

Proinflammatory cytokines in the saliva of patients with active and nonactive Crohn's disease

Katarzyna Szczeklik¹, Danuta Owczarek², Jolanta Pytko-Polończyk¹, Barbara Kęsek¹, Tomasz H. Mach²

¹ Institute of Dentistry, Jagiellonian University Medical College, Kraków, Poland

² Department of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Kraków, Poland

KEY WORDS

Crohn's disease, interleukin 1 β , interleukin 6, oral cavity, tumor necrosis factor α

ABSTRACT

INTRODUCTION Crohn's disease (CD) involves the entire gastrointestinal tract, including the mouth. Numerous cytokines play a role in the regulation of inflammatory process in CD.

OBJECTIVES The aim of the study was to examine the prevalence of oral lesions in adult patients with CD and to investigate whether salivary concentrations of interleukin 1 β (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF- α) are associated with the activity and oral manifestations of CD.

PATIENTS AND METHODS A prospective study included 95 adult patients: 52 with active CD and 43 with inactive CD. The control group involved 45 subjects without CD. We performed blood tests, careful oral examination, and measurement of IL-1 β , IL-6, and TNF- α in unstimulated whole saliva by enzyme-linked immunosorbent assays.

RESULTS IL-1 β , IL-6, and TNF- α were significantly elevated in patients with active CD. IL-1 β levels were 289.8 ± 52.7 in patients with active CD vs. 196.7 ± 42.9 pg/ml in patients with inactive CD ($P < 0.039$), and 196.7 ± 42.9 pg/ml ($P < 0.01$) in controls. IL-6 levels were 13.8 ± 4.2 vs. 7.2 ± 3.1 pg/ml ($P < 0.041$), respectively, and 6.3 ± 1.4 pg/ml ($P < 0.001$) in controls. TNF- α levels were 32.5 ± 8.7 vs. 10.2 ± 6.3 pg/ml ($P < 0.002$), respectively, and 6.8 ± 2.8 pg/ml ($P < 0.001$) in controls. We observed CD-specific oral lesions: diffuse asymptomatic buccal swelling in 12 patients (23%) and cobblestoning in 5 patients (11.3%). CD-nonspecific lesions were observed in 17 patients (32.7%) with active CD, in 11 patients (25.6%) with inactive CD, and in 6 controls (13.3%). In active CD, higher salivary IL-6 and TNF- α and serum C-reactive protein levels correlated with specific oral lesions.

CONCLUSIONS In patients with active CD, salivary IL-1 β , IL-6, and TNF- α levels are higher than in patients with inactive disease and controls. Elevated salivary IL-6 and TNF- α levels correlate with specific oral lesions. These cytokines may be used as markers of active CD, but the finding should be confirmed in a larger group of patients.

Correspondence to:

Prof. Tomasz H. Mach, MD, PhD,
Katedra Gastroenterologii, Hepatologii
i Chorób Zakaźnych, Uniwersytet
Jagielloński, Collegium Medicum,
ul. Śniadeckich 5, 31-531 Kraków,
Poland, phone: +48-12-424-73-40,
fax: +48-12-424-73-80,
e-mail: tmach@su.krakow.pl

Received: February 1, 2012.
Revision accepted: April 16, 2012.

Published online: April 24, 2012.

Conflict of interest: none declared.

Pol Arch Med Wewn. 2012;
122 (5): 200-208
Copyright by Medycyna Praktyczna,
Kraków 2012

INTRODUCTION Crohn's disease (CD) is a chronic granulomatous inflammatory disease of the entire gastrointestinal tract. Its etiology is unknown. The disease manifests itself with exacerbation (and then remission) of such symptoms as abdominal pain, fever, and weight loss, and is associated with many general and gastrointestinal complications.¹ Therapy involves the use of mesalazine and azathioprine. In active CD, corticosteroids and anti-tumor necrosis factor α (TNF- α) are

introduced.^{2,3} Since the first description of inflammatory lesions in the oral cavity of patients with CD, it has been well established that the mouth may be involved in the disease.⁴ The prevalence of oral lesions is particularly common in children (48%–80%) compared with adults (0.5%–20%), but only a small proportion of lesions with granulomatous inflammation is characteristic of oral CD.⁵⁻¹⁰ Nonspecific lesions, including aphthous stomatitis, ulcerations, and atrophic glossitis,

were reported in 4% to 37% of the patients with active CD.^{11,12} However, the prevalence of aphthous ulcers and other nonspecific lesions is high in other diseases and the general population.⁵ Because of a relatively easy access to direct visual examination of the oral cavity and biopsy, the mouth is recognized as a very useful part of the gastrointestinal tract in the diagnostic procedures of CD, especially in children.^{5,7-15}

Inflammatory process in CD involves the entire digestive tract from the mouth to the anus. Therefore, some alterations in the saliva, which is a secretion of the salivary glands of the mouth, may possibly reflect the severity of not only oral but also systemic CD.¹⁶ A noninvasive collection and easy access to the whole unstimulated saliva have some obvious advantages compared with blood-based analyses.^{17,18} The salivary concentrations of drugs or various biochemical compounds can be easily applied in the diagnosis or monitoring of numerous diseases.¹⁷ Several authors reported changes in the salivary levels of selected cytokines in inflammatory diseases.¹⁹⁻²³ Nielsen et al.²⁴ demonstrated higher levels of salivary interleukin (IL-6) compared with serum concentrations in CD, and they speculated that it was caused by the disease process involving the mouth as part of inflammation of the entire gastrointestinal tract.

The aim of the study was to examine the prevalence of oral lesions in adult patients with CD and to investigate whether the salivary levels of interleukin 1 β (IL-1 β), IL-6, and TNF- α are associated with the activity and oral manifestations of CD.

