TITLE The application of Hyperbaric Oxygen Therapy in Non-ventilated COVID-19 patients – Randomized Controlled Trial

INTRODUCTION

The 2019-20 coronavirus disease, caused by COVID-19, is an ongoing pandemic. The outbreak started in Wuhan, Hubei province, China, in December 2019 and the World Health Organization (WHO) recognized it as a pandemic on March 11, 2020. Up to Apr 9, 2020, there are more than 2 million confirmed cases, and over 140,000 deaths. In Israel, COVID-19 was confirmed in more 12,000 cases and took the life of 140 victims. There are 323 mild admitted cases, 170 moderate admitted cases and 170 severe admitted cases (16.04.2020).

Even though the general mortality rate is low (0.2-7%, country based), patients who develop Acute Respiratory Distress Syndrome (ARDS) have a significantly higher mortality rate, up to 61-90%]1[. COVID-19 ARDS is different, causing a rapidly progressive disease including respiratory insufficiency and pulmonary fibrosis]2[. The mechanism behind isn't clear yet, but evidence points to the direction of an acute cytokines storm which include: IL-2, IL-7, GCSF, InterferonGamma, TNF-alpha, Macrophage chemoattractant protein]3[. Poor prognosis include high levels of IL-6 and Ferritin]3[.

The SARS-CoV-2 virus is a positive RNA strand with the following structural proteins: Spike protein (S), Envelope protein €, Membrane protein (M) and nucleocapsid. In addition the virus is encoded to several other protein: orflab, ORF1, OR3a, ORF6, ORF7a, ORF8 [4, 5]. A recent bioinformatic study analyzed the pathogenic function of these proteins. The study found the ORF8 protein and another glycoprotein are able to bind to the Porphyrin molecule (the ring surrounding the iron atom in the Heme component). Parallelly, the orflab, ORF10, ORF3a proteins are able to harm the HEME component. Located on the 1-beta chain of the hemoglobin molecule, unbinding the iron atom needed for the Porphyrin]6[.

More than 160 clinical trials have been registered, but as of April 2020, there is no proven effective treatment. In a recent randomized controlled trial including 199 patients, anti-viral drugs (Lopinavir-Ritonavir) did not show efficacy]7[. Moreover, the use of extracorporeal membrane oxygenation (ECMO) did not prove efficacy compared to the standard treatment on an analysis of 17 patients]8[.

BACKGROUND AND SIGNIFICANCE

The use of hyperbaric oxygen therapy (HBOT) includes breathing 100% oxygen in pressures higher than 1 absolute atmospheres (ATA), increasing the amount of oxygen dissolved in the plasma and the different tissues. During the session, oxygen partial pressure can increase from 100mmHg to 1700-2500mmHg (according to the applied pressure) [9]. In the last month, Chen et al. reported a case series of 5 severe COVID-19 patients treated with 3-8 HBOT sessions in addition to the standard therapy]10[. In all cases, they reported an increase in oxygen saturation, arterial oxygen content, lactate levels reduction, fibrinogen levels decrease and increase in lymphocytes number. In addition, the patients' chest CT showed improved signs. Symptomatic relief started following the 2nd session. No significant adverse events were reported [9].

These findings may be explained by the known physiological effects of HBOT, related to the SARS-CoV-2 virus pathogenesis:

- **Increased competitive binding of oxygen to the hemoglobin molecule** – it has been postulated recently the SARS-CoV-2 bind to the heme component in the hemoglobin molecule and reduces the oxygen affinity to hemoglobin. During HBOT, the increased amount of available oxygen molecules increases the binding to the hemoglobin molecules. This has shown significant beneficial effects in cases of another competitive molecule such as carbon monoxide intoxication, where HBOT induces carbon monoxide unbinding and release of the red blood cells [12, 11]. If the virus indeed affects the Heme component (as suggested above) in a competitive affinity, HBOT may significantly improve and normalize the red blood cells oxygen carrying capability.

- **Tissue oxygenation** – The oxygen content in the different tissues is multiplied by 25-30 times [13]. This effect has two therapeutic aspects: First, overcoming pulmonary hypoxia (either shunt or VQ mismatch) by increasing the FiO2 significantly. By increasing the pulmonary oxygen gradient, oxygen diffusion increases and can overcome the inflammation in the alveoli and the thickened fibrosis caused by ARDS. These effects have been demonstrated in patients who developed ARDS after suffering a blunt chest trauma who were treated with HBOT. In these patients, HBOT induced improvement and rapid normalization of PaO2, PaO2/FiO2 in addition to cardiac parameters CI and SVI]14[. Second, during HBOT, the amount of oxygen dissolved in the plasma

becomes significant and enables tissue oxygenation without the need of red blood cells]15[.

