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Short communication

Low serum erythropoietin levels are associated with fatal COVID-19 cases at 4,150 meters above sea level

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A B S T R A C T

Previous studies suggested that erythropoietin (EPO) may protect against severe COVID-19-induced injuries, ultimately preventing mortality. This hypothesis is based on the fact that, in addition to promoting the increase in red blood cells, EPO is an anti-inflammatory, anti-apoptotic and protective factor in several non-erythropoietic tissues. Furthermore, EPO promotes nitric oxide production in the hypoxic lung and stimulates ventilation by interacting with the respiratory centers of the brainstem. Given that EPO in the blood is increased at high-altitude, we evaluated the serum levels of EPO in critical patients with COVID-19 at “Hospital Agramont” in the city of El Alto (4150 masl) in Bolivia. A total of 16 patients, 15 men, one woman, with a mean age of 55.8 ± 8.49 years, admitted to the Intensive Care Unit were studied. All patients were permanent residents of El Alto, with no travel history below 3000 m asl for at least one year. Blood samples were collected upon admission to the ICU. Serum EPO concentration was assessed using an ELISA kit, and a standard technique determined hemoglobin concentration. Only half of the observed patients survived the disease. Remarkably, fatal cases showed 2.5 times lower serum EPO than survivors (2.78 ± 0.8643 mU/mL vs 7.06 ± 2.713 mU/mL; p = 0.0096), and 1.24 times lower hemoglobin levels (13.96 ± 2.56 g/dL vs 17.41 ± 1.61 g/dL; p = 0.0159). While the number of cases evaluated in this work is low, our findings strongly warrant further investigation of EPO levels in COVID-19 patients at high and low altitudes. Our results also support the hypothesis that exogenous EPO administration could help critically ill COVID-19 patients overcome the disease.

1. Introduction

The COVID-19 pandemic, caused by the coronavirus type 2 (SARS-CoV-2), has reached alarming global dimensions. As such, health systems are being hit hard, especially in developing countries. In Latin America, preventing public and private hospital institutions collapse is an enormous challenge. This materializes in Intensive Care Units (ICU): the battlefield against disease. Despite the advances in the development of vaccines, their arrival and delivery in emergent countries is taking much longer than it was initially planned. On the other hand, regrettably, treatments to alleviate the disease, particularly in critically ill patients, are still controversial. Thus, the most serious cases rapidly evolve into pneumolysis (lung destruction) with severe hypoxemia (Zubieta-Calleja et al., 2020), requiring the administration of oxygen and even mechanical ventilation (Ehrenreich et al., 2020). Erythropoietin (EPO) is essential to promote tissue oxygenation. Indeed, EPO is the main growth factor involved in increasing the number of red blood cells (Jelkmann, 2007). However, in addition to this "classical endocrine task", EPO fulfills several other non-erythropoietic functions. EPO counteracts pulmonary vasoconstriction by increasing the endothelial capacity to produce the vasodilator nitric oxide (NO), thus facilitating the oxygen supply to the brain, heart, and other tissues (Beleslin-Cokic...
et al., 2011). Other studies in mice demonstrated that EPO protects against acute lung injury induced by renal ischemia-reperfusion, playing a key role in suppressing pulmonary edema and attenuating inflammation of alveolar epithelial cells (Moehini et al., 2013; Zhu et al., 2019). In neural pathological contexts, studies in animal models of ischemic stroke and head trauma have shown that EPO is a potent neuroprotective factor that activates anti-apoptotic, anti-inflammatory, anti-cytotoxic, and antioxidant molecular mechanisms (Gassmann et al., 2003; Ghezzi and Brines, 2004). Studies in our laboratory have shown that EPO stimulates normoxic and hypoxic ventilation by interacting directly with the respiratory centers in the brainstem (Ballot et al., 2015; Khemiri et al., 2011; Soliz, 2013; Soliz et al., 2020). In the context of the COVID-19 pandemic, it was suggested that treating critically ill patients with EPO could be highly beneficial (Hadadi et al., 2020; Soliz et al., 2020; Zubieta-Calleja and Zubieta-DeUrioste, 2020; Zubieta-Calleja et al., 2020). In fact, within high altitude medicine, the altered lung gas exchange has similarities between High Altitude Pulmonary Edema (HAPE) and COVID-19 (Zubieta-Calleja et al., 2020). An attenuated stimulation of the neural circuits that control respiration has also been suggested to occur in COVID-19 (Soliz et al., 2020). HAPE, however, is rapidly reversible under treatment, leaving no sequelae whereas COVID-19 can result in pulmonary fibrosis due to pneumolysis (Zubieta-Calleja and Zubieta-DeUrioste, 2021). Furthermore, it was reported that prophylactic injections of EPO could at least partly prevent Acute Mountain Sickness (AMS) within 14 days prior to ascent (Heo et al., 2019). On the other hand, a blunted erythropoietic response (evidenced by anemia) has been reported to be rather common in critically ill patients, regardless of the disease type that led to that condition (Rogiers et al., 1997). In addition, recombinant human EPO was used as a final treatment option for an 80-year-old COVID-19 patient who recovered from the severe stage. In this case, one week before therapy, the patient was already hemoglobin deficient, further decreasing as the disease progressed. With the EPO treatment, her hemoglobin levels finally improved to physiological levels, with evident clinical, laboratory, and radiological improvement. An accelerated reversal of acute respiratory distress due to SARS-CoV-2 infection was also described (Hadadi et al., 2020). Finally, an increasing number of clinical reports show that SARS-CoV-2 infections can also cause kidney and heart failure due to a general excessive inflammation (cytokine storm) (Alert et al., 1988; Wang et al., 2021). In this context, EPO could prevent and/or protect against tissue damage and inflammation in several tissues (Nairz et al., 2012). EPO-mediated anti-inflammatory effects have been demonstrated in chronic inflammatory diseases and infectious diseases, including stimulation with bacterial lipopolysaccharide (LPS) and Salmonella sp. infection (Cuzzocrea et al., 2005; Nairz et al., 2012; Yuan et al., 2008). In addition, it has been shown that there is a positive effect of EPO in patients suffering from a critical systemic infection (Corwin, 2007; Napolitano et al., 2008). Taking into account that EPO is physiologically increased in high-altitude residents (Basu et al., 2007), we reasoned that the evaluation of EPO levels in critically ill COVID-19 patients could shed light on the protective effects of EPO mentioned above. This study was carried out in COVID-19 critically ill patients admitted to the “Hospital Agramont” in the city of El Alto (4150 masl) in Bolivia.

