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ANALYSIS OF HISTOMORPHOLOGICAL PARAMETERS OF ODONTOGENIC INFLAMMATORY DISEASES IN CHILDREN WITH PREMORBID CONDITIONS

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Abstract.

Odontogenic inflammatory diseases (OID) in children with premorbid conditions follow a more severe and protracted clinical course, necessitating objective morphological assessment of the inflammatory process. Standardised histological and cytological diagnostic methods enable detailed characterisation of pathomorphological changes in the tissues of the pathological focus.

Objective. *To evaluate the histomorphological parameters of odontogenic inflammatory diseases in children with a premorbid history, employing Romanowsky–Giemsa and Van Gieson staining methods.*

Materials and Methods. *A prospective clinico-morphological study was conducted. A total of 239 children aged 3-17 years with odontogenic inflammatory diseases were examined; all had received treatment at the Department of Paediatric Maxillofacial Surgery, Bukhara Regional Children's Multidisciplinary Medical Centre, between 2021 and 2025. The study group comprised 127 children with premorbid conditions (recurrent acute respiratory viral infections, chronic diseases), and the control group comprised 112 patients without comorbid pathology. Biological specimens were collected intraoperatively and on days 3-5 of treatment; Romanowsky–Giemsa and Van Gieson staining with Weigert's haematoxylin and picrofuchsin were applied. Histological sections were prepared according to the standard paraffin technique with fixation in 10% neutral formalin. The study was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki (2013 revision). The protocol was approved by the Local Ethics Committee of Bukhara State Medical Institute. Written informed consent was obtained from the legal representatives of all paediatric participants. The confidentiality of personal data was maintained. Intergroup differences in categorical variables were assessed using the chi-square (χ^2) test. Statistical significance was set at $p < 0.05$. Data were analysed with SPSS Statistics, version 26.0. The study was conducted as part of the research plan of Bukhara State Medical Institute entitled «Improving Diagnostic and Treatment Methods for Inflammatory Diseases of the Maxillofacial Region in Children» (2021-2025).*

Results. *In the study group, Romanowsky–Giemsa staining revealed a predominance of neutrophils and macrophages, accompanied by an attenuated lymphocytic response. Van Gieson staining demonstrated pronounced fibrosis and destruction of collagen fibres. In the control group, the inflammatory cellular composition corresponded to a typical acute reaction with earlier lymphocytic transformation. The morphological differences between the groups were statistically significant ($p < 0.05$).*

Conclusions. *Premorbid conditions significantly affect the histomorphological pattern of odontogenic inflammation in children, manifesting in more pronounced destructive changes. The combined application of Romanowsky–Giemsa and Van Gieson staining methods enables objective assessment of the nature of the inflammatory process and serves as a basis for developing individualised treatment protocols.*

Keywords: *Odontogenic Inflammatory Diseases; Children; Premorbid Conditions; Histomorphological Parameters; Romanowsky–Giemsa Staining; Van Gieson Staining; Cytological Examination.*

Introduction

Odontogenic inflammatory diseases (OID) – periostitis, osteomyelitis, phlegmon, and facial abscesses – constitute a major group of conditions in paediatric maxillofacial surgery. Systematic studies attribute the prevalence of OID in children to early caries development, the anatomical and topographic characteristics of the paediatric facial skeleton, and an increasing burden of somatic disease [1, 2, 3]. Paediatric odontogenic infections disproportionately affect children with compromised immune function, chronic disease, and unfavourable premorbid conditions [4].

Premorbid conditions – including chronic upper respiratory tract infections, allergic diseases, endocrine pathology, and immunodeficiency states – significantly influence the nature of the inflammatory response, producing a more severe and protracted course of OID together with a higher risk of phlegmonous forms and complications [5, 6, 7]. The morphological pattern of the inflammatory process in such patients frequently differs from the typical responses observed in somatically healthy children, requiring the application of reliable and reproducible methods of histological and cytological diagnostics [8].