- **Mitochondrial function restoration** – HBOT can restore the normal function of the mitochondria and the cellular metabolism, decrease apoptosis and reduce the oxidative injury [16].

- **Anti-inflammatory** – HBOT reduces the following inflammatory cytokines both in the protein level and genes expression (mRNA): IL-2, TNF-alpha, IL-6, IL-1beta, mediated by several transcription factors: Hypoxia induced factor 1alpha (HIF1alpha), Nrf2, NFkB [17-19] . The anti-inflammatory effect has been shown in chronic diseases as well as models of acute infection and massive hemorrhage [20, 21].

Apart from Chen et al. case series, no prospective study was performed on the effect of HBOT on COVID-19 patients. In addition to the safety report in that case series, there's more evidence for the level of safety of HBOT in patients with acute pulmonary insufficiency:

- There is an ongoing debate in regards to the use of high inhaled oxygen level for patients with ARDS. In a recent randomized controlled trial (LUNGSAFE), there was no association between high oxygen levels and bad prognosis]22[.

- The use of HBOT in patients with ARDS after blunt chest trauma, showed promising results in oxygenation and cardiac function, with no safety issues]14[.

STUDY OBJECTIVES

The purpose of the current study is to evaluate the efficacy of HBOT in moderate-severe non ventilated COVID-19 patients.

The primary objectives of study will be to assess the following indicators:

- Oxygenation index = PaO2/FiO2 one day after the last session.
- SpO2 – room air saturation one hour before and after a session, 6 hours after a session and one day after the last session.
- NES – Early warning score, before and after each session, 6 hours after each session and one day after the last session
- Inflammatory parameters one day after the last session: WBC, Immune profile, CRP, ESR, Procalcitonin, TNFalpha, IL1, IL2, IL6, IL10.

The secondary objectives of study will be to assess the following indicators:

- Oxygenation index = PaO2/FiO2 one week after the last session.
- SpO2 – room air saturation one week after the last session.
- NES – Early warning score, one week after the last session
- Inflammatory parameters one week after the last session: WBC, Immune profile, CRP, ESR, Procalcitonin, TNFalpha, IL1, IL2, IL6, IL10.
- Symptoms level one day and one week after the last session
- Viral load one day and one week after the last session
- Chest Xray changes one day and one week after the last session
- Percentage of patients with positive IgG serology one day and one week after the last session
- Percentage of patients with positive IgM serology one day and one week after the last session
- Pulmonary function test one day and one week after the last session
- Time to symptoms recovery
- Percentage of patients who required invasive intubation
- Time to recovery (negative PCR twice) – TTCR
- Mortality rate in 30 days
- Safety – percentage of adverse events

STUDY DESIGN
Subjects: Patients at Maimonides Medical Center (MMC)

Eligibility Criteria:
Patients who are (1) within 7 days of need of oxygen supply, (2) positive to SARS-CoV-2 through RT-PCR, (3) have at least one risk factor for bad prognosis of COVID-19: hypertension, diabetes mellitus, ischemic heart disease, smoking, age>50, etc., (4) have respiratory insufficiency: Room Air SpO2 <94% or PaO2/FiO2<300mmHg, (5) are older than 18 years of age and (6) have ability to sign an informed consent will be included in the study.

Patients who are (1) negative to SARS-CoV-2 through RT-PCR, (2) have a HBOT contraindication: pneumothorax, pneumomediastinum, claustrophobia, ear/sinus disease which aren't allowed in HBOT, known chronic pulmonary disease: severe emphysema or known pulmonary bullae, (3) have pregnancy and/or (4) have inability to sign an informed consent will be excluded from study.

Design:
This study is a prospective randomized controlled, double blind clinical trial performed on laboratory confirmed COVID-19 infection admitted patients in MMC. It involves the use of MMC monoplace HBO chambers. The trial will include 30 patients who will undergo either hyperbaric oxygen therapy (HBOT) or Normobaric oxygen therapy (NBOT), randomized on a 2:1 ratio, within 4 days in addition to the standard treatment including oxygen, drugs, steroids, bronchodilators, antibiotics and others.

The evaluation procedure includes symptom monitoring, room air saturation, vital signs monitoring, pulmonary function and blood tests at baseline, one day and one week after the last session. In addition, one hour prior to and post session saturation and vitals will be monitored.

Data Collection Procedures:
Upon signing an informed consent, the patients will be randomized 2:1 to hyperbaric oxygen therapy group (HBOT) and normobaric oxygen therapy group (NBOT) (Randomization will be performed using a computer software based on patient id). Following the randomization, the patients will undergo evaluation including symptoms, vitals, pulmonary function, blood tests.

