Methemoglobin Role in the Subdural and Epidural Hemorrhage Recentness Evaluation in Hot Climate

B.M. Daljanov[1], O.I. Khvan[2]

[1]PhD, MD, Department of Forensic Medicine and Medical Rights, Tashkent Pediatric Medical Institute, 223 Bogishamol Str., Yunusobod District, Tashkent, Uzbekistan, [2]Doctor of Medical Sciences, Teaching Assistant, Department of Forensic Medicine and Medical Rights, Tashkent Pediatric Medical Institute, 223 Bogishamol Str., Yunusobod District, Tashkent, Uzbekistan

Abstract

Methemoglobin (MetHb) is a physiological blood component that performs protective functions and neutralizes the endogenous hydrocyanic acid, hydrogen sulfide and other poisons. MetHb is slowly formed in the blood, its surplus is constantly restored while the content in blood remains low.

The spectrophotometric method has been used to measure the methemoglobin concentration in 72 traumatic subdural and epidural hematomas of various prescriptions. Based on the data obtained, the limitation of subdural and epidural hematomas has been determined by the methemoglobin concentration. The results are recommended for use in the forensic expert examinations.

Keywords: *Subdural and epidural hematomas, methemoglobin level, statute of limitations, forensic medicine, blood, hematoma, methemoglobin, intracranial.*

Introduction

Information on the methemoglobin concentration in the blood of healthy persons is very controversial. According to some researchers, MetHb is found in the blood of healthy adults in 23-25% of cases; however, its average concentration in the blood does not exceed 3 - 5%. Moreover, the concentration is higher in females than in males[1]. Many researchers believe that the MetHb content in human blood does not exceed 1%. According to others, MetHb formed in red blood cells during lifetime is reverted into hemoglobin by enzymes contained in red blood cells[2]. Moreover, the intracellular MetHb concentration is increased in corpses when the reducing enzyme substrates disappear from the red blood cells. 70% of red blood cells do not contain any methemoglobin.[3] With that, the more MetHB in cells, the less often they are found in the bloodstream.[4] Such distribution pattern indicates the active elimination of red blood cells with methemoglobin from the hematopoietic stream.

The MetHb content is increased depending on the erythrocyte aging. It is triggered by the direct changes in hemoglobin molecules. The electronic configuration of hemoglobin protein molecules is varying, therefore, the methemoglobin formation and reverse recovery are not uniform and depends on the medium pH.[5] In an acidic environment, oxidation is easier than in an alkaline one. Supposedly, it leads to the higher methemoglobin content in capillary blood than in venous blood. The MetHb increase in the blood is possible in various morbid conditions. However, under normal conditions, it is formed in the body in small quantities while quickly reverting into hemoglobin.[6]

Some researchers point out that assimilation and dissimilation in the body are not interrupted immediately and that the cadaveric red blood cells retain their gaseous metabolism for some time. As time passes after death, the MetHb content in the blood is increased both in the human cadavers and animal carcasses. An increase in ambient temperature accelerates the methemoglobin spontaneous generation in the blood of the dead, as well as the methemoglobin reduction and disintegration.

The processes of MetHb generation and reduction in the blood are directly dependent on the storage temperature requirements. Its most intense formation was recorded at a temperature of t18±24ºC.

As a result, the identification of posthumous biochemical patterns is of interest, both for determining the time of death and for assessing the recentness of brain injury in relation to the intracranial hematomas. Moreover, special emphasis was put on the temperature conditions in our Republic when studying the methemoglobin generation in the intracranial hemorrhages.[7]

In this regard, *the research objective* is to analyze the increase in MetHb concentration in the intracranial hematomas depending on the time of injury, as well as to reveal the trends in the process development based on the data obtained for determination of the injury time.[8]

The research materials and method: In order to determine the methemoglobin concentration in the blood, a spectrophotometric method was used. Spectrophotometry is based on measuring the monochromatic light flux attenuation degree as a result of selective light absorption by a dissolved substance.

The spectrophotometric method are based on the Beer-Lambert-Bouguer law of light absorption that determines the ratio between the impinging light intensity passed through the absorbed solution and monochromatic radiation. The log ratio between the light flux intensity entering the solution and the light flux intensity escaping from the solution is directly proportional to the substance concentration and the absorbing layer thickness: $D=lgIo/I=Cl\Sigma$. The lq value specifies the attenuation degree of the radiation intensity after its transmission through the solution. It is called the solution transmission density - D (A or Σ). The law indicates the linear dependence of D on the absorbing substance concentration (C), the absorbing layer thickness (I), the absorption coefficient or extinction coefficient (Σ).

