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# The role of NF-kB in the mechanisms of inflammation of the stomach's mucosa in children

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**Introduction.** One of the important and unresolved issues of pediatric gastroenterology remains study of inflammation mechanisms of the stomach's mucosa, as well as factors that regulate inflammatory reactions. Nowadays, there are many studies devoted to the research of the nuclear role of factor kappa B (NF-kB) in the regulation of the level of gene expression that control proliferation, cell apoptosis, angiogenesis, and determine the nature and expressiveness of the inflammatory process in adults. However, there are no conclusion regardless the role of NF-kB in the regulation of the mechanisms of inflammation development in the stomach's mucosa of chronic gastritis (CG) in children.

**Purpose** — to study the level of NF-κB activity depending on the degree of gastric mucosa inflammation of chronic gastritis in children.

**Materials and methods.** We observed 76 children aged 8–16 years with verified chronic gastritis. To verify the diagnosis, all children underwent a morphological examination of the stomach's mucosa in the fundal and antral departments of the stomach. An indirect streptavidin-peroxidase method of protection using polyclonal antibodies to NF-kB was used for immunohistochemical research.

**Results.** On the basis of the morphological study of gastric biopsies, we discovered inflammatory changes based of lymphocytic infiltration, microcirculatory disorders of the stomach's mucosa with the subsequent development of stroma lamina propria fibrosis. During the immunohistochemical study of gastric biopsies, a high level of NF-kB expression was noted in the cytoplasm with a pronounced degree of inflammation, atrophy, and lymphocytic infiltration of the stomach's mucosa.

**Conclusions.** The results of the study indicate the morphological signs of the formation of an early chronic inflammatory process in gastritis in children. The transcription factor NF-kB determines the level of inflammatory activity and plays a leading role in the mechanism of development of chronic gastritis in children.

The research was carried out in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Local Ethics Committee of the participating institution. The informed consent of the patient was obtained for conducting the studies.

No conflict of interests was declared by the author.

**Keywords:** children, chronic gastritis, nuclear factor kappa-B.

## Роль NF-kB у механізмах розвитку запалення слизової оболонки шлунка в дітей

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**Вступ.** Одним із важливих і невирішених питань дитячої гастроентерології залишається вивчення механізмів запалення слизової оболонки шлунка (СОШ), а також факторів, які регулюють запальні реакції. На тепер проведено значну кількість досліджень, присвячених вивченню ролі ядерного фактора капа В (NF-kB) у регуляції рівня експресії генів, що контролюють проліферацію, апоптоз клітин, ангіогенез, визначають характер і виразність процесу запалення в дорослих. Водночас не існує єдиної точки зору на роль NF-kB у регуляції механізмів розвитку запалення СОШ при хронічному гастриті (ХГ) у дітей.

**Мета** — вивчити рівень активності NF-κB залежно від ступеня запалення СОШ при ХГ у дітей.

**Матеріали та методи.** Під нашим спостереженням було 76 дітей віком від 8 до 16 років із верифікованим діагнозом ХГ у періоді загострення. Для верифікації діагнозу всім дітям проведено морфологічне дослідження СОШ фундального, антрального відділів. Для імуногістохімічного дослідження застосовано непрямий стрептавидин-пероксидазний метод забарвлення з використанням поліклональних антитіл до NF-kB («DAKO», Данія)

**Результати.** На підставі проведеного морфологічного дослідження біоптатів СОШ виявлено виражені запальні зміни на тлі лімфоцитарної інфільтрації, мікроциркуляторні порушення СОШ з подальшим розвитком фіброзу строми власної пластинки. За даними імуногістохімічного дослідження біоптатів шлунка відмічено високий рівень експресії NF-kB у цитоплазмі при вираженому ступені запалення, атрофії та лімфоцитарній інфільтрації СОШ.

**Висновки.** Результати проведеного дослідження вказують на морфологічні особливості формування раннього хронічного запального процесу при гастриті в дітей. Фактор транскрипції NF-kB визначає рівень активності запалення і відіграє провідну роль у механізмі розвитку ХГ у дітей.

Дослідження виконано відповідно до принципів Гельсінської декларації. Протокол дослідження ухвалено Локальним етичним комітетом зазначеної в роботі установи. На проведення досліджень отримано інформовану згоду батьків, дітей.

Автор заявляє про відсутність конфлікту інтересів.