PATIENTS AND METHODS **Patients** A prospective study was conducted in 95 adult patients with CD, including 52 patients with active (exacerbation) and 43 inactive (remission) disease. The control group comprised 45 individuals – students, staff, and patients with dyspepsia or irritable bowel syndrome. All patients were from the University Hospital in Kraków, Poland. CD was diagnosed according to the clinical, radiological, endoscopic, and histological criteria described in the European Crohn's and Colitis Organization (ECCO) guidelines.¹ Deterioration of CD was classified according to the CD activity index (CAI),²⁴ which is a composite scoring system based on selected clinical symptoms, such as the number of liquid stools, severity of abdominal pain, general well-being, extraintestinal CD manifestations, abnormal abdominal mass, use of loperamide or opiates for diarrhea, as well as hematocrit and body weight. The CAI values of 150 or less are associated with quiescent disease (remission). Values above 150 indicate active disease with 150–219 points considered as mild exacerbation, 220–450 as moderate, and above 450 points as severe.^{1,25} Patients were on maintenance therapy with mesalazine and azathioprine according to the ECCO guidelines.² We excluded patients with a history or the signs and symptoms of systemic infections,

cardiovascular disease, pulmonary and kidney diseases, allergy, autoimmune diseases, diabetes, patients with chronic periodontitis or oral lichen planus and those treated with anti-inflammatory drugs (except azathioprine), antioxidants, or statins, which can affect the levels of cytokines in the saliva.^{19-21,23}

The study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki. The study protocol was approved by the Commission of Bioethics at the Jagiellonian University in Krakow, and all participants gave their informed consent before enrollment.

Laboratory tests performed in all patients included blood cell counts, platelet count, and serum C-reactive protein (CRP). In patients with active CD, blood and saliva samples were taken before the start of therapy with corticosteroids.

Oral examination Oral examinations were performed in each subject by 2 investigators (K.S., B.K.) who were blind to CD activity. Oral examinations involved detailed anamnesis and careful systematic assessment of the oral cavity, including the submandibular lymph nodes, lips, labial mucosa and sulci, commissures, buccal mucosa and sulci, gingiva, tongue, floor of the mouth, and hard and soft palate.^{11,12} Oral lesions were identified and described according to the previously reported criteria, photographed and, if necessary, biopsied in order to establish the diagnosis of CD.^{12,15,26}

Saliva collection In the sialometric evaluation, whole unstimulated saliva was collected between 8 and 10 a.m. using standard techniques in fasting patients.²⁷ All subjects were requested to swallow first, tilt their head forward, and then expectorate saliva for 15 minutes into a sterile centrifuge calibrated tube placed on ice. The saliva volume and flow rate were calculated. The samples were centrifuged for 20 minutes at 3500 rpm, and supernatants were separated and immediately frozen at –80°C for further analysis.

Cytokine assay Saliva supernatants were stored for 30 minutes at room temperature to defrost. Cytokine levels in the salivary supernatants were determined by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using the human kits for IL-1 β , IL-6, and TNF- α (R&D Systems Inc., Minneapolis, United States), according to the manufacturer's instructions. The sensitivity of the methods were as follows: below 1 pg/ml for IL-1 β , below 0.7 pg/ml for IL-6, and 1.6 pg/ml for TNF- α . Cytokines were measured in the Laboratory of Biochemistry, 2nd Department of Internal Medicine, Kraków, Poland.

Statistical analysis The results were analyzed using the Statistica v. 9.0 software (StatSoft, Inc., Tulsa, United States). The results were expressed as means $\pm$ standard deviation. Statistical comparison was performed between patients with

TABLE 1 Characteristics of patients with active and inactive Crohn's disease and the control group

		Crohn's disease			Control (n = 45)
		active (n = 52)	inactive (n = 43)	P	
age, y		33.8 ± 2.2	36.0 ± 3.1	NS	35.1 ± 3.2
women, n (%)		16 (30.7)	14 (32.6)	NS	17 (37.8)
men, n (%)		36 (69.3)	29 (67.4)	NS	28 (62.2)
smokers, n (%)		14 (27)	13 (30.2)	NS	12 (27)
duration of disease, y		6.4 ± 1.7	7.3 ± 2.4	NS	0
BMI, kg/m ²		20.2 ± 1.4	22.1 ± 1.0	0.01	24.2 ± 1.8 ^a
hemoglobin, g/dl		11.6 ± 0.6	13.9 ± 0.4	0.001	14.2 ± 0.3 ^a
red blood cell count, × 10 ⁶ /mm ³		3.8 ± 0.3	4.2 ± 0.2	0.01	4.3 ± 0.2 ^a
white blood cell count, × 10 ³ /mm ³		8.4 ± 2.0	7.1 ± 1.5	NS	6.4 ± 1.2 ^a
platelet count, × 10 ³ /mm ³		370.9 ± 60.2	258.3 ± 36.0	0.001	234.8 ± 31.5 ^b
CRP, mg/l		26.5 ± 4.2	5.3 ± 1.0	0.001	4.3 ± 0.8 ^b
location of lesions, n (%)	small intestine	13 (25)	9 (20.9)	NS	0
	large intestine	3 (5.8)	4 (9.0)	NS	0
	small and large intestines	36 (69.2)	30 (69.8)	NS	0
	perianal lesions	10 (19.2)	6 (14)	NS	
disease activity	CDAI, points	256.5 ± 36.9	107.5 ± 30.2	0.001	0
	low CDAI (150–220), n (%)	14 (27)	0	0.0001	0
	moderate CDAI (220–450), n (%)	38 (73)	0	0.0001	0

Data are presented as mean ± standard deviation.

a $P < 0.01$ vs. active CD; χ^2 test and Mann-Whitney test

b $P < 0.001$ vs. active CD; χ^2 test and Mann-Whitney test

Abbreviations: BMI – body mass index, CD – Crohn's disease, CDAI – Crohn's disease activity index, CRP – C-reactive protein, NS – nonsignificant

active and inactive CD and the control group. The nonparametric Mann-Whitney test, χ^2 test, and the Kruskal-Wallis one-way analysis of variance were used to compare variables between different groups. Correlations between salivary parameters and oral lesions were calculated using the Spearman's rank correlation coefficient. In multiple comparisons of the data, the Bonferroni

correction was taken into account. P values less than 0.05 in the 2-sided test were considered statistically significant.

