

Immunohistochemical Characteristics of The Adrenal Glands in Newborns Who Died from Birth-Related Brain Injury

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Abstract: Hsp70 was used as an immunohistochemical marker to examine the adrenal glands of newborns who died from birth-related brain injuries. This marker helps identify cellular stress by interacting with receptors located on the epithelial cell membranes, mainly in the zona glomerulosa of the adrenal cortex. Hsp70 is a 70-kDa protein produced in response to various stress conditions, including heat shock, traumatic shock, cardiogenic shock, infectious-toxic shock, and other harmful factors. Its expression is mainly observed on the surface of the cell membrane. Hsp70 also plays an important role in several biological processes, including cancer development, neurodegeneration, apoptosis, sleep regulation, and cellular protein synthesis.

Keywords: Adrenal gland, immunohistochemical method, birth-related brain injury, necrosis, dystrophy.

Introduction: Birth injuries in newborns, especially birth-related brain injuries, are among the major causes of perinatal illness and death. The incidence of this condition ranges from 0.2 to 41.2 cases per 1,000 births in different countries and regions. Therefore, it remains an important problem in modern neonatology [1,7,8,9]. According to the World Health Organization, approximately 2.5–3 million newborns worldwide develop severe neurological disorders each year due to birth asphyxia and mechanical injuries. A fatal outcome during the perinatal period is observed in 20–25% of these cases [2,4,5].

In the United States and European countries, the incidence of birth injuries is reported to be 6–7 cases per 1,000 births, while the mortality rate is 1–3 cases

per 1,000 births. Worldwide, different types of birth injuries are diagnosed in an average of 4.6 cases per 1,000 births. This rate is higher in the Russian Federation and the Commonwealth of Independent States, where it ranges from 25 to 38 cases per 1,000 births. The highest rates have been reported in Turkmenistan, Armenia, and Uzbekistan [3,6,10].

Birth-related brain injuries affect not only the central nervous system but also the general adaptive mechanisms of the newborn's body, particularly the activity of the hypothalamic–pituitary–adrenal (HPA) axis. Traumatic injury, hypoxia, asphyxia, and haemodynamic disturbances cause a strong stress response and activate the HPA axis. The adrenal glands act as important effector organs in this process by increasing the secretion of catecholamines and

corticosteroids in response to stress. Therefore, morphological and functional changes in the adrenal glands are important for assessing the body's adaptive response to stress.

However, the combined assessment of pathomorphological, morphometric, and immunohistochemical changes in the adrenal glands of newborns who died from birth-related brain injuries has not yet been studied sufficiently. Available studies mainly describe morphological changes in the adrenal cortex and medulla separately. Research that provides a comprehensive assessment of these changes and their relationship with morphometric parameters and immunohistochemical markers remains limited.

The relatively high incidence of birth injuries in Uzbekistan, their complications and fatal outcomes during the perinatal period, and the need for a comprehensive examination of autopsy material using modern morphological and immunohistochemical methods determine the relevance of this research. Identifying pathomorphological, morphometric, and immunohistochemical changes in the adrenal glands is of significant scientific and practical importance. It may contribute to a better understanding of the pathogenesis of birth-related brain injury, assessment of stress-adaptive responses, and improvement of the morphological criteria used to evaluate perinatal deaths.

Aim of the study

To investigate and analyse immunohistochemical changes in the adrenal glands of newborns who died from birth-related brain injuries.

METHODS

The study included adrenal gland tissue samples obtained during the autopsies of 69 full-term newborns who died from birth-related brain injuries. The tissue samples were examined using immunohistochemical methods.

DISCUSSION AND RESULTS

The HSP70 marker was used to study the immunohistochemical features of the adrenal glands in newborns who died from birth-related brain injuries. This protein marker helps identify cellular responses to various stress factors, particularly in the epithelial cells

of the zona glomerulosa of the adrenal cortex. HSP70 is a 70-kDa heat-shock protein produced in response to heat shock, traumatic shock, cardiogenic shock, infectious-toxic shock, and other stressful conditions. Its expression is mainly observed on the cell membrane. HSP70 plays an important role in several biological processes, including cancer development, neurodegeneration, apoptosis, sleep regulation, and cellular protein synthesis.

