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*The significance of radiological and biochemical examinations  
in discovering latent focuses of infection in the oral cavity  
and function of renal graft*

Chronic apical periodontitis is the state of dynamic stability between such irritative factors as bacteria, their byproducts, root filling materials etc. and the defence mechanism of the host organism. To date, the knowledge of the pathophysiology of focal infections is incomplete, although it is believed that cytokines produced by the cells located in periapical lesions can cause inflammation in distant tissues and organs which have a negative influence on the health of patients (7, 9).

The fundamental element in preparing a patient for renal transplantation and in taking medical care of patients after renal transplantation is to discover the focuses of infection in the oral cavity (also asymptomatic) and its immediate treatment (8, 10, 20).

IL-6 (interleukine-6) is the main cytokine, which plays a significant role in influencing the onset of several immunological reactions, inflammatory processes and the rejection of renal graft. It has been suggested that IL-6 is released locally in periapical lesions (1, 4, 5, 13, 16). Some researchers have observed that TNF- $\alpha$  (tumor necrosis factor  $\alpha$ ) is present in small amounts in chronic periapical periodontitis lesions. It is important to mention that the level of this cytokine in serum increases in the case of allogeneal process of rejecting renal graft (4). It is a known fact that in cases of chronic apical periodontitis lesions level of acute phase proteins, including CRP (C - reactive protein) in serum is increased (11).

The aim of the present study was to determine the potential connection between latent focuses of infection located in the oral cavity, discovered by radiological methods, the level of some cytokines, acute phase protein in serum as well as in unstimulated whole saliva and the function of renal graft.

#### MATERIAL

The study group consisted of 27 patients (9 women and 18 men) after renal transplantation. The mean age of the patients was 41 years and 8 months  $\pm$  10 years and 9 months. The mean period of time between the renal transplantation and examination was 37  $\pm$  33.14 months. The causes for chronic kidney insufficiency were: chronic kidney inflammation (n=18), congenital kidney disease (n=4) tubulo-interstitial kidney disease (n=1), unknown/uncertain reasons (n=4). Patients were treated by three-drug immunosuppressive therapy – cyclosporine, azathioprine and corticosteroids

(n=22) or by two-medicated draft (n=5). During examination the patients did not have any infection focuses (based on physical examination) or any feature of acute process of the rejection of a renal transplant.

## METHODS

Clinical, radiological examinations, laboratory tests and statistical analyses were carried out on the group. To determine possible latent focuses of infection in the oral cavity panoramic radiograms were taken. According to WHO criteria and using panoramic radiograms, the state of dentition and the level of dental caries were determined using the DMFs Index (Decayed, Missing or Filled surfaces of teeth). Incipient lesions were included. The state of periapical parodontium and the number of root canal treated teeth were determined using panoramic radiograms. CPITN (Community Periodontal Index and Treatment Needs) and DIGO, according to *Ingles et al.* (Drug-Induced Gingival Overgrowth) (6) were used to determine the state of marginal parodontium. The subjects had been instructed to refrain from eating (particularly sweets), drinking or using breath fresheners for a minimum of 2 hrs before the saliva was collected. Unstimulated whole saliva was collected on ice during morning hours into sterile test tubes and maintained at 4°C during centrifugation. The saliva was not collected in fractions. The TNF- $\alpha$ , IL-6 and CRP levels in serum and unstimulated whole saliva were determined by means of ELISA (Enzyme Linked Immune Sorbent Assay). The level of creatinine was determined by using a conventional method and the level of creatinine clearance was calculated according to Cockcroft- Gault formula (14). The methods employed for statistical analysis of the obtained clinical and biochemical data were as follows: mean value for quantitative (measurable) results and the standard deviation test for the data. The obtained results were tested for normality by  $\chi^2$  test. For comparison of the results, the following statistical hypothetical tests were used: Kruskal-Wallis test and median test. The multiple regression test, both in the linear model and in polynomial functions, and especially in the quadratic functions was also used. Significance was accepted according to estimated p-levels of <0.05. Statistical analysis was carried out on an IBM PC using Statistica Version 5 '97.

