Clinical and Experimental Health Sciences

The Effects of Therapeutic Intermittent Hypoxia Implementation on Complete Blood Count Parameters: An Experimental Animal Model

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ABSTRACT

Objective: Intermittent hypoxia (IH) implementation is a method performed by intermittently decreasing oxygen concentration in inhaled air at specific rate. This method varies between studies in terms of its application. This study aims to examine the changes in Complete Blood Count (CBC) parameters caused by IH implementation at therapeutic dose ranges with a single model.

Methods: Ten Sprague Dawley type adult male rats were divided into two groups. In the study group, FiO_2 level of inhaled air, was reduced to 10% in hypoxic cycle. 5 minutes normoxia-hypoxia cycle was used in each 30 minutes experiment period for study group. Control group remained in normoxic air for 30 minutes. 1 cc of blood was taken from mandibular vein from all rats at the end of 6th day. CBC analyzes were performed and differences between two groups were investigated.

Results: Significant differences were detected in some CBC parameters between the two groups. It was determined that significant increase in MONO ($p<0.001$), MONO% ($p<0.001$), MCH ($p=0.03$), PLT ($p=0.013$) and PCT ($p=0.007$) parameters and significant decrease in MPV ($p=0.02$) parameters, in favor of study group.

Conclusion: IH implementation was caused significant changes in MONO, MONO%, MCH, PCT, PLT and MPV parameters in the CBC analysis of rats. Considering the study results, therapeutic IH implementation may thought to have important effects in terms of lung protection and regeneration. Further research may focus on this point for precising and supporting of this study' results.

Keywords: Intermittent hypoxia, complete blood count tests, animal model

1. INTRODUCTION

Using the complete blood count test (CBC) is spread for predicting respiratory system status in the last years (1). This method is also started using in studies on intermittent hypoxia (IH) implementation.

IH implementations are classified as chronic and acute implementations. IH occurs after the decrease of arterial partial pressure of oxygen (PaO_2) in the blood of the living organism when the inspired oxygen level reached to the range of 35-60 mmHg (2). Although knowledge on the dosage and method of implementation for IH is not clear, it is generally implemented with varying episodes of lower oxygen levels for 30 minutes. It has been reported that the principle of "moderate hypoxia, few episodes" could increase the effectiveness of IH and could help avoid harmful effects (3).

Many useful effects of intermittent hypoxia implementation have been investigated until today. These studies include that phrenic long-term facilitation, hypoglossal long-term facilitation, ventilatory long-term facilitation, brain-derived neurotrophic factor (BDNF) and serotonin release, erythropoietin, hemoglobin levels and monocyte counts (4,5,6,7,8,9).

CBC is a group of tests that analyse cells circulating in the blood along with white blood cells (WBC), red blood cells (RBC) and platelets (PLT). The methods of implementation of intermittent hypoxia varies according to studies in the literature. In addition, differences are observed between studies for CBC analysis. This study aims to examine the changes in CBC parameters caused by IH implementation at therapeutic dose ranges with a single model. In this way, we think that results will shed light on the expected positive or negative changes in CBC parameters for future studies on IH.

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2. MATERIALS AND METHODS

2.1. Experimental Animals

Sprague Dawley type 7 – 10 weeks old male rats constituting the sample of our study constituted the study sample. Their weight was between 321 and 408 grams. Rats were randomly selected into groups. The sample size of our study was calculated using the PS Power and Sample Size version 3.1.2 Software Program (United States). The data of Linnarsson et al. were used for the sample size calculation (10). When the power analysis of the study was determined to be "p" less than 0.05 ($p<0.05$) and 80% reliability, the sample size was determined as 10.

The experimental animals were divided into two groups.

(1) Study group (n = 5): Intermittent Hypoxia group

(2) Control group (n = 5): Normoxic atmosphere group

During the experiment, rats were kept under controlled environmental conditions (12 hr light/dark cycle, temperature 23°C) and standard laboratory food and water were provided ad libitum until the end of the study. Rats were kept in different cages until the end of the study.

2.2. Ethical Approval

The research was conducted in accordance with the Declaration of Helsinki. Ethical approval for animal experiments was obtained from the Local Ethics Committee of Bezmialem Vakif University (Date: 27.09.2019, No: 2019/227).

