Evaluation of Oxidative Stress

M. Arif AKŞİT
Neonatology Department of Osmangazi University Medical Faculty, Eskişehir, TURKEY

SUMMARY

The main structure of human body is cells. To evaluate the function of the cell (especially mitochondria) is nowadays indirectly estimated from the perspective of blood. The values are different in arterial, capillary, venous blood and in intercellular structure. In order to make a correct estimation, all blood values must be altogether discussed under the patronage of clinical evaluation (including neurological, respiratory and other organ system functions, also concerning gut/liver, immune response).

Blood gases are classified as; a)Blood gases; pH, pCO_2, pO_2, b)Oxygenation: ctHb (Total blood hemoglobin concentration = cO_2Hb-oxy + cHHb-deoxy + cCOHb-carboxy + cMetHb-met), Hctc, sO_2 (Make correlation with ctHB, oxygen saturation = cO_2Hb/cHHb + cO_2Hb), FO_2Hb (Oxyhemoglobin ratio = cO_2Hb / cO_2Hb + cHHb + cCOHb + cMetHb), FHHb, FmetHb, FetalHb, c)Electrolytes: Na, K, Ca, Cl, d)Metabolic values: Glucose, lactate, bilirubin, mOsm, e)Status of oxygen: ctO_2 (Content = Hb (g/dl) x 1.34 ml O_2 / g Hb x saO_2 x (0.003 ml O_2/mmHg/dl), p50, f)Acid-base status: cBase, $cHCO_3$, ABE, SBE, AG (Anion gap = [Na + K] – [Cl + HCO_3]).

The values will be taken arterial and venous simultaneously. After the treatment the values can be affected between 2-5 minutes. If you don't obtained any response, then change your approach. Don't just give intravenous fluid, but make reperfusion, prevent the baby from ischemic perfusion complications and edema.

The values are not taken individually. We have to discuss the correlations with the concerning parameters. E.g. baby A with paO_2 85 mmHg, saO_2 95%, Hb 7 g/dl, is more hypoxemic than the other baby B with paO_2 55 mmHg, saO_2 85%, Hb 15 g/dl. CtO_2 is 8.9 in baby A, but in baby B 17.1 mlO_2/dl.

All for one, one for all will be the main topic for evaluation of blood gases. All the components will be systematically examined and must be correlated with the clinical findings.

The main unit for living organisms is cell. The vital importance is primarily the viability of the cell. All organ systems were established for proper cell functions. The functional status of the cell have to be in good coordination and correlations with the organ systems. For oxygenation at least respiratory, circulation, metabolism, hematological and osmolarity of the body must altogether work well. One cell must be in coordination with all the body and with other cells. Therefore the organ systems must be in balance.

We cannot directly look inside of the cell. We can only estimate the functions, by clinically (activity, cerebral functions etc), by equipments like EEG, EKG etc., by the balance of the living status. The main tool for evaluation of the cell is nowadays by the blood gases.

In the presence of alkalosis or acidosis, one must think that the defense mechanisms are in action. Due to the systemic inflammatory/oxidative response, several unexpected events take place. The main goal is to protect the cell. If the condition is inevitable, then the first step is to overcome the first stage of hypoxia (Table 1), before the dysfunction begins.

There is a close correlation between the causative factor (e.g. oxidative stress) and the tissue reactions. This interaction is indicated at the Table 2. There are at least 9 different clinical presentations of problems (9 is the severest, 1 is the slightest). X-ray, ultrasound and several other diagnostic methods are used to get information about tissue changes. Severities of the conditions are numbered 1 to 9 levels. Tissues in organ systems are also vulnerable at different severity.

In oxidative stress syndrome multiple organ involvement is noticed. CNS injury (HIE) is occurred in 72%, renal in 42%, gastrointestinal system in 29%, myocardium in 29% and pulmonary in 26% of the infants (1).

