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RESULTS OF INFLAMMATORY AND IMMUNOLOGICAL PARAMETERS OF THE ORAL CAVITY AFTER CYSTECTOMY WITH DIFFERENT METHODS OF CONNECTING THE EDGES OF THE OPERATED AREA

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ABSTRACT

The aim: To analyze the results of inflammatory and immunological parameters of the oral cavity after cystectomy with different methods of connecting the edges of the operated area.

Materials and methods: The research was conducted in 87 patients who sought surgical treatment of odontogenic cysts of the jaws. Patients were divided into groups depending on the method of wound closure after surgery. We analyzed the results of laboratory tests (leukocytes, ESR, IL-1β, IL-6, TNF-α, IL-8, NO synthase, MMP-9).

Results: The analysis of the effectiveness of using different methods of approximation of the oral mucosa wound on inflammatory and immunological parameters showed that when using welding of the edges of the operated area using the EKVZ-300 "Patonmed" apparatus, we get positive results of laboratory indicators of inflammatory markers faster (leukocyte counts are normal on day 30, ESR, IL-1β – on the 14th day, TNF-α – on the 7th day, IL-6, IL-8, NO synthase, MMP-9 – on the 30th day), that is, healing in such patients is faster than in patients with wound closure with classic suture material or laser.

Conclusions: When comparing the methods of approximation of postoperative oral mucosa wounds by different methods, based on the results of these inflammatory and immunological parameters, the best results were obtained when using electric welding of tissues. Further research and use of the proposed method will facilitate and shorten the rehabilitation period of patients after surgery.

KEY WORDS: odontogenic cyst, cystectomy, electric welding of tissues, laboratory tests

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INTRODUCTION

Odontogenic cysts of the jaws are one of the most common diagnoses requiring surgical treatment. Among them, radicular cysts are the most common. They are the result of an inflammatory process in the periapical tissues associated with necrotic and infected pulp [1]. Humoral and cellular immune responses play a central role in the pathogenesis of these lesions. The most important role in the growth of these lesions is played by the proinflammatory cytokines TNF-alpha, IL-1, and IL-6 [2]. The cytokine can be secreted by macrophages, monocytes, and other cells of the immune system and may be involved in skeletal homeostasis, including osteoclast formation, and bone resorption in the maxillofacial region [3]. For a more in-depth study of this issue, it is necessary to first give a generalized overview of the mechanism of growth of maxillofacial radicular cysts with an indication of the clinical aspect, as well as to clarify the role of cytokines in this pathophysiological process [4, 5].

Radicular cysts are the most common among jaw cysts, and, according to various authors [6, 7, 8], they account for 6 to 84 % of all periapical lesions. At the same time, tooth-containing cysts (follicular cysts) and keratocysts are diagnosed in 37 % and 6-32 % of patients, respectively. When studying the prevalence of radicular cysts by gender, a small difference was found between men and women. According to some authors, odontogenic cysts were more commonly detected in men (55.9-65.0 %) than in women (44.1-35.0 %) [9, 10]. Other authors report that odontogenic cysts are more common in women (52.37-65.0 %) and less common in men (35.0-47.62 %) [7].

Surgical operations performed at present and associated with cystectomy are based on two main points: tissue separation and subsequent connection [11, 12]. One of the conditions for a properly performed operation is the ability to achieve rapid wound healing, provide a good cosmetic effect, and prevent complications [13, 14, 15]. During many surgical interventions in the oral cavity, the failure of a single suture in the postoperative period can lead to significant complications that usually require repeated operations [16, 17, 18, 19].

THE AIM

To analyze the results of inflammatory and immunological parameters of the oral cavity after cystectomy with different methods of connecting the edges of the operated area.

MATERIALS AND METHODS

In accordance with the aim, laboratory tests were performed in patients after surgical treatment of odontogenic cysts of the jaws at the Educational-Treatment Center "University Clinic" (Chernivtsi, Ukraine).

The patients were divided into three groups: Group I (20 patients) – cystectomy with wound suturing using suture material «Vicryl»; Group II (28 patients) – cystectomy with wound edge consolidation using the laser scalpel LSP – "IRE-POLUS" and Group III (39 patients) – the edges of the postoperative defect were connected by using the electric welding apparatus EKVZ-300 "Patonmed".