RESULTS Patient characteristics The characteristics of patients are given in **TABLE 1**. Study groups did not differ in terms of number, age, sex, smoking status, and duration of the disease. Patients with active CD had a significantly lower body mass index (BMI), red blood cell count, and blood hemoglobin levels and higher platelet count and serum CRP levels compared with patients with inactive CD and the control group. The CDAI of patients with active CD was 256.5 ± 36.9 points, and in patients with inactive disease – 107.5 ± 30.2 points ($P < 0.001$). The majority of patients (73%) had moderate disease activity (CDAI > 220 points), while the remaining 27% had low CD activity (CDAI < 220 points). In almost 70% of the patients in both groups, inflammatory lesions were located in the terminal intestine and proximal large intestine (**TABLE 1**). Patients did not have a history of surgery for CD.

Oral lesions The prevalence of oral lesions in patients with active CD, inactive CD, and in the control groups is presented in **FIGURES 1** and **2**. The following CD-specific oral lesions, classified according to Scheper and Brand,³⁸ were observed in patients with active CD: indurated polypoid tag lesions on vestibular and retromolar fossae in 13.5%

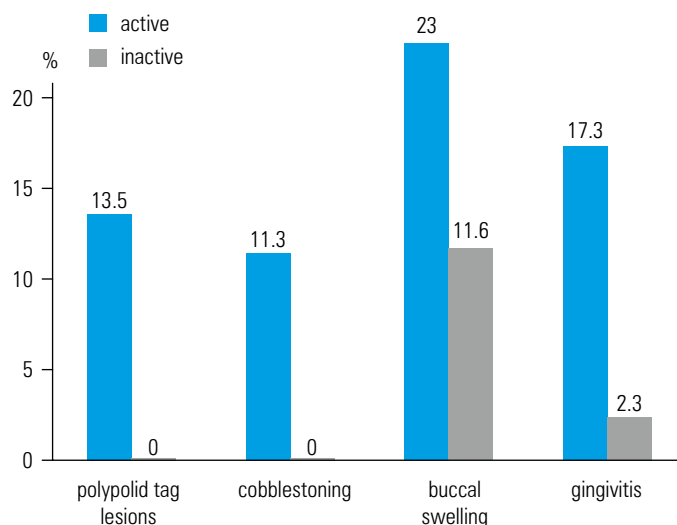


FIGURE 1 Prevalence of specific oral lesions classified according to Scheper and Brand³⁸ in patients with active and inactive Crohn's disease; active vs. inactive: $P < 0.01$; χ^2 test

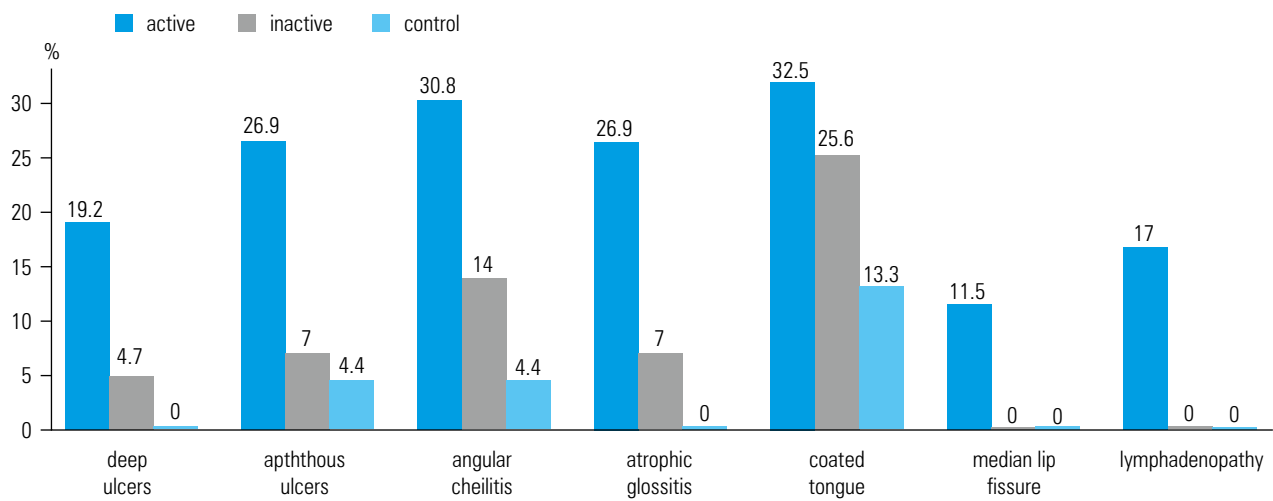


FIGURE 2 Prevalence of nonspecific oral lesions classified according to Scheper and Brand³⁸ in patients with active and inactive Crohn's disease and controls; active vs. inactive: $P < 0.01$ (except coated tongue, $P < 0.05$); active vs. controls: $P < 0.01$; inactive vs. controls (angular cheilitis): $P < 0.01$ (except aphthous ulcers, $P > 0.05$); χ^2 test

of the patients, cobblestoning in 11.3%, diffuse asymptomatic buccal swelling in 23%, and gingivitis in 17.3% (FIGURE 1). Diffuse asymptomatic buccal swelling was diagnosed in 11.6% and gingivitis in 2.3% of the patients with inactive CD (FIGURE 2). Nonspecific lesions in the oral cavity were observed in 11.5% (median lip fissure) to 32.5% (coated tongue) of the patients active CD and up to 25.6% (coated tongue) of the patients with inactive disease (FIGURE 2). Nonspecific lesions were rarely observed in the control group (from 4.4% to 13.3%; FIGURE 2). The Spearman rank test showed a positive correlation between the presence of specific lesions and blood hemoglobin, platelet count, serum CRP, and BMI in patients with active CD (TABLE 2). However, no correlation was observed between oral lesions and the CDAI in active CD (TABLE 2).