The patients will undergo 8 sessions of either hyperbaric/normobaric oxygen therapy, 2 sessions per day, in 4 consecutive days. During the sessions, the symptoms and vitals will be monitored. 1 day and 1 week following the last session, revaluation will be performed.

Protocol Day 1

1. Baseline evaluation: blood test including: arterial blood gases, 2 CBC tubes, 2 Gel tubes (10-15 cc total), vitals (temperature, blood pressure, heart rate, room air saturation), symptoms questionnaire, pulmonary function test.
2. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour prior to session
3. One-hour session NBO/HBO
4. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour after the session.
5. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour before the 2nd session.
6. One-hour session NBO/HBO (8 hours following the first session)
7. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour after the session.
8. Daily oxygen supply dose monitoring.

Protocol Day 2-4

1. Daily symptoms questionnaire
2. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour prior to session
   One-hour session NBO/HBO
3. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour after the session.
4. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour before the 2nd session.
5. One-hour session NBO/HBO (8 hours following the first session)
6. Vitals (temperature, blood pressure, heartrate, room air saturation) one hour after the session.
7. Daily oxygen supply dose monitoring.

Follow up Protocol

1. Repeat evaluations one day after the last session and one week after the last session:
   - Blood test including: arterial blood gases, 2 CBC tubes, 2 Gel tubes (10-15 cc total)
   - Vitals (temperature, blood pressure, heart rate, room air saturation)
   - Symptoms questionnaire
   - Pulmonary function test.
2. Oropharyngeal swab for SARS-CoV-2 RT-PCR every 3 days for 1 week
3. Clinical monitoring for 30 days

Data Analysis:

Continuous data will be expressed as means ± standard-deviations. Independent and dependent t-test with two-tail distribution will be performed to compare variables between and within the two groups, when a normality assumption holds according to Kolmogorov-Smirnov test. Categorical data will be expressed in numbers and percentages and compared by chi-square tests. Univariate analyses were performed using Chi-Square/Fisher's exact test to identify significant variables. A value of $p<0.05$ will be considered significant.

Data analysis will be performed with Matlab R2019b (Mathworks, Natick, MA) Statistics Toolbox.

Sample Size: 30

Based on a previous study on HBOT effect on ARDS]14[, with 95% power and significance level of 5%, and a groups difference of 30 (SD 15-18) in the PaO2/FiO2 and a 2:1 groups allocation, the sample requires 7 NBOT patients and 13 HBOT patients. Considering 15% dropout, we will recruit 10 NBOT patients and 20 HBOT patients, 30 in total.

Expected Outcomes:
The primary outcomes measures that will be accessed in the study are:

- Oxygenation index = PaO2/FiO2 one day after the last session.
- SpO2 – room air saturation one hour before and after a session, 6 hours after a session and one day after the last session.
- NES – Early warning score, before and after each session, 6 hours after each session and one day after the last session
- Inflammatory parameters one day after the last session: WBC, Immune profile, CRP, ESR, Procalcitonin, TNFalpha, IL1, IL2, IL6, IL10.

The secondary outcomes measures that will be accessed in the study are:

- Oxygenation index = PaO2/FiO2 one week after the last session.
- SpO2 – room air saturation one week after the last session.
- NES – Early warning score, one week after the last session
- Inflammatory parameters one week after the last session: WBC, Immune profile, CRP, ESR, Procalcitonin, TNFalpha, IL1, IL2, IL6, IL10.
- Symptoms level one day and one week after the last session
- Viral load one day and one week after the last session
- Chest Xray changes one day and one week after the last session
- Percentage of patients with positive IgG serology one day and one week after the last session
- Percentage of patients with positive IgM serology one day and one week after the last session
- Pulmonary function test one day and one week after the last session
- Time to symptoms recovery
- Percentage of patients who required invasive intubation
- Time to recovery (negative PCR twice) – TTCR
- Mortality rate in 30 days
- Safety – percentage of adverse events

Timetable: 12 months

Adverse Event Reporting

Subjects will be monitored for adverse events (AEs) during the entire study and more particularly, during and after each treatment session by clinical inquiry, clinical observation and (when applicable) by clinical examination. AE information will be documented and all subjects will be questioned about AEs at each visit during the treatment period and at the follow-up visits. AEs will also be recorded whenever spontaneously reported by the subject. Event description, date of onset, intensity,

duration, treatment for AE, whether the AE was resolved and probable relationship to the study product will be recorded onto case report forms (CRFs).

The investigator must record all serious adverse events regardless of treatment or relationship to investigational product as soon as s/he is informed of the event.