After cystectomy, 20 patients (group A, 1 control) had their surgical wounds sutured with suture material «Vicryl». In 28 patients (group B, 2 control), after cystectomy, the wound edges were approximated using the LSP laser – "IRE-POLUS", with an irradiation wavelength of 0.97±1.56 μm, maximum irradiation power of 30/60 W; continuous, pulsed, pulsed-periodic mode of operation [20]. A laser with a wavelength of 1.06 μm, a power of 0.71-0.85 W, a continuous irradiation mode, a light guide diameter of 0.6 mm was used to join the wound edges in the area of contact by moving the source along the surface of the wound edge at a distance of 1.0-3.0 mm, with a light guide movement speed of no more than 1.0 mm/sec [21].

In 39 patients (group C, main), the edges of the postoperative wound, after cystectomy, were welded using the apparatus EKVZ-300 "Patonmed".

Patients in this group were treated with the wound edges using the apparatus EKVZ-300 "Patonmed" in the automatic welding mode N2 using the following parameters: voltage – 25 W, duration of exposure to high-frequency current on tissues no more than 2-6 s/per point, duration of voltage amplification – 150 ms, specific pressure force on tissues in the range of 0.3-0.7 N/cm², current frequency – 66 kHz, temperature in the exposure zone 55-65 °C. Patients of all research groups were given recommendations for wound care in the postoperative period and prescribed drug therapy: nimesulide – 100 mg, 1 sachet twice a day for three days after the intervention; rinsing the oral cavity with chlorhexidine 0.06 % after each meal; topical cold for 5 minutes three times a day for the first three days after the intervention.

RESULTS

As a result of our studies, we found that in healthy subjects of the control group, the content of leukocytes in the oral fluid was 276.80±45.72 x 10^9/L (Table I). On the first postoperative day, the content of leukocytes in the oral fluid increased in patients of all Patients of all research groups compared with the data in the control group: by 63.87 %, $p<0.05$ in group A, by 100 %, $p<0.01$ in group B, and by 81.94 % in group C. At the same time, the values of the obtained indicators did not differ in statistical significance in the intergroup comparison. On the 5th postoperative day, the quantitative content of leukocytes in the oral fluid in the patients of all research groups continued to increase and was significantly higher than in the control group in all Patients of all research groups, $p<0.01$. However, the minimum values were determined in patients of groups C and A – 523.90±61.80 x 10^9/L and 554.20±60.15 x 10^9/L, respectively, $p<0.01$, $p1>0.05$. The maximum value was studied in patients of group B – 763.90±62.18 x 10^9/L, which was, on average, 29.43 % higher than in patients of groups A and C, $p1<0.05$, $p2<0.05$.

On day 7th, 14th and 30th day of postoperative follow-up, a moderate decrease in the number of leukocytes in the oral fluid was determined in patients of the research groups, which, however, remained significantly higher than in patients of control group B, $p<0.01$, $p<0.05$, than in patients of groups A and C, $p1>0.05$, $p2<0.05$, on average.

The investigation of ESR parameters in the research groups (Table II) revealed a tendency to increase the data of this parameter on the 1st-5th postoperative day, which reached maximum values on the 5th day of observation and was higher than in the control group: in 3.3 times in patients of group A; in 3.7 times in patients of group B and 3.0 times in patients of group C, $p<0.01$; p1, $p2>0.05$. It should be noted that on the 7th day of postoperative follow-up in patients of group C, the ESR was minimal (8.34±1.58 ml/h) and equal to that of the control group, p, p1, $p2>0.05$. At the same time, in

Table I. Dynamics of the number of leukocytes in the oral fluid in the patients of the patients of all research groups at different follow-up periods

Research groups	1st day	5th day	7th day	14th day	30th day
Control group			276.80±45.72 x 10^9/l		
Group A	453.60± ±56.20°°	554.20± ±60.15°	454.0± ±56.20°°	353.50± ±51.18	273.50± ±49.82
Group B	553.60± ±58.42°	763.90± ±62.18°,**	603.70± ±57.83°	503.20± ±50.00**	400.70± ±49.97
Group C	503.60± ±57.28°	523.90± ±61.80°,ΔΔ	423.70± ±55.19°°,ΔΔ	323.00± ±49.86ΔΔ	272.50± ±48.22

Notes:
1.°p<0.01; °p<0.05 – significant difference in values relative to the average statistical norm.
2.**p1<0.05 – significant difference in values relative to group A.
3.ΔΔp2<0.05 – significant difference in values relative to group B.