Cytokine levels in saliva The results of saliva analysis, including IL-1 β , IL-6, and TNF- α levels, in patients with CD and the control group are presented in FIGURES 3–6. The salivary flow rate was not significantly different between patients with active and inactive CD (0.41 ± 0.1 and 0.38 ± 0.1 ml/min, respectively) and healthy controls

(0.36 ± 0.1 ml/min.). The rate did not correlate with the prevalence of oral lesions in both CD groups (FIGURE 3).

The ELISA analysis of the whole unstimulated saliva in this cohort revealed a significant increase in the levels of IL-1 β , IL-6, and TNF- α in active CD compared with inactive CD and controls. Specifically, the results analyzed by the Mann-Whitney test showed significant differences in the salivary levels of IL-1 β : 289.8 ± 52.7 pg/ml in active CD vs. 196.7 ± 42.9 pg/ml in inactive CD ($P < 0.039$) and 168.2 ± 39.1 pg/ml in controls ($P < 0.011$ vs. active CD; $P < 0.095$ vs. inactive CD; FIGURE 4); IL-6: 13.8 ± 4.2 pg/ml in active CD vs. 7.2 ± 3.1 pg/ml in inactive CD ($P < 0.041$) and 6.3 ± 1.4 pg/ml in controls ($P < 0.01$ vs. active CD; $P < 0.142$ vs. inactive CD; FIGURE 5); and TNF- α : 32.5 ± 8.7 pg/ml in active CD vs. 10.2 ± 6.3 pg/ml in inactive CD ($P < 0.002$) and 6.8 ± 2.8 pg/ml in controls ($P < 0.001$ vs. active CD; $P < 0.850$ vs. inactive CD; FIGURE 6). There were no statistically significant differences in salivary IL-1 β , IL-6, and TNF- α levels between patients with inactive CD and controls.

No correlations were found between IL-1 β , IL-6, and TNF- α levels in the saliva and age, sex, duration of the disease, red and white blood counts, blood hemoglobin levels, and the intestinal location of CD. A positive correlation by the Spearman rank test was observed between elevated IL-6 and TNF- α levels in the saliva and specific lesions in active CD ($r = 0.328$, $P = 0.012$ and $r = 0.413$, $P = 0.023$, respectively; TABLE 3), while no such relationship was observed for IL-1 β ($r = 0.172$, $P = 0.282$; TABLE 3).

DISCUSSION The study is the first to have analyzed the salivary levels of 3 important proinflammatory cytokines, IL-1 β , IL-6, and TNF- α , in patients with active and inactive CD. Our results show that patients with active CD have higher salivary IL-1 β , IL-6, and TNF- α levels compared with patients with inactive CD and healthy controls. Higher levels of these proinflammatory cytokines correlated with clinical and biochemical markers of disease activity. Our results confirm that salivary IL-1 β , IL-6, and TNF- α are involved

TABLE 2 Correlations between specific oral lesions and blood tests, Crohn's disease activity index, and body mass index in patients with active Crohn's disease

Oral lesions and blood tests, CDAI, and BMI	Spearman's rank correlation coefficient		
	<i>r</i>	t(n–2)	<i>P</i>
white blood cell count	0.212	1.745	0.086
red blood cell count	–0.202	–1.663	0.101
hemoglobin	–0.420	–3.728	0.0001
hematocrit	–0.448	–4.035	0.0001
platelets	0.344	2.953	0.004
CRP	0.453	4.095	0.001
fibrinogen	0.430	3.841	0.0003
albumin	–0.358	–3.087	0.003
CDAI	0.217	1.296	0.204
BMI	0.324	2.752	0.008

Abbreviations: see TABLE 1

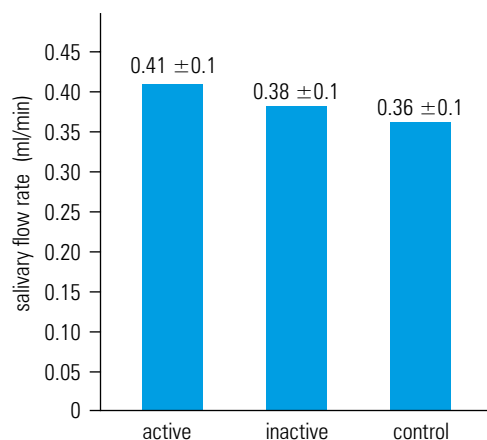


FIGURE 3 Salivary flow rate in patients with active and inactive Crohn's disease and controls; differences between groups are not statistically significant; Mann-Whitney test

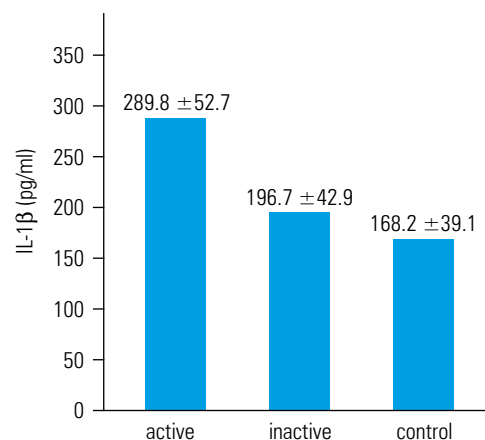


FIGURE 4 Interleukin 1β levels in the saliva of patients with active and inactive Crohn's disease and controls; active vs. inactive: $P < 0.039$; active vs. controls: $P < 0.011$; inactive vs. controls: $P = 0.095$; Mann-Whitney test
Abbreviations: IL-1β – interleukin 1β

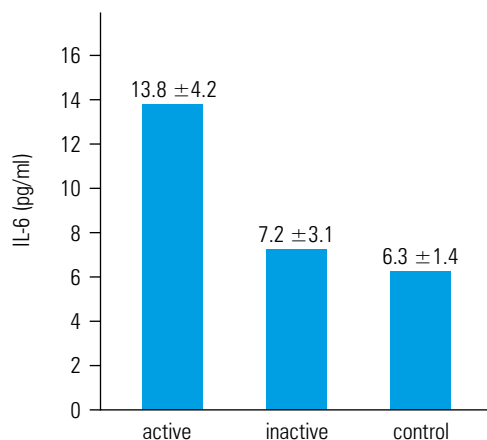


FIGURE 5 Interleukin 6 (IL-6) levels in the saliva of patients with active and inactive Crohn's disease and controls; active vs. inactive: $P < 0.041$; active vs. controls: $P < 0.001$; inactive vs. controls: $P = 0.142$; Mann-Whitney test
Abbreviations: IL-6 – interleukin 6