Any serious adverse event (SAE) which occurs after a patient has entered the study (after signing informed consent), must be reported. The original copy of the serious adverse event form must be kept with the documentation at the site.

Patients who have had an SAE during the treatment period must be followed clinically until all parameters (including laboratory), have either returned to normal, have stabilized or are otherwise explained.

SAEs should be reported by the investigator to competent authorities and ethics committees according to local requirements.

For more information, see research protocol from overall study.

REFERENCES

1. Goh, K.J., et al., *Rapid Progression to Acute Respiratory Distress Syndrome: Review of Current Understanding of Critical Illness from COVID-19 Infection.* Ann Acad Med Singapore, 2020. **49**(3): p. 1-9.
2. Mehta, P., et al., *COVID-19: consider cytokine storm syndromes and immunosuppression.* Lancet, 2020. **395**(10229): p. 1033-1034.
3. Ruan, Q., et al., *Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China.* Intensive Care Med, 2020.
4. Zhu, N., et al., *A Novel Coronavirus from Patients with Pneumonia in China, 2019.* N Engl J Med, 2020. **382**(8): p. 727-733.
5. Wu, F., et al., *A new coronavirus associated with human respiratory disease in China.* Nature, 2020. **579**(7798): p. 265-269.
6. liu, w. and h. Li, *COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism*. 2020.
7. Cao, B., et al., *A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19.* N Engl J Med, 2020.
8. Henry, B.M. and G. Lippi, *Poor survival with extracorporeal membrane oxygenation in acute respiratory distress syndrome (ARDS) due to coronavirus disease 2019 (COVID-19): Pooled analysis of early reports.* J Crit Care, 2020. **58**: p. 27-28.
9. Fosen, K.M. and S.R. Thom, *Hyperbaric oxygen, vasculogenic stem cells, and wound healing.* Antioxid Redox Signal, 2014. **21**(11): p. 1634-47.

10. Ruiyong Chen 1*, X.Z., Yanchao Tang 3*, Yi Liang 4, Bujun Li 5, Xiaolan Tao 2, Changbo Liao 1., *The Outcomes of Hyperbaric Oxygen Therapy to severe and critically ill patients with COVID-19 pneumonia.*
11. Ernst, A. and J.D. Zibrak, *Carbon monoxide poisoning.* N Engl J Med, 1998. **339**(22): p. 1603-8.
12. Weaver, L.K., et al., *Hyperbaric oxygen for acute carbon monoxide poisoning.* N Engl J Med, 2002. **347**(14): p. 1057-67.
13. Rockswold, S.B., et al., *A prospective, randomized clinical trial to compare the effect of hyperbaric to normobaric hyperoxia on cerebral metabolism, intracranial pressure, and oxygen toxicity in severe traumatic brain injury.* J Neurosurg, 2010. **112**(5): p. 1080-94.
14. Rogatsky, G.G. and A. Mayevsky, *The life-saving effect of hyperbaric oxygenation during early-phase severe blunt chest injuries.* Undersea Hyperb Med, 2007. **34**(2): p. 75-81.
15. Boerema, I., et al., *[Life without blood].* Ned Tijdschr Geneeskd, 1960. **104**: p. 949-54.
16. Palzur, E., et al., *Neuroprotective effect of hyperbaric oxygen therapy in brain injury is mediated by preservation of mitochondrial membrane properties.* Brain Res, 2008. **1221**: p. 126-33.
17. Novak, S., et al., *Anti-Inflammatory Effects of Hyperbaric Oxygenation during DSS-Induced Colitis in BALB/c Mice Include Changes in Gene Expression of HIF-1alpha, Proinflammatory Cytokines, and Antioxidative Enzymes.* Mediators Inflamm, 2016. **2016**: p. 7141430.
18. Aricigil, M., et al., *Anti-inflammatory effects of hyperbaric oxygen on irradiated laryngeal tissues.* Braz J Otorhinolaryngol, 2018. **84**(2): p. 206-211.
19. Benson, R.M., et al., *Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages.* Clin Exp Immunol, 2003. **134**(1): p. 57-62.
20. Halbach, J.L., et al., *Early hyperbaric oxygen therapy improves survival in a model of severe sepsis.* Am J Physiol Regul Integr Comp Physiol, 2019. **317**(1): p. R160-R168.
21. Yamashita, M. and M. Yamashita, *Hyperbaric oxygen treatment attenuates cytokine induction after massive hemorrhage.* Am J Physiol Endocrinol Metab, 2000. **278**(5): p. E811-6.
22. Madotto, F., et al., *Hyperoxemia and excess oxygen use in early acute respiratory distress syndrome: insights from the LUNG SAFE study.* Crit Care, 2020. **24**(1): p. 125.