The attenuation degree of the light intensity passing through the substance (solution) can describe the transmission: $T= Io/I= Io- \Sigma Cl.$ T (transmission) is the ratio of the radiation intensity transmitted through the absorbing solution to the initial radiation intensity.

Transmission can be ranged from 0 to 100%.

During the spectrophotometric analysis, the qualitative spectrophotometry is widely distributed in addition to the quantitative spectrophotometry, i.e. identification of dissolved matters by the light absorption curve shape or the so-called absorption spectrum.

In general, these curves are shown in a Cartesian coordinate system so that the optical density values (D) or extinction coefficients are plotted on the ordinate, and the length (in microns) is plotted on the abscissa. During the curve assessment, attention is brought to availability of the maximum and minimum light absorption values, its relative intensity, as well as the wavelengths that correspond to the spectrophotometric curve knees.

For this purpose, we took hematoma (subdural and epidural) and blood from the cerebral sinuses (to control the methemoglobin generation). The hematoma was ground in a glass homogenizer to a homogeneous liquid state for a hemolysate preparation. We used the cyanide method developed by M.S. Kushakovsky (19680 based on the indirect method by Evellyn and Malloy (1938).[9]

This method has a fairly high sensitivity and accuracy. It is notable for the following advantages:

1. It is simple and affordable.
2. It can also be used when, in addition to oxyhemoglobin (HbO^2) and methemoglobin (MetHb), other hemoglobin derivatives are available in the blood solution.

The repeatability of the results is very high. The single-measurement uncertainty did not exceed 0.2–0.3% methemoglobin of total hemoglobin.

The method is based on the well-known fact that the methemoglobin absorption band (acidic medium) disappears in the red region when cyanite is added. In this case, methemoglobin is turned into cyanmethogemoglobin.

If we take the total Hb (hemoglobin) concentration as 100%, then the MetHb concentration (x%) will be: $X= C\ MetHb\ x\ 100/C\ total\ Hb\%.$

In such a way, the calculation formula for the MetHb concentration is as follows: $X=(D_1-D_2)x11x100/3.6xD_3.$

The dependence of methemoglobin concentration on the time of injury was determined by the univariable linear regression analysis. According to this method, the constraint equation is as follows: $M=a_0+a_1t$, where M is the MetHb concentration, t is the time elapsed since the injury; a_0 and a_1 are the equation variables.

In order to perform quantitative methemoglobin determination in the substrates of intracranial hemorrhages, the spectrophotometric analysis for

methemoglobin concentration in the blood from the intracranial hematomas was performed in relation to 72 cadavers with the established terms of injury who were subject to a forensic medical study. The bodies were dissected according to the generally accepted method 24-36% after death. The blood from the superior sagittal cerebral sinus was used as a control.

The spectrophotometric study results, obtained as a difference in the MetHb concentration from the intracranial hemorrhages and the control blood group from the superior sagittal cerebral sinus at various times of brain injury, were subject to evaluation. The MetHb concentration between the study groups was compared in relation to 7 periods of post-traumatic hematoma development: up to 3 hours; up to 12 hours; up to 24 hours; up to 48 hours; up to 60 hours; up to 72 hours; more than 3 days.

Study Results

In the first group of the injured who suffered the brain injury and died within 3 hours after the injury (16 cases), it was found that the MetHb concentration in the studied hematomas ranged from 1.03 to 1.7, and the average value was 1.26±0.17.

The statistical processing of the results in 7 study groups revealed the statistically significant differences in concentrations at different times after the brain injury compared with the control group. Thus, in the 2nd group of injured with the period of up to 12 hours after the brain injury, the MetHb concentration was equal to 7.78±0.3% compared with the control values of 1.04±0.04% (P<0.01).

In the third group, where death after the brain injury occurred within one day, we observed the noticeable increase in the MetHb concentration up to 10.6±0.50% (P<0.01), in the fourth group, respectively, the MetHb concentration increased up to 15.21±0.7% (1.6±0.07% for the control group) (P<0.01).

The same trend is observed in other groups (V, VI, VII) where the methemoglobin concentration is gradually increased with increase in the body survivability period after the brain injury.