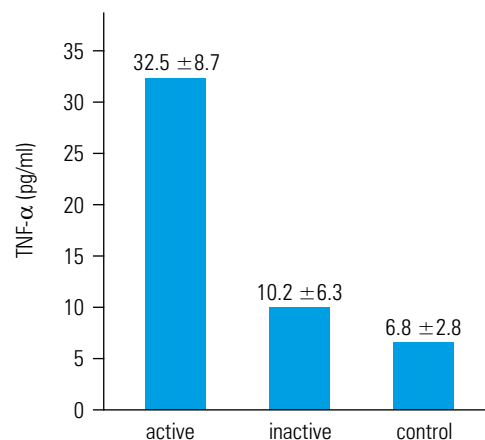


FIGURE 6 Tumor necrosis factor α (TNF-α) levels in the saliva of patients with active and inactive Crohn's disease and controls; active vs. inactive: $P < 0.002$; active vs. controls: $P < 0.001$; inactive vs. controls: $P = 0.850$; Mann-Whitney test
Abbreviations: TNF-α – tumor necrosis factor α

in gastrointestinal inflammation and suggest that they could be used as additional markers of active CD. Furthermore, increased salivary IL-6 and TNF-α levels correlated with active but not inactive CD as assessed by the CDAI.

The biomarkers of an inflammatory process in the saliva of patients with active CD have been rarely studied.²⁸⁻³⁰ Rezaie et al.²⁸ and Jahanshahi et al.²⁹ reported increased salivary epidermal growth factor, transforming growth factor-β1 (TGF-β1), oxidative stress markers, and nitric oxide in relation to disease activity in patients with CD. Nielsen et al.²⁴ observed a significant difference in salivary IL-6 levels between patients with CD and reference persons (median, 16.9 ng/l vs. 6.3 ng/l; $P < 0.05$). The authors concluded that higher IL-6 levels in the saliva of patients with CD confirmed the general involvement of the gastrointestinal tract extending to the mouth cavity

and that measuring IL-6 might be an additional method of evaluating and monitoring disease activity.²⁴

Higher salivary levels of IL-1β, IL-6, interleukin 8 (IL-8), and TNF-α in patients with other inflammatory diseases of the oral cavity, such as periodontitis, and in patients with oral cancer have been observed.^{19-23,27,31,32} Tales et al.²³ reported opposite results; they found no statistically significant differences in salivary IL-1β, IL-6, TNF-α, and other cytokines between patients with periodontitis and healthy controls. Recently, Stein et al.³⁰ have observed that patients with CD have increased prevalence of periodontitis, and that periodontal microbiota may affect mucosal lesions. Considering the fact that periodontal inflammation may alter cytokine levels in the saliva, we decided to exclude patients with periodontal disease from our study. Of note, cytokine levels

TABLE 3 Correlations between specific oral lesions and proinflammatory cytokines in the saliva of patients with active Crohn's disease

Specific oral lesions and cytokine levels in the saliva	Spearman's rank correlation coefficient		
	<i>r</i>	<i>t</i> (<i>n</i> -2)	<i>P</i>
IL-1 β , pg/ml	0.172	1.091	0.282
IL-6, pg/ml	0.328	2.602	0.012
TNF- α , pg/ml	0.413	2.960	0.023

Abbreviations: see FIGURES 4–6

in the whole unstimulated saliva of healthy controls reported by other authors were similar to our results. Specifically, the mean IL-1 β levels were between 61.1 and 173.2 pg/ml vs. 168 $\pm$ 39.1 pg/ml in our study; IL-6 levels were between 1.35 and 47.46 pg/ml vs. 6.3 $\pm$ 1.4 pg/ml in our study; and TNF- α levels were between 2.24 and 4.06 pg/ml vs., 6.8 $\pm$ 2.8 pg/ml in our study.^{19,20,22,27,31} Despite the heterogeneity of the results and different study populations, it may be concluded that patients with active CD have altered cytokine production, which is reflected by the levels of proinflammatory cytokines in the saliva.

Saliva plays an important role in maintaining proper oral environment.¹⁸ Results from our sialometric study confirm a previous finding that the saliva flow rate in patients with active and inactive CD is within the normal range, i.e., between 0.36 and 0.41 ml/min.¹¹ Our study showed that the levels of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) in the whole unstimulated saliva of patients with active CD were significantly higher compared with patients with inactive disease and healthy controls. Our results are consistent with the study of Nielsen et al.,²⁴ who observed elevated IL-6 levels in the saliva of patients with CD²⁴ and with the studies by other investigators who reported increased salivary cytokines in other inflammatory diseases.^{19-23,27,31,32}

Studies in patients with various oral disorders, such as periodontal disease, lichen planus, and oral carcinoma, demonstrated that the levels of certain proinflammatory and proangiogenic nuclear factor- κ B (NF- κ B)-dependent cytokines are increased in the whole unstimulated saliva.^{19,22,27}

The analyzed NF- κ B-dependent cytokines (IL-1 β , IL-6, and TNF- α) could play an important and complex role in the induction of an inflammatory process such as CD.³³⁻³⁵ NF- κ B is a primary transcription factor that controls the expression of genes encoding a series of cytokines with proinflammatory, immunoregulatory, and proangiogenic activity, including TNF- α , interleukin 1 α , IL-6, and IL-8.^{19,22,27,34} Activation of NF- κ B also contributes to the expression of genes that mediate inflammatory and immune response.^{22,27,34} TNF- α and IL-6 are secreted by T and B lymphocytes, monocytes, endothelial cells, epithelial cells, and macrophages and play a significant role in the stimulation of T and B lymphocytes, induction of acute-phase proteins, stimulation of hematopoiesis and

thrombopoiesis, as well as tumor growth induction and suppression.^{32,34} TNF- α and IL-6, in combination with some other cytokines, exhibit immunoregulatory activities through a complex cytokine network and have been reported to be involved in the pathogenesis of several chronic inflammatory diseases, such as inflammatory bowel diseases, rheumatoid arthritis, asthma, and others.^{22,27,34} Rhodus et al.¹⁹ showed that NF- κ B-dependent cytokines are elevated in the saliva of patients with several diseases.^{19,27,33} CD is a chronic inflammatory disorder characterized by not fully elucidated T-cell-mediated immune response against the epithelial cells with persistent accumulation of T lymphocytes and epithelial cell damage.³⁵