Romanowsky–Giemsa staining is a standard cytological tool that enables identification of the cellular composition of the inflammatory infiltrate in biological specimens obtained from the pathological focus [9]. Van Gieson staining using Weigert's haematoxylin and picrofuchsin is employed to assess connective tissue status, the degree of fibrosis, and destructive changes in collagen fibres [10]. The combined use of these methods provides opportunities for objective pathomorphological characterisation of OID in children with varying premorbid conditions, which is of importance for disease severity stratification and the development of personalised treatment approaches [11, 12].

Study Objective

To evaluate the histomorphological parameters of odontogenic inflammatory diseases in children with

a premorbid history, employing Romanowsky–Giemsa and Van Gieson staining methods.

Materials and Methods

Study Design and Setting. A prospective clinico-morphological study was conducted at the Department of Paediatric Maxillofacial Surgery, Bukhara Regional Children's Multidisciplinary Medical Centre, between 2021 and 2025.

Patient Characteristics. A total of 239 children aged 3-17 years with odontogenic inflammatory diseases were enrolled in the study. The study group comprised 127 children with premorbid conditions (recurrent acute respiratory viral infections occurring more than 4 times per year, chronic diseases, allergic conditions); the control group comprised 112 somatically healthy patients. The distribution of patients by age group is presented in Table 1.

Table 1

Distribution of Patients by Age Group

Age	Study group (n = 127), abs. (%)	Control group (n = 112), abs. (%)	Total (n = 239), abs. (%)
3-6 years	38 (29.9)	31 (27.7)	69 (28.9)
7-12 years	54 (42.5)	49 (43.7)	103 (43.1)
13-17 years	35 (27.6)	32 (28.6)	67 (28.0)
Всего	127 (100)	112 (100)	239 (100)

Note: $p > 0.05$ – no statistically significant intergroup differences in age distribution were detected (χ^2 test).

Biological Specimen Collection. Cytological specimens were collected from the cavity of the pathological focus by two methods: intraoperatively (during surgical debridement) and from the wound area on days 3-5 of treatment. Prior to specimen collection, the oral cavity was cleansed with a sterile cotton swab to remove food debris, mucus, and necrotic tissue; local anaesthesia was administered, after which the surface of the pathological focus was thoroughly scraped with a sharp spatula. The biopsy material was immediately placed in 10% neutral formalin (4% formaldehyde). The volume of fixative exceeded the volume of the biological material by a factor of 25.

Staining Methods. Two staining methods were employed for biological specimen processing.

1. Romanowsky–Giemsa Method. The ready-to-use liquid dye was diluted at a ratio of 1-2 drops per 1 mL of distilled water; given the limited solubility of the staining agent, the solution was further diluted to 300 mg/100 mL when required. Fixed smears were stained in a humid chamber at 37 °C for 20-25 min, rinsed with running water, and air-dried. The method enables identification of the cellular composition of the inflammatory infiltrate (neutrophils, macrophages, lymphocytes, eosinophils).

2. Van Gieson Method. Deparaffinised sections were stained sequentially with Weigert's iron haematoxylin (3-5 min), rinsed with running water (10-15 min until nuclear bluing), and then placed in distilled water (3-5 min). Cytoplasmic staining was carried out with picrofuchsin (0.5-2 min). Dehydration was conducted in graded ethanol solutions, clearing in a carbolic acid–xylene mixture, and mounting in Canada balsam. Sections were dried in a thermostat for 24 h at 37 °C.

Histological Processing. Biological material was fixed in 10% neutral formalin at room temperature (22-24 °C). Dehydration was carried out in graded ethanol solutions (Table 1), after which the tissues were embedded in paraffin. The approximate protocol for preparation of ethanol solutions of the required concentration is presented in Table 1; the dehydration schedule is presented in Table 2.

