THE ANALYSIS OF HEMOLYSIS AND THE RISK FACTORS IN THE PATIENTS WHO UNDERWENT CORONARY ARTERY SURGERY WITH CARDIOPULMONARY BYPASS

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Objective: In patients who underwent coronary artery surgery are studied. Hemolysis and the risk factors due to the extraphysiological procedures in cardiopulmonary bypass (CPB).
Material and method: Fifty-two sequential patients who underwent coronary artery by-pass grafting (CABG) in Turkiye Yuksek Ihtisas hospital (TYIH) between March 2001 and April 2001 were studied. They did not have any hemolytic disorders and they did not use hemolytic drugs. The patients were evaluated on the 1st , 3rd and 5th post-operative days for the hematological parameters.
Results: As the extraphysiological procedures in CPB are short and mild, their effects on hemolysis are not statistically significant. However, some of the hematological parameters, like the hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), reticulocyte, haptoglobin and thrombocyte counts were increased, bilirubin and lactate dehydrogenase (LDH) were decreased until the 5th post-operative day.
Conclusion: In our study, the hemolytic process appeared minimal. However, the avoidance of the from the risk factors of hemolysis and the utilisation of hemorheological (hemoprotective) drugs would be useful for preventing hemolysis. Thus less blood and blood products will be transfused and mortality, morbidity rates and cost will be lowered.

Key Words: Coronary artery surgery; coronary artery by-pass graft; cardiopulmonary by-pass, hemolysis.

In open heart surgery, inflammatory processes that have multisystemic effects are triggered by toxins, mediators and cytokines liberated by the extraphysiological procedures in cardiopulmonary bypass (CPB)[1]. One of the most important systems affected by the inflamatory process is the hematological system. Although all constituents of blood are affected, red blood cells (RBC), since they have the largest volume and the longest renewal time, are mostly affected (i.e. hemolysis). Hemolysis is caused by the physical (roller pumps, lines) and the chemical factors during CPB. The chemical factors in the inflammatory process are the humoral activation systems (kinin-kallikrein, complement, coagulation, fibrinolytic systems), proteolytic enzymes, endogenous mediators and the free oxygen radicals [2].

In the post-operative period, Hct will decrease because of the hemodilution that occurred during CPB. In addition, hemolysis decreases Hct, too. This will increase the mortality and morbidity in newly revascularised patients who are fragile for ischemia [2]. This will highlight the importance of efforts for preventing hemolysis.

MATERIAL AND METHOD

Study characteristics of a population of fifty-two sequential patients who underwent coronary artery by-pass grafting (CABG) in TYIH between March 2001 and April 2001 were taken into consideration. They did not have any hemolytic disorders and did not use any hemolytic drugs.

Age, sex, pre-operative used drugs, anesthetic protocols, cross-clamping periods, hypothermia degree, total CPB periods, hemodilution degree (after CPB), amount of post-operative transfusion (packed red cells, fresh frozen plasma, packed whole blood), intensive care time, ventilator support periods, intraaortic baloon pump (IABP) usage and period for each patient were carefully examined and noted. Besides, every patient was evaluated for free hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV) reticulocyte count, haptoglobin, lactate dehydrogenase (LDH), plasma iron, total iron binding capacity, transferrin saturation, mean platelet volume (MPV), thrombocyte count, bilirubin (conjugated & unconjugated), Coombs test (direct & indirect). Urine samples were detected for red blood cells and urobilinogen. The heart rates of each patient (at the time that the samples are taken) and their pre-operative and post-operative rhythms were also noted.

Statistical Analysis

Parameters that identify hemolysis were stated by percentages and means. Temporal changes of data, where data do not fit in to normal distribution, were evaluated by "Friedman test" (two way Anova). When the difference was ascertained, "Wilcoxon test" was done.

Temporal changes of data, where data fit in to normal distribution "variation analysis" was evaluated. When the difference was ascertained, the "paired t test" was done. Temporal changes of data, where data has been obtained by survey, was done by "McNemar test". The statistical p value smaller than 0,05 was accepted as significant. Besides, p value in "Wilcoxon test" smaller than 0,05/3(comparison number)=0,017 instead of 0,05 that was done in order to find out significant difference in which time in "Friedman test" was accepted significant.

RESULTS

The Anesthesia protocol of fentanyl + pavulon (I.V.) was applied to all of the patients. Forty-four of the patients were males (84,6%) and 8 of them were females (15,4%). Mean age was 55,12±10,15.

The cross-clamping periods, hypothermia levels, total CPB periods, hemodilution levels (after CPB), post-operative transfusions, intensive care periods, ventilator supporting periods and IABP usage and periods were shown in Table-1. Results of hematological parameters on the 1st , 3rd and the 5th post-operative days are shown in Table-2.

Direct and indirect Coombs tests in the post-

operative period were "negative".
The presence of urobilinogen and RBC in urine is shown in Table-3.
Mean heart rates of the patients in time that the blood samples are taken are 98,4±11,34 beat/min. Pre-operative and post-operative rhythms of the patients are shown in Table-4.