Higher salivary levels of proinflammatory cytokines may be caused either by the local production from the cells of inflammatory infiltrations of the oral mucosa and/or salivary glands, or by the loss of structural barriers of the oral mucosa, particularly in the active inflammatory process.²² Our results confirm that patients with active CD have higher levels of IL-1 β , IL-6, and TNF- α compared with patients with inactive CD and controls. In another, so far unpublished study conducted in the same group of patients with CD, we have shown much lower serum levels of IL-6 and TNF- α compared with their salivary levels, which may suggest increased secretion of these proinflammatory cytokines to the saliva. Specifically, serum IL-6 levels were 6.1 $\pm$ 3.8 pg/ml in active CD vs. 2.47 $\pm$ 1.7 pg/ml in inactive CD (*P* < 0.05) and 2.07 $\pm$ 1.2 pg/ml in controls; serum TNF- α levels were 4.6 $\pm$ 5.8 pg/ml in active CD vs. 1.6 $\pm$ 0.9 pg/ml in inactive CD (*P* < 0.05) and 1.62 $\pm$ 1.3 pg/ml in controls. Nielsen et al.²⁴ demonstrated higher levels of IL-6 in the saliva of patients with CD compared with serum levels, most likely caused by local secretion of IL-6.²⁴ The authors speculated that the inflammatory process of the entire gastrointestinal tract including the mouth may subsequently affect the levels of inflammatory markers in the saliva.²⁴ Our studies have confirmed this observation in patients with active CD. Moreover, we observed increased levels of other proinflammatory cytokines in the saliva, namely, IL-1 β and TNF- α .

IL-6 is the major mediator of inflammation, and together with IL-1 β and TNF- α , it stimulates the synthesis of acute-phase proteins, including CRP, and activates bone marrow cells with the release of platelets to the blood.²⁴ Elevated CRP and increased platelet count observed in our study in patients with active CD may be caused by higher salivary and blood levels of proinflammatory cytokines.^{24,35} Our study also confirmed a close correlation between the elevated salivary levels of IL-6 and TNF- α , but not IL-1 β , and specific oral lesions in patients with active CD as assessed by the CDAI. Lack of association between increased salivary IL-1 β and specific oral lesions in patients with active disease is difficult to explain in this study, particularly because IL-1 β , similarly to IL-6

and TNF- α , correlated with other inflammatory markers. We can only speculate that the antagonistic effect of CD medication on salivary IL-1 β may partially explain the lack of a statistically significant correlation between oral lesions in active CD as assessed by the CDAI and IL-1 β levels in the saliva. Our findings should be confirmed in a larger group of patients.

The study has several limitations. First, we did not analyze other parameters of inflammatory process or oxidative stress markers. The importance of oxidative stress in the pathophysiology of CD is well known and was studied with respect to the mucosal inflammation of the oral cavity by several authors. Jahanshahi et al.²⁹ analyzed the whole saliva of patients with CD and observed oxidative stress in the saliva with increased values of nitric oxide and lipid peroxide markers as well as decreased antioxidant power assessed by ferric-reducing ability. Rezaie et al.²⁸ measured oxidative capacity, specific antioxidants in saliva such as uric acid, albumin, transferring, and thiol molecules as well as lipid peroxidation, nitric oxide, and TGF- β in the saliva of patients with active CD.²⁸ They observed significant reduction in salivary levels of total antioxidant capacity, albumin, and uric acid in active CD, and the CDAI correlated with the antioxidant capacity and lipid peroxidation.²⁸

We also did not analyze the effect of smoking on salivary cytokines. Several authors reported higher systemic levels of IL-1 β , IL-6, and TNF- α in healthy smokers compared with healthy non-smokers but it was not confirmed by other investigators.^{32,36,37} Conflicting data regarding these cytokines in the saliva of smokers with CD require further research.

Another limitation of our study is the fact that the patients with CD were chronically treated with mesalazine and azathioprine, and we could not exclude the effect of these anti-inflammatory drugs on the measured cytokines. Both drugs are currently recommended as maintenance therapy of patients with CD.²

The present study showed that more than 11% of the patients with active CD had disease-specific lesions in the oral cavity. Oral lesions specific for CD have been described in several studies and confirmed by histological examination with characteristic granuloma in inflammatory lesions.^{10-12,38} Types of CD-specific oral lesions have been described by numerous authors and included diffuse swelling of the lips, buccal mucosal swelling, mucogingivitis, cobblestoning and granulomatous cheilitis, deep linear ulcerations, and mucosal tags.^{12,38} Halme et al.¹¹ studied 53 patients with CD located in the small and large intestine and observed specific oral inflammatory lesions confirmed by histopathological examination in 9 patients with active disease. Nonspecific lesions occur more often in CD and can be found in patients without gastrointestinal diseases or even in the general population.^{8,11-15,39,40} The high prevalence of these lesions may be caused by a specific

diet, malabsorption, adverse effects of drugs, as well as anemia and deficiencies of iron, fat-soluble vitamins, folic acid, or vitamin B₁₂, which are typically associated with CD.¹²

We observed CD-specific lesions, classified according to Scheper and Brand,³⁸ in 23% of the patients with active CD. Indurated polypoid tag lesions on vestibular and retromolar fossae were found in 13.3% of the patients, cobblestoning in 11.3%, and gingivitis in 17.3%. The most characteristic and the most common lesion is the inner cheek swelling, which was observed in 23% of the patients in our study.^{6,12,38}

