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Morphological changes in salivary glands of neonatal rats after intra-abdominal hypertension

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Abstract. – OBJECTIVE: Given the overall prevalence of elevated Intra-abdominal pressure (IAP), along with earlier detection and appropriate therapy of Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS), a significant reduction in patient morbidity and mortality is currently achieved by modern medicine. This article assesses the long-term degree of salivary gland damage in rats depending on the severity of experimental IAH during the neonatal period.

MATERIALS AND METHODS: To simulate IAH, newborn rats, under the control of intravesical manometry, were injected into the abdominal cavity with bulking collagen filler in the amount necessary to create a given level of IAP.

RESULTS: As shown by the results obtained, rats exposed to intra-abdominal hypertension for ten days had pathological changes in their salivary glands within 120 days. The severity of sialadenitis revealed a correlation with the severity of IAH. Some rats had individual reactions expressed in relative resistance to their organs' abnormalities under hypoxia.

CONCLUSIONS: It was concluded that children with severe IAH history might need the disease prevention of the CNS, kidneys, digestive, and respiratory systems and oral diseases, particularly diseases involving the salivary glands. Future research is supposed to investigate further the IAH effect on various organs and tissues, including the dentofacial system.

Key Words:
Intra-abdominal hypertension, Intra-abdominal pressure, Hypoxia, Abdominal compartment syndrome, Salivary glands.

Introduction

Intra-abdominal hypertension (IAH) is a persistent increase in intra-abdominal pressure (IAP) above 12 mmHg[1]. About 30% of critically ill children have an elevated IAP, which may be accompanied by abdominal compartment syndrome (ACS) development. ACS is a persistent elevation of IAP above 20 mmHg associated with new organ dysfunction/failure[2,3]. The most common consequences of ACS are brain, digestive, respiratory, cardiovascular, and renal systems dysfunctions[1,4]. Thanks to continuously updated clinical guidelines developed by the World Society of the Abdominal Compartment Syndrome (WSACS), mortality among adult specimens have been reduced from 64% to 37%[3,5]. The mortality rate for children with ACS remains high, ranging from 40% to 60% 6 and reaching 70-90% in newborns[7,8].

The critical IAP of most patients is in the range of 10-15 mmHg[9]. At this pressure, the microcirculatory blood flow decreases, and the progression of target organ dysfunction begins, and ACS develops if IAH is not adequately recognized[10]. Increased IAP significantly reduces blood flow in the renal artery and compresses the renal vein leading to renal dysfunction. Oliguria develops at an IAP of 15 mmHg and anuria at 25 mmHg. Thus, as evidenced by the oliguria development, decreased renal function is one of the first detectable signs of IAH. Indeed, some clinical studies[11] showed that IAH (15 mmHg) is directly associated with renal failure.

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One of the first clinical aspects of IAH is hypoxia and accompanying hypercapnia[12]. The previous studies' results show that rat long-lasting hypoxia causes tissue damage of salivary glands, decreased secretory function, and changes in saliva composition[13,14]. Histological analysis of rat salivary glands reveals micro structural changes in secretory cells[15]. However, there is no articles describing the influence of IAH on salivary glands. This unique research could contribute to the development of IAH diagnostics, dentistry, and science in general. This study plans to show the long-term effect of experimental IAH during the neonatal period in rats on the morphological structure of salivary glands.

According to our hypothesis, the extent of rats' salivary gland damage depends on the severity of experimental IAH during the neonatal period. To confirm this hypothesis, the original IAH model of various degrees of severity in newborn rats was chosen due to the similarity of salivary glands with human ones, especially regarding the structural organization and secretions[16].

Materials and Methods

Institutional Review Board Statement (Animal Ethics)

The study was approved by the Institutional Review Board. Care and handling of the animals were following the National Institute of Health guidelines. The Local Ethics Committee approved the study of the Sechenov University, Ministry of Health of the Russian Federation (protocol No. 16-19 as of 2019-12-04). Studies at the experimental animals were carried out following GOST 33215-2014 as of 2016-07-01, GOST 33216-2014 as of 2016-07-01, and the Guide for Care and Use of Laboratory Animals (8th edition).

Female rats with broods (7-10 offspring in each brood) were used for IAH experimental simulation. Each female with a brood was kept in a separate polycarbonate cage (previously disinfected and treated) at 19-23°C and under the 24-hour light regime (12 hours of light, 12 hours of darkness), with free access to water and food. Feedstuff for rats and mice "ProKorm" was used for feeding females. It contained granules d=11-14 mm with vitamins, macro elements, and microelements. Ingredients match the state standards (GOST R 50258-92). The brood was fed naturally.