Table 1

Approximate Protocol for Preparation of Ethanol Solutions of Required Concentration (Starting Volume: 95° Ethanol)

No.	Ethanol concentration (100 mL)	95° ethanol (mL)	Water (mL)
1	40°	42	58
2	45°	47	53
3	50°	52	43
4	60°	63	37
5	70°	73	27
6	80°	83	17
7	90°	94	6

Table 3

Approximate Dehydration Schedule Following Fixation of Histological Material in Formalin

No.	Dehydration stage	Dehydration time
1	40% ethanol	12 h
2	50% ethanol	12 h
3	60% ethanol	12 h
4	70% ethanol	12 h
5	80% ethanol	12 h
6	95% ethanol	12 h
7	100% (I) ethanol	6 h
8	100% (II) ethanol	6 h

Composition of Weigert's Haematoxylin. Solution I: 1% haematoxylin solution in 95% ethanol. Solution II: 4 mL of $FeCl_3$ solution + 1 mL of concentrated HCl + 95 mL of distilled water. Picrofuchsin composition: saturated picric acid solution (1.2 g per 100 mL of water) combined with 1% aqueous acid fuchsin solution at a ratio of 10:1.

The study was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki (2013 revision). The protocol was approved by the Local Ethics Committee of Bukhara State Medical Institute. Written informed consent was obtained from the legal representatives of all paediatric participants. The confidentiality of personal data was maintained.

Intergroup differences in quality indicators were assessed using the chi-square (χ^2) test. Statistical significance was set at $p < 0.05$. Data were analysed with SPSS Statistics, version 26.0.

The study was conducted as part of the research plan of Bukhara State Medical Institute entitled «Improving Diagnostic and Treatment Methods for Inflammatory Diseases of the Maxillofacial Region in Children» (2021-2025).

Results and Discussion

Cytological examination of biological specimens stained by the Romanowsky–Giemsa method revealed, in the study group (children with premorbid conditions), a predominance of neutrophils and macrophages at the early stages of inflammation, with delayed lymphocytic transformation. The smears were characterised by pronounced degeneration of cellular elements, abundant background microflora, and evidence of impaired phagocytic function of neutrophils. In the control group, the cellular composition of the inflammatory infiltrate corresponded to a typical acute inflammatory reaction: the initial predominance of neutrophils during the first 1-2 days was succeeded by a progressive increase in the proportion of lymphocytes and macrophages by days 3-5, indicating an adequate immune response.

Histological examination of Van Gieson-stained sections in the study group revealed pronounced stromal fibrosis, destruction and disorganisation of collagen fibres, extensive inflammatory infiltration with a predominance of neutrophils, and focal necrosis. In the control group, changes in collagen structures were less pronounced, with preservation of the distinct connective tissue architecture.

The morphological differences between the groups with respect to the degree of destructive changes, cellular composition of the infiltrate, and extent of fibrosis were statistically significant ($p < 0.05$, χ^2 test).

The findings are consistent with clinical studies demonstrating that odontogenic infections in children with compromised immune function and chronic diseases follow a more severe course and are accompanied by more pronounced destructive tissue changes [13]. The predominance of neutrophils with evidence of impaired phagocytosis and the delayed lymphocytic transformation observed in the study group are consistent with the pathophysiological mechanisms of immunosuppression characteristic of children with premorbid conditions [14].

The combined application of Romanowsky–Giemsa and Van Gieson methods enables characterisation of the cellular composition of the inflammatory infiltrate alongside objective assessment of connective tissue structures, both of which are essential for predicting outcomes and determining the timing of active surgical intervention [15, 16]. The findings underscore the importance of considering premorbid conditions when planning treatment of OID in children.

Conclusions

1. Premorbid conditions significantly alter the histomorphological pattern of odontogenic inflammation in children, manifesting in a delayed immune response, pronounced neutrophilic infiltration with evidence of impaired phagocytosis, and destructive changes in collagen fibres ($p < 0.05$).
2. Romanowsky–Giemsa staining enables accurate identification of the cellular composition of the inflammatory infiltrate in biological specimens from the pathological focus in children; Van Gieson staining enables objective assessment of connective tissue status and the degree of fibrosis.
3. The combined application of both staining methods represents an informative tool for pathomorphological stratification of odontogenic inflammation severity in children with premorbid conditions and may serve as a basis for developing individualised treatment protocols.