Intracellular HSP70 proteins are an important part of the cellular protein-folding system and help protect cells from the harmful effects of physiological stress. Membrane-bound HSP70 proteins have also been identified as potential targets for cancer therapy. Extracellular forms of HSP70 may be present in both membrane-bound and membrane-free forms.

Thus, HSP70 is an important molecular marker widely used in immunohistochemical studies to assess cellular responses to acute and chronic stress, hypoxia, ischaemia, and mechanical injury, including birth trauma. In our study, HSP70 acted as an anti-apoptotic regulator that supported the survival of cells in the zona glomerulosa of the adrenal cortex.

The pattern of HSP70 expression in response to early hypoxic stress in the adrenal tissue of newborns who died from birth-related brain injuries within the first 24 hours of life was of considerable scientific importance. The characteristics and significance of HSP70 expression were assessed at eight-hour intervals.

Group 1: 0–8 hours. This period represented the early adaptive stress-response and protective phase. Morphological and morphometric parameters differed from those of the control group by approximately 10–15%.

Immunohistochemical examination showed a marked increase in HSP70 expression, which reached a high intensity during this period.

A clear accumulation of HSP70 protein was detected in the apical cytoplasmic regions of the zona glomerulosa cells and spongiocytes of the adrenal cortex. This finding indicated an adaptive response characterised by increased production of the stress protein HSP70.

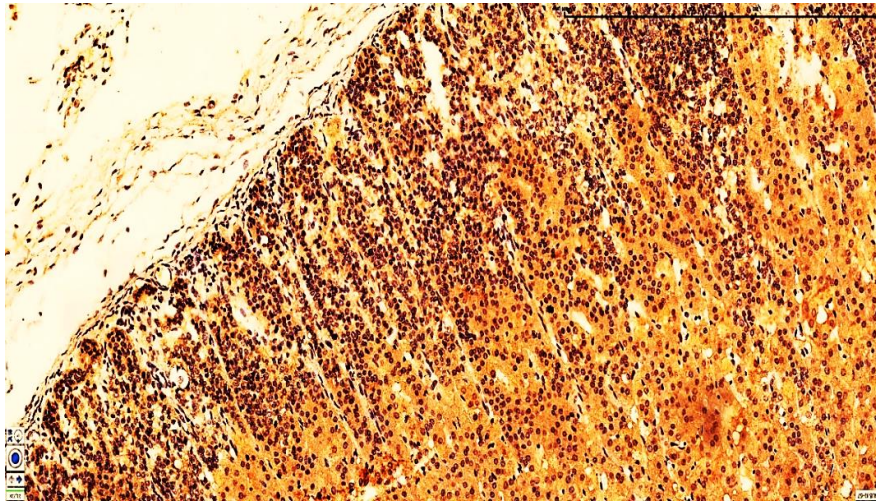


Figure 1. Group 1, 0–8 hours. Adrenal gland of a newborn with birth-related brain injury. High HSP70 expression was detected in the cytoplasm of all cells in the zona glomerulosa and zona fasciculata of the adrenal cortex. Marked expression was also observed in the fetal zone of the cortex and appeared as brown staining. Stain: DAB chromogen. Magnification: $\times 100$.

