

## ORIGINAL ARTICLE

## THE BALANCE OF IL-1 $\beta$ , IL-10 AND THE LEVEL OF I $\kappa$ B $\alpha$ EXPRESSION IN CHILDREN WITH CHRONIC CATARRHAL GINGIVITIS AND GASTRODUODENITIS

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### ABSTRACT

**The aim:** To define the level of pro- and anti-inflammatory IL-1 $\beta$ , IL-10 in the oral fluid of children with chronic gastroduodenitis, depending on the level of I $\kappa$ B $\alpha$  expression.

**Materials and methods:** Studying the oral fluid and scraping of the gums of 50 children 6-12 years old was carried out to determine the level of IL-1 $\beta$ , IL-10 and I $\kappa$ B $\alpha$ . The children were divided into 3 groups. Group 1 - 10 children with healthy periodontium and without somatic diseases. 2nd group - 20 somatically healthy children with chronic catarrhal gingivitis. The third group (20 children) of children with gastroduodenitis and chronic catarrhal gingivitis. The determination of the mRNA expression of the I $\kappa$ B $\alpha$  gene and the level of IL-10 and IL-1 $\beta$  in the oral fluid was carried out by real-time PCR.

**Results:** We determined that the level of pro-inflammatory IL-1 $\beta$  in the oral fluid of primary school children had different levels in accordance with the state of dental and somatic health. It was the lowest in somatically healthy children without signs of gingivitis. We revealed that the level of IL-10 has anti-inflammatory properties, and an inverse relationship with the concentration of IL-1 $\beta$  in the oral fluid of examined children. Analysis of I $\kappa$ B $\alpha$  expression made it possible to reveal its suppression in children of the second and third groups compared with healthy children.

**Conclusions:** We found changes in the levels of I $\kappa$ B $\alpha$  in the gums of children with chronic catarrhal gingivitis; attenuated I $\kappa$ B $\alpha$  expression may lead to the deregulation of NF- $\kappa$ B pathways in the pathogenesis of periodontitis. Decreased I $\kappa$ B $\alpha$  expression may affect cytokine production and inflammatory response associated with chronic catarrhal gingivitis and with chronic gastroduodenitis.

**KEY WORDS:** children, gingivitis, gastroduodenitis, cytokines, I $\kappa$ B $\alpha$

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### INTRODUCTION

Lesions of the gastrointestinal tract (chronic gastroduodenitis) occupy one of the leading places among other general somatic pathologies. Chronic gastroduodenitis plays great role in the development of gingivitis and periodontitis [1,2,3,4].

The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity can be explained by similar mechanisms of cellular damage for the whole organism. The cytokine profile of immunobiological processes plays the leading role in the occurrence of these changes [5].

The level of cytokines reflects the pro- and anti-inflammatory activity of any inflammatory process [6]. Cytokines are subdivided into pro-inflammatory (they are involved in the initiation of inflammation), and anti-inflammatory, depending on the nature of the effect on the inflammatory process. The key pro-inflammatory cytokine is interleukin-1 (IL-1), the main anti-inflammatory cytokine is interleukin-10 (IL-10) [7].

Recent studies show that the level of cytokines in saliva does not correlate with their level in the blood. This fact indicates a certain independence of the local immunity of the oral cavity, and reflects the general tendencies of the cytokine cascade in the patient's body [8].

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a cytokine-inducible factor that plays a significant role in the transcriptional regulation of genes involved in inflammatory reactions and cell survival [9,10].

NF- $\kappa$ B is associated with many autoimmune diseases, chronic inflammation, metabolic disorders [11]. Normally, NF- $\kappa$ B is present in the cytoplasm in an inactive form due to its complex with I $\kappa$ B, which prevents the penetration of NF- $\kappa$ B into the nucleus. Regulation of the NF- $\kappa$ B and the I $\kappa$ B interaction plays a key role in NF- $\kappa$ B. NF- $\kappa$ B activation is initiated by extracellular signals, recorded by membrane receptors, and transmitted into the cell. In addition, I $\kappa$ B $\alpha$  blocks the ability of NF- $\kappa$ B transcription factors to bind to DNA, which is necessary for the correct functioning of NF- $\kappa$ B [12]. Understanding the molecular mechanisms that regulate NF- $\kappa$ B signaling and its functioning is important for finding new approaches in the treatment of gingivitis and periodontitis.