## RESULTS

On the ground of dental and radiological significance, it was defined that the mean DMFs Index was  $72.92 \pm 45.71$ ; mean Ms Index (Missing surfaces)  $52.96 \pm 47.34$ . The Ds Index (Decayed surfaces of teeth) and the Fs Index (Filling surfaces of teeth) were not normally distributed; the results of these parameters are given as median (Me) and vary. The Ds Index ranged from 0–19 (Me=3.0), the Fs Index was within the limit 0–63 (Me=9). None of the patients had pathological lesions on oral mucosa. All of them had drug-induced hypertrophic inflammation of periodontium (grade 0 to 3, according to the DIGO index, elaborated by *Ingles et al.* <6>). It was observed that in six patients marginal gingiva bled after a delicate probe. Twenty-one patients had supragingival and subgingival calculus. The mean level of creatinine in serum was  $1.69 \pm 0.90$  mg/dl, mean creatinine clearance was  $68.26 \pm 21.73$  ml/min. The levels of IL-6, TNF- $\alpha$ , CRP in serum and in mixed, unstimulated whole saliva were not normally distributed. The level of IL-6 in blood serum was within the limit 0.0 pg/ml – 19.60 pg/ml (Me=3.6 pg/ml) and in mixed, unstimulated whole saliva, 0.0 pg/ml – 85.70 pg/ml (Me=7.10 pg/ml). The median level of TNF- $\alpha$  in serum was Me=11.00 pg/ml (within the range 0.0 pg/ml – 435.00 pg/ml), in mixed unstimulated saliva Me=3.0 pg/ml (within the range 0.0 pg/ml – 12.00 pg/ml). In the case of CRP, in serum Me=11.34 ng/ml (within the range 0.0 ng/ml – 145.12 ng/ml), in mixed, unstimulated whole saliva Me=13.40 ng/ml (within the range 0.0 ng/ml – 486.55 ng/ml). Statistical results of biochemical significance in serum and mixed, unstimulated whole saliva are given in Table 1.

It was not noted that there is an important relationship between biochemical parameters in mixed, unstimulated whole saliva, as well as in serum, and between the number of latent foci of infection in the oral cavity.

Table 1. Number and percent of patients after renal transplantation with and without latent foci in the oral cavity with TNF- $\alpha$ , IL-6, CRP levels in serum and whole unstimulated saliva higher and lower than Me

Examined sample	Factor	Patients				Statistical results
		with latent foci in oral cavity		without latent foci in oral cavity		
		Number	%	Number	%	
Serum	IL - 6 $\leq$ Me	7	58.3	8	53.3	Me=3.60 $\chi^2=0.067$ p=0.795
	IL - 6 $>$ Me	5	41.7	7	46.7	
	TNF - $\alpha$ $\leq$ Me	7	58.3	7	46.7	Me=11.00 $\chi^2=0.363$ p=0.546
	TNF - $\alpha$ $>$ Me	5	41.7	8	53.3	
	CRP $\leq$ Me	6	50.0	8	53.3	Me=11.340 $\chi^2=0.296$ p=0.863
	CRP $>$ Me	6	50.0	7	46.7	
Whole unstimulated saliva	IL - 6 $\leq$ Me	7	58.3	7	46.7	Me = 7.10 $\chi^2 = 0.363$ p = 0.546
	IL - 6 $>$ Me	5	41.7	8	53.3	
	TNF - $\alpha$ $\leq$ Me	6	50.0	10	66.7	Me=3,00 $\chi^2=0,767$ p=0.381
	TNF - $\alpha$ $>$ Me	6	50.0	5	33.3	
	CRP $\leq$ Me	4	33.3	10	66.7	Me=13.40 $\chi^2=2.967$ p=0.850
	CRP $>$ Me	8	66.7	5	33.3	