Investigators report a varying prevalence of oral lesions in patients with active and inactive CD.^{10-12,26} It may be caused by numerous factors including age, ethnicity, and the severity of active CD as assessed by the CDAI.¹² Experience of the investigator and definition of disease-specific lesions should also be considered.¹² Numerous studies described nonspecific lesions as characteristic of oral CD.^{15,38} Therefore, it is important to describe oral lesions in patients with CD according to a classification that is based on a careful evaluation of the lesions.^{10,38} The prevalence of CD-specific lesions in adult patients reaches 32%^{4,12} and is much lower than in children (up to 80%).^{8,12}

Our study showed an association between oral CD in patients with active disease and increased levels of erythrocytes, hemoglobin, platelets, serum CRP, and BMI. However, no correlation was observed between the CDAI and oral CD. Interestingly, several case reports confirmed that severe oral lesions may occur in patients without clinical symptoms of active CD.^{13,16,40}

In conclusion, the present study demonstrated that proinflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , are elevated in the whole unstimulated saliva of patients with active CD compared with patients with inactive disease and controls. Cytokine levels correlated with several markers of the inflammatory process and the presence of oral lesions, confirming that the mouth cavity is involved in patients with active CD. The observation that IL-1 β , IL-6, and TNF- α in the whole saliva are significantly elevated in active CD compared with inactive CD and the control group may have diagnostic and prognostic value. Furthermore, salivary cytokine levels in saliva may be useful sensitive biomarkers of CD activity. Moreover, considering that over 11% of the patients with active CD had disease-specific lesions in the oral cavity, careful oral examination of the lesions should be included in the clinical evaluation of patients with CD.

Acknowledgements The study was supported by the grants from the Jagiellonian University Medical College in Kraków (K/ZDS/002284 and K/DSC/000019).

REFERENCES

- 1 Van Assche G, Dignass A, Panes J, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis*. 2010; 4: 7-27.
- 2 Dignass A, Van Assche G, Lindsay JO, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohn's Colitis*. 2010; 4: 28-62.
- 3 Owczarek D, Cibor D, Szczepanek M, Mach T. Biological therapy of inflammatory bowel disease. *Pol Arch Med Wew*. 2009; 119: 84-89.
- 4 Dudeney TP. Crohn's disease of the mouth. *Proc R Soc Med*. 1969; 62: 1237.
- 5 Greenstein AJ, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. *Medicine (Baltimore)*. 1976; 55: 401-412.
- 6 Cosnes A, Dupuy A, Revuz J, et al. Long-term evolution of oral localisation of Crohn's disease. *Gastroenterology*. 1998; 114: A956.
- 7 Dupuy A, Cosnes J, Revuz J. Oral Crohn's disease: clinical characteristics and long-term follow-up of 9 cases. *Arch Dermatol*. 1999; 135: 439-442.
- 8 Pittock S, Drumm B, Fleming P, et al. The oral cavity in Crohn's disease. *J Pediatr*. 2001; 138: 767-771.
- 9 Mach T, Szczeklik K, Garlicka M, Owczarek D. [Oral ulceration in patient with active Crohn's disease]. *Przegl Gastroenterol*. 2007; 2: 210-213. Polish.
- 10 Michailidou E, Arvanitidou S, Lombardi T, et al. Oral lesions leading to the diagnosis of Crohn disease: report on 5 patients. *Quintessence Int*. 2009; 40: 581-588.
- 11 Halme L, Meurman JH, Laine P, et al. Oral findings in patients with active or inactive Crohn's disease. *Oral Surg Oral Med Oral Pathol*. 1993; 76: 175-181.
- 12 Rowland M, Fleming P, Bourke B. Looking in the mouth for Crohn's disease. *Inflamm Bowel Dis*. 2010; 16: 332-337.
- 13 Scully C, Cochran KM, Russell RI, et al. Crohn's disease of the mouth: an indicator of intestinal involvement. *Gut*. 1982; 23: 198-201.
- 14 Stricker T, Braegger CP. Images in clinical medicine. Oral manifestations of Crohn's disease. *N Engl J Med*. 2000; 342: 1644.
- 15 Harty S, Fleming P, Rowland M, et al. A prospective study of the oral manifestations of Crohn's disease. *Clin Gastroenterol Hepatol*. 2005; 3: 886-891.
- 16 Logan RM. Links between oral and gastrointestinal health. *Curr Opin Support Palliat Care*. 2010; 4: 31-35.
- 17 Lawrence HP. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J Canad Dental Assoc*. 2002; 68: 170-174.
- 18 Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. *Oral Dis*. 2002; 8: 69-76.
- 19 Rhodus NL, Cheng B, Myers S, et al. A comparison of the pro-inflammatory, NF-kappaB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. *Clin Immunol*. 2005; 114: 278-283.
- 20 Rhodus NL, Cheng B, Bowles W, et al. Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. *Oral Dis*. 2006; 12: 112-116.
- 21 Tobón-Arroyave SI, Jaramillo-González PE, Isaza-Guzmán DM. Correlation between salivary IL-1beta levels and periodontal clinical status. *Arch Oral Biol*. 2008; 53: 346-352.
- 22 Zhang Y, Lin M, Zhang S, et al. NF-κB-dependent cytokines in saliva and serum from patients with oral lichen planus: A study in an ethnic Chinese population. *Cytokine*. 2008; 41: 144-149.
- 23 Teles RP, Likhari V, Socransky SS, Haffajee AD. Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *J Periodontol Res*. 2009; 44: 411-417.
- 24 Nielsen A, Nielsen NJ, Schmedes A, et al. Saliva Interleukin-6 in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 2005; 40: 1444-1448.
- 25 Best WR, Beckett JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology*. 1976; 70: 439-444.
- 26 Zervou F, Gikas A, Merikas E, et al. Oral manifestations of patients with inflammatory bowel disease. *Ann Gastroenterol*. 2004; 17: 395-401.
- 27 Rhodus NL, Ho V, Miller CS, et al. NF-κB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detect Prev*. 2005; 29: 42-45.
- 28 Razaie A, Ghorbani F, Eshgork A, Zamani MJ et al. Alterations in salivary antioxidants, nitric oxide, and transforming growth factor-beta 1 in relation to disease activity in Crohn's disease patients. *Ann N Y Acad Sci*. 2006; 1091: 110-122.
- 29 Jahanshahi G, Motavasel V, Rezaie A, et al. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig Dis Sci*. 2004; 49: 1752-1757.
- 30 Stein JM, Lammert F, Zimmer V, et al. Clinical periodontal and microbiologic parameters in patients with Crohn's disease with consideration of the CARD15 genotype. *J Periodontol*. 2010; 81: 535-545.
- 31 Brailo V, Vucicevic-Boras V, Cekic-Arambasin A, et al. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. *Oral Oncol*. 2006; 42: 370-373.
- 32 Brailo V, Vucicevic-Boras V, Lukac J, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. *Med Oral Patol Oral Cir Bucal*. 2012; 17: e10-e15.
- 33 Rhodus NL, Cheng B, Myers S, et al. The feasibility of monitoring NF-kappaB associated cytokines: TNF-alpha, IL-1alpha, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus. *Mol Carcinog*. 2005; 44: 77-82.