### THE AIM

To study and to compare the level of pro- and anti-inflammatory IL-1 $\beta$ , IL-10 in the oral fluid of children with chronic gastroduodenitis, depending on the level of I $\kappa$ B $\alpha$  expression.

## MATERIALS AND METHODS

The oral fluid and scraping of the gums of 50 children 6-12 years old were studied to determine the level of IL-1 $\beta$ , IL-10 and I $\kappa$ B $\alpha$ . Children were divided into 3 groups. Group 1 (control) consisted of 10 schoolchildren, who had a healthy periodontium according to a clinical dental examination and did not have somatic diseases according to a pediatrician examination. Group 2 consisted of 20 children and they had chronic catarrhal gingivitis, they were somatically healthy. The third group (20 children) had chronic gastro-duodenitis and chronic catarrhal gingivitis. Children with chronic gastroduodenitis had an inpatient treatment at the Poltava Regional Children's Clinical Hospital and were treated according to the order of the Ministry of Health of Ukraine No. 59 dated January 29-th, 2013 «An approval of unified clinical protocols for medical care for children with digestive diseases.»

Dental examination was carried out according to the WHO method, 1989, the results were recorded in the examination cards. The severity of gingivitis was assessed by the papillary-marginal-alveolar index (PMA) modified by Parma, 1960. The papillary bleeding index (PBI) was also determined as an indicator of the severity of gum inflammation. This index is assessed in 30 seconds after probing the interdental area. 1 degree - single punctate bleeding; 2 degree - linear / punctate mild bleeding along the edge of the apex of the papilla; 3 degree - moderate bleeding from the interdental papilla (in the form of triangle); 4 degree - severe bleeding that occurs immediately after probing the gum in the interdental spaces.

The level of I $\kappa$ B $\alpha$  expression was determined in the material by scraping the marginal part of the gum. The

epithelium was taken from the marginal part of the gums with a disposable plastic spatula early in the morning (before the meal). The tip of a disposable spatula was cut off with sterile scissors and put to sterile eppendorf. The samples were stored and transported within 2 hours. These eppendorfs were transported to the laboratory in thermal containers with refrigerant.

The level of mRNA expression of the I $\kappa$ B $\alpha$  gene was determined by real-time PCR. Total RNA was isolated from a biological sample using the RIBO-sol-V reagent kit (AmpliSens, Russia). A set of reagents for carrying out the reverse transcription reaction (Syntol, Russia) was used to obtain cDNA.

The determination of the mRNA expression of the I $\kappa$ B $\alpha$  gene was carried out by real-time PCR using a DT-Light detector (DNA-Technology, Russia).

The level of IL-10 in the oral fluid was determined to characterize inflammation in the organs of the oral cavity. Unstimulated oral fluid was taken at fixed time, in the morning, on an empty stomach. Patients were previously offered to rinse the mouth. The collection of oral fluid was made by spitting 4 ml into plastic sterile tubes. They were hermetically closed and carried out in 30 minutes. This oral fluid was delivered to the laboratory.

The determination of IL-10 and IL-1 $\beta$  in the oral fluid was carried out by polymerase chain reaction using the «Interleukin-10-IFA-BEST», «Interleukin-1-IFA-BEST» kits.

The measurement of optical density (it is automatically converted to concentration) was carried out at a wavelength of 450 nm on a STATFax 303 Plus enzyme immunoassay analyzer (USA). The research results were processed using the generally accepted methods of medical statistics.

**Table I.** Condition of periodontal tissues in children

Contingent of children	Number of children	Papilla_bleeding index (PBI)	PMA
Healthy children	10	1,0	0 %
Healthy children with chronic catarrhal gingivitis	20	1,35 $\pm$ 0,11*	22,25 $\pm$ 0,57*
Children with chronic gastroduodenitis and chronic catarrhal gingivitis	20	2,5 $\pm$ 0,12** ***	34,85 $\pm$ 1,23** ***

Notes:

\* - the difference is significant when we compare group 1 and group 2,  $p < 0.05$

\*\* - the difference is significant when we compare group 1 and group 3,  $p < 0.05$

\*\*\* - the difference is significant we compare group 2 and group 3,  $p < 0.05$