**Ключові слова:** діти, хронічний гастрит, ядерний фактор капа В.

Analysis of inflammation mechanisms and factors mediating inflammatory reactions remains one of the most important and non-conclusive pediatric gastroenterology points. Inflammation is the defense mechanism of an organism to infection or tissue injury, characterized by involvement of immune cells and plasma proteins. In case of timely termination, it is a useful process; but non-regulated inflammatory reactions can impose excessive or long-term tissue damage, leading to acute or chronic inflammatory diseases. According to the literature, there are plenty of inflammation mediators, including nuclear factor kappa B (NF-kB) as a key mediator, that induces pro inflammatory genes and functions at both innate and adaptive immune cells [3,5,7,9]. Analysis of scientific data has revealed activation of NF-kB transcription factor by the inflammatory or non-inflammatory triggers on epitheliocytes, dendritic cells, macrophages, neutrophils of gastrointestinal tract. NF-kB directly mediates the level of expression of mRNA bioactive

molecules, such as chemokines and molecules of adhesion, that play a key role in the development of inflammation [1–3,11–13].

According to NF-kB role in the development of *Helicobacter pylori* (*H. pylori*) associated gastritis (HAG), the level of expression of NF-kB was much higher in HAG, compared to non-HAG. The authors pointed out the correlation between the level of NF-kB expression and histological changes of gastric mucosa [1,6,8].

In the past few years, a lot of research has taken place in the role of NF-kB in regulating the level of gene expression, that control cell proliferation, apoptosis, angiogenesis and determine type and severity of inflammation in adult patients [10,14]. As a matter of fact, there is no common point of view on a role of NF-kB in regulation of inflammation in children. There are specific differences between clinical course of children and adults. Proliferation and differentiation of cells that take place during childhood create a background of decreased ability of a child to localize inflammation [1,3,6,8].

Listed data is a proof to study morphological and immunohistochemical patterns of gastric mucosa inflammation due in chronic gastritis (CG) in children.

**The purpose** of the work — to study the level of NF-κB activity depending on the degree of gastric mucosa inflammation of CG in children.

## Materials and methods of the research

We investigated 76 children aged from 8 to 16 with verified diagnosis of acute exacerbation of CG.

Investigations have been carried out with a strict adherence to basic statements of the GCP Council of Europe Convention on Human right and biomedicine and basic statements of Ethical principles for medical research involving human subjects of World Medical Association Declaration of Helsinky. Esophagogastroduodenoscopy (upper endoscopy — UE) with a target biopsy of stomach's mucosa has been performed in all children to verify the diagnosis with the help of morphological and immunohistochemical investigation. To assess histological changes of stomach's mucosa a tissue fragments were stained with hematoxylin, eosin and picrofuchsin by van Gieson's. For immunohistochemical examination, sections 4–6 μm thick were applied to Super Frost Plus adhesive slides and an indirect streptavidin peroxidase staining method was used.

When interpreting immunostaining using monoclonal antibodies to NF-kB (DAKO, Denmark), the prevalence and intensity of the reaction was evaluated by a semi-quantitative method in points from 0 to 3 as follows:

a) prevalence:

1) 0 — no coloration;

2) 1 — less than 10% of positively stained cells;

3) 2 — more than 10% and less than 50% of positively stained cells;

4) 3 — homogeneous staining of more than 50% of cells;

b) the intensity of the reaction:

1) 0 — no visible color;

2) 1 — weak color;

3) 2 — moderate color;

4) 3 — expressive color.

Visualization of *H. pylori* was performed with Romanovsky–Himze stain.

Statistical processing of the results was performed according to generally accepted methods of variation statistics.

## Results and discussion of the research

The diagnosis is verified by histological examination of fundal and antral mucosa. Morphological assessment of stomach's mucosa bioptates revealed, that 41.4±9.1% of examined patients had significant extent of mucosal inflammation, 37.9±9% — moderate extent of inflammation and 20.7±7.5% — mild extent of mucosal inflammation. Atrophic changes were found in 6.9±4.7% of patients. According to the analysis of the cellular composition of the infiltrate, almost half of the tissue specimen (52.2±10.4%) consisted mainly of lymphocytes, 30.4±9.6% were presented with combination of neutrophilic and plasmocytic infiltration, 17.4±4.6% — lymphocytic and eosinophilic infiltration.

Mild extent of mucosal inflammation was associated with mucosal abnormalities presented with shallowing of foveolae, flattening of rollers, perivascular swelling signs. Lamina propria is mildly infiltrated with neutrophils and plasma cells, localized mainly at superficial layers of mucosa and inside the rollers, glands are densely located with signs of edema (Fig. 1).