Table 1: Comparative values of methemoglobin concentration in the intracranial hematomas

Study groups according to the prescription of death coming	Methemoglobin concentration, %		
	Compared group	Control value	P<
1st group – death within up to 3 hours after the brain injury	1,7±0,06	0,82±0,02	0,01
2nd group – death within up to 12 hours after the brain injury	7,78±0,3	1,04±0,04	0,01
3rd group – death within up to 24 hours after the brain injury	10,6±0,50	1,5±0,06	0,01
4th group – death within up to 48 hours after the brain injury	15,21±0,7	1,6±0,07	0,01
5th group – death within up to 60 hours after the brain injury	16,7±0,63	1,8±0,12	0,01
6th group – death within up to 72 hours after the brain injury	18,2±0,74	1,8±0,14	0,01
7th group – death after 3 days	23,52±0,92	2,61±0,19	0,01

The 6th and 7th groups of post-traumatic intracranial hematoma development, indicated by a period of 3 or more days, demonstrated a slow increase in the methemoglobin concentration to an average value of 19.72%. When the injury survivability time is increased up to more than 10 days due to the organizational processes in hematomas (formed sacs and vascular neoplasms, fresh hemorrhages in the hematoma substrates), these MetHb concentrations have the quantitative variation and are not subject to adjustment.[10]

In such a way, the body survivability period after the brain injury and formation of intracranial hematomas leads to the increase in methemoglobin content in red blood cells. In part, the reasons for this is a decrease in methemoglobin reductive activity in the senescent cells. Contrarily, there are direct changes in the hemoglobin molecules. The spectrophotometric data obtained can undoubtedly serve as an additional criterion for recentness assessment of the resulting brain injury.[11]

At the next stage, we performed the recentness assessment of intracranial hematomas by the structural changes.[12]

Our observations were divided into seven periods, depending on the body survivability periods after the brain injury.[13]

The first period included 16 observations, the second period consisted of 12 observations, the third, fourth, fifth, sixth, seventh periods consisted of 8, 9, 10, 6 and 5 observations, respectively. During the first time period after the injury, the hemorrhages were represented by liquid blood with almost similar color regardless of localization and bleeding point. It should be noted that such a condition of hematomas remained unchanged during rather long-term storage of a corpse in the refrigerator.[14] The specific features of this study group were as follows: changes in the tinctorial collagen fiber properties in the form of pronounced basophilia, eosinophilia and insignificant cerebral edema, severity of vascular responses in the form of angioparesis and angiospasm. The hematoma substrates had the ratio of normal and abnormal red blood cells in favor of the former, moderate leukocytosis and lymphocytosis (Table 2).

The first post-traumatic period with the duration of up to 3 hours is represented by the pathomorphological changes typical for the inflammatory conditions: the changes in the tinctorial collagen fiber properties of the dura matter included basophilia (13.0±1.2%), eosinophilia (11.7±1.2%) and edema (6.5±0.7%) without any necrosis.

Table 2: Structural changes in the intracranial hematomas depending on the injury limitation

Morphological features of hematomas		Study groups						
		I – up to 3 hours	II – up to 12 hours	III – up to 24 hours	IV – up to 48 hours	V – up to 60 hours	VI - up to 72 hours	VII – after 3 days
Tinctorial properties of fibers (area, %)	Basophilia	13,0±1,2	12,7±1,4	8,7±1,3	0	0	0	0
	Eosinophilia	11,7±1,2	8,4±1,4	2,3±0,4	0	0	0	0
	Edema	6,5±0,7	7,6±1,5	10,2±1,3	10,4±0,6	11,1±1,2	1,5±0,4	1,6±0,3
	Necrosis	0	0	55,6±6,9	13,6±1,1	0	0	0
Vascular response, %	Tetanus	46,5±3,4	27,9±3,2	6,5±1,6	2,2±0,6	0	0	0
	Plasmorrhagia	0	30,4±3,2	78,7±4,2	82±5,6	0	0	0
	Fibrinoid necrosis	0	0	20,5±1,6	54,6±2,6	16,7±3,2	8,6±1,6	0
Normal red blood cells, %		95,6±6,4	92,5±5,2	87,0±3,8	56,8±1,8	32,6±2,6	27,8±1,2	25,6±1,2
Abnormal red blood cells, %		4,3±1,2	7,4±1,5	10,7±1,3	44,0±1,8	69,6±4,2	62,6±3,9	62,7±4,2
Normal white blood cells, %		28,1±2,8	30,4±1,2	24,0±2,6	55,8±3,2	16,7±1,8	14,0±0,8	18,6±0,6
Abnormal white blood cells, %		0	6,4±1,2	16,8±1,8	25,4±1,8	36,4±2,3	46,2±3,4	12,1±1,8
Macrophages, %		0	1,4±0,2	15,8±1,6	25,2±1,4	36,4±1,8	44,7±3,2	73,2±1,8
Hemosiderin, %		0	0	1,3±0,4	7,5±0,8	3,6±0,8	4,3±1,2	4,6±0,4
Fibrin strands, %		0	83,0±6,2	8,2±0,6	0	0	0	0
Fibrin mesh, %		0	0	76,4±4,1	14,2±1,6	0	0	0
Fibrin clot, %		0	0	0	15,7±0,5	76,6±4,4	8,6±0,8	0
Fibrin bodies, %		0	0	0	6,1±0,7	92,4±6,2	74,8±2,4	85,5±6,4
Lymphocytes, %		1,4±0,3	1,6±0,7	4,3±0,6	5,6±0,6	16,4±2,1	12,4±1,8	14,4±6,8
Fibroblasts, %		0	0	0	10,3±0,8	38,4±3,0	26,7±2,6	45,0±4,3
New vessels, %		0	0	0	0	35,4±3,2	44,6±4,0	82,4±4,6
Fascia, %		0	0	0	0	54,2±6,8	64,4±7,4	94,1±13,6