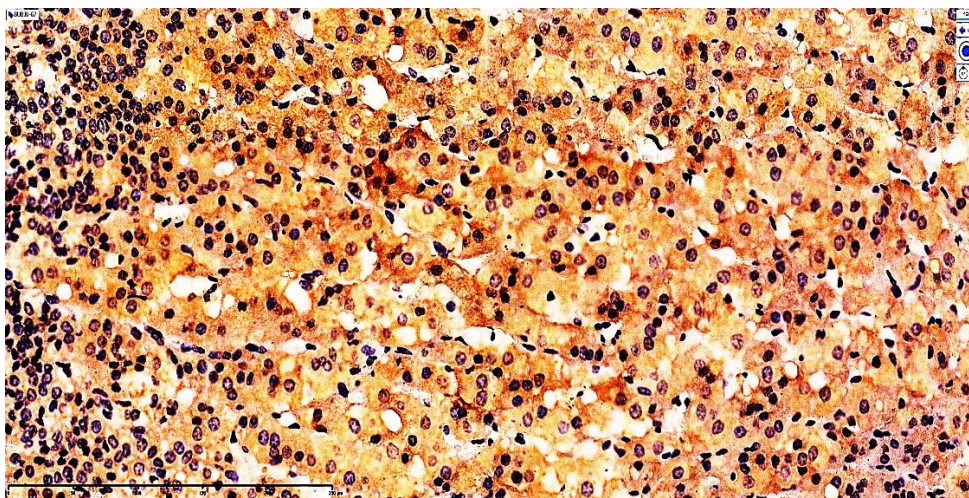


Figure 2. Group 1, 0–8 hours. Adrenal gland of a newborn with birth-related brain injury. High HSP70 expression was detected in the cytoplasm of all cells in the zona glomerulosa and zona fasciculata of the adrenal cortex. Marked expression was also observed in the fetal zone of the cortex and appeared as brown staining. Stain: DAB chromogen. Magnification: $\times 200$.

This process indicates that birth trauma-related stress, haemodynamic disturbances, and cerebral hypoxia cause marked activation of the sympathoadrenal system. In response to mechanical trauma and shock, the synthesis of early protective proteins is activated in the adrenal gland cells. This represents the first adaptive cellular response to injury.

The high level of HSP70 expression during the first 0–8 hours of life indicates that the tissue remains viable and that an active protective mechanism against acute stress has been activated.

In Group 1, high positive HSP70 expression was

detected in 14 of the 16 examined cases (87.5%), while moderate positive expression was found in 2 cases (12.5%). These findings indicate that the compensatory mechanisms had reached their highest level of activity. From a clinical and morphological point of view, delayed medical care during the early period after birth-related brain injury may lead to irreversible changes in the brain and internal organs. This period reflects the compensatory and subcompensatory stages of the adrenal gland response.

Group 2. During the period of 8–16 hours, the adrenal response to birth trauma was related to the duration of the pathological process. During this period,

intracellular resources became depleted, the synthesis of stress-adaptive hormones decreased markedly, and severe cellular degeneration and structural breakdown developed. As a result, the production of stress proteins declined, leading to reduced HSP70 expression.

This condition indicates the depletion of intracellular

protective reserves. It may be considered a transitional subcompensatory stage occurring within the first 24 hours of life. The intensity of HSP70 expression decreased significantly, and positive reactions became focal. At $\times 200$ magnification, weak expression was preserved in some cells, while it began to disappear completely in others.

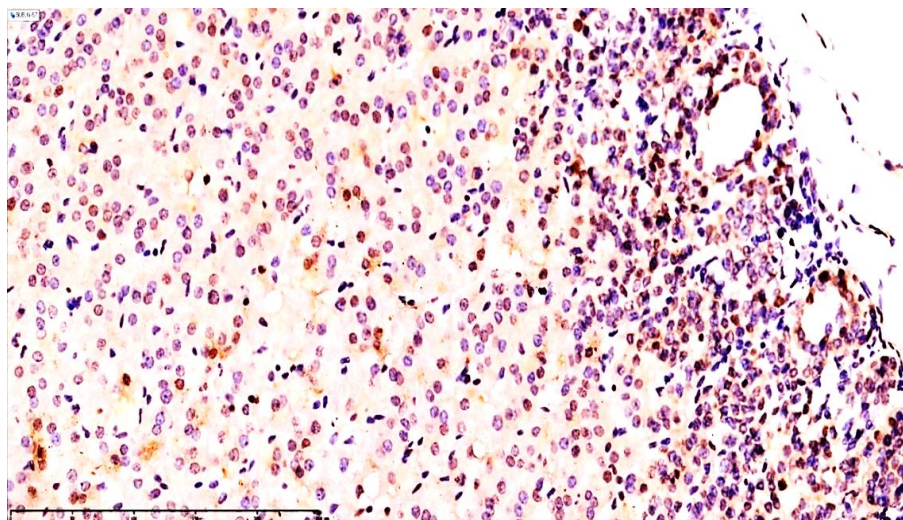


Figure 3. Group 2, 8–16 hours. Adrenal gland of a newborn with birth-related brain injury. Moderate-to-low HSP70 expression was detected in the cytoplasm of the adrenal cortical cells. Stain: DAB chromogen. Magnification: $\times 200$.