Model of IAH in Newborn Rats

A total of 30 newborn Wistar rats (age range, 2-6 h) were equally randomized into three groups. Rats were anesthetized with isoflurane-oxygen mixture and subjected to urinary catheterization and intravesical manometry using a 24 G peripheral venous catheter. IAP was measured in cm-H_2O and converted to mmHg. IAH was created by intraperitoneal injection of cross-linked bovine collagen (Zyplast; Allergan, Irvine, CA, USA) in the amount necessary to create a given level of IAP under control of intravesical manometry (Figure 1)[17]. In the control group, the abdominal cavity was pierced without further injection of the collagen gel. Then, they had free access to water and laboratory rat chow, their height, weight, and perimeter of the abdomen, and frequency of

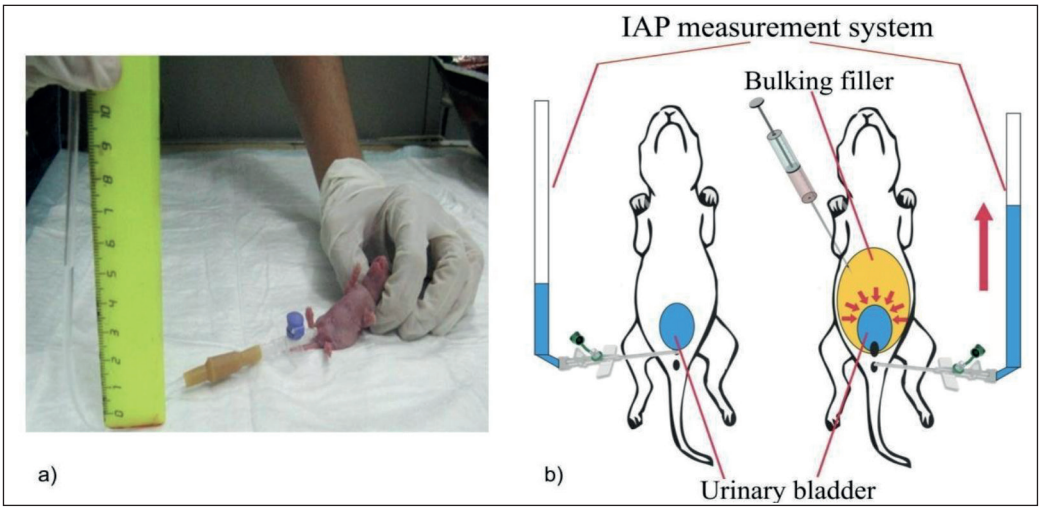

Figure 1. IAH simulation: **a)** photo of IAH control, **b)** diagram of the IAP measurement system.

evacuations being monitored. Intra-abdominal pressure (IAP) was evaluated in 24 hours and for 10 days following cross linked bovine collagen intraperitoneal injection. IAP measurements were performed at 8.00 a.m. In all cases, each measurement was repeated twice, and if the difference between measurements was >1 cmH_2O, measurements were repeated. When the level of IAP was lower than the target level, we added the cross-linked bovine collagen. IAP was measured by a 24 G catheter positioned in the urinary bladder under sterile conditions and then connected to an H_2O pressure system. During all the experiment period (120 days), pain was monitored by analyzing rats' daily behavior, such as the amount of food and drink, changes in body weight, sleeping, grooming, and coat appearance.

Newborn rats (n = 30) were divided into three groups (Table I):

- Group 1 (n = 10) – newborn rats with experimental early-stage IAH (IAP = 6-13 mmHg) lasting 10 days;
- Group 2 (n = 10) – newborn rats with experimental severe IAH (IAP = 14-20 mmHg) lasting 10 days;
- Group 3 (n = 10) – control group.

Animal Sacrifice and Harvesting of Biological Material

At the end of the experiment period (120 days), adult four-month-old rats that underwent IAH during the neonatal period got general anesthesia with a combination of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg)[18]. When the absence of the corneal reflex and the paw withdrawal reflex was stated, animal sacrifice was performed by decapitation. A parotid gland harvesting operation was performed after that. The surgical technique of rat parotid salivary gland harvesting was carried out as follows. The ear was pulled upwards, and a scalpel incision was made from behind the ear to the corner of the mouth. Then, the wound edges are parted with tweezers, and the skin was separated from the underlying tissues by sharp dissection with microsurgical scissors. In the projection of the jaw angle in front of the auricle, a salivary gland of a dull-transparent color was located. It had soft consistency, covered with fascia. The fascia with the gland were separated from the underlying bone and muscle, then dissected off and placed in formalin.