2.3. Experimental Procedure

To implement the hypothesis, two completely identical cabinets of 20x30x40 cm were used. The oxygen level, pressure, temperature and humidity sensors were placed that inaccessible to the rats, in the study cabin and insulated cables were taken out of the box and computer connection was established. Arduino UNO (Ivrea, Italy) was used as an intermediate module and sensors were operated on this platform. The oxygen level of the cabin was determined with the Winsen electrochemical cell (Zhengzhou Winsen Electronics Technology Co., Ltd. Zhengzhou, China), placed on the Grove brand sensor card (Seeed Technology Co.,Ltd. Shenzhen, China). The highly sensitive sensor was working in the range of 0 to 25% oxygen concentration. The voltage information sent from the sensor was transformed into its real value via the microcontroller. During the experiments, this information was continuously displayed on a screen. At the same time, the information recorded in the serial port was put into the prepared report.

The control of oxygen ratio in the cabin was provided by nitrogen gas released into the cabin. When the oxygen ratio wants to be reduced to 10%, a 1.5 bar pressure was given from the nitrogen tank compressed into the cabin. The Grove sensor was continuously controlled and the gas flow was stopped when it reached its real value. When there was a decrease in the oxygen rate, air flow was provided from the environment through a cover that could be opened from the outside and so the oxygen value was kept at the desired value. All ports, opening to the outside, were covered with silicone for preventing air passage. Thus, the cables were prevented from being damaged by rats, and the rats are prevented from harming themselves through cables and devices. During the normoxia period, the door of the cabinet was opened and the environment in which the rats were located was provided to atmospheric conditions. It was shown that atmospheric conditions were formed in line with the data from the oxygen sensor as shown in Figure 1. Holes were drilled in the cabin of the control group in order not to disturb the atmospheric weather conditions. In this way, the atmospheric conditions were protected in their environment.

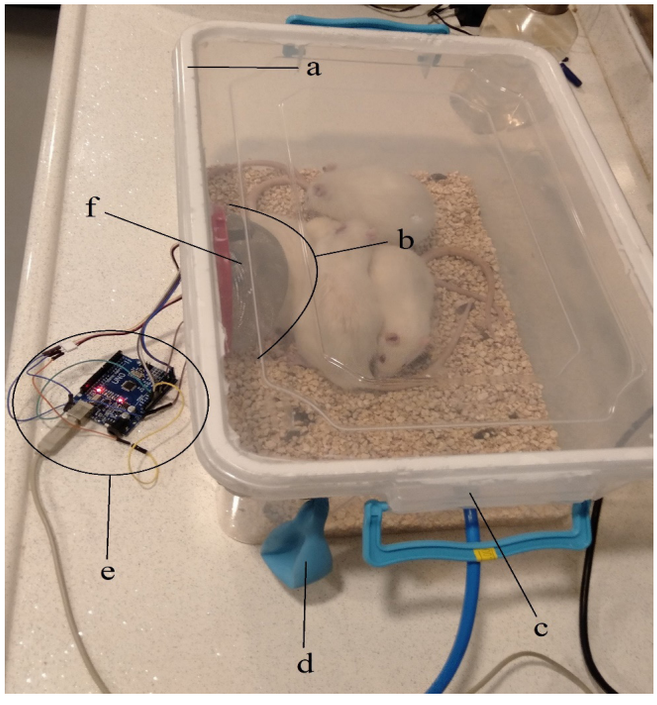

Figure 1. *Cabinet environment of study group rats (a; Insulating material to prevent air passage, b; Protection of sensors, c; Nitrogen gas inlet, d; Pressure balancing balloon, e; Data transfer module, f; Sensors).*

During the experiment, five rats were placed in each cabin. The experiment ended six days and the rats were put in the cabin at the same time each day. Sensors transferred data to the computer every five seconds. The experiment was implemented for 30 minutes, once a day. During the experiment, both rat groups were kept in the box for the same time. The experimental procedure was continued by the same researcher. At the end of the experiment on 6th day, rats were anesthetized with isoflurane in 50% O_2 (balance N_2) for CBC measurement followed by 1 cc blood drawn from the jugular vein. The experimental procedure is shown in Figure 2..