Prevention always comes before treatment. As you can see in the Table 2, blood gases may indicate severe acidosis, but clinical picture may be

Corresponding Author: *M. Arif AKŞİT*
Neonatology Department of Osmangazi University Medical Faculty, Eskişehir, TURKEY
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Table 1. The Evaluation of the Cell/Tissue Status

STAGE		STATUS FUNCTION
1.	Biological variation	Variations between the gestational ages and infants.
2	Physiological adaptation	Adaptation mechanisms, stimulus and feedback can control the body.
3	Functional disturbance Metabolic activity increases.	Increase in respiration, deep breathing, heart rate etc. No any injury.
4	Compensation period	Compensatory phase of acidosis and alkalosis. Metabolic problems.
5	Reaction of tissues started	Vasoconstriction, pooling, interstitial edema, central flowing of blood and systemic inflammatory reactions started.
6	Disturbances begin	Cellular functions will be delayed, halted, ineffective and reactive states (e.g. Hypoxic Ischemic Encephalopathy (HIE) begin.
7	Degeneration	Vacuolar, hydropic cells and vasogenic edema develops. Histopathological findings are noticed. Changes in mitocondria
8	Rupture of the membranes	Erythrocytes; burr and acantocytosis or degenerated. Cell organelles are in the circulation, bursting of cells by complement.
9	Tissue reactions edema, Graft Versus Host, fibrosis.	Tissue reactions, degenerations, hemorrhages, scleredema, cytostatic
10	Cell and/or tissue death	Lyses of erythrocytes, necrosis.

Table 2. The Correlation of Tissue Reactions and Severity of the Cause (9 point is the severest, 1 point is the slightest condition)

SEVERITY OF	Severe	5	6	9
THE CAUSATIVE	Medium	2	4	8
FACTOR	Slight	1	3	7
		Slight	Medium	Severe
		DIFFERENT	TISSUE	REACTIONS

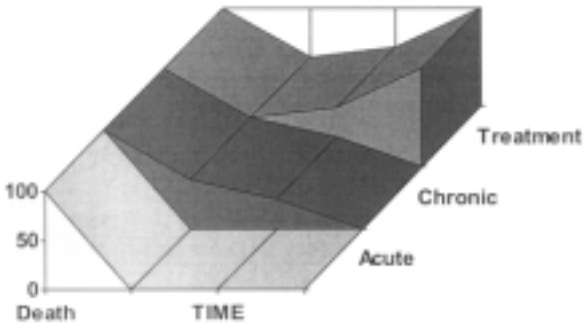

Figure 1. *The duration of tissue reactions in acute, chronic and treatment phases. (100 value means healthy, 0 means death)*

mild. It's vice versa. Therefore clinical evaluation must be carefully performed, not once, but for every 15 minutes. After the perfusion, we can aware of the change within 2 minutes, and in 10 minutes the action can be fully noticed. Try to prevent the baby from oxidative stress, not to treat the hypoxia. First rule is not to be harmful (primum non nocera) for every medical application.

If we consider the time factor, the reactions can be categorized as acute, sub-acute or chronic.

When the problems develop very fast and severe, death is inevitable before the tissue reactions develop. In chronic state or in the case of treatment, the symptoms and clinical findings (tissue reactions = systemic inflammatory and/or oxidative response syndrome) were encountered. The healing have to be needed a time. This duration is symbolized in Figure 1.

The clinical features, like cyanosis, inactivity, edema etc. can be noticed in the condition of the compensation, the critical period. But nowadays the main important issue is the estimate the oxidative stress before symptomatic state. Hypoxia triggers the other mechanisms. In the compensation period, it's hard to estimate the clinical severity. We have to go into cell to evaluate. But at current practice, it's not possible to get recordable values from outside of the cell membrane. They are indirect values.

Parameters of blood gases can be classified in 4 parameters. They are; a) Blood gases; pH, pCO_2, pO_2, b) Oxygenation: ctHb (Total blood hemoglobin concentration), Hctc, sO_2 (oxygen saturation), FO_2Hb (Oxyhemoglobin ratio), FHHb, FmetHb, FetalHb, c) Electrolytes: Na, K, Ca, Cl, d) Metabolic values: Glucose, lactate, bilirubin, mOsm, e) Status of oxygen: ctO_2 (Content), p50, f) Acid-base status: cBase, $cHCO_3$, ABE, SBE, AG (Anion gap).