2. Methods

2.1. Study design

We conducted an observational, descriptive, and transversal study at Hospital Agramont’s Intensive Care Unit (exclusive for COVID-19 adult patients) in El Alto city (4,150 m above sea level), La Paz - Bolivia.

2.2. Ethics declaration

The study was approved by the Bioethics Institutional Committee of the hospital and carried out in accordance with the Helsinki declaration.

2.3. Patients

Sixteen patients were studied based on the following inclusion parameters: 1) 18 years old or older, 2) high-altitude permanent residents (no history of migration from lower lands during the last year), 3) “pneumonia” diagnosis due to SARS–COV-2 infection, 4) admitted to ICU. Patients with one or more of the following criteria were not considered: 1) non-COVID-19 related pneumonia diagnosis, 2) hematological disease (including anemia), 3) blood transfusion during the last six months.

2.4. Computed tomography (CT)

All the patients underwent chest CT prior to ICU admission. The percentage of lung involvement, the tomography severity score, and the COVID-19 Reporting and Data System (CO-RADS) score were calculated from the imaging data. Patients were categorized into one of six categories, from very low to very high (CO-RADS 1 to CO-RADS 6) probability of being COVID-19 positive (Ozel et al., 2021).

2.5. Determination of EPO levels

Blood samples were taken from patients prior to UCI admission and were analyzed by the standard ELISA methods using the DRG EPO ELISA EIA3646 kit (DRG Instruments, Marburg-Germany) in the MR-96A microplate reader (Mindray, Shenzhen-China) to read the EPO levels. Analyses were performed in the clinical laboratory of “Hospital Agramont”.

2.6. Hemoglobin (Hb) concentration

From the same blood samples, the concentration of hemoglobin was measured using an automated spectrometric system (Coulter Hematology Analyzer, Brea CA, U.S.A.).

2.7. Data and statistical analysis

For each patient, the following data were collected at the moment of UCI admission: age, comorbidities, lung compromise percentage, tomography severity score, CO-RADS score, number of days of COVID-19 evolution, EPO levels in the blood, and hemoglobin concentration. The number of days in ICU was accounted for each patient at the moment of discharge or death. The final data set, including the final outcome for each patient (survival or deceased), was anonymized entirely for statistical analysis. Two subsets of data were formed separating the survivors (n = 8) and the deceased (n = 8) patients. Differences in the percentage of lung involvement, tomography severity score, CO-RADS score, days of evolution of COVID-19 before admission to ICU, days in ICU, levels of EPO, and hemoglobin concentration, between surviving and deceased patients were evaluated by Mann-Whitney U tests in GraphPad Prism version 9.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. Test significances were set to p < 0.05. Values are presented as mean ± S.D. unless stated differently.

3. Results

The study recruited 16 patients (1 woman and 15 men), all adults (46–75 years old). The most common comorbidities found in the cohort were overweight/obesity (62 %), systemic arterial hypertension (18.75 %), diabetes mellitus type 2 (6.25 %), and pulmonary tuberculosis sequelae (6.25 %). By the end of the study, 50 % (8/16) of the patients survived.

When comparing surviving versus deceased patients, no differences
were found in the number of days of evolution of COVID-19 before UCI admission \( (U = 22, p = 0.31) \) or the number of days patients remained in ICU \( (U = 31.5, p = 0.971) \) (Table 1).

3.1. Lung involvement and injury severity

The percentage of lung involvement and the tomography severity score were lower (better) in patients who survived compared to those who died (Fig. 1A, B; Table 1). However, the CO-RADS score was not different between both groups (Fig. 1C; Table 1).