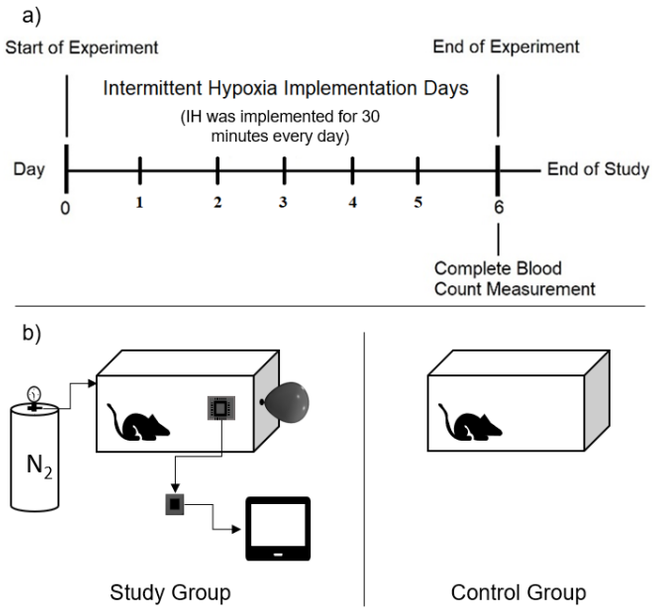

Figure 2. *(a) Flow chart of the experiment; (b) Illustration of the study and control groups.*

2.4. IH Implementation

The study group was exposed to hypoxia every five minutes. Hypoxia was provided by supplying nitrogen gas into the box till oxygen level reaches to 10% (11). The balloon was fixed to the box to balance the pressure of the compressed air and the pressure inside was kept constant. Following each period of hypoxia, the study box was restored to a five-minutes 21% oxygen level, completing the cycle. 15 minutes of hypoxia and 15 minutes of normoxia were implemented to the rats every day during the experiment as shown in Figure 3.

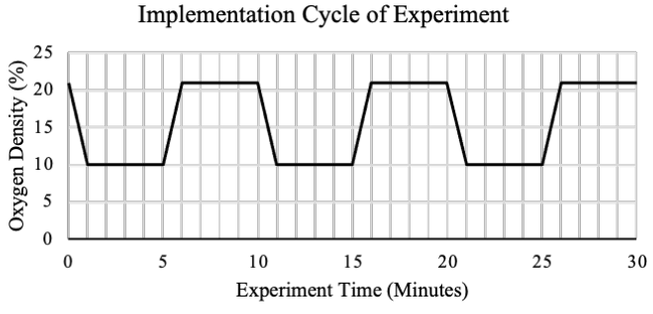

Figure 3. *Graphical form of hypoxia episodes.*

The rats in the control group were provided with a box same to the one in the study group. During the experiment, the rats in the control group were also taken into the experimental boxes. It was aimed not to originate an environment difference between the two groups. While hypoxic time periods were established in the study group box, the control group box was kept in normoxic state for 6 days. The subjects in this group were taken from their cages in the laboratory as in the study group and placed in a box equivalent to that of the study group for 30 minutes. No other procedure was implemented to the rats in this group as shown in Figure 3.

CBC measurements: CBC measurements were made with the same device located in the same laboratory (Abacus Junior Vet 5, Seico Scientific ltd. Punjab, Pakistan). Performed CBC measurements included WBC count, RBC count, lymphocyte (LYM), lymphocyte percentage (LY%), monocytes count (MONO), monocyte percentage (MONO%), granulocytes count (GRA), granulocyte percentage (GR%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc), platelets count (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) (1,12,13,14).

2.5. Statistical Analysis

Descriptive statistical analyzes were used to present the obtained data. The Mann Whitney U test was used to evaluate the statistical significance of the CBC measurement values of the study and control groups. Analyzes were performed using Instat Statistical Package Program (GraphPad Prism Version 8 Software Program San Diego, CA) and $p<0.05$ was considered as statistically significant.

3. RESULTS

One cc of blood was drawn from the rats three hours after the end of the experiment and placed in tubes to use in CBC analysis. The results were listed as expressed as shown in Table 1.