**Table II.** The level of IL-1, IL-10 and expression of I $\kappa$ B $\alpha$  in elementary school children

Contingent of children	Number of children	IL-1 $\beta$ , pg/ml	IL-10, pg/ml	I $\kappa$ B $\alpha$ 2 <sup>-<math>\Delta</math>ct</sup>
Healthy children	10	3,72 $\pm$ 1,12	1,44 $\pm$ 0,1	0,045 $\pm$ 0,07
Healthy children with chronic catarrhal gingivitis	20	183,82 $\pm$ 15,76*	1,07 $\pm$ 0,14*	0,022 $\pm$ 0,003*
Children with chronic gastroduodenitis and chronic catarrhal gingivitis	20	282,33 $\pm$ 6,82** ***	0,57 $\pm$ 0,16** ***	0,026 $\pm$ 0,04***

Notes:

\* - the difference is significant when we compare group 1 and group 2,  $p < 0.05$

\*\* - the difference is significant when we compare group 1 and group 3,  $p < 0.05$

\*\*\* - the difference is significant we compare group 2 and group 3,  $p < 0.05$

## RESULTS AND DISCUSSIONS

The group of children with chronic gastroduodenitis had the most severe inflammation of gums (Table I). We determined that children with gastroduodenitis had chronic catarrhal gingivitis of moderate severity and the PMA index was  $34.85 \pm 1.23\%$ . Somatically healthy children with chronic catarrhal gingivitis had mild severity of gum inflammation ( $22.25 \pm 0.57\%$ ,  $p < 0.05$ ).

Children with chronic gastroduodenitis had significantly worse state of gum inflammation. It is also confirmed by high papillary bleeding index -  $2.5 \pm 0.12$  points. Somatically healthy children had a low degree of PBI, namely,  $1.35 \pm 0.11$  points.

Condition of periodontal tissues in children

The balance of pro- and anti-inflammatory IL-1 $\beta$ , IL-10, depending on the level of expression of I $\kappa$ B $\alpha$  is presented in Table II.

We determined that the level of pro-inflammatory IL-1 $\beta$  in the oral fluid of primary school children had different levels in accordance with the state of dental and somatic health. Thus, IL-1 $\beta$  level was low in somatically healthy children without signs of gingivitis or periodontitis -  $3.72 \pm 1.12$  pg/ml. We determined a significantly higher concentration of IL-1 $\beta$  in the group of children with chronic catarrhal gingivitis, which reached  $183.82 \pm 15.76$  pg/ml, and it was almost 45 times higher. The level of IL-1 $\beta$  was 1.5 times higher and reached  $282.33 \pm 6.82$  pg/ml in the third group of examined children with chronic gastroduodenitis and chronic catarrhal gingivitis.

We found an inverse relationship with the concentration of IL-1 $\beta$  and the level of IL-10 (it has anti-inflammatory properties) in the oral fluid of children of the examined groups. We determined the concentration of IL-10 was  $1.44 \pm 0.17$  pg/ml in healthy children, and it fell to  $1.07 \pm 0.14$  pg/ml in somatically healthy children with chronic catarrhal gingivitis ( $p < 0.05$ ), due to increase of the severity of gingivitis. The level of IL-10 dropped almost 2 times in children with chronic gastroduodenitis and chronic catarrhal gingivitis, compared with children of the 2nd group and 3 times compared with healthy children and amounted to  $0.57 \pm 0.16$  pg/ml ( $p < 0.05$ ,  $p < 0.05$ ).

We found the suppression of I $\kappa$ B $\alpha$  expression in children of the second and third groups compared with healthy children ( $0.022 \pm 0.003$  2- $\Delta$ ct and  $0.026 \pm 0.04$  2- $\Delta$ ct compared with  $0.045 \pm 0.07$  2- $\Delta$ ct). So, both somatically healthy children and children with chronic gastroduodenitis and chronic catarrhal gingivitis had a lower level of I $\kappa$ B $\alpha$  expression ( $p < 0.05$ ).

## CONCLUSIONS

Our results demonstrate changes in I $\kappa$ B $\alpha$  levels in the gums of children with chronic catarrhal gingivitis. We suggest that attenuated I $\kappa$ B $\alpha$  expression may contribute to deregulation of NF- $\kappa$ B pathways in the pathogenesis of gingivitis and periodontitis. Decreased I $\kappa$ B $\alpha$  expression may affect cytokine production and inflammatory response associated with chronic catarrhal gingivitis in children with chronic gastroduodenitis.

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**Conflict of interest:**

*The Authors declare no conflict of interest.*

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