The right gland was removed after perfusion (the passage of blood through the vascular system of the parotid salivary gland) and used for morphological analysis with formalin (10%) fixation and paraffin embedding. Paraffin sections were stained using hematoxylin and eosin. The material after the biopsy was examined under a microscope (Olympus). The photos were taken with a built-in digital camera.

Head of the Department of Pathological Anatomy of Sechenov First Moscow State Medical University, professor, member of the International Academy of Pathology, and experienced pathologist E.A. Kogan conducted the histopathological examination.

Statistical Analysis

Data were compared using Fisher's exact test for categorical variables or the *t*-test or a distribution-free method for small samples (Mann-Whitney U test) for continuous variables as appropriate. All statistical tests were 2-sided. A result was considered statistically significant when $p<0.05$.

Results

Newborn rats in the acute period had clinical signs of hypoxia: tachycardia, lividity of mucous membranes, tachypnea.

Microscopic Characterization of Changes in Rat Salivary Glands against the Background of Intra-abdominal Hypertension

Morphology of control group (group 3)

Alveolar adenomeres represented mixed-type salivary glands with signs of protein-synthetic activity (protein type). Excretory ducts surrounded by thin connective tissue were among adenomeres. In three cases, there were protein demilunes (Figure 2).

Morphology of Group with Early-Stage Intra-abdominal Hypertension (Group 1)

The morphology of the group with early-stage intra-abdominal hypertension was identical to the morphology of the control group.

Alveolar adenomeres represented mixed-type salivary glands with signs of protein-synthetic activity (protein type). Excretory ducts surrounded by thin connective tissue were among adenomeres (Figure 3).

Morphology of Group with Severe Intra-abdominal Hypertension (Group 2)

Salivary glands had signs of sialadenitis. Stroma (mainly periductal) was infiltrated by lympho-

Table I. Newborn rats with experimental IAH.

Group Quantities	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)
Age, days	9	8	9
Weight, gr.	7.1±0.03	6.9±0.01	7.3±0.04
IAP, mmHg.	7.9±0.01	16.3±0.02	1.9±0.06

histiocytic elements with an admixture of plasma cells. There was lymphoid hyperplasia in regional lymph nodes (Figure 4). Figure 5 shows the presence of sialadenitis.

Three features were chosen as samples comparing criteria: the presence of inflammation signs, dyscirculatory disorders, and changes in lymphoid tissue. The comparison results are in Figure 6. Statistical analysis was implemented by using a distribution-free method for small samples Mann-Whitney U test.

Discussion

We decided to study the parotid gland for many reasons, particularly, the ease of surgical access and adequate size. We aimed to research the morphology of parotid salivary glands as a step towards a bigger study. There are no other studies of morphological changes in salivary glands of neonatal rats after intra-abdominal hypertension. That is why we are discussing the mechanisms of pathological processes caused by IAH based on previous studies.

Development Mechanisms of Multiple Organ Failure and Systemic Hypoxia in the Case of IAH and ACS

All organ systems of the human body are prone to IAH-induced injuries and damage. Increased IAP leads to dysfunction of all abdominal organs due to arterial circulation disorders, venous outflow tract obstruction, and microcirculatory disorders[11].

Increased IAP initiates pathological changes in abdominal organs. Compression of tissues leads to microcirculatory disorders, thrombosis, and, consequently, increased ischemia of hollow organ walls, increased volume of transudation and exudation, and aggravation of IAH. As a result of renal ischemia and relative hypovolemia, the levels of antidiuretic hormone, renin, and aldosterone increase. This causes a further increase in the swelling of abdominal organs[19].

IAH leads to decreased absorption of peritoneal fluid into the lymphatic vessels due to their compression. Increased IAP is accompanied by elevation of the hemidiaphragm and increased intrathoracic pressure, which hinders lymph outflow through the thoracic duct flow into the superior vena cava and stops when it reaches 22 mmHg[20].