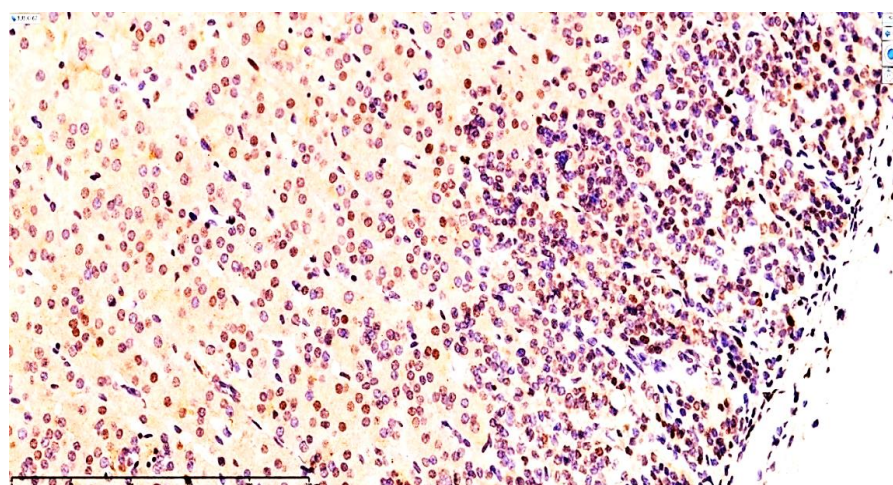


Figure 4. Group 2, 8–16 hours. Adrenal gland of a newborn with birth-related brain injury. Moderate-to-low HSP70 expression was detected in the cytoplasm of the adrenal cortical cells. Stain: DAB chromogen. Magnification: $\times 200$.

This finding indicates that, under continuous traumatic hypoxia and shock, the protective reserves of the cells were gradually depleted and the cells were approaching functional failure. This transitional period may be described as the stage in which cellular defence mechanisms begin to decline and destructive changes start to develop. Of the 16 cases examined in this

group, low positive HSP70 expression was detected in 11 cases (68.75%), moderate positive expression in 3 cases (18.75%), and a negative reaction in 2 cases (12.5%). These differences in expression indicate that the adrenal glands do not show the same response in all newborns. The response depends on the duration of the pathological process and the severity of brain injury caused by birth trauma. Thus, low and moderate HSP70

expression in the immunohistochemical examination of Group 2 indicates that the remaining viable cells were in the subcompensatory stage. In Group 3, during the period of 16–24 hours, the adrenal glands of newborns with birth-related brain injury showed an almost complete loss of morphofunctional activity. HSP70

protein expression was detected only in isolated focal areas. These findings were also confirmed by morphological and morphometric examinations. Destructive changes were observed in both the cortical and medullary regions of the adrenal glands.

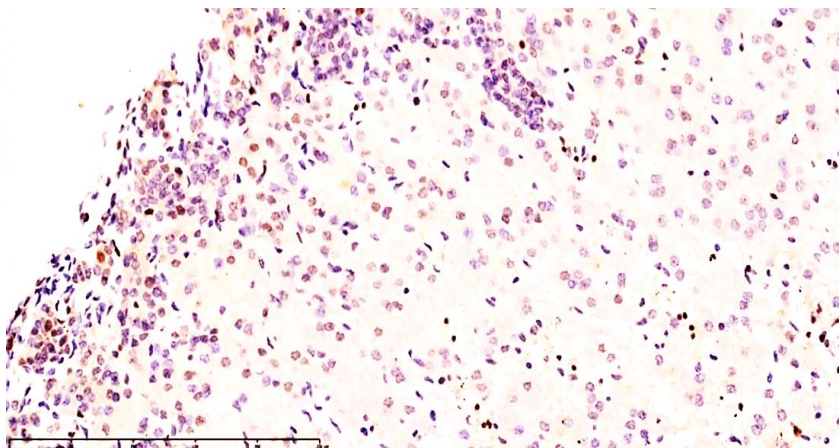


Figure 5. Group 3, 16–24 hours. Adrenal gland of a newborn with birth-related brain injury. No HSP70 expression was detected in the adrenal cortical cells (negative expression). Stain: DAB chromogen. Magnification: ×200.