The vascular response was represented by spasms (46.5±3.4%). The hematoma substrates had the ratio of normal red blood cells (95.6±6.4%) with a small admixture of abnormal red blood cells (4.3±1.2%). The leukocytosis was noted mainly at the border with the dura mater due to the normal white blood cells (28.1±2.8%). This case is represented by the single lymphocytes (1.4±0.3%). According to the above specifications, there are statistically significant differences from the subsequent study group (Table 3).

Table 3: Changes in blood properties (in the intracranial hematoma substrates) during the first period after an injury (up to 3 hours)

Indicators of blood and hematoma properties	Degree of incidence in %
Basophilia	13,0±1,2
Eosinophilia	11,7±1,2
Edema	6,5±0,7
Spasm	46,5±3,4
Normal red blood cells	95,6±6,4
Abnormal red blood cells	4,3±1,2
Normal white blood cells	28,1±2,8
Abnormal white blood cells	0

The second injury survivability period (up to 12 hours) was described by an insignificant increase in cerebral edema (7.6±1.5%) and decrease in eosinophilia up to (8.4±1.4%) collagen fibers, decrease in the number of vessels with spasm (27.8±9.2%). The hematoma substrates adjacent to the dura mater showed an insignificant change in the ratio of normal (92.5±5.2%) and lysed red blood cells (7.4±1.5%), an increase in leukocytosis up to 30.4±1.2% cells with the leukocyte debris (6.40±1.2%).

The macrolesions (1.4±0.2%) and fibrin in the form of individual strands were detected for the first time in 83.0±6.2% of cases. The number of leukocytes is hardly varying. In the hematoma substrates on the periphery of the dura mater, the inflammatory response is increasing much more slowly (Table 4).

The survivability period after the injury (up to 24 hours) was described by the occurrence of necrosis in the collagen fibers (55.6±6.9%) in the setting of increasing cerebral edema (10.2±1.2%), decrease in basophilia (8,7±1.3%) and eosinophilia (2.3±0.4). The vascular response is represented by plasmorrhagia of the vascular walls (78.7±4.2%) and the first appeared fibrinoid necrosis. The normal red blood cells remain almost at the same level (87.0±3.8%), the number of both normal (55.8±2.6%) and destructed white blood cells (16.8±1.8%) is increased. The number of macrophages has been increased up to 15.8±1.6% of the cells, the lymphocytosis was accumulated up to 4.3±0.6% of the cells. Free hemosiderin was found with the value of up to 1.3±0.4% for the first time. Fibrin is available predominantly in the form of a mesh (76.4±4.1%).

Table 4: Changes in blood properties (in the intracranial hematoma substrates) during the death period after an injury (up to 12 hours)

Indicators	Degree of incidence in %
Basophilia	12,7±1,4
Eosinophilia	8,4±1,4
Edema	7,6±1,5
Spasm	27,8±9,2
Plasmorrhagia	30,4±3,2
Normal red blood cells	92,5±5,2
Normal white blood cells	30,4±1,2
Leukocyte debris	6,4±1,2
Macrophages	1,4±0,2
Lymphocytes	1,6±0,2
Fibrin strands	83,0±6,2
Abnormal red blood cells	7,4±1,5

The hematoma substrates located on the cerebral periphery demonstrate an insignificant increase in the number of leukocytes, as well as the first occurrence free hemosiderin (Table 5).