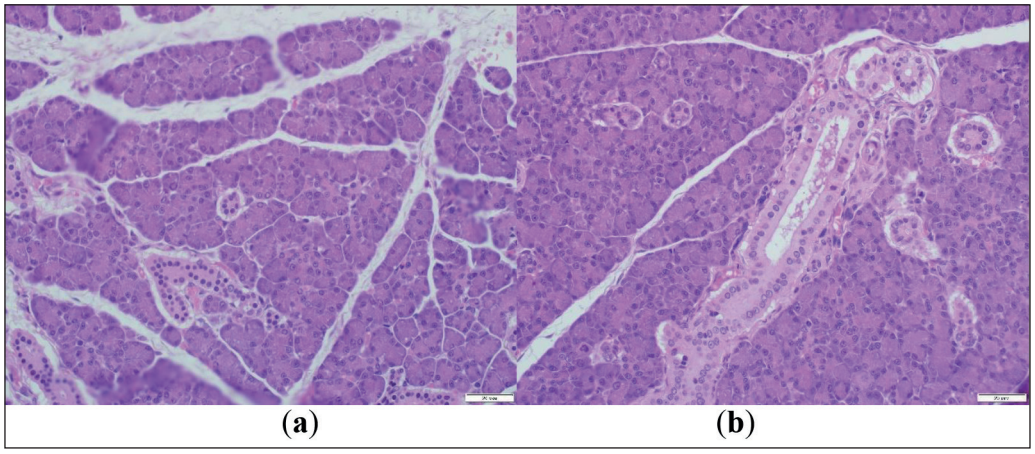

Figure 2. Micrograph of normal rat salivary glands. Hematoxylin-eosin staining; **(a)**, **(b)** – the structure of salivary glands represented by alveolar and ductal structures.

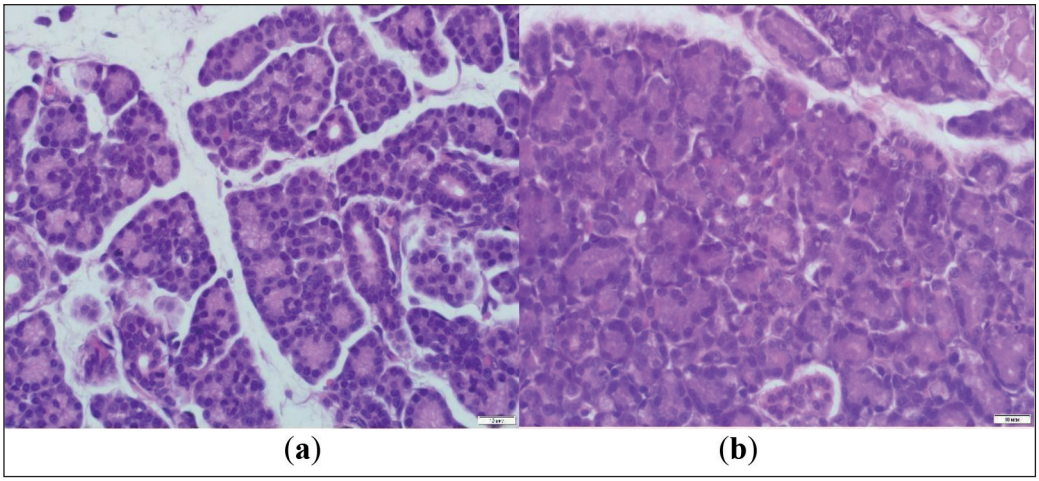

Figure 3. Micrograph of rat salivary glands with early-stage IAH. Hematoxylin-eosin staining; **(a), (b)** – the structure of salivary glands represented by alveolar and ductal structures is unchanged.

Compression of the great vessels of the abdominal cavity leads to changes in central hemodynamics. High IAP shifts the diaphragm and increases intrathoracic pressure, leading to compression of the heart and vessels, reducing diastolic ventricular filling[21]. Blood flow through the inferior vena cava decreases in proportion to IAP increase while decreasing venous return[22,23]. Pulmonary capillary pressure and total peripheral vascular resistance increase as IAP increases due to the large vascular bed compression. Decompression of abdominal organs leads to a fairly rapid recovery of cardiovascular activity, but in some cases, it requires inotropic support[19].

Respiratory disorders in children manifest themselves faster in younger children. Oxygen saturation of blood deteriorates due to decreased respiratory volumes and collapse of the alveoli adjacent to the diaphragm. Hypoxemia, hypercapnia, and respiratory acidosis gradually develop[19,21].