3.2. EPO and hemoglobin levels

The average EPO level in the deceased patients was roughly 2.5 times lower than in the survivors (Fig. 2A; Table 1). Similarly, the mean hemoglobin concentration was 25% lower in the deceased compared to the survivors (Fig. 2B; Table 1).

4. Discussion

In the present study, we have investigated the potential involvement of EPO in the survival of critically ill COVID-19 patients at high-altitude (4150 masl, El Alto-Bolivia). Taking into account that EPO is physiologically increased in high-altitude residents and that EPO is an ubiquitous protective factor against inflammation, the main findings of the present report are that: (i) the serum level of EPO (and hemoglobin) in all tested patients is significantly lower than standard values reported for this altitude, (ii) serum EPO levels (and hemoglobin) in deceased subjects was about 2.5 times lower than in survivors, and (iii) lung involvement was significantly higher in deceased subjects than in survivors.

Several works (Cano-Pérez et al., 2020; Quevedo-Ramírez et al., 2020), including ours (Arias-Reyes et al., 2021, 2020; Zubieta-Calleja et al., 2020) clearly suggested that the virulence of SARS-CoV-2 decreases significantly with altitude. In a first report, we showed that at global and region specific scale (Tibetan Autonomous Region of China, Bolivia and Ecuador) COVID-19 cases were notably decreased at high altitudes (above 2500 masl) (Arias-Reyes et al., 2020). Later, we expanded this information through the epidemiological analysis of 23 American countries. Our results showed that both the incidence, the transmission capacity and the severity of COVID-19 significantly decrease with altitude, with a turning point that begins at 1000 masl (Arias-Reyes et al., 2021). Although the causes of this effect could be related to environmental and physiological factors, we proposed that high levels of EPO (naturally increased at altitude) could be a mayor factor. Indeed, apart from stimulating the production of red blood cells and high levels of EPO (naturally increased at altitude) could be one major protective factor against inflammation, the main findings of the present report are that: (i) the serum level of EPO (and hemoglobin) in all tested patients is significantly lower than standard values reported for this altitude, (ii) serum EPO levels (and hemoglobin) in deceased subjects was about 2.5 times lower than in survivors, and (iii) lung involvement was significantly higher in deceased subjects than in survivors.

Author statement

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References

Alert, J., Longchong, M., Valdés, M., Menéndez, J., 1988. Cranial irradiation of children with soft-tissue sarcomas arising in parameningeal sites. Neoplasma 35, 627–633.
Hadadi, A., Morteza-zadeh, M., Kolah-douzan, K., Alavian, G., 2020. Does recombinant human Erythropoietin administration in critically ill COVID-19 patients have miraculous therapeutic effects? J. Med. Virol.

Heo, K., Kang, J.K., Choi, C.M., Lee, M.S., Noh, K.W., Kim, S.B., 2014. Prophylactic effect of erythropoietin injection to prevent acute mountain sickness: an open-label randomized controlled trial. J. Korean Med. Sci. 29, 416–422.

Jellmann, W., 2007. Erythropoietin after a century of research: younger than ever. Eur. J. Haematol. 78, 183–205.

Kakavas, S., Demestiha, T., Vasileiou, P., Xanthos, T., 2011. Erythropoetin as a novel agent with pleiotropic effects against acute lung injury. Eur. J. Pharmacol. 67, 1–9.

Khemiri, H., Seaborn, T., Gestreau, C., Soliz, J., 2011. Erythropoietin and soluble erythropoietin receptor regulate the neural control of hypoxic respiration in newborn mice. Resp. Phys. Neurobiol. 183, 151.

Moeini, M., Nematabakhsh, M., Fazlali, M., Talehi, A., Pilevarian, A.A., Azarkish, F., Esraghi-Jazi, F., Pezeshki, Z., 2013. Protective role of recombinant human erythropoietin in kidney and lung injury following renal bilateral ischemia-reperfusion in rat model. Int. J. Prev. Med. 4, 648–655.

Nair, M., Sonnweber, T., Schroll, A., Theurl, I., Weis, G., 2012. The pleiotropic effects of erythropoietin in infection and inflammation. Microbes Infect. 14, 238–246.

Napolitano, L.M., Fabian, T.C., Kelly, K.M., Bailey, J.A., Block, E.F., Langhoff, W., Enzy, C., Corwin, H.L., 2008. Improved survival of critically ill trauma patients treated with recombinant human erythropoietin. J. Trauma 65, 285–297 discussion 297–289.

Özel, M., Aslan, A., Araç, S., 2021. Use of the COVID-19 Reporting and Data System (CO-RADS) classification and chest computed tomography involvement score (CT-IVS) in COVID-19 pneumonia. Radiol. Med.

Phrommintikul, A., Haas, S.J., Eltik, M., Kram, H., 2007. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. Lancet 369, 381–388.

Quevedo-Ramirez, A., Al-Kassab-Córdova, A., Mendez-Guerra, C., Cornejo-Venegas, G., Alva-Chavez, K.P., 2020. Altitude and excess mortality during COVID-19 pandemic in Peru. Respir. Physiol. Neurobiol. 281, 103512.