Table 1. *Complete blood count analysis results.*

Parameters CBC Measurement	Study Group Mean ± SD	Control Group Mean ± SD	p
WBC (10^9/L)	14.856 ± 0.69	14.302 ± 0.76	>0.05
LYM (10^9/L)	10.438 ± 1.05	10.628 ± 1.02	>0.05
MONO (10^9/L)	0.99 ± 0.8887	0.204 ± 0.06989	<0.001***
GRA (10^9/L)	3.574 ± 0.832	3.474 ± 0.36	=0.13
LY%	69.38 ± 7.516	74.18 ± 3.473	=0.16
MONO%	6.66 ± 5.953	1.4 ± 0.4637	<0.001***
GR%	23.96 ± 4.862	24.4 ± 3.564	>0.05
RBC (10^9/L)	8.356 ± 0.318	8.592 ± 0.124	=0.09
HGB (g/dL)	15.38 ± 0.497	15.36 ± 0.2074	=0.12
HCT (%)	46.15 ± 1.738	46.76 ± 0.7592	=0.14
MCV (fL)	55.2 ± 0.836	54.4 ± 0.894	=0.18
MCH (pg)	18.4 ± 0.3317	17.88 ± 0.2864	=0.03*
MCHC (g/dL)	33.34 ± 0.61	32.84 ± 0.492	=0.19
RDWc (%)	15.98 ± 0.216	15.82 ± 0.37	>0.05
PLT (10^9/L)	908.4 ± 106.2	872.6 ± 23.64	=0.013*
PCT (%)	0.586 ± 0.0789	0.584 ± 0.01517	=0.007**
MPV (fL)	6,46 ± 0,114	6,66 ± 0,114	=0.02*
PDWc (fL)	33.1 ± 1.072	33.32 ± 0.96	>0.05

(, p<0.05; **, p<0.01; ***, p<0.001, CBC: complete blood count, WBC: white blood cells, LYM: lymphocyte, MONO: monocyte count, GRA: granulocytes count, LY%: lymphocyte percentage, MONO%: percentage of monocytes, GR%: granulocyte percentage, RBC: red blood cells, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDWc: red cell distribution width, PLT: platelets count, PCT: plateletcrit, MPV: mean platelet volume, PDWc: platelet distribution width)*

3.1. MONO and MONO% Values

In the CBC analyzes of the rats, it was observed that the MONO ($p<0.001$) and MONO% ($p<0.001$) values increased significantly in the study group compared to the control group as shown in Figure 4a, 4b.

The mean MONO value was determined as 0.99x10^9/L (±0.88887) in the study group, and 0.204 x10^9/L (±0.06989) in the control group, respectively. In addition, the minimum MONO value was measured as 0.09 x10^9/L and the maximum MONO value was measured as 1.95 x10^9/L. In the control group, the minimum MONO value was measured as 0.0 9 x10^9/L and the maximum MONO value was measured as 0.28 x10^9/L. For the MONO% parameter, the mean value detected in the study group was 6.66% (±5.953), and the determined value in the control group was 1.4% (±0.4637). The MONO% value measured in the study group was determined as minimum 0.6% and maximum 13.3%. In the control group, the measured minimum MONO% value was 0.6% and the maximum MONO% value was 1.8%.

3.2. MCH Values

According to the analysis, the MCH value in the study group increased significantly compared to the control group ($p=0.03$). In the study group, the mean MCH value was determined as 18.4 pg (±0.3317), the minimum was 18.1 pg, and the maximum was 18.8 pg as shown in Figure 4c. In the control group, the mean MCH value was determined as 17.88 pg (±0.2864), the minimum was 17.5 pg, and the maximum was 18.3 pg as shown in Figure 4c.

3.3. PCT Values

It was found that PCT values increased significantly in the study group compared to the control group ($p=0.007$). The mean PCT values were determined as 0.586% (±0.07893) in the study group and determined as 0.584% (±0.01517) in the control group. On the other hand, the minimum PCT value was measured as 0.49% and the maximum PCT value was measured as 0.7% in the study group, while the minimum PCT value was measured as 0.57% and the maximum PCT value was measured as 0.61% in the control group.

3.4. MPV Values

There was a significant decrease in MPV values in the study group ($p=0.02$). The mean MPV values was determined as 6.46 fL (±0.114) and 6.66 fL (±0.114) in the study and control groups, respectively. The minimum MPV value was measured as 6.3 fL and the maximum MPV value was 6.6 fL in the study group. In the control group, the minimum MPV value was detected as 6.5 fL and the maximum MPV value was 6.8 fL as shown in Figure 4d.

3.5. PLT Values

PLT value in the study group increased significantly compared to the control group ($p=0.013$). While the mean PLT value in the study group was determined as 908.4 x10^9/L (±106.2), it was determined as 872.6 x10^9/L (±23.64) in the control group. In the study group, the minimum PLT value was detected as 786 x10^9/L and the maximum PLT value was 1066 x10^9/L. In the control group, the minimum PLT value was measured as 844 x10^9/L and the maximum PLT value was determined as 901 x10^9/L as shown in Figure 4e.

