Milosava. M. Djelkapic*[1], Bozidar. D. Nesevic[1],
Snezana. Dj. Sunderic[2], Snezana. J. Lesovic[3]

# EFFECTS OF ALTITUDE TO BLOOD PARAMETERS

**Abstract:** Reducing of partial pressure of oxygen in the air leads to a reduced arterial oxygen saturation and increases secretion of erythropoietin, wich stimulates erythropoiesis.

Study included 63 healthy children aged 7 years,devided into 3 groups. I group consists of 21 children from suburb of altitude of 370 m, II group of 22 children from the village on 822 m, III group of 21 children from the town on 411 m. Complete blood count was determined on a Hematology analyzer HmX ( Beckman Coulter).

Statistical analysis of data showed that children from II group have a higher average values of erythrocytes than children from the I ($p<0.01$) and III ($p < 0.05$),and the higher values of hemoglobin than children from I and III ( $p<0.01$). II and III group had lover average values of leukocytes,related to I group ($p <0.01$).

The boys from II group had more erythrocytes and hemoglobin then boys from I ( $p<0.01$), and more platelets than boys from the III group ($p<0,05$), but less leukocytes than boys from I and III group ($p < 0.01$). Girls from II group had higher values of erythrocytes, hemoglobin, hematocrit and platelets ( $p<0.01$) than the girls from I group.

It is not found statistically significant variation of parameters between boys and girls within the same group nor erythrocytes and hemoglobin of I and II and platelets of all groups ($p>0.05$).

Results show that stay in the village is usefull for stimulation of erythropoiesis.

**Key words:** altitude, erythropoietin, Complete Blood Count, hemoglobin.

* [1]Deparment of Laboratory Diagnostics, [2] Department of occupational safety and health, Health Center, Uzice, [3] Special Hospital Zlatibor, The Serbia. E-mail: mila13@ptt.rs

## *Introduction*

Blood is a liquid tissue that has the role of the most important transport system in organism and consists of blood plasma with blood cells erytrocytes (RBC), leucocytes (WBC) and platelets (PLT) suspended. Special rouls of blood are mostly conected to its cells so that the blood diseases are almost always consequences of the changes in blood cells but the relationship between blood cells and plasma (hematocrit) is in very arrow borderlines.

Intrauterine hematopoiesis is going on in liver, spleen, bone marrow and lymphatics but after the bearth it is going on in active red bone marrow, thymus, lymphatics and spleen.

Immediatelly after the bearth active bone marrow is placed in all bones because in the first four years of life the mass of blood cells increase so that the actual volumen or bone marrow can not fulfill the needs of normal erytropoiesis. Because of that when there is a need of increased production of blood cells in that term of life a new haematopoiesis starts in liver and spleen what is known as embryonic haematopoiesis. After that period of life the increase of the volumen of bone marrow is faster and bigger than needs for normal haematopoiesis so that the lipid cells start to replace the haematopoietic cells in long bones, and in puberty is replaced all the haematopoietic tissue in the bone marrow of all long bones.That is the reason why the haematopoiesis in adults is active only in bone marrow of ribs, sternum, vertebras, bones of cranium, pelvis and in the proximal parts of humerus et femur. In adults the volume of all bone marrow is almost twice bigger than the volume of the liver and it owns a big reserve capacity.When there is a need of increased production of blood cells lipid marrow can converte into active red bone marrow. This way is increased the number of the stem cells of some bloodlines and together with fast maturing end differentiation of cells and its fast cross into blood contribute to needed increase of production of blood cells. It is found that the erythropoiesis in bone marrow can increase 6-8 times, plateletopoiesis 8 times and granulocytopoiesis 4 times than normal. There is normally the balance between the number of lost cells in perifer blood and the number of the cell which at the same time go from bone marrow to blood so that the number of blood cells in perifer blood is changed in narrow borderline. There are normally only mature, functional cells in perifer blood: erythrocytes, granulocytes, lymphocytes, monocytes and platelets (1).