Exposure to hypoxia is considered a stress stimulus that activates physiological compensatory mechanisms to ensure homeostasis. Acclimation is the most common phenotypic modification under hypoxia and includes hematological, cardiovascular, renal, and metabolic changes that help the body cope with lower O_2 levels[24]. At the molecular level, hypoxia induces highly coordinated cellular responses to maintain cell viability. Hypoxia activates transcription of genes that encode glucose transporters and glycolytic enzymes increasing the conversion of glucose to pyruvate. PDK1 gene encodes pyruvate dehydrogenase enzyme complex kinase, a mitochondrial enzyme that converts pyruvate to acetyl-CoA to enter the tricarboxylic acid cycle. It also activates lactate dehydrogenase that converts pyruvate to lactate. Shunting the substrate from the mitochondria reduces adenosine triphosphate production but prevents excessive active oxygen species (AOS) production due to inefficient electron transport under hypoxia. Hypoxia-inducible factors (HIFs), the master regulators of the hypoxic response[25], control all these adaptive responses.

Although the adaptive response aims to help the body cope with low oxygenation levels, hypoxia can also lead to pathological processes if the ability to maintain O2 homeostasis fails[26]. Hypoxia enhances intracellular reactions that contribute to phagocytosis, leukocyte activation, and adaptive immunity. It means the activation of HIF-1α is necessary to eliminate pathogens[27]. In addition, it is known that exposure to hypoxia increases the oxidative stress of cells, leading to the production of AOS with harmful effects on lipids, proteins, and DNA[28].

Although hypoxia's effects on the body are well studied, its role in oral health is still unclear. Studies of salivary glands have shown that hypoxia reduces submandibular gland secretion[29].

The elevated prostaglandin E2 (PGE2), the inflammatory mediator, was associated with decreased salivary discharge rates in rats[30]. Saliva is an essential oral fluid since its chemical composition is closely related to basic functions contributing to oral health.

In one study, Argentinian scientists found that periodic hypoxia simulated in rats inside a pressure chamber showed increased catabolism of HIF-1α[31]. It means that less gene transcription is

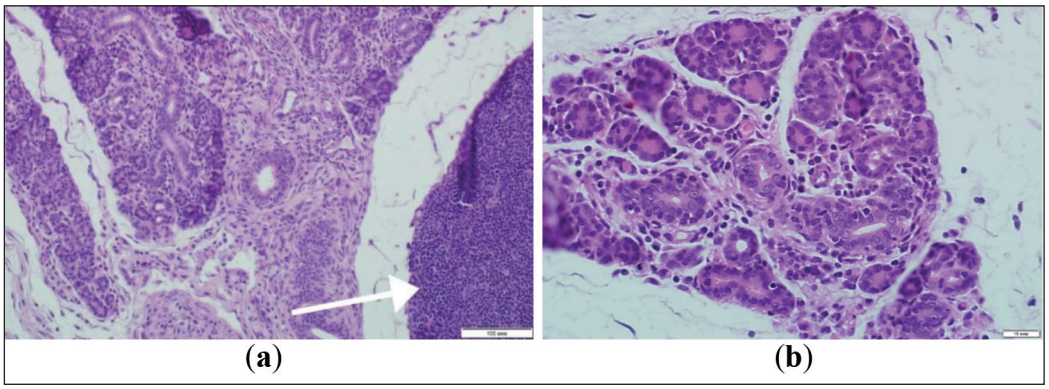

Figure 4. Micrograph of rat salivary glands with severe IAH. Hematoxylin-eosin staining; **(a)**, **(b)** – sialadenitis with lymphocyte infiltration and perialveolar and periductal sclerosis; **(a)** – lymphoid hyperplasia in regional lymph nodes (arrow), **(b)** – X600.

needed for the adaptation process, thus leading to a poorer coordinated response to ensure proper gland function. In this case, the inflammatory response is more pronounced. A high level of PGE2 was observed, which may explain hyposalivation in adult rats in the experiment. In addition, analysis with use of electronic microscopy revealed apoptotic cells in the acini and intercalated ducts, and a smaller number of secretory granules, which are unequally distributed in the acini of the submandibular salivary gland[31].

In their studies, Scott and Gradwell[15,32] showed that the long-term hypoxic state caused structural changes in the heart and lungs of laboratory rats. Severe hyperemia was observed in their parotid and submandibular salivary glands. The average proportional volume of vascular tissue has increased by 57% in parotid glands and 30% in submandibular glands.

This research also studied ultrastructural changes caused by chronic hypoxia in the cells of rats' parotid salivary glands. There were disorders in an organization, reductions in functions, fragmentations (or even destruction) in the Golgi apparatus, the rough endoplasmic reticulum, mitochondria, and their matrix and cristae. The nucleus revealed a fibrillar pattern and a reduced amount of heterochromatin.