Analysis of variance for multiple regression showed essential changes of the level of creatinine in serum during synchronous changes of TNF- $\alpha$ , IL-6, CRP levels in serum, and changes of the number of latent foci of infection in the oral cavity ( $F=10.767$ ,  $p<0.001$ ). Coefficient of regression  $R=0.826$  showed that there exists a correlation between these features. Lower creatinine levels were characteristic when the levels of TNF- $\alpha$ , IL-6, and CRP in serum were also lower, and there were less latent foci of infection in the oral cavity. The closest connection was noticed between the levels of creatinine and IL-6 in serum and the number of latent foci of infection in the oral cavity (Fig. 1 a, c). Similar dependences were noticed in the case of changes in the level of creatinine in serum and levels of TNF- $\alpha$ , IL-6 in whole unstimulated saliva as well as the number of latent foci in oral cavity ( $F=19.107$ ,  $p<0.001$ ). In this case, the closest relationship existed between the level of creatinine, the number of latent foci, and the level of CRP in saliva (Fig. 1 b, c).

Multiple regression analysis showed that the creatinine clearance decreased when the levels of TNF- $\alpha$ , IL-6, CRP increased in serum; at the same time, the number of latent foci of infection in the oral cavity increased. Analysis of variance for multiple regression showed a significant relationship among the creatinine clearance level and the levels of TNF- $\alpha$ , IL-6, CRP in serum as well as the number of asymptomatic latent foci in the oral cavity ( $F=3.848$ ,  $p<0.017$ ). Analysis of variance F Snedecor showed that there existed the significant dependence between the decreasing level of creatinine clearance and the increasing levels of TNF- $\alpha$ , IL-6, CRP in whole unstimu-

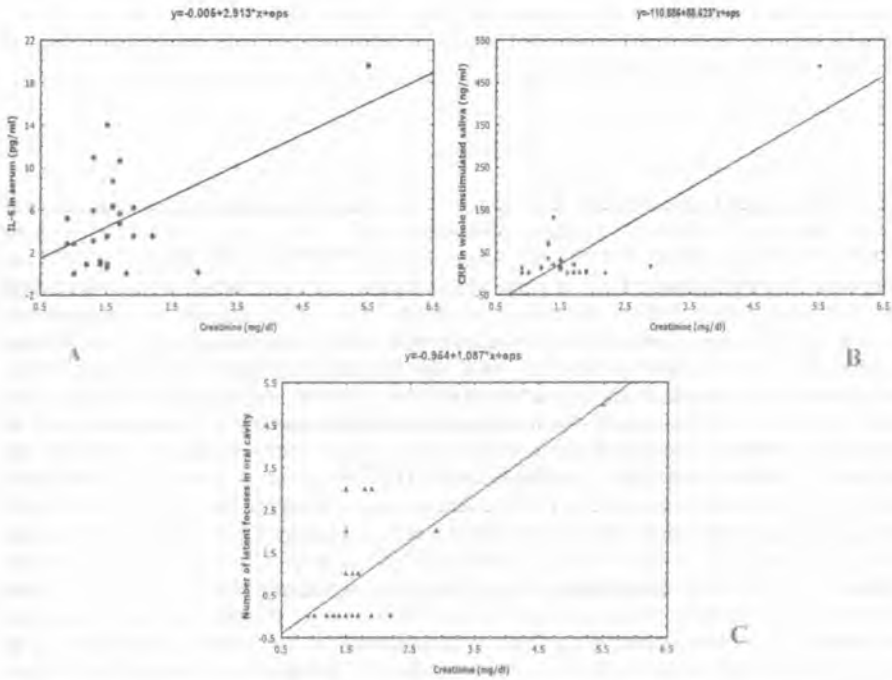


Fig. 1. A – Relationship between the level of creatinine and the level of IL-6 in serum of the patients in this study; B – relationship between the level of creatinine and the level of CRP in whole unstimulated saliva in the examined patients; C – relationship between the level of creatinine and the number of latent foci in oral cavity in the studied group of patients