Table 5: Content of distinctive features in the intracranial hematoma substrates during the survivability period after injury (up to 24 hours)

Features	Degree of incidence in %
1	2
Basophilia	8,7±1,3
Eosinophilia	2,3±0,4
Edema	10,2±1,3
Necrosis	55,6±6,9
Spasm	6,5±1,6
Plasmorrhagia	78,7±4,2
Fibrinoid necrosis	20,5±1,6

Features	Degree of incidence in %
Normal red blood cells	87,0±3,8
Abnormal red blood cells	10,7±1,3
Normal white blood cells	24,0±2,6
Leukocyte debris	16,8±1,8
Macrophages	15,8±1,6
Hemosiderin	1,3±0,4
Lymphocytes	4,3±0,6
Fibrin mesh	76,4±4,1

The following survivability periods (24-72 hours) were described by a sharp increase in the collagen fiber necrosis (13.6±1.1%) on the setting of pronounced cerebral edema (11.1±1.2%). The basophilia and eosinophilia are not found. The vascular walls are subject to the fibrinoid necrosis (54.6±2.6%). The hematoma substrates adjacent to the dura mater showed a decrease in the number of normal red blood cells up to 32.6±2.6%, decrease in the number of white blood cells up to 16.7±1.8%, including 25.4±1.8% of destructed cells.

The level of macrophages (25.2±1.4%) and lymphocytes (16.4±2.1%) is increasing. Free hemosiderin reaches a maximum level of up to 7.5±0.8% of the lump. The fibrin clots are shown in 76.6±4.4% of cases. The fibroplasts are found for the first time (38.4±3.0%).

The injury survivability period of more than 3 days is described by the cerebral productive processes. The fascia formation was noted in 64.4±7.4% of cases. The number of fibroblasts, macrophages and lymphocytes reached the levels of 45.0±4.3%, 23.2±1.8%, and 14.4±6.8%, respectively.

The hematoma substrates demonstrate a sharp decrease in the normal red blood cells (up to 15.6±1.2%). The number of leukocytes is decreased up to 8.6±0.6%. Fibrins have the form of granular mass (85.5±6.4%). In the hematoma substrates on the cerebral periphery, the inflammatory response dynamics is slowed down. The macrophages (Table 6) in large numbers are found for the first time.

As a result, our studies using the spectrophotometric and pathomorphological data indicate specific changes in the methemoglobin concentration and structural parameters in the intracranial hematoma substrates, depending on the body survivability period after the severe brain injury.

Table 6: Morphological indicators in the intracranial hematoma substrates during the period after the injury (after 3 days)

Indicators	Degree of incidence in %
Edema	1,6±0,3
Normal red blood cells	25,6±1,2
Abnormal red blood cells	62,7±4,2
Normal white blood cells	15,6±0,6
Leukocyte debris	12,1±1,8
Macrophages	23,2±1,8
Hemosiderin	4,6±0,4
Lymphocytes	14,4±6,8
Fibroblasts	14,5±4,3
Fibrin grain bodies	85,5±6,4

While using the obtained data, we conducted the forensic medical studies based on the spectrophotometric and pathomorphological studies presented above.

When studying the brain injury cases with intracranial hemorrhage in the mortuaries of the Tashkent urban and Karakalpakstan branches of the Republican Scientific Center for Forensic Medical Examination, it was found that in 45.5% of studies, the injury terms were not determined. In this regard, we have attempted to determine the brain injury limitation period using the spectrophotometric and pathomorphological data obtained in relation to the people with the known injury terms.

Based on the data obtained as a result of structural and spectrophotometric studies of autopsy material of intracranial hemorrhages and the adjacent dura mater and the known brain injury terms, we have revealed the statistically significant differences between a number of time intervals that demonstrate specific manifestations of the inflammatory process.

Conclusions

1. The intracranial hematomas reach their maximum volume during the first few minutes after the injury, and one of the main factors determining the clinical implications is its generation rate.
2. The identification of post-mortem biochemical patterns is of concern, both for determining the death term and for assessing the brain injury limitation in the study of intracranial hematomas.

3. During the spectrophotometric study of the intracranial hematoma substrates, it is necessary to use the structural studies of intracranial hematomas and the adjacent dura mater according to our recommendations provided in this chapter in order to correctly interpret the results obtained and to avoid possible errors in determining the injury terms.

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