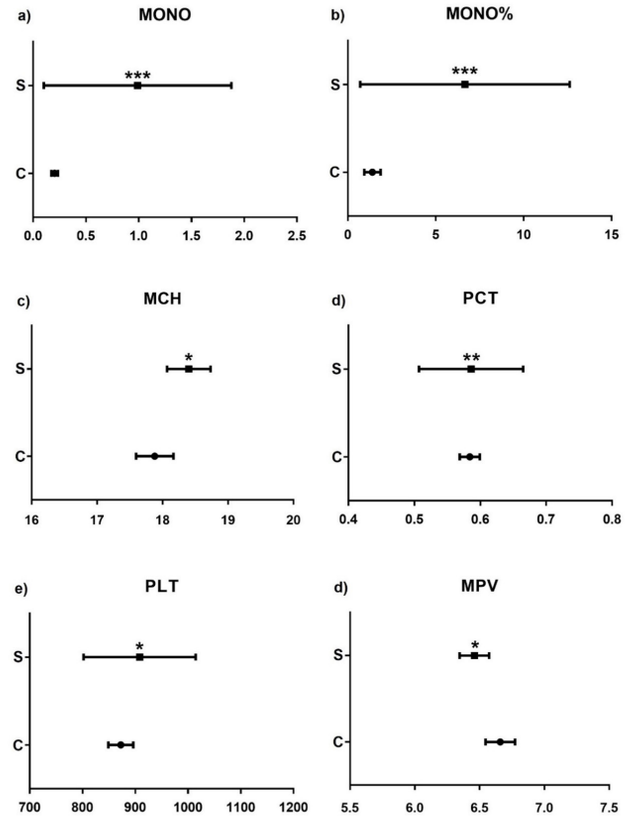

Figure 4. *(a) Monocyte count (MONO) ($p<0.001$); (b) Percentage of monocytes (MONO%) ($p<0.001$); (c) Mean corpuscular hemoglobin (MCH) ($p=0.03$); (d) Plateletcrit (PCT) ($p=0.007$); (e) Platelet (PLT) count ($p=0.013$); (f) Mean platelet volume (MPV) ($p=0.02$); S, study group; C, control group.*

3.6. HGB and HCT Values

There was no significant difference in HGB and HCT values between the control and study groups. The mean HGB values were found as 15.38 g/dL (±0.497) and 15.36 g/dL (±0.2074) in study and control groups, respectively. In addition, the mean HCT values were determined as 46.15% (±1.738) and 46.76% (±0.7592) in study and control groups, respectively.

No significant difference was found between the two groups' WBC, LYM, GRA, LY%, GR%, RBC, MCV, MCHC, RDWc and PDWc parameters as shown in Table 1.

4. DISCUSSION

The purpose of this study is to observe results that IH implementation causes in CBC analysis. Evaluations were made after 10% FiO_2, 5-minutes normoxia-hypoxia cycles,

daily 30-minutes implementations for 6 days. As a result of CBC analysis, changes were found in MONO, MONO%, MCH, PCT, PLT and MPV parameters. While there was a significant increase in the values of MONO, MONO%, MCH, PCT and PLT parameters in the study group, the opposite was found in the MPV value.

In the study, the IH protocol was implemented using a special cabinet. There is no consensus in the literature regarding the IH implementation method in rats. In some studies, this point is not clearly stated in the methodology; In some publications, the use of masks is stated. Since we wanted to eliminate the risk of leakage in the use of masks in the rat, IH was implemented using a special cabin in this study. The cabin used in the research was specially designed by the research team during the preparation phase. With special sensors used in the cabin design, the oxygen density in the cabin was checked and verified before each implementation.

IH implementation does not have a standard protocol. According to literature, FiO_2, normoxia-hypoxia cycles, daily implementation times and total implementation time differ. Considering these differences, it has been observed that beneficial effects can be obtained from IH without pathological effects when moderate hypoxia (9-16% inspired FiO_2) and low cycle number (3-15 episodes per day) are used. On the other hand, severe hypoxia (2-8% inspired FiO_2) and high cycle number (48-2,400 episodes per day) were found causing pathological effects (3). In addition to achieve standardization, it is necessary to reduce the PaO_2 value in the blood from 90 (± 5,5) mmHg to 35 – 60 mmHg (2).

In line with this information, we evaluated the responses of IH implemented in CBC analyzes without causing pathological effects in the study.