Blood Gases: 1) Oxygen: Atmospheric oxygen

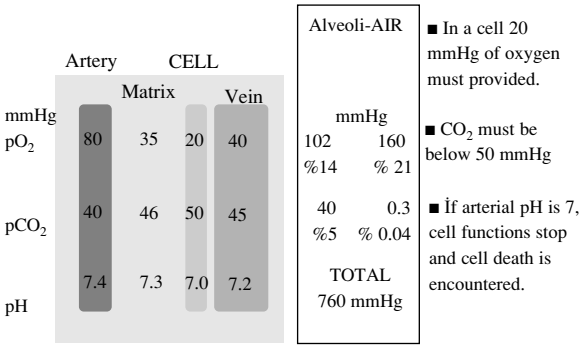

Figure 2. *Oxidative pressures in tissues (3).*

pressure which is 160 mmHg is needed to be reduced at 20 mmHg in the endoplasm (Figure 2). For the passage of oxygen, from alveolar space to blood, 11 mmHg gas pressure difference is required. This pressure difference must be 24 mmHg in edema or premature state. Ventilation to perfusion ratio is not 0.8 for every condition. Hypoventilation, inter-alveolar space widening (prematurity is a factor), especially inter-alveolar shunts, atalectasis, closing capacity, less residual volume problems and surfactant insufficiency create problems in oxygenation. The oxygen exchange can be performed 1/3 of time of ventilation. This means that, giving oxygen to a baby by ventilators, doesn't mean that you can increase the pO_2 levels of the blood. Since PaO_2 reflects only free oxygen molecules dissolved in plasma and not those bound to hemoglobin. PaO_2 cannot tell us 'how much' oxygen is in the blood, for that you need to know how much oxygen is also bound to hemoglobin, information given by the SaO_2 and hemoglobin content (2).

2) Carbon dioxide: Organic substances are composed of carbon. There are chemical bonds between the carbon atoms. These bonds are transferred to ATP at the mitochondria. The inorganic CO_2 is the end result of energy metabolism. This must be carried to the atmosphere from the cell. For diffusion 1 mmHg pressure difference is enough. CO_2 is 20 times more potent than O_2. Therefore the main problem is oxygen transport. If CO_2 is increased in the blood, the tissue is badly damaged. But in Heldan effect CO_2 is also transported by the hemoglobin. The diffusion period is nearly takes 2/3 of ventilation. It's mainly in 1/3, but because of HCO_3 buffering system, it needs time for transportation (4).

3) pH: Function of the buffering system is to maintain normal pH value. For HCO_3 diffusion the pK is 6.1. Therefore for pH 7.4 value (pH = pK + Base/Acid) exactly 24 mmol base / and 1.2 mmol CO_2 is needed.

4) Acid-base status: If the pH is within the normal range, but the $PaCO_2$ and/or HCO_3 (or both) are abnormal, then there is compensation. An acid-base derangement exists. Respiratory compensation is very fast, occurring within seconds or minutes. This compensation occurs via the body's control of respiratory rate, through the brain respiratory center. Thus respiratory compensation for metabolic abnormalities is seen almost immediately. Metabolic compensation, on other hand, is slow. It occurs through elimination of acid or alkali by the kidney. Hours go by, before significant compensation is seen. Metabolic correction through the kidney will be seen for metabolic disturbances (5).

Oxygen transport of Hemoglobin (Oxygenation): Diffusion of gases is not satisfactory for the 20 mmHg oxygen content of the cell. Hemoglobin is required for the transportation. Every hemoglobin molecule has different oxygen transport capacity and biologically different molecular actions. Oxygen must bind not in chemically but in physically for easy transportation. Blood hemoglobin concentration is very important. Total blood hemoglobin concentration (ctHb) indicates oxyhemoglobin (cO_2Hb), deoxyhemoglobin (cHHb), carboxyhemoglobin (cCOHb), methemoglobin (cMetHb) (6). We have also noticed saturation and oxygen contents. E.g. baby A with paO2 85 mmHg, saO_2 95%, Hb 7 g/dl, is more hypoxemic than the other baby B with paO_2 55 mmHg, saO_2 85%, Hb 15 g/dl. Oxygen content (ctO_2) is 8.9 in baby A, but in baby B 17.1 mlO_2/dl. Oxygenation is 2 times better than baby A.