Cytokiny prozapalne w ślinie chorych z aktywną i nieaktywną postacią choroby Leśniowskiego i Crohna

Katarzyna Szczeklik¹, Danuta Owczarek², Jolanta Pytko-Polończyk¹,
Barbara Kęsek¹, Tomasz H. Mach²

1 Instytut Stomatologii, Uniwersytet Jagielloński, Collegium Medicum, Kraków

2 Katedra Gastroenterologii, Hepatologii i Chorób Zakaźnych, Uniwersytet Jagielloński, Collegium Medicum, Kraków

SŁOWA KLUCZOWE

choroba
Leśniowskiego
i Crohna, czynnik
martwicy
nowotworów α ,
interleukina 1 β ,
interleukina 6, jama
ustna

STRESZCZENIE

WPROWADZENIE Choroba Leśniowskiego i Crohna (ChLC) dotyczy całego przewodu pokarmowego, w tym jamy ustnej. W regulacji procesu zapalnego w ChLC odgrywa rolę wiele cytokin.

CELE Celem badania była ocena częstości występowania zmian w jamie ustnej u dorosłych chorych na ChLC oraz zbadanie, czy stężenie w ślinie interleukiny 1 β (IL-1 β), interleukiny 6 (IL-6) i czynnika martwicy nowotworów α (*tumor necrosis factor α* – TNF- α) zależy od aktywności choroby i zmian w jamie ustnej.

PACJENCI I METODY Do badań prospektywnych włączono 95 dorosłych chorych na ChLC: 52 z aktywną postacią choroby oraz 43 z nieaktywną postacią choroby. Grupę kontrolną stanowiło 45 osób bez ChLC. Wykonano badania laboratoryjne krwi, szczegółowe badanie jamy ustnej oraz pomiar stężenia IL-1 β , IL-6 i TNF- α w niestymulowanej pełnej ślinie za pomocą testu immunoenzymatycznego.

WYNIKI Stężenia IL-1 β , IL-6 i TNF- α były znacznie większe u chorych z aktywną postacią ChLC. Stężenie IL-1 β wynosiło: $289,8 \pm 52,7$ pg/ml u chorych z aktywną postacią ChLC vs $196,7 \pm 42,9$ pg/ml u chorych z nieaktywną postacią ChLC ($p < 0,039$) oraz $196,7 \pm 42,9$ pg/ml ($p < 0,01$) w grupie kontrolnej. Stężenie IL-6 wynosiło odpowiednio: $13,8 \pm 4,2$ pg/ml vs $7,2 \pm 3,1$ pg/ml ($p < 0,041$) oraz $6,3 \pm 1,4$ pg/ml ($p < 0,001$) w grupie kontrolnej. Stężenie TNF- α wynosiło odpowiednio: $32,5 \pm 8,7$ pg/ml vs $10,2 \pm 6,3$ pg/ml ($p < 0,002$) oraz $6,8 \pm 2,8$ pg/ml ($p < 0,001$) w grupie kontrolnej. Obserwowano swoiste dla ChLC zmiany w jamie ustnej: rozlany niesymetryczny obrzęk policzka u 12 chorych (23%) i nierówności błony śluzowej u 5 chorych (11,3%). Nieswoiste dla ChLC zmiany obserwowano u 17 (32,7%) chorych z aktywną postacią choroby, u 11 (25,6%) z chorych z nieaktywną postacią choroby oraz u 6 (13,3%) osób z grupy kontrolnej. W aktywnej postaci ChLC większe stężenia IL-6, TNF- α i białka C-reaktywnego w surowicy korelowały ze swoistymi zmianami w jamie ustnej.

WNIOSKI U chorych z aktywną postacią ChLC stężenie IL-1 β , IL-6 i TNF- α w ślinie jest większe niż u chorych z nieaktywną postacią choroby i u osób z grupy kontrolnej. Zwiększone stężenie IL-6 i TNF- α w ślinie koreluje ze swoistymi zmianami w jamie ustnej. Cytokiny te mogą być stosowane jako markery aktywnej postaci ChLC, lecz wymaga to potwierdzenia w większej grupie chorych.

Adres do korespondencji:
prof. dr hab. med. Tomasz Mach,
Katedra Gastroenterologii, Hepatologii
i Chorób Zakaźnych, Uniwersytet
Jagielloński, Collegium Medicum,
ul. Śniadeckich 5, 31-531 Kraków,
tel.: 12-424-73-40, fax: 12-424-73-80,
e-mail: tmach@su.krakow.pl

Praca wpłynęła: 01.02.2012.

Przyjęta do druku: 16.04.2012.

Publikacja online: 24.04.2012.

Nie zgłoszono sprzeczności
interesów.

Pol Arch Med Wewn. 2012;

122 (5): 200-208

Copyright by Medycyna Praktyczna,

Kraków 2012