Communal stem cell of haematopoiesis (pluripotent) under the influence of the factors of microsurrounding products stem cells which are predetermined for determine bloodlines, and they devide and differentiate under the influence of its poetins (humoral stimulating factors) to mature blood cells. The process of maturing erythrocytes from thair stem cells is called erythrocytopoiesis. Normally it is produced daily about 3 milliards of erythrocytes in kilogram of body mass, cause almost the same number of erythrocytes daily end. As the mature erythrocyte is a cell without a nucleus and can not synthesize proteins, it is necessary ( in the time of maturing young cells of erythrocytes) to be synthesized all proteins in them that are necessary for the function of mature erythrocyte by the time of 120 days . Normal erythrocytopoiesis it is needed special conditions in bone marrow (temperature, hormons, etc.) as well as build maters (iron and aminoacids). The regulation of intensity of erythrocytopoiesis depends on the level of partial pressure of oxygen in kidneys. The reduction of this pressure increase the erythrocytopoiesis and the increase of it reduces the erythrocytopoiesis. Unipotent cell for erythrocytic bloodline become from the pluripotent stem cell, and it differenttiates under the influence of erythropoietin to the first morfological known cell of erythrocytic bloodline-proerythroblast. The influence to erythrocytopoiesis has hypothalamus, androgen hormons, cobalt (stimulating) estrogens (inhibiting). Maturing of stem cell is helped by iron, proteins, vitamins of B complex, C, E, copper et zinc. The most important factors of erythrocytopoiesis are iron, vitami $B_{12}$ and folic acid (2). The most important function of erythrocyte is acceptance of oxygen in lung and its delivering in tissues (3). The main consistents of erythrocyte is hemoglobin (32% of 35% of dry residue of erythrocyte) so it is considered as membranic sack for hemoglobin. The membrane of the erythrocytes can be got by osmotic lysis when the contents leave the cell, and the rest membrane structure is signed as „shadow“ (ghost) of erythrocyte, what is used in authomatic count of erythrocytes. Hemoglobin has many important roles and the most important of them is transport of molecular oxygen from lung to tissues, transport of carbon dyoxide from tissues to lung and function of buffer in regulation of pH of blood.

Leucocytes are mobile cells of immunological sistem of organism.there are normally three classes of leucocytes in blood: granulocytes, lymphocytes and monocytes. By morphological characteristics granulocytes are devided in neutrophiles, basophiles and eosinophiles. By immunological and functional characteristics lymphocytes are devided in two types: B and T lymphocytes. B- lymphocytes syntetisize immunoglobins and are responsible for humoral immunity, and T-lymphocytes for cellular immunity. Among these two group of lymphocytes there is possible a great number of immunal reactions and responses. The most of all leucocytes are neutrophils (62%), and their most important role is destroying of bacterys.

Platelets are the smallest cells of blood, and their most important functions are in haemostasis, maintaining of structure of endotel, detoxication, phagocytosis, inflamattory reactions, rejection of transplant, thromboembolic phenomenons and metastasis of carcinoma (4,5).

## *The aim*

The aim of the study was to aprove the influence of altitude on parameters of Complete Blood Count ( CBC).

## *Clinical matherial and methods*

Study included 63 healthy children aged 7 years, divided into 3 groups. I group consists of 21 children from suburb Sevojno of altitude of 370 m, II group consists of 22 children from the village Kremna on altitude of 822 m and III group consists of 21 children from the town Uzice on the altitude of 411 m. It was used the sample of phleb blood with $K_3$EDTA as anticoagulant. Complete Blood Count was determined on a Hematology analyzer HmX (Beckman Coulter).