Status of oxygen: Transfer of the oxygen to the cell is important. In this case the oxygen content is important. Fetal hemoglobin oxygen content is more than the adult hemoglobin. But oxygen transferring capacity of adult hemoglobin is higher than the fetal Hb. Adult hemoglobin is generous, but fetal hemoglobin is miser. Fetal hemoglobin can take oxygen at low concentrations which is necessary for fetal life.

Electrolytes and osmolarity: Living organisms are mostly composed of water. Water can allow the molecular activity by biochemical ways, without energy consumption. By means of hydrogen covalent bonds, diffusion is easily performed. There must be osmotic balance. This ionic effect causes the attraction between negative (oxygen) to positive (Na, K, Ca) and positive (Hydrogen) to negative (Cl) (7). This makes a great diffusion activity in the fluid matrix, about 100 million times per se-

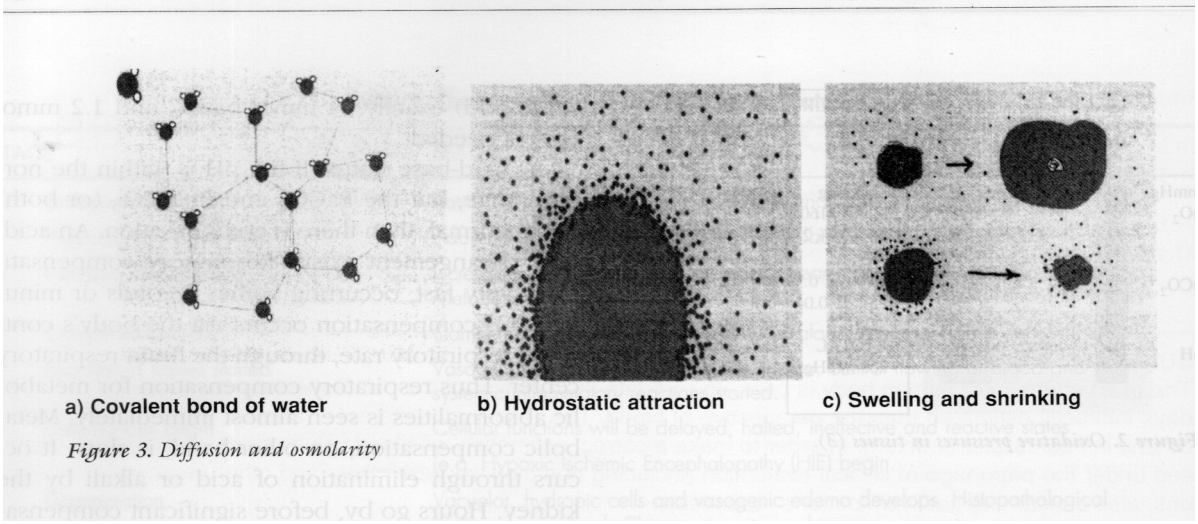

Figure 3. Diffusion and osmolarity

cond. If the balance is not established, swelling or shrinking of the cells are noticed (Figure 3). The macromolecules and the membranes of cells attract the molecules and form a hydrostatic zone. Electrolytes are important for this attraction.

Free fluid pressure in the matrix is nearly minus 8 cmH2O. If it increases, to + values, we notice the edema. When the free fluid increases, the diffusion decreases. The lymphatic drainage develops after 34-36 gestational ages. This indicates that, great pressure is required to save the fluid into the vascular bed. Capillary colloidal osmotic pressure is important, at this stage. Hydrostatic osmotic pressure mainly depends on electrolytes. Electrolytes are highly transferable to capillary, to matrix and to cell. 300 mOsmol must be obtained in every circumstance (Figure 4).

When the intercellular matrix is degenerated, first interstitial (increase of fluid), than vasogenic (escape of plasma) and cytostatic (extravasations of blood components) edema develops (Figure 5).

Metabolic values: Blood values are not always good indicators for cellular function. We therefore try to estimate the function inside of the cell. For example the blood glucose is increased in diabetic mellitus but the cell is lack of energy. The glucose combines with phosphorus and forms Glucose 6 Phosphate. After the consumption of glucose for energy requirement, lactate, private, ammonium, ketone bodies are formed, due to the different metabolic pathways, whether oxidative or anaerobic (8). All are good indicators. Comparisons of values are more important.

mmol	CELL	MATRIX
Na	14	146
K	140	4.2
Cl	4	105
HCO3	10	27
Glucose	(8.7)	5.6
Protein	40	5
Urea	4	4
TOTAL	302.2	302.9
in mmHg	5430	5453

• All living organisms must perform an osmotic balance.