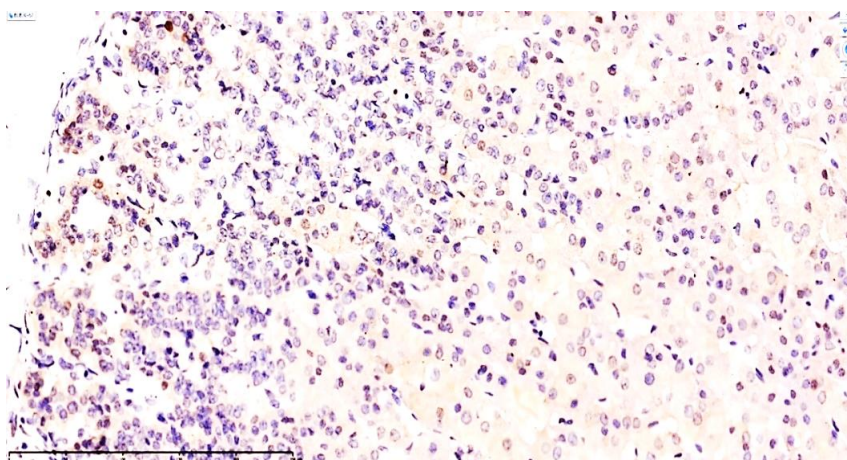


Figure 6. Group 3, 16–24 hours. Adrenal gland of a newborn with birth-related brain injury. No HSP70 expression was detected in the adrenal cortical cells (negative expression). Stain: DAB chromogen. Magnification: ×200.

In Group 3, a negative HSP70 reaction was detected in 10 of the 16 examined cases (62.5%), low positive expression in 4 cases (25%), and moderate positive expression in 2 cases (12.5%).

Clinical history and instrumental examination findings in newborns with low positive HSP70 expression showed that severe stress initially caused a critical increase in blood cortisol levels. This was followed by a marked decrease in blood pressure that did not respond to vasopressor therapy. Multiple organ failure was identified as the main cause of death in these

newborns.

Post-mortem examination revealed cerebral oedema, adrenal haemorrhage and autolysis, pulmonary oedema, and shock-related changes in the internal organs. Morphological examination of Group 3 showed predominantly destructive changes in the adrenal cortex and medulla, together with areas of autolysis and haematoma formation.

Thus, during the final 18–24 hours after birth-related brain injury, the protective reserves of the adrenal cells against stress were completely depleted. Lipid

inclusions in the spongiocytes were lost, and irreversible cellular damage developed. Post-mortem findings confirmed that the loss of HSP70 expression during the 16–24-hour period indicated complete failure of the compensatory and protective mechanisms of the adrenal glands due to acute shock caused by birth-related brain injury. This condition represented a critical and irreversible stage that directly contributed to death.

CONCLUSIONS

In newborns who experienced birth-related brain injury and died within the first 24 hours of life, HSP70 stress protein expression in the adrenal cortex changed markedly according to the time after birth. During the first 0–8 hours of life, the protective response reached its peak, with high positive HSP70 expression observed in 87.5% of cases. This finding indicates a strong adaptive and compensatory cellular response to acute hypoxia and shock.

During the period of 8–16 hours, HSP70 expression decreased significantly because of the depletion of intracellular reserves and the development of destructive changes. Low positive expression was observed in 68.75% of cases. This period may be considered a stage of subcompensation and gradual failure of the cellular defence system, indicating the beginning of irreversible tissue damage.

During the period of 16–24 hours, the protective reserves of the adrenal cells against stress were completely depleted, and negative HSP70 expression was observed in 62.5% of cases. This finding provides morphological evidence that the compensatory mechanisms of the adrenal glands had completely failed under conditions of acute shock and multiple organ failure, indicating progression to a critical and irreversible stage that directly contributed to death.

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