The Coulter´s system of determining blood cells is based on registration and measurement of changes in electric resistance which is produced by blood cells suspended in conductive solution while go through orifice by average diameter (aperture). By both side of aperture there is one electrode and electric current between them.By passing of cell through orifice electrical resistance between electrodes increases, what is registered as electric impulse. The number of impulses correlate to number of cells, and the value of impulse is determined by the size of the cell. Haematology analyzer HmX use triplicated counting in three consecutive periods of four seconds and after analyzing of datas of all countings gives one result.This number of cells in a semple of blood is almost hundred times bigger than mycroscopic counting and statistic false reduces approximately ten times. The cells are devided by size in many regions: leucocytes 35-450fL, erythrocytes 36-360fL, platelets 2-20fL. HmX Haematology analyser is based on VCS technology ( Volume, Conductivity, Light-Scatter) for differentiation of leucocytes. In this technology it is achieved more exact analysis of morphology of cells ( by combination of different principes of measurement). Together with measurement of volume of cells it is included measurement of intensity of light scatter and high frequent conductivity. Measurement of conductivity gives informations about size, internal structure and density of cells. Laser light scatter (of the light which goes through cells) is related on surface morphology of cell, her granulation and does not depend on size of cell. This technology is specially important for differentiation of cells of white bloodline.

Determination of hemoglobin is based on hemoglobincyanide method, which is referent method suggested by International Committee for Standardisation in Haemathology (ICSH). Release of hemoglobin from erythrocytes is achieved by using of lysing reagent which haemolyses, and released hemoglobin is converted to stabil pigment . This pigment is determined by spectrophotometry and its apsorbency is proportional to concentration of hemoglobin in sample in g/L.

## *Statistical analysis*

Data were statistically analysed. Mean, standard deviation, Student´s t-test were used for statistical analysis. High significant difference is signed as $p < 0.01$, significant difference is signed as $p < 0.05$, and not significant difference as $p > 0.05$.

## *Results*

By this exploration within sistematic examination of children for the first class of the primary school it is determined the number of erytrocytes (RBC), leucocytes (WBC), platelets (PLT) as well as value of hemoglobin (HGB) and hematocrit (HCT). Study included 63 healthy children aged 7 years, devided into 3 groups by the altitude of the place of living. I group consists of 21 children from suburb Sevojno of altitude of 370 m, II group of 22 children from the village Kremna on 822 m (mountain Tara), III group of 20 children from the town of Uzice on 411 m.

Statistical analysis of data show that children from II group have a higher average values of erythrocytes than children from the I ( high statistically significant difference, $p < 0.01$) and III (statistically significant difference, $p < 0.05$), and the higher values of hemoglobin than children from I and III ( $p < 0.01$). II and III group had lover average values of leukocytes, related to I group ($p < 0.01$). The results are shown on the table 1.

*Table 1. Values of parameters of CBC et groups (mean ±standard deviation)*

| PARAMETER | GROUP | |
|---|---|---|
| | I | II |
| RBC × $10^{12}$/L | 4,46 ± 0,26 | 4,76 ± 0,29 |
| HGB (g/L) | 125,23 ± 5,41 | 131,72 ± 5,62 |
| HCT (L/L) | 0,035 ± 0,016 | 0,38 ± 0,019 |
| WBC ×$10^{9}$/L | 8,78 ± 1,95 | 7,44 ± 1,24 |
| PLT ×$10^{9}$/L | 316,6 ± 67,11 | 335,68 ± 47,93 |
| n | 21 | 22 |

n – number of examined

Higher value of hemoglobin in II group than in I group ($p < 0,01$) are shown on figure 1.

The boys from II group have more erythrocytes and hemoglobin then boys from I group ( $p<0.01$), and more platelets than boys from the III group ($p<0,05$), but less leukocytes than boys from I and III group ($p < 0.01$). The results are shown on the table 2.