Alvarez-Martins et al. reported that chronic IH implementation caused an increase in MONO numbers in rats with obstructive sleep apnea (9). However, chronic IH implementations consist of severe hypoxia (5% inspired FiO_2) and a prolonged implementation period (10.5 h / day). In addition to this study, an increase in monocyte was found in both acute and chronic IH implementations. The increase in MONO, which occurs without any infection, may correlate improvement of the lungs. The reason for this situation is thought to be the physiological response of the body, it is thought to be an infectious response due to hypoxia inducible factor (HIF) synthesis due to IH administration (15,16). The reason of the increase in MONO% ($p<0.001$) parameter was due to the increase in MONO ($p<0.001$).

MCH refers to the amount of hemoglobin per erythrocyte, and low-level MCH has been found to be strongly associated with intensive care admission due to myocardial infarction (17). In a study, low level MCH was found to be highly correlated with mortality rate, and a significant relationship was pointed out between very high level of MCH and mortality (18). In our study, in terms of the CBC analysis, there was no significant difference in hemoglobin and hematocrit levels between the two groups, but it was found that MCH increased significantly in the study group ($p=0.03$). In other words, IH implemented for 30 minutes for 6 days increased the amount of hemoglobin per erythrocyte. The increase of the MCH levels, can be interpreted as increasing the hemoglobin number in the erythrocyte for to meet the oxygen demand of the tissue against the decrease in PaO_2 levels, and supporting the efficient transport and use of oxygen.

McDonald et al. showed that increased PLT levels were demonstrated and related to the hypoxia (19). Berg et al. reported that platelets play an important role in the repair of the lung parenchyma (20). In their studies, it was observed that with the increase of alveolar hypoxia, platelet-derived growth factor-B production increased to protect the lung tissue. Other studies, high levels of PCT were reported in pediatric cases with chronic lung disease, and the PCT value has been reported to show a positive correlation with C – reactive protein (CRP). In addition, in the pediatric mortality studies in the hospital, they were reported that the survival rate was higher in cases with high PCT values (21,22,23). According to our study, increased PCT ($p=0.007$) values can be interpreted as a reaction developed against hypoxia. Recently literature has reported that individuals' who was affected from COVID-19, the MCH levels have diminished (24). Additionally, it was found that the decrease continued until the end of the treatment and continued after the therapy. In our study, we found that the MCH levels increased significantly after IH. We think that alternative IH implementations may provide positive respiratory effects in patients with critically pulmonary diseases. Comprehensive studies associated with IH implementation are needed on this area.

MPV, which indicates the mean size of the platelets in the bloodstream, is one of the indicators of platelet activity (25). There are many studies investigating the change of MPV levels in non-communicable diseases. Slavka et al. defended the relationship between MPV and thromboembolic complications and cardiovascular events (26). Kodiatte et al. have suggested a high-level MPV relationship with diseases such as diabetes mellitus and hypertension, while Przygodzki et al. defended the opposite view (27,28). Kanbay et al. reported that the high MPV values in obstructive sleep apnea associated with the severe hypoxemic periods experienced by these cases (29). In the current study, a decrease was observed in MPV value in rats treated with IH as shown in Figure 4f. This result suggests that MPV may change according to period, duration and severity of hypoxic episodes. There are require to further research on this subject.

Brito et al. were found that there was an increase in HGB and HCT values in adults who were exposed to chronic IH (30). According to the study conducted by Zhang et al. it was observed that there was a significant increase in HGB, HCT and RBC levels in IH implemented rats compared to the control group (31). The experiment period in this study lasted 28 days (31). In our study, there was an insignificant decrease in HCT ($p=0.14$) and RBC ($p=0.09$) parameters compared to the control group. On the other hand, there was

an insignificant increase in the HGB parameter (p=0.12). No significant difference was found in other values measured in CBC analysis. Our experiment completed in 6 days. However, if the duration of IH implementation is extended, we think that HGB, HCT and RCB values may change.

This study contains some limitations. Since saturation monitoring can be performed under anesthesia in rats, saturation measurements could not be performed in our study due to the direct effect of anesthesia. The lack of measurement of blood gas parameters was another limitation. The distance between the evaluation and test laboratories on our campus has prevented the preservation of the samples taken. An additional device could not be purchased due to high costs. On the other hand, since the rats should not enter into hypovolemia, the location of the rats was not changed and this measurement could not be made.