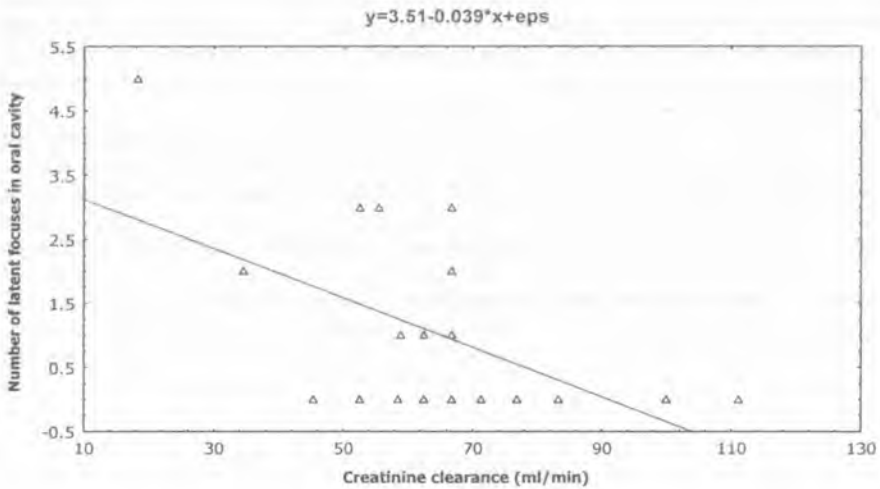


Fig. 2. The relationship between creatinine clearance and the number of asymptomatic latent foci in oral cavity

lated saliva and the number of latent focuses of infection ( $F=3.446$ ,  $P<0.026$ ). The closest relationship existed between the decreasing level of creatinine clearance and the increasing number of latent focuses of infection in oral cavity (Fig. 2).

## DISCUSSION

Data of the Expert Group on Renal Transplants shows that the frequency of new cases of end-stage renal disease in 1996 was 118 patients per million inhabitants of the European Union countries; 34.1% of patients were treated by renal transplantation (17). Proper dental care is one of the most important factors in patients after renal transplantation; it is important to discover latent focuses of infection (most of all asymptomatic) in the oral cavity (3). In the case of asymptomatic and chronic latent focuses of infections there is no single biochemical marker of chronic inflammatory process (11). Orzędala-Koszeł et al. showed that the chronic periapical periodontitis lesions caused an increase in the concentration of CRP in serum, and that the increase in concentration of this protein had diagnostic and prognostic significance as well as significance in monitoring the course of the disease. It is worth mentioning that 96% of patients with chronic periapical periodontitis lesions had an increased level of CRP in serum, within the range 625 mg/l – 78.48 mg/l (11). In our studies Me of CRP in serum was 11,34 ng/ml (range 0,0 ng/ml to 145, 12 ng/ml), in mixed, unstimulated whole saliva  $Me=13.40$  ng/ml (range 0.0 ng/mg – 486.55 ng/ml). The differences in results could be possibly caused by various groups of patients being examined. Orzędala-Koszeł's patients were healthy people, whereas our patients were patients after renal transplantation. An essential fact is that the level of CRP in saliva depends on the condition of periodontium (12). In our studies all patients had drug-induced hypertrophic inflammation of periodontium, but it is important to mention that according to Thomas et al. the grade of hypertrophy of the gingiva caused by cyclosporine and the function of renal graft is independent (18).

In our previous studies of healthy 17-year-olds the level of IL-6 in mixed, unstimulated whole saliva in 40.7% of the study group was below the lowest detection limit of ELISA test. Me of IL-6 was 9.0 pg/ml (15). In this studies the median level of IL-6 in whole unstimulated saliva in patients after renal transplantation was 7.10 pg/ml; in serum, however, it was on the level of 3.60 pg/ml.

Winkler et al. consider that saliva is the perfect research medium, rendering the discovering of the markers of inflammatory processes and asymptomatic infections of oral cavity. The authors examined 167 children aged 8–10 and discovered that the concentration of TNF- $\alpha$  in saliva was within the limit 0.3–40.6 pg/ml (19). In our studies median of TNF- $\alpha$  was 11.00 pg/ml in serum and 3.00 pg/ml in saliva.

