidation in the cell membrane with DNA damage.

Some metabolomics have been used successfully in assessing the health status of workers at environmental exposures.5 8-Hydroxy-2’-deoxyguanosine (8-OHdG), which is the most
common metabolomics in the oxidative damage of DNA, is a sensitive biomarker that can show even low-level DNA damage of oxidative stress. It is formed when the hydroxyl radical damages the guanidine nucleoside at the C8 position on the DNA or when the hydroxyl radical binds to deoxyguanidine with receiver electron.

The studies on DNA damage in the hyperbaric environment remain challenging. In fact, this study is the first to examine 8-OHdG levels after air diving. In a previous study, we showed that single deep diving in the dry hyperbaric environment can cause thiol-disulfide imbalance in divers. In addition to the high oxygen levels, the increasing number of workers in pressurized environments and the water temperature can also cause increase oxidative DNA damage. Therefore, this study aims to investigate and identify the negative impact of environmental factors on health and cellular aging of individuals exposed to hyperbaric environment throughout their professional life.

**METHODS**

The experimental protocol was approved by the local ethics committee of the Harran University (2018/09/12) and was planned in accordance with the Helsinki Declaration. The study was planned with the control group, and the samples were taken before and after the application.

**Study population**

A total of 35 healthy volunteers (34 males and 1 female) participated in this study. Based on the demographic characteristics, participants were divided into study and control groups. The study group consisted of 17 volunteer professional divers (16 males and 1 female, 20.6±0.8 years) graduated from professional underwater diving school and were exposed to hyperbaric hyperoxic environment. The control group consisted of 18 volunteer male medical students (19.4±0.3 years) who did not do any diving and were included as the control group. Informed consent with personal information and health status was obtained from all volunteers and was kept confidential. Inclusion criteria of participants were nonsmokers, nonalcoholics, without any chronic disease (e.g., orthopedic, heart, kidney, liver, autoimmune, and metabolic diseases), and no history of any medication use. A diet and sleep plan was prepared for all volunteers 7 days prior to the study. All participants should not have any travel history during this period. Before the dive, only light breakfast was given to the participants (17 divers). Volunteer divers had 162 h of self-contained scuba apparatus, 192 h surface supplied breathing apparatus, and 28 h pressure room diving experience.

**Study design and diving session**

Fifteen minutes before the hyperbaric environment exposure, a blood sample was taken from all divers to examine whether the chronic hyperbaric exposure caused DNA damage. Then, blood samples taken 1 h after diving and before diving were compared to examine whether the dive caused acute damage to DNA.

Hyperbaric hypoxic exposure was planned inside the decompression chamber as a dry (Barotech Multi DK 1700, Istanbul, Turkey). The Dive Plan was planned according to the U.S. Navy Air dive profile with zero deco limits. The divers were planned as a group of quaternaries and the first dive was made at 40 meter sea water (msw) at 11:00 a.m. with 5 min bottom time. The ascent rate was set at 10 m/min, at the end of the dive; a 3-min safety deco was applied at 5 msw. The first dive was completely ended at 11:15 a.m. The other groups were taken to the chamber in turn according to the plan.

During the dive, the temperature was kept constant at 25°C depending on the temperature in the pressure chamber and the indoor air was continuously analyzed and ventilated during the dive. A light and tempo walking and on-site movement were planned for volunteer divers during the chamber dive to imitate the stress factors in wet diving.

**Sample collections and 8-hydroxy-2’-deoxyguanosine analysis**

The antecubital vein was selected for sample collection from all participants. Samples were taken from the divers 90 min before the dive (T1) and 90 min after the dive (T2). The samples of the control group (C1) are the same as the time of T1 samples. Immediately after the blood was taken into tubes, it was centrifuged at 2,000 g (Hettich, Universal 320R, Tuttingen, Germany) and the sampling serum was obtained. It was stored at -80°C for commercial kits application.

To measure the levels of 8-OHdG in samples, the commercial enzyme-linked immunosorbent assay (ELISA) (Lot No: WXCy7EGD8D, Elabscience, Houston, TX, USA) commercial kit procedure was applied.

The sera stored in suitable storage conditions were dissolved to room temperature (+18–25°C). After the samples were loaded into 50 μl wells, 50 μl of biotin was added and incubated at +37°C for 45 min. After aspirating and washing three times, 100 μl of conjugate liquid was added and incubated for 30 min at +37°C. The sera were aspirated again, washed three times, and finally incubated with 90 μl of the substrate at +37°C for 15 min. In the final stage, 50 μl of stop solution was added to the sera and the results were calculated by measuring at 450 nm.
Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows software version 22.0 (IBM Inc., Chicago, IL, USA). Data are expressed as mean and standard deviation. Laboratory findings were evaluated with the “Shapiro-Wilk” test to determine whether they were normally distributed.

RESULTS

The demographic and anthropometric characteristics of the volunteers participated in the study are given in Table 1. There was no statistically significant difference in the demographic and anthropometric values between the two groups (p>0.05).

To examine the chronic effects of hyperbaric environment exposure on DNA damage, the 8-OHdG levels in serum taken from the C1 group and the T1 group were compared and found to be statistically insignificant (C1: 47.48±3.73; T1: 38.86±4.7, p=0.05) (Table 2).

To examine the acute effects of hyperbaric environment exposure on DNA damage, the 8-OHdG levels in serum samples taken from the T1 group and the T2 group were compared. The levels after the dive were higher than those before the dive, and this difference was statistically significant (T1: 38.86±4.7; T2: 51.77±4.53, p<0.05) (Table 3).