Table I: Operative and postoperative datas in patients

Cross-clamping periods	35,46 15,52 min
Hypothermia levels	31,40 1,64 C
Total CPB periods	59,81 22,46 min
Hemodilution levels	%25,63 3,67
Post-op transfusion	
Whole blood	0,65 0,74 units
Packed red cells	1,64 0,46 units
Fresh frozen plasma	2,15 0,75 units
Intensive care periods	1,18 0,90 days
Ventilator support periods	12,16 5,16 hours
IABP usage	None

Table III: The presence of urubilinogen and red blood cell in urine.

	1st day	2nd day	3rd day
RBC in urine			
(+)	22(42,3%)	12(23,1%)	10(19,2%)
(-)	30(57,7%)	40(76,9%)	42(80,8%)
Urobilinogen			
(+)	4(7,7%)	16(30,8%)	5(9,6%)
(-)	48(92,3%)	36(69,2%)	47(90,4%)

Table IV: Pre-operative and post-operative rhythms of the study groups.

	Pre-op rhythm	Post-op rhythm
Sinusal rhythm	51(98,1%)	48(92,3%)
Atrial fibrillation	1(1,9%) 4(7,7%)	

Table II: Hematological parameters on the 1st , 3rd and the 5th post-operative days.

	Pre-op	Post-op 1	Post-op 3	Post-op 5	
Hb	14,1±1,49	9,69±0,9	9,47±1,11	10,12±1,14	NS
Hct	42,6±4,01	29,2±3,11	29,02±3,42	31,39±3,03	NS
MCV	86,17±4,03	87,22±4,48	88,11±5,96	89,01±4,99	*
Reticulocyte	46,75±18,25	68,95±25,6	84,74±22,51	179,01±66,71	*
Reticulocyte (%)	1,1±0,08%	2,5±0,11%	3,5±0,24%	5,7±0,74%	*
LDH	378,24±89,41	602,58±145,45	584,35±216,06	538,81±175,01	*
Haptoglobin	3,01±0,81	0,72±0,3	2,25±0,64	2,91±0,71	*
Free Hb	2,86±1,01	3,35±1,84	2,92±1,034	3,48±1,7	NS
Bilirubin(conj)	0,11±0,06	0,16±0,08	0,14±0,06	0,12±0,08	*
Bilirubin (unconj)	0,41±0,18	0,66±0,38	0,56±0,2	0,56±0,23	NS
Thrombocyte	375,61±81,61	202,38±56,37	203,52±50,24	360,25±79,17	*
MPV	9,52±1,12	9,47±1,06	9,32±0,75	9,03±0,87	NS
Plasma iron	46,05±41,62	45,05±40,58	41,26±27,11	41,44±12,71	NS
TIBC	321,16±81,36	319,13±70,43	291,77±53,66	355,14±93,79	*
Transferrin sat	16,35±19,81	16,3±19,7	15,6±10,56	14,27±6,55	NS

*=significant, NS= non-significant, TIBC= total iron binding

DISCUSSION

Red blood cells are 7-9 μ in diameter and flexible blood corpuscles; their basic functions are tissue oxygenation, removal of CO2 and buffering the plasma. RBC need energy to maintain these functions and their shapes and flexibility; 80% of this energy is gained by the Embden-Meyerhoff pathway and 20% by the hexose-monophosphate pathway. They are biconcave in pattern and their cell membrane is trilaminary; glycoprotein layer between two phospholipid layers. Their mean half life is 120 days [2]. All kinds of negative physical and/or chemical trauma to construction and functions of red blood cells cause degradation, i.e. hemolysis.

Parameters used to evaluate hemolysis are increased free Hb, increased reticulocyte count, decreased Haptoglobin, hyperbilirubinemia, increased LDH levels, increased osmotic fragility, hemoglobinuria, hemosiderinuria and increased urobilinogen levels[2].

Chemical and physical factors that cause hemolysis in the extraphysiological procedures of CPB; are hypothermia (moderate and deep), hemodilution, mechanical stress, excessive venous drainage, priming solution and they cause hemolysis via shear stress, turbulence and increased osmotic fragility. [1,3]

The type of the pump is an important factor in hemolysis (mechanical stress). In cardiac surgery; generally roller, non-occlusive roller, and centrifugal pumps are used. Standard roller pumps are the most hemolytic of all [4,5]. Mechanical trauma, of the pumps, because of increased pressure and flow is the main problem [6,7]. In centrifugal pumps hemolysis, mostly because of the irregular and rough surface of the impeller, is minimal [8,9]. Oxygenators and tubular lines are also important and they cause hemolysis either by shear stress (mechanical) or by affecting the inflammatory elements and the cascades statically (chemical). The most hemolytic oxygenators are the "Bubble Oxygenators"[10,11]. "Membrane oxygenators" have variable effects regarding their materials, and the less hemolytic ones are silicon coated polypropylene hollow fiber oxygenators [12,13]. Silicon and polyvinyl carbon lines used in CPB have little hemolytic and cytotoxic effects; however, the least leukocyte adhesion and cascade activation occurs using platinum coated silicon tubes [14]. Recent studies show that heparin coated lines have minimal effects on cascades and blood corpuscles [9,14,15].