5. CONCLUSION

It has been observed that the parameters of the CBC were evaluated a very limited level in the studies on IH. The target of this study is based on what kind of changes will be discussed in the CBC analysis with IH implementation at therapeutic levels. In this study, changes in CBC parameters were detected with IH, which was applied systematically. Each rat in the study group was exposed to a uniform IH pattern. As a result of IH implementation, significant changes were observed in MONO, MONO%, MCH, PCT, PLT and MPV parameters in the CBC analysis of rats. We think that the effects of therapeutic IH implementation on MONO and MONO% levels may be effective in lung regeneration and lung protection. In addition, our results support that the PLT activity is inhibited by the decrease in the MPV level despite the increase in the PLT level. In this way, we can say that the risk of thrombosis is reduced. The increase in PCT expected due to the decrease in MPV appeared as a normal result. However, the expected reduction in PDWc in correlation with the decrease in MPV was not statistically significant.

The intermittent hypoxia method used in this study, which was defined as “Therapeutic dose” and consisted of moderate hypoxia (9-16% FiO2) – low cycles (3-15 episodes per day), did not cause a pathological effect and gave positive results on CBC. The changes revealed by IH implementation on CBC parameters suggested that this dose protocol used could provide minimum negative effect and maximum gain. This supports the applicability of the IH model in our study in clinical trials under controlled conditions. In future studies, the effects of therapeutic IH implementation on lung tissue healing, increasing blood oxygen transport efficiency and immune system can be investigated in appropriate patient population and clinical conditions.

Considering all these conditions, therapeutic IH implementation may thought to have important effects in terms of lung protection and regeneration. Further research may focus on this point for precising and supporting of this study’ results.

Authors Contributions

Conception and design of the experiments: T.K., M.S. and A.Y.O. Collection, analysis and interpretation of data for the experiments: T.K., M.S., S.U., A.K., H.D. and A.Y.O. Drafting and revising the article critically for important intellectual content: T.K. and A.Y.O. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing Interests

None

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REFERENCES

[1] Guang Y, Jie Z, Feng D, Hui L. Surrogate scale for evaluating respiratory function based on complete blood count parameters. J Clin Lab Anal 2018;32(5):e22385.

[2] Xing T, Pilowsky PM. Acute intermittent hypoxia in rat in vivo elicits a robust increase in tonic sympathetic nerve activity that is independent of respiratory drive. J Physiol 2010;588(Pt 16):3075-3088.

[3] Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol 2014;307(10):R1181-1197.

[4] Baker TL, Mitchell GS. Episodic but not continuous hypoxia elicits long-term facilitation of phrenic motor output in rats. J Physiol 2000;529 Pt 1(Pt 1):215-219.

[5] Ryan S, Nolan P. Episodic hypoxia induces long-term facilitation of upper airway muscle activity in spontaneously breathing anaesthetized rats. J Physiol 2009;587(Pt 13):3329-3342.

[6] Nakamura A, Olson EB Jr, Terada J, Wenninger JM, Bisgard GE, Mitchell GS. Sleep state dependence of ventilatory long-term facilitation following acute intermittent hypoxia in Lewis rats. J Appl Physiol 2010;109(2):323-331.

[7] Lovett-Barr MR, Satriotomo I, Muir GD, Wilkerson JE, Hoffman MS, Vinit S, Mitchell GS. Repetitive intermittent hypoxia induces respiratory and somatic motor recovery after chronic cervical spinal injury. J Neurosci 2012;32(11):3591-3600.

[8] Nagel MJ, Jarrard CP, Lalande S. Effect of a single session of intermittent hypoxia on erythropoietin and oxygen-carrying capacity. Int J Environ Res Public Health 2020;17(19):7257.

[9] Alvarez-Martins I, Remédio L, Matias I, Diogo LN, Monteiro EC, Dias S. The impact of chronic intermittent hypoxia on hematopoiesis and the bone marrow microenvironment. Pflugers Arch 2016;468(5):919-932.

[10] Linnarsson S, Björklund A, Ernfors P. Learning deficit in BDNF mutant mice. Eur J Neurosci 1997;9(12):2581-2587.

[11] Wen MH, Wu MJ, Vinit S, Lee KZ. Modulation of serotonin and adenosine 2A receptors on intermittent hypoxia-induced respiratory recovery following mid-cervical contusion in the rat. J Neurotrauma 2019;36(21):2991-3004.

[12] Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H, Hohlfeld R. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 1999;189(5):865-870.

[13] Lommatzsch M, Niewerth A, Klotz J, Schulte-Herbrüggen O, Zingler C, Schuff-Werner P, Virchow JC. Platelet and plasma BDNF in lower respiratory tract infections of the adult. Respir Med 2007;101(7):1493-1499.