*Table 2. Values of parameters of CBC et groups of boys (mean ±standard deviation)*

| PARAMETER | SUBGROUP | |
|---|---|---|
| | $I_b$ | $II_b$ |
| RBC × $10^{12}$/L | 4,47 ± 0,27 | 4,8 ± 0,37 |
| HGB (g/L) | 122,75 ± 5,606 | 131,4 ± 5,87 |
| HCT (L/L) | 0,348 ± 0,016 | 0,382 ± 0,0203 |
| WBC ×$10^9$/L | 9,26 ± 1,95 | 7,22 ± 0,97 |
| PLT ×$10^9$/L | 325,12 ± 43,87 | 323,7 ± 37,76 |
| n | 8 | 10 |

n – number of examined; b- boys

Girls from II group have higher values of erythrocytes, hemoglobin, hematocrit and platelets than the girls from I group ( $p<0.01$). The results are shown on the table 3.

*Table 3. Vallues of parameters of CBC et groups of girls (mean ±standard deviation)*

| PARAMETER | SUBGROUP | |
|---|---|---|
| | $I_g$ | $II_{dg}$ |
| RBC× $10^{12}$/L | 4,49 ± 0,25 | 4,73 ±0,16 |
| HGB (g/L) | 126,76 ± 4,67 | 132 ± 5,63 |
| HCT (L/L) | 0,0358 ± 0,013 | 0,385 ± 0,018 |
| WBC×$10^9$/L | 8,48 ± 1,89 | 7,63 ± 1,37 |
| PLT×$10^9$/L | 287 ± 71,45 | 347,9 ± 42,02 |
| n | 13 | 12 |

n – number of examined; g- girls

It is not found statistically significant variation of parameters between boys and girls within the same group nor between values of erythrocytes and hemoglobin of I and III group nor between values of platelets of all groups ($p>0.05$).

Results show that stay in the village is usefull for stimulation of erythropoiesis.

## *Discussion*

On the high altitude where the oxygen level in the air is very low it is not transported enough oxygen in tissues, so that the erythrocytes are producted so fast to increase their number. It is obvious that the production of erythrocytes does not depend on their concentration in blood, but it depends on their functional abillity to transport oxygen to tissues as much as it is needed. Total pressure of all gasses in the air (barometric pressure) reduces while the altitude increases. Reduction of barometric pressure is the main reason of all problems which are caused by hypoxia on high altitudes. By reducing barometric pressure it is reduced the pressure of oxygen proportionally, and it always consist of less than 21% of total barometric pressure. When the function of mechanism of erythropoietin is normal, hypoxia causes increased secretion of erythropoietin and it stimulate the production of erythrocytes . When a man is exposed to atmosphere with low concentration of oxygen, the production of erythropoietin starts after a few minutes or hours, and the maximum is reached within 24 hours. But new erythrocytes are present in blood after five days. According to this and other researchs it is considered that the main effect of erythropoietin is to stimulate the production of erythroblasts from haematopoietic cells of bone marrow. When proerythroblasts become erythropoietin helps them to pass through all erythroblastic developing phases faster than usual, and this way helps the production of new cells. It lasts as long as man stays in the atmosphere with low level of oxygen or until it is producted enough number of erythrocytes which transport enough oxygen to tissues. After that the level of erythropoietin reduces to the value which can maintain the number of erythrocytes on needed level . When man is moved from the low oxygen atmosphere , the value of oxygen that is transported to tissues increases above normal value.That is why the erythropoietin production stops immediatelly, and in the next few days the production of erythrocytes reduces completely, and stays this way until the destruction of enough erythrocytes and until the level of oxygen transported to tissues reach normal level again (6). Hypoxia is a main stimulans for encrising of production of erythrocytes, of concentration of haemoglobin, hematocrit and volumen of blood. The increase of haemoglobin and volumen of blood is very slow, so that it is not significant to the end of the second of third week. In almost whole month it reaches half of value and is completely developed in a few months.