The mechanical dynamic factor for hemolysis in CPB is how venous drainage is made. Smaller lines affect the RBC membrane in various degrees by kinetic, vacuum and gravitational causes. The major cause of hemolysis in CPB is cardiotomy suction [16]. Thus excess venous drainage and negative pressure are important parameters. The negative pressure should be - 43±14 mmHg. Pressures below this limit correlate with hemolysis in a linear way [4,16]. Besides, the venous blood meets air bubbles and this causes destruction either directly or by the turbulence it produces [17,18].

The type of the priming solution in CPB is important in hemolysis for cosmotic reasons[19]. The chemical agents produced during CPB cause hemolysis either by attacking the membrane (peroxydation e.g.) or by ruining the metabolism. Functional and constitutional damage occurs by osmotic changes in hemodilution and by metabolically slowing in hypothermia.

The negative "lytic" effects of CPB are usually seen in red blood cells, because they are seen in great numbers. White blood cells are mostly affected (because they are bigger) but their turn-over time is shorter (in hours) and the damage can be neglected.

Thrombocytes are the smallest corpuscles so they are less affected. The changes in thrombocytes are functional and quantitative (because of hemodilution) and can be neglected, too [5].

Hb and haptoglobin levels are important in detecting hemolysis. Less or no haptoglobin shows hemolysis but normal values do not mean that the possibility of hemolysis can be excluded. The hemolysis can be proceeding in the extravascular compartment. Besides, steroid usage and inflammatory process can increase the haptoglobin levels as an acute phase reactant [2].

It is recently shown that RBC derived Neuron Specific Enolase (NSE) increases in hemolysis. It is noted that NSE can be used as a marker of hemolysis besides neuron-glial cell damage [22].

"Superoxide" production catalyzed by the iron ion derived from free Hb, is considered responsible for tissue damage [22]. Free Hb decreases coronary flow and causes detrimental effects on left ventricular contractility, whether in ischemia-reperfusion period or in normal individuals [1,22].

Excess free Hb is seen in the urine if the Hb binding proteins in plasma (haptoglobin, hemopexin, methemalbumin) are spent. When small amounts of Hb reaches urine, this is changed into hemosiderine by tubular absorption. If the epithelium is shed, hemosiderinuria occurs. This is an absolute marker of intravascular hemolysis. However, if Hb reaches very high levels, this change is not enough and Hb sediments are seen in the tubuli. So acute tubular necrosis and renal failure takes place [2].

Red blood cells protect themselves by their own hemorrheological reactions by 68,5%-74,8%[23,24]. However, in the case of hemolysis these compensation mechanisms are not enough and hemolysis proceeds. Hemolytic effects will not disappear shortly after CPB, so it needs long term monitorization [25].

Studies are being made on hemorrheological (hemoprotective) agents to prevent from hemolytic effects. Best known of these are PGE1 and Haptoglobin. PGE1 protects the erythrocyte membrane and hasten regeneration [28]. Haptoglobin prevents cardiac and renal functions from negative effects of excess Hb by binding Hb.

In our study, as the cross-clamping and total CPB periods are short and hypothermia is mild, they do not appear to be risk factors for hemolysis.

In our study post-op Hb and Hct levels are lowered due to pre-operative values. We suppose that hemodilution is the cause. MCV values increase following the operation. Increased reticulocytes cause MCV to be measured relatively bigger because they are bigger than the red blood cells.

Haptoglobin levels decrease on the first post-operative day and increase on the 3rd and the 5th days to normal values. Free Hb keeps its limits. Bilirubin levels increase on the 1st post-operative day and returns to normal by the 5th day within the limits. These show indefinite clinical hemolysis.

Urobilinogen levels are high on the 3rd day and decrease on the 5th day. The reticulocyte crisis seen on the 3rd -5th days is for Hct levels that fell because of minimal hemolysis and hemodilution.

LDH levels rise on the 1st day and fall on 3rd-5th days, and this is multifactorial. Perioperative cardiac and hepatic changes and minimal hemolysis cause this variation.

Thrombocyte counts fall on the first 1-3 days in normal limits and increase, on the 5th day, to the pre-operative values. This is parallel to 3-5 days that is needed for the regeneration of the thrombocytes.

Other parameters, like plasma iron, total iron binding capacity and transferrin saturation, are in normal limits, and this supports the mild hemolysis.

With these results we conclude that the hemolysis is minimal, in our study. Keeping the risk factors for hemolysis in minimal and using hemorheological agents when necessary, we can prevent hemolysis. Thus less transfusion of blood and blood products will be needed, and mortality, morbidity and cost will fall.

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