[14] Yamamoto H, Gurney ME. Human platelets contain brain-derived neurotrophic factor. J Neurosci 1990;10(11):3469-3478.

[15] Scott MKD, Quinn K, Li Q, Carroll R, Warsinske H, Vallania F, Chen S, Carns MA, Aren K, Sun J, Koloms K, Lee J, Baral J, Kropski J, Zhao H, Herzog E, Martinez FJ, Moore BB, Hinchcliff M, Denny J, Kaminski N, Herazo-Maya JD, Shah NH, Khatri P. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. Lancet Respir Med 2019;7(6):497-508.

[16] Colgan SP, Furuta GT, Taylor CT. Hypoxia and innate immunity: keeping up with the HIFsters. Annu Rev Immunol 2020;38:341-363.

[17] Huang YL, Hu ZD. Lower mean corpuscular hemoglobin concentration is associated with poorer outcomes in intensive care unit admitted patients with acute myocardial infarction. Ann Transl Med 2016;4(10):190.

[18] Honda H, Kimachi M, Kurita N, Joki N, Nangaku M. Low rather than high mean corpuscular volume is associated with mortality in Japanese patients under hemodialysis. Sci Rep 2020;10(1):15663.

[19] McDonald TP. Platelet production in hypoxic and RBC-transfused mice. Scand J Haematol 1978;20(3):213-220.

[20] Berg JT, Breen EC, Fu Z, Mathieu-Costello O, West JB. Alveolar hypoxia increases gene expression of extracellular matrix proteins and platelet-derived growth factor-B in lung parenchyma. Am J Respir Crit Care Med 1998;158(6):1920-1928.

[21] Draz IH, Shaheen IA, Youssef EA. Platelets count and platelets indices; mean platelet volume and plateletcrit in pediatric chronic lung disease. Egypt Pediatric Association Gaz 2020;68:69.

[22] Sayed SZ, Mahmoud MM, Moness HM, Mousa SO. Admission platelet count and indices as predictors of outcome in children with severe sepsis: a prospective hospital-based study. BMC Pediatr 2020;20(1):387.

[23] Golwala ZM, Shah H, Gupta N, Sreenivas V, Puliyel JM. Mean platelet volume (MPV), platelet distribution width (PDW), platelet count and plateletcrit (PCT) as predictors of in-hospital paediatric mortality: a case-control study. Afr Health Sci 2016;16(2):356-362.

[24] Zhao G, Su Y, Sun X, Cui X, Dang L, Zhao L, Tan X, Wang H, Yang M. A comparative study of the laboratory features of COVID-19 and other viral pneumonias in the recovery stage. J Clin Lab Anal 2020;34(10):e23483.

[25] Tsiara S, Elisaf M, Jagroop IA, Mikhailidis DP. Platelets as predictors of vascular risk: is there a practical index of platelet activity? Clin Appl Thromb Hemost 2003;9(3):177-190.

[26] Slavka G, Perkmann T, Haslacher H, Greisenegger S, Marsik C, Wagner OF, Endler G. Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease. Arterioscler Thromb Vasc Biol 2011;31(5):1215-1218.

[27] Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK, Lakshmaiah V. Mean platelet volume in type 2 diabetes mellitus. J Lab Physicians 2012;4(1):5-9.

[28] Przygodzki T, Luzak B, Kassassir H, Mnich E, Boncler M, Siewiera K, Kosmalski M, Szymanski J, Watala C. Diabetes and hyperglycemia affect platelet GPIIIa expression. Effects on adhesion potential of blood platelets from diabetic patients under in vitro flow conditions. Int J Mol Sci 2020;21(9):3222.

[29] Kanbay A, Tutar N, Kaya E, Buyukoglan H, Ozdogan N, Oymak FS, Gulmez I, Demir R. Mean platelet volume in patients with obstructive sleep apnea syndrome and its relationship with cardiovascular diseases. Blood Coagul Fibrinolysis 2013;24(5):532-536.

[30] Brito J, Siqués P, León-Velarde F, De La Cruz JJ, López V, Herruzo R. Chronic intermittent hypoxia at high altitude exposure for over 12 years: assessment of hematological, cardiovascular, and renal effects. High altitude medicine & biology 2007;8(3):236–244.

[31] Zhang CY, Zhang JX, Lü XT, Li BY. Effects of intermittent hypoxic exposure on the parameter of erythrocyte and serum hypoxia inducible factor-1 alpha and erythropoietin levels. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2009;25(10):932-934.

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