Our results shows that levels of erythrocytes, haemoglobin, hematocrit and platelets are higher at the group of children that live on the area of Tara mountain, on the altitude of 822m, than the other two groups, and the number of leucocytes is lower. One of the reason for that is the fact that stay in higher altitude stimulates erythropoiesis, what is often used for sportists condition cause the concentration of haemoglobin starts to increase in the first days of stayng in high altitude mostly because of increasing of haemoglobin and because of haemoconcentration secundary. The increased erythropoiesis increase the amount of haemoglobin so that the level of oxygen on liter of blood in aclimated person on altitude of 4500 meters is the same as in person on the sea level. The increased amount of haemoglobin in aclimated person make possible that one liter of blood in hogh altitude can hold out the same level of oxygen as in the sea level ( unaclimated person). There are datas that in the process of aclimatisation the level of myoglobin in muscle and the number of capillarys increase what also helps the using of oxygen. Although many researches are done to define optimal conditions of aclimatisation, that matter is not solved completely until now. According to nowadays results it can be supposed that with appropriate training on high altitude it is attained extra stimulans and this way is increased aerobic capacity as well as ability for long term load on lower altitude. Optimal last of training on high altitude is three weeks, or longer if it is possible (7). The faster accomodation can be attained by often trainings on high altitude. Training with long term load under extra influence of hypoxia on high altitude lead to higher increase of ability for lasting load than the same training on sea level (8).

It is known that reducing of concentration of hemoglobin and hematocrit are most important markers and the number of erythrocytes is secundarly haematological marker in diagnostic of anaemia (9,10). As we have proved by our research lower values of these parameters in children which lives on lower altitude it is recommendable stay on higher altitude in order to stimulate erythrocytopoiesis, cause the tissue hypoxia stimulates synthesis of erythropoietin no matter if became be cause of reduced saturation of arterial blood by oxygen (because of reduced partial pressure of oxygen in atmospheric air) or reduced capacity of oxygen in blood in anemia (11).

## *Conclusion*

Our research showed that higher altitude has stimulative effects to erythrocitopoiesis and plateletpoiesis. Considering the fact that examined children are lasting settled in the areas that are included and that high significant difference is found between the groups of children where the difference in altitude of places of living is more than 400 m, it is left the possibility of research of period of time that is needed for making appearance of this effects at children which changes the place of living and the smallest changes of altitude that are needed for appearance of this effects.

## *References*

1) Stefanovic S., Interna medicina. Medicinska knjiga Beograd–Zagreb,1989; 11: 1085–1090.
2) Petrovic M, Dopsaj V, Rajic M, Milojevic Z., Laboratorijska hematologija, Beograd, 2002; III: 77-83.

3) Liberman M, Marks DA, Smit K., Marksove osnove medicinske biohemije, klinički pristup. Data status, Beograd, 2008; 5: 71-73.

4) Koraćevic D, Bjelakovic G, Djordjevic BV, Nikolic J, Pavlovic DD, Kocic G., Biohemija, Savremena administracija, Beograd, 2000; XII; 940-955.

5) Baklaja R, Elezovic I, Miljic P, Mikovic D, Lucic MA., Laboratorijska dijagnostika poremećaja hemostaze. Interlab exim Beograd 2008; I: 8-10.

6) Guyton A., Medicinska fiziologija, Medicinska knjiga Beograd–Zagreb, 1989; 4: 59-69.

7) Medved R., Sportska medicina, Jumena, Zagreb, 1987; III: 141-144.

8) Melerovic H, Meler V. Trening biološki i medicinski principi i osnovi treninga. Beograd 1975; 79-83.

9) Vukusic Z, Macukanovic-Golubovic L., Diferencijalna dijagnostika sindroma anemije. Sven-Nis 2007; I: 15-23.

10) Dopsaj V, Spasojevic-Kalimanovska V, Marisavljevic D, Terzic-B, Memon L. Osnovi laboratorijske dijagnostike i lecenja anemija. Farmaceutski fakultet, Beograd 2006; III: 72-84.

11) Stefanovic S. Specijalna klinička fiziologija. Medicinska knjiga Beograd–Zagreb, 1990; 13: 671.