• Cell and surrounding matrix must be in physio-biochemical balance.

Figure 4. *Inter and extra cellular interactions (3) (osmolarity in mmol, pressure in mmHg)*

Perfusion, reperfusion: Adequate circulation of blood is vital importance. Blood is composed of fluid matrix in which molecules are transported. If diffusion is impaired, the circulation is directed at the central sites. Capillary and peripheral circulation is nearly stopped. In vasoconstriction only plasma flows. Erythrocytes are cumulated and forms cloth. Fibrinolysis, consumption coagulopathy, chain reactions begin. There will be no oxidative stress at the beginning, later the most advanced develops. It is nearly the same in vasodilatation, in which impaired circulation develops due to the pooling of blood.

Administration of intravenous fluids has a direct effect on the electrolyte balance. To support the capillary diffusion, osmolarity controlled fluids, like diluted dextran's or plasma, can be added to the i.v. fluids. While considering the diffusion of capillary, we should care of the ischemic perfusion damage to the tissues at this reperfusion stage. One must consider the prevention of reperfusion injury at the beginning of the perfusion.

Dopamine like cardiovascular drugs must be selected. Dosage must be justified due to the condition of the baby.

The other important one is portal system circulation. Attention must be drawn to the portal system. If you reduce the flow, toxins and microorganisms are passed from intestine to blood. NEC must be prevented.

Bilirubin is one of the first encountered liver functions of the body. Therefore if we considered

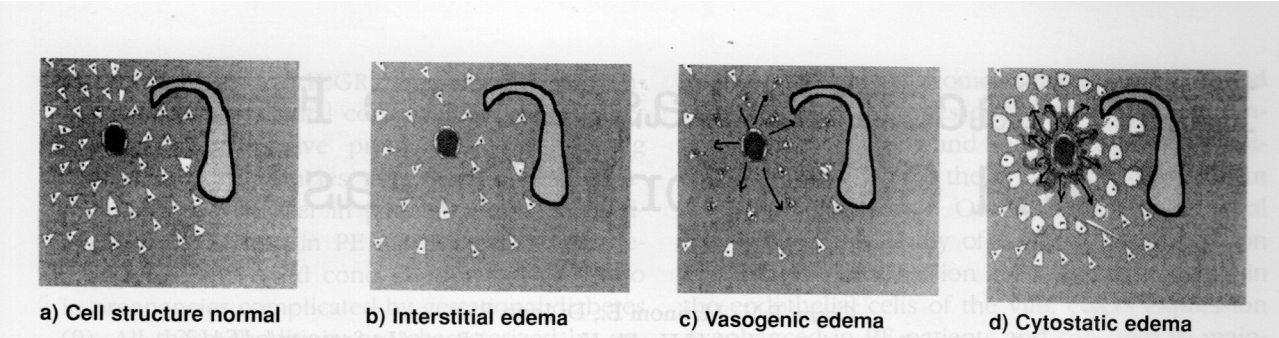

Figure 5. Types of edema.

the bilirubin for the metabolism of the liver and entero-hepatic circulation system, we will not try to overcome hyperbilirubinemia at the first week of life.

Clinical Findings (Apgar score): APGAR is a clinical scoring system which is a combination of several organ systems. It's a good predictor for clinical status of the baby. We have to protect the baby before the tissue reactions begin. Brain functions give us clues about the cell function. If a baby is depressed, it means that something is going to wrong to the baby (9).

Close Bedside Physician Care (CBPC): If you want to be a good doctor for your baby, please sit near to the incubator and try to understand what is going on. The books, consultants, professors of Neonatology can help the physicians and they will give some information. But the reality is the baby.

CONCLUSİON: From outside of the house (cell), it's hard to see the inside. From inside, outside can be seen. Values from the blood are only an indicator of the vessels. For good estimation and evaluation, all for one, one for all will be the main philosophy. All the components will be systematically examined and must be correlated with the clinical findings. Close Bedside Physician Care is the prime important.

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