The moderate extent of fundal and antral mucosa inflammation is presented with more severe (in comparison to mild extent) lamina propria infiltration with lymphocytes, neutrophils, eosinophils. Areas of lamina propria comprised solitary glands. Superficial epithelium had foci of flattening. Distrophic changes of epitheliocytes, areas of perivascular swelling, erosions,

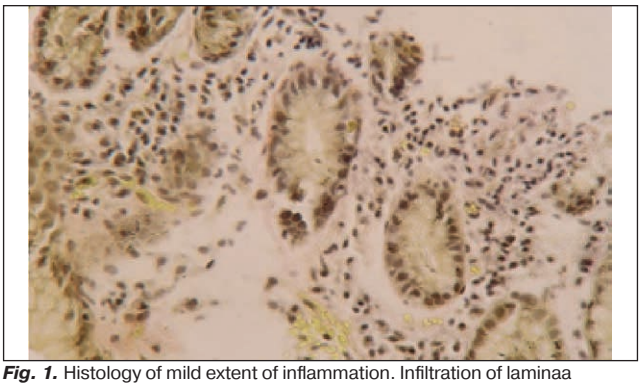

***Fig. 1.*** Histology of mild extent of inflammation. Infiltration of laminaa propria with solitary lymphocytes and plasma cells, ×200

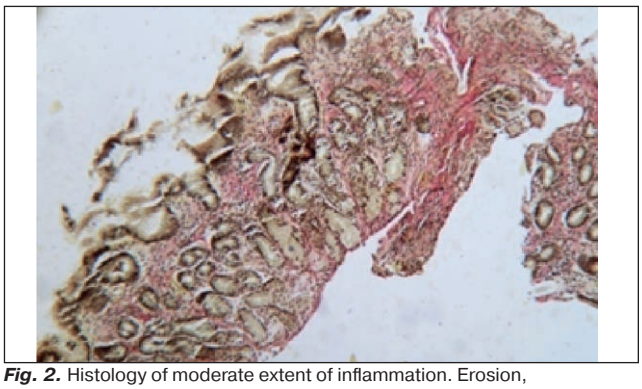

***Fig. 2.*** Histology of moderate extent of inflammation. Erosion, hemorrhages, ×200

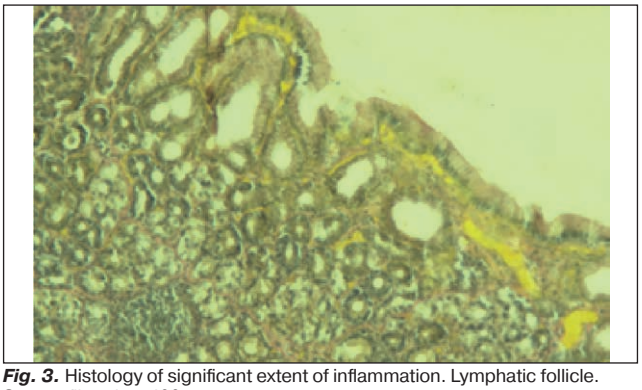

***Fig. 3.*** Histology of significant extent of inflammation. Lymphatic follicle. Stroma fibrosis, ×400a

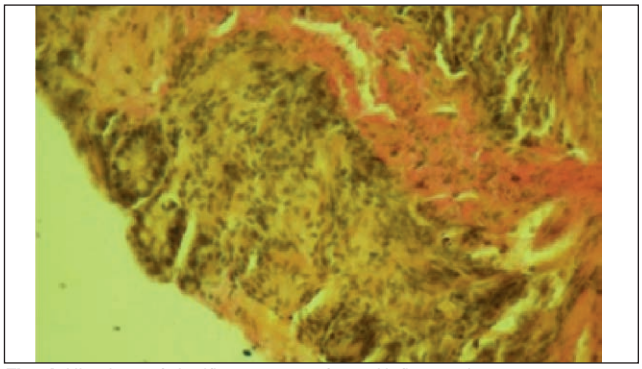

***Fig. 4.*** Histology of significant extent of antral inflammation. Focal atrophy, ×100

hemorrhages and microtromboses were specific for moderate extent of inflammation. Foci of mucosal hypotrophy with decreased number of glands, impaired architectonics, shallowing of pits and flattening of rollers were detected (Fig. 2).