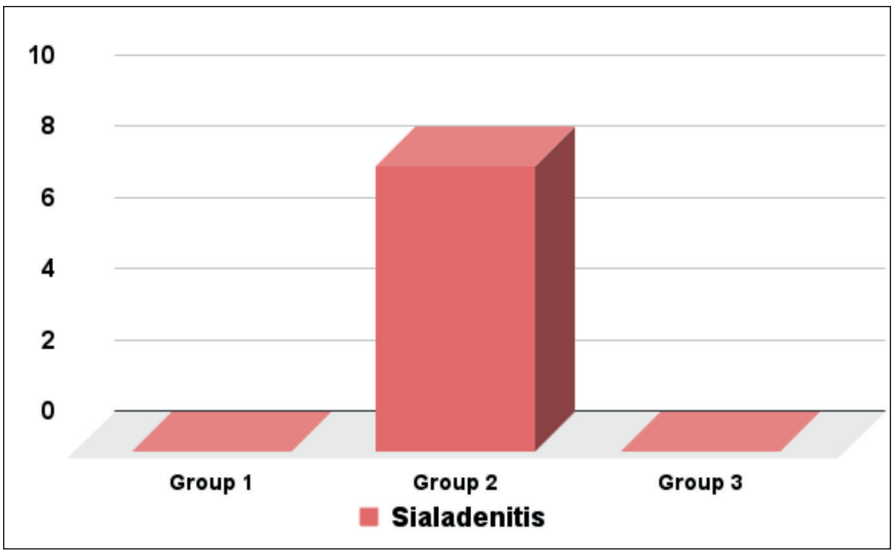

Figure 5. Presence of sialadenitis in rat salivary glands. Sialadenitis was detected in eight samples with severe IAH and not in two cases ($p < 0.05$).

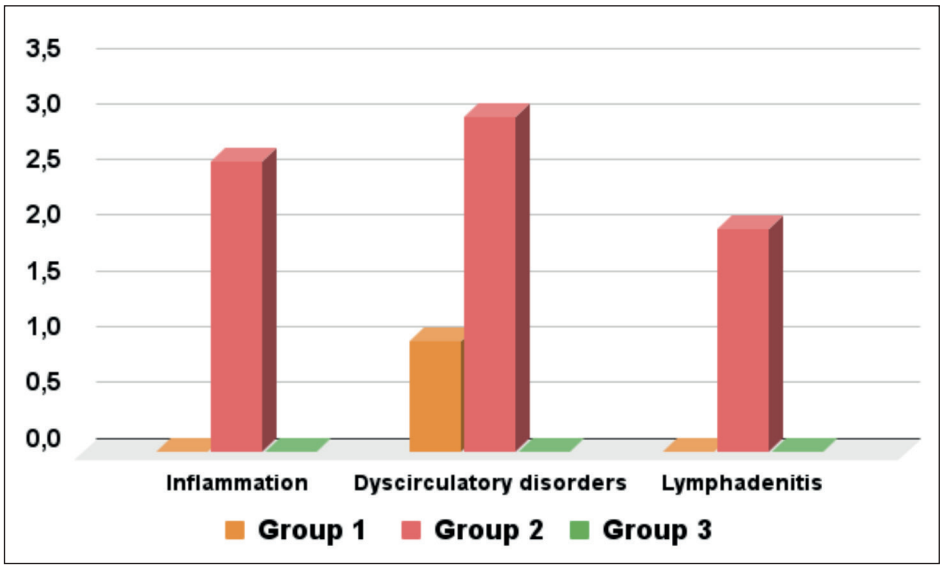

Figure 6. Comparison of samples ($p < 0.05$).

Biochemical results show that hypoxic cells contain only 55% amylase and 84% DNA compared to the control group, thus leading to a sharp decrease in exported protein and cell growth[33].

Conclusions

After being exposed to Intra-abdominal hypertension for ten days, rats endured pathological changes in their salivary glands within 120 days. The severity of sialadenitis revealed its correlation with the severity of IAH. Some rats showed individual reactions expressed in relative resistance to their organs' abnormalities under hypoxia.

It is expected that long-term hypoxia associated with severe IAH will cause pathological changes in the entire structure of the newborn rat salivary gland, from the circulatory system to the secretory elements.

Conflict of Interests

The authors declare that they have no conflict of interest.

Funding

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