In the case of the patients undergoing bone marrow transplant, Bishay et al. suggested that it is essential to utilise pantograms for radiological examination of dentition as well as full intraoral radiograms and this is necessary for suitable diagnosis of diseases of the oral cavity (2). It seems that in the case of patients after renal transplantation it is enough to perform panoramic radiograms, especially that they provide a large coverage of the maxilla, mandible, the teeth, maxillary sinuses and nasal fossa. They not only show which teeth are present in the dental arch and their relative state of development, but also whether any pathologic abnormalities are present. Panoramic radiograms carried out in patients after renal transplantation are the essential examination. They make possible to determine the occurrence of any latent focus of infection in the oral cavity.

## CONCLUSIONS

1. Radiological evaluation is fundamental and most important procedure for the patients after renal transplantation, especially for detecting latent focuses of infection in the oral cavity.

2. None of the examined markers of inflammatory processes (TNF- $\alpha$ , IL-6, CRP) can provide the answer whether latent focuses of infections are present in the oral cavity or not in the group of patients after renal transplantation.

3. Latent focuses in the oral cavity negatively influence the state and function of renal graft.

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#### SUMMARY

The aim of the study was to determine the potential relationship between latent focuses of infection located in the oral cavity, the levels of some cytokines, acute phase protein in serum and unstimulated whole saliva and the renal graft function. 27 renal transplanted patients were studied. Clinical, radiological examinations, laboratory tests (TNF- $\alpha$ , IL-6 and CRP levels in serum and unstimulated whole saliva) and statistical analyses were carried out. Lower creatinine levels were

characteristic when the levels of TNF- $\alpha$ , IL-6, and CRP in serum were also lower, and there were less latent foci of infection in the oral cavity. It was also established that the closest relationship existed between the decreasing level of creatinine clearance and the increasing number of latent foci of infection in oral cavity. Radiological evaluation is fundamental and most important procedure for the patients after renal transplantation, especially for detecting latent foci of infection in the oral cavity; none of the examined markers of inflammatory processes can provide the answer whether latent foci of infections are present in the oral cavity; latent foci in the oral cavity negatively influence the state and function of renal graft.

#### Znaczenie badań radiologicznych i biochemicznych w wykrywaniu utajonych ognisk zakażenia w jamie ustnej a funkcja graftu nerki

Celem pracy było wykrycie potencjalnych związków między utajonymi ogniskami zakażenia w jamie ustnej wykrywanymi metodami radiologicznymi, poziomem wybranych cytokin i białek ostrej fazy w surowicy krwi i w ślinie a funkcją graftu nerki. Badaniami objęto 27 pacjentów po transplantacji nerki. Przeprowadzono kliniczne badania stomatologiczne, badania radiologiczne, biochemiczne (TNF- $\alpha$ , IL-6, CRP w surowicy oraz ślinie mieszanej niestymulowanej), a uzyskane wyniki poddano analizie statystycznej. Niższe wartości kreatyniny były charakterystyczne przy niższych wartościach TNF- $\alpha$ , IL-6, CRP w surowicy krwi oraz mniejszej liczbie utajonych ognisk zakażenia w jamie ustnej. Stwierdzono występowanie związku między spadkiem wartości klirensu kreatyniny a zwiększaniem się liczby utajonych ognisk zakażenia, występujących w jamie ustnej. W przypadku pacjentów po przeszczepie nerki badania radiologiczne umożliwiają wykrycie większości utajonych ognisk zakażenia, zlokalizowanych w jamie ustnej; żaden z badanych markerów procesu zapalnego nie jest charakterystyczny dla utajonych ognisk infekcji występujących w jamie ustnej; zlokalizowane w jamie ustnej utajone ogniska zakażenia wywierają negatywny wpływ na stan nerki przeszczepionej.