Significant extent of inflammation was associated with mucosal disorders presented with shallowing of foveolae and flattening of rollers in all samples. Superficial epithelium was found to be with areas of desquamation and foci of flattening. Lamina propria was densely infiltrated with lymphocytes, solitary eosinophils and neutrophils with areas of swelling anad hemorrhages in all bioptates Fibrotic foci sized from 50–70 μm to 100–150 μm, areas of proliferating fibroblasts and thin collagen fibers with unclear outlines in basal and superficial parts were detected in lamina propria. Lamina propria glands were situated unevenly, some of which had deteriorated architectonics (Fig. 3).

Atrophic changes of mucosa were detected mainly in antrum, associated with significant extent of inflammation, presented with mucosal thinning, frequently diffuse. Epithelium is considerably flattened, lamina propria glands are located sparsely and unevenly. Areas of fibrous tissue were detected instead of glands, with size ranging between 1–2 rollers (Fig. 4).

Diagnosis of *H. pylori* infection was performed histologically in 76 patients. As a result, the

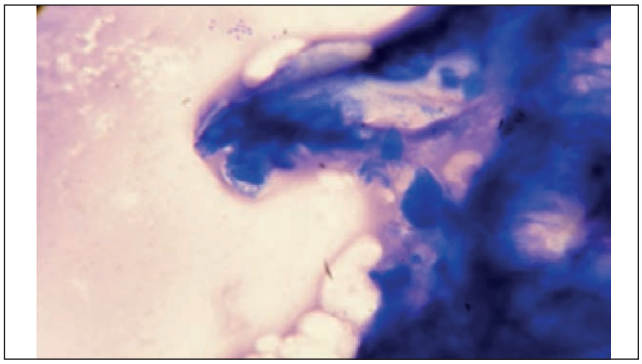

***Fig. 5.*** Histology of moderate extent of mucosal inflammation of antrum. Mild extent of *H. pylori* carriage. Hymze staining, ×100

level of contamination by *H. pylori* was 27.6%. By implementing Hymze stain to antral biopsy, 57.1% of patients were found to have moderate extent of *H. pylori* carriage, 42.9% — mild extent of *H. pylori* carriage associated with moderate or mild extent of mucosal inflammatory activity (Fig. 5).

*H. pylori* infection was detected in 14.3% of patients with atrophic changes of stomach mucosa.

So based on our research, mucosal morphological changes specific for CG in children, characterized by significant extent of inflammation with lymphocytic infiltration, fibrosis of lamina propria stroma, areas of gland deterioration with multiple hemorrhages, trombosis and erosions were detected. Results indicate early chronic inflammatory process specific for gastritis in children.

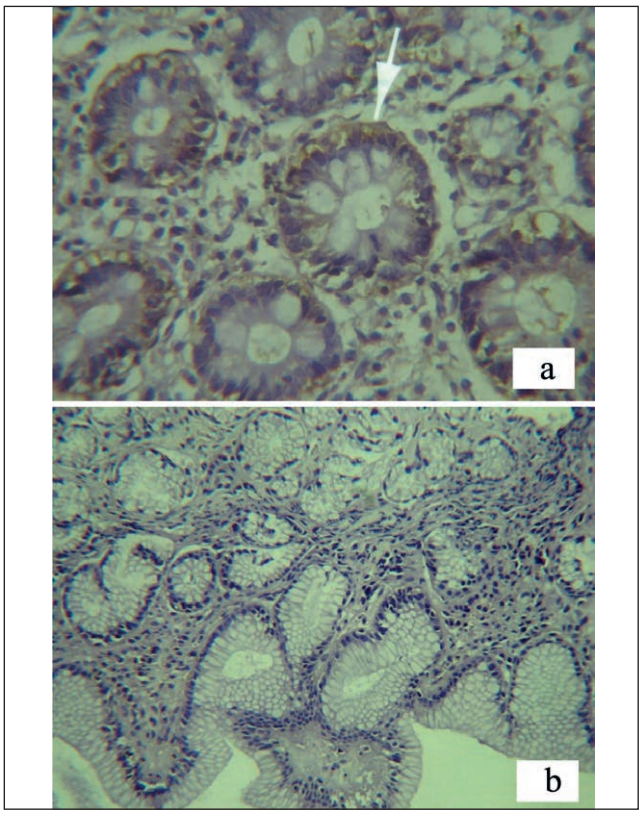


***Fig. 6.*** Histology of mucosa with polyclonal antibodies expression to NF-kB (3 points): a — considerable lymphocytic infiltration, ×400; b — absence of expression with polyclonal antibodies to NF-kB, ×200