DISCUSSION

The aim of this study is to show whether hyperbaric environment exposure causes damage to DNA in divers. We planned a single air dive to a depth of 40 msw to assess DNA damage during acute hyperbaric exposure in divers. The reason for choosing 40 msw is to measure the partial pressure of oxygen in the inhaled air as 1 atm. Also, 40 msw depth is the upper limit of recreational diving. We also aimed to make inferences about sport, recreational, and professional diving. According to the results, although single-session deep diving caused acute damage to DNA, this damage did not continue when divers were not involved in diving.

Factors causing increased DNA damage include stress factors such as increased environmental pressure, compressed gas breathing, and claustrophobia in dry hyperbaric environment exposure, and stream-cloudy water and hot-cold water in wet diving. Similar to underwater exercise, during the dive, the divers do a light walk and on-site movement in the pressure chamber. Tranfo et al. found that hypoxanthine levels were higher in the wet environment than in the dry hyperbaric environment1. Bosco et al. showed that the exposure to wet hyperbaric environment caused stress with hot diving water and underwater cycling, and increased urinary 8-OHdG levels10. As mentioned previously, the exercise given to the divers in a single-session hyperbaric environment exposure caused an increase in 8-OHdG levels, leading to DNA damage.

8-OHdG is a biomarker of oxidative stress and aging which can be analyzed through urea, organs, and leukocyte DNA11. 8-OHdG is the most common product of oxidative DNA damage12. In addition, increased levels of 8-OHdG are responsible for cardiovascular risk and endothelial dysfunction6. High levels of 8-OHdG have been shown to impair renal function, with inflammatory markers6.

Table 1. Demographic and anthropometric characteristics of study population.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Height (m)</th>
<th>Mass (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (medical students) n=18</td>
<td>19.4±0.3</td>
<td>1.74±10.6</td>
<td>66±12.5</td>
<td>23.7±2.2</td>
</tr>
<tr>
<td>Study group (professional diver group) n=17</td>
<td>20.6±0.8</td>
<td>1.71±11.8</td>
<td>63±11.9</td>
<td>22.8±1.6</td>
</tr>
<tr>
<td>p-value</td>
<td>0.081</td>
<td>0.057</td>
<td>0.61</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD. Statistically significant at p>0.05.

Table 2. Comparison of serum 8-hydroxy-2´-deoxyguanosine levels before the dive (T1) of control group and study group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control group (C1)</th>
<th>Study group (T1)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Hydroxy-2´-deoxyguanosine serum level</td>
<td>47.48±3.73</td>
<td>38.86±4.7</td>
<td>0.20</td>
</tr>
</tbody>
</table>

T1/C1=p>0.05. Values are presented as mean±SD.

Table 3. Comparison of serum 8-hydroxy-2´-deoxyguanosine levels of study group 1 h before diving and 1 h after diving.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Study group (T1 before exposure)</th>
<th>Study group (T2 after exposure)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Hydroxy-2´-deoxyguanosine serum level</td>
<td>38.86±4.7</td>
<td>51.77±4.53</td>
<td>0.02</td>
</tr>
</tbody>
</table>

T1/T2=p<0.05. Values are presented as mean±SD.
It has been shown that homocysteine increases due to oxidative stress in saturation dives where exposure to a hyperbaric environment lasted for days and weeks, and circulating vitamins B6 and B12 and folate levels decrease. This hyperoxic environment causes increased iron stores and decreased erythropoietin levels. The decrease in folate acts as an antioxidant in the oxidative stress environment. A previous study showed that oxidative stress and DNA damage return to normal values with the end of the dive. Our study shows that the 8-OHdG levels of C1/T1 were statistically insignificant, which is consistent with the results of the previous study.

Oxidative stress causes damage by disrupting the structure of enzymes in the mitochondrial membrane and the tertiary structure and functions of proteins. Brain aging occurs with the formation of 8-OHdG and increased oxidative damage to mtDNA. In addition, mitochondrial damage due to oxidative stress in brain tissue has been associated with aging and Alzheimer’s disease.

Aerobic exercise in a hyperbaric environment has been shown to increase serum lactate levels. In another study, oxidative DNA damage has been shown to cause pulmonary injury. It has also been shown that DNA damage and subsequent serum 8-OHdG levels increase chronic fatigue syndrome. As can be seen, DNA damage caused by oxidative stress results in aging that affects the whole body.

Some restraints should be considered in the assessment of our study. In this regard, our study was mostly conducted on male samples. To simulate warm water diving, oxidative DNA damage could be increased by turning off the air conditioner in the pressure chamber. Finally, since it is a cross-sectional study, no conclusion can be drawn between 8-OHdG levels and long-term hyperbaric exposure.

CONCLUSIONS

We studied the effect of occupational and environmental hyperbaric exposures on the formation of 8-OHdG. From a practical point of view, it may be suggested that DNA damage occurs in the early stages of diving and its effects are seen in many tissues in our body. Although there is no DNA damage in daily life in divers with high dive time record in this study, there is a need for multicenter studies with wider interpretation.

ACKNOWLEDGMENTS

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AUTHORS’ CONTRIBUTIONS

AEG: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. OY: Conceptualization, Methodology, Resources, Supervision. CE: Investigation, Supervision. SND: Data curation, Formal analysis, Writing – review & editing. HC: Data curation, Formal analysis, Resources, Software, Validation, Visualization.

REFERENCES