Directions for Future Research. A priority area for future research is the development of standardised morphological criteria for predicting the course and outcomes of OID in children with varying premorbid conditions. Multicentre studies with larger sample sizes and the inclusion of immunohistochemical markers of inflammatory activity are warranted. Of particular interest is investigation of the relationship between specific types of premorbid pathology – allergic disease, immunodeficiency, and chronic infection foci – and the pattern of histomorphological changes in the tissues of the pathological focus.

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АНАЛІЗ ГІСТОМОРФОЛОГІЧНИХ ПАРАМЕТРІВ ОДОНТОГЕННИХ ЗАПАЛЬНИХ ЗАХВОРЮВАНЬ У ДІТЕЙ З ПРЕМОРБІДНИМИ СТАНАМИ

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Резюме.

Одонтогенні запальні захворювання (ОЗЗ) у дітей з преморбідними станами мають більш важкий та тривалий клінічний перебіг, що вимагає об'єктивної морфологічної оцінки запального процесу. Стандартизовані гістологічні та цитологічні методи діагностики дозволяють детально охарактеризувати патоморфологічні зміни в тканинах патологічного вогнища.

Мета дослідження. Оцінити гістоморфологічні параметри одонтогенних запальних захворювань у дітей з преморбідним анамнезом, використовуючи методи забарвлення за Романовським-Гімзою та Ван Гізоном.

Матеріали та методи. Було проведено проспективне клініко-морфологічне дослідження. Загалом було обстежено 239 дітей віком від 3 до 17 років з одонтогенними запальними захворюваннями; всі вони проходили лікування у відділенні дитячої щелепно-лицевої хірургії Бухарського обласного дитячого багатопрофільного медичного центру з 2021 по 2025 рік. Досліджувану групу склали 127 дітей з преморбідними станами (рецидивуючі гострі респіраторно-вірусні інфекції, хронічні захворювання), контрольну групу – 112 пацієнтів без супутньої патології. Біологічні зразки збирали інтраопераційно та на 3-5 день лікування;

застосовували забарвлення за Романовським-Гімзою та Ван Гізоном гематоксиліном Вейгерта та пікрофуксином. Гістологічні зрізи готували за стандартною парафіновою методикою з фіксацією у 10% нейтральному формаліні. Дослідження було проведено відповідно до принципів Гельсінської декларації Всесвітньої медичної асоціації (редакція 2013 року). Протокол був схвалений Місцевим етичним комітетом Бухарського державного медичного інституту. Письмова інформована згода була отримана від законних представників усіх учасників-педіатрів. Конфіденційність персональних даних була збережена. Міжгрупові відмінності за категоріальними змінними оцінювалися за допомогою тесту хі-квадрат (χ^2). Статистичну значущість було встановлено на рівні $p < 0,05$. Дані були проаналізовані за допомогою SPSS Statistics, версія 26.0. Дослідження було проведено в рамках дослідницького плану Бухарського державного медичного інституту під назвою «Удосконалення методів діагностики та лікування запальних захворювань щелепно-лицевої області у дітей» (2021-2025 рр.).

Результати. У досліджуваній групі фарбування за Романовським-Гімзою виявило переважання нейтрофілів та макрофагів, що супроводжувалося ослабленою лімфоцитарною відповіддю. Фарбування за Ван-Гізоном продемонструвало виражений фіброз та руйнування колагенових волокон. У контрольній групі запальний клітинний склад відповідав типовій гострій реакції з більш ранньою лімфоцитарною трансформацією. Морфологічні відмінності між групами були статистично значущими ($p < 0,05$).

Висновки. Преморбідні стани суттєво впливають на гістоморфологічний патерн одонтогенного запалення у дітей, проявляючись більш вираженими деструктивними змінами. Спільне застосування методів забарвлення за Романовським-Гімзою та Ван-Гізоном дозволяє об'єктивно оцінити характер запального процесу та служить основою для розробки індивідуалізованих протоколів лікування.

Ключові слова: одонтогенні запальні захворювання; діти; преморбідні стани; гістоморфологічні параметри; забарвлення за Романовським-Гімзою; забарвлення за Ван-Гізоном; цитологічне дослідження.

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