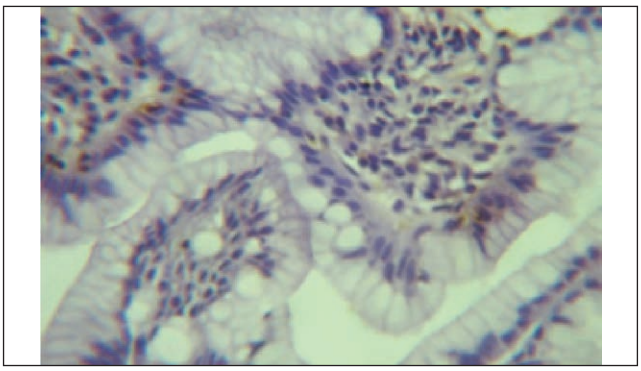

***Fig. 7.*** Histology of mucosa polyclonal antibodies expression to NF-kB (2 points), ×200

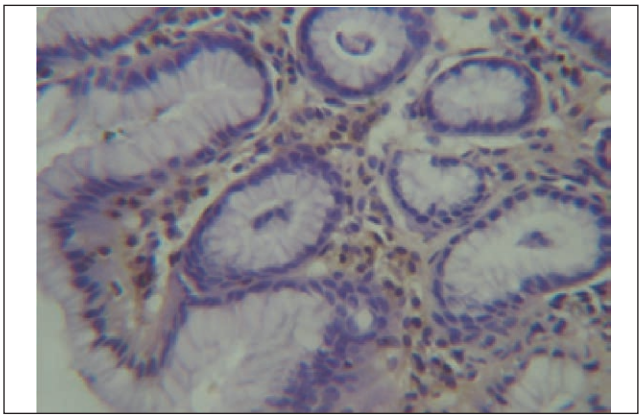

***Fig. 8.*** Histology of mucosa with polyclonal antibodies expression to NF-kB (1 point), ×200

Aforementioned data points out the relevance of studying NF-kB, that directly affect the regulation of the extent of mucosal inflammation. The NF-kB expression in cytoplasm and nuclei of epithelyocytes was irregular. Severity and distribution of reaction depended on the extent of activity of mucosal inflammatory process. The highest level of expression of NF-kB was associated with the significant extent of mucosal inflammation, as the distribution of reaction was more than 50% of positive cells with considerable intensity of colouration. We found interdependence of NF-kB expression with lymphocytic infiltration: all children with positive NF-kB reaction had lymphoid infiltration of lamina propria (Fig. 6a), while the absence of NF-kB expression resulted in no lymphocytic infiltration (Fig. 6b).

Due to neutrophil-rich infiltrate, NF-kB distribution was more than 10% and less than 50% of positively stained cells with mild intensity of staining (Fig. 7).

Due to mucosal eosinophil-rich infiltration, distribution of NF-kB reaction was less than 10% of positively stained cells with mild intensity of staining (Fig. 8).

Due to mucosal atrophic changes, considerable expression of NF-kB was detected with homogenous staining of more than 50% of cells.

According to our research, no interdependence between NF-kB staining and extent of mucosal *H. pylori* carriage was identified.

Specific features of distribution and intensity of NF-kB immunostaining were found by immunohistochemical examination of bioptates. High level of NF-kB in cytoplasm was associated with significant extent of inflammation and, mainly, considerable lymphocytic infiltration; patients with neutrophil-rich infiltration had weak immunostaining reaction in the form of separate fragmented foci in superficial epithelium basal cells and solitary glands. Received data indicates, that NF-kB activation is the main factor, responsible for the mechanism of development of chronic mucosal inflammation in children. Low level of NF-kB expression in the case of eosinophil-rich infiltration is likely to indicate, that NF-kB activation is not the main factor for involvement of eosinophils to inflammation. Also, we identified high level of distribution of positively stained cells in the case of mucosal atrophy. We did not identify the interdependence

between the level of NF-kB expression and *H. pylori* infection, that is likely due to small size of sampling and the fact, that none of the histology slides had more than moderate extent of mucosal *H. pylori* carriage.

## Conclusions

Analysis of morphological and immunohistochemical mucosal changes due to CG in children indicated microcirculatory disturbances and signs of mucosal stromal-epithelial rebuild associated with significant lymphocytic infiltration.

Results of investigation indicate the important role and multidirectional functional role of NF-kB in the development of CG in children. NF-kB transcription factor identifies the level of intensity of inflammatory process reaction and is a main factor, responsible for a mechanism of chronic mucosal inflammation development in children

*No conflict of interests was declared by the author.*

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