Table II. Changes in ESR in patients of the research groups in different postoperative periods

Research groups	1st day	5th day	7th day	14th day	30th day
Average statistical norm			5.5±1.10 mm/h		
Group A	13.28±1.46°	18.33±1.72°	10.56±1.62°°	7.23±1.60	5.00±1.21
Group B	14.39±1.49°	20.45±1.98°	12.55±1.70°	9.20±1.66	7.18±1.24
Group C	13,84±1.47°	16.42±1.83°	8.34±1.58	5.29±1.57	5,10±0.93Δ

Notes:
1.°p<0.01; °p<0.05 – significant difference in values relative to the data in the control group.
2.Δp2<0.05 – significant difference in values relative to the data in group B.

Table III. Dynamics of IL-1β content in the oral fluid of patients after cystectomy at different follow-up periods

Research groups	1st day	5th day	7th day	14th day	30th day
Control group			68.34±6.03 pg/ml		
Group A	98.65± ±6.68°	118.76± ±9.13°	88.46± ±6.13°°	78.63± ±6.12	68.40± ±6.11
Group B	110.20± ±6.90°	130.42± ±6.15°	100.12± ±6.14°,**	90.00± ±6.13°°	75.15± ±6.13
Group C	104.42± ±6.69°	114.62± ±6.14°	84.32± ±6.12ΔΔ	64.12± ±6.11ΔΔ	64.50± ±6.12

Notes:
1.°p<0.01; °p<0.05 – significant difference in values relative to the control group.
2.**p1<0.05 – significant difference in values relative to the data of group A.
3.ΔΔp2<0.05 – significant difference in values relative to the data of group B.

patients of groups A and B, the value was in 1.9 times, p<0.05, and in 2.3 times, p<0.01 higher, respectively, compared to the control data. On the 7th-14th day of observation, the ESR value decreased compared with the control data, p>0.05, with the most pronounced positive dynamics in patients of group C.

The early action of interleukin IL-1β plays a key role in the development and regulation of innate and acquired immunity, being one of the first to be involved in the defense response to pathogenic factors, activating neutrophils, and stimulating the synthesis of acute phase proteins [12, 22].

It was established (Table III) that on the 1st post-operative day, the content of IL-1β in the oral fluid of patients of the research groups increased compared to the data in the control group: by 44.35 % in group A, by 61.25 % in group B, and by 52.79 % in group C, p<0.01, p1, p2>0.05.

On the 5th postoperative day, the maximum increase in values was noted relative to the data in the control group: by 73.78 % in group A, by 90.83 % in group B and by 67.72 % in group C, p<0.01, p1, p2>0.05. It should be noted that on the 7th, 14th, 30th postoperative days, the level of IL-1β in the oral fluid of the subjects decreased to the maximum and reached normal levels in the patients of group C.

The concentration of the proinflammatory cytokine IL-6, which plays a leading role in the induction of acute

phase proteins [24], increased in the oral fluid of the subjects on the 1st day after cystectomy compared with the control data: in 3.0 times in group A, in 3.3 times in group B, p1<0.05, and in 3.1 times, p1, p2>0.05, (Table IV).

On the 5th day of the postoperative period, in groups A and B, the level of IL-6 in the oral fluid increased and was higher than in the control group by 3.5 times and 3.8 times, p1<0.05, respectively, p<0.01. At the same time, in patients of group C, a decrease in the content of IL-6 in the oral fluid was studied relative to the data of the previous research period, but the obtained values remained in 2.7 times higher than in the control group, p<0.01. It should be noted that at this time of the investigation, the content of IL-6 in the oral fluid of patients in group C was, on average, in 1.3 times lower than in patients in groups A and B. On the 7th, 14th and 30th days of observation in patients of group C, IL-6 in the oral fluid did not differ statistically from the data in the control group, p>0.05, and was lower in patients of groups A and B.

Table IV. Dynamics of IL-6 content in the oral fluid of patients after cystectomy in different periods of observation

Research groups	1st day	5th day	7th day	14th day	30th day
Control group			2.20±0.10 pg/ml		
Group A	6.65± ±0.19°	7.76± ±0.21°	6.00± ±0.15°	3.48± ±0.12°	2.95± ±0.10°
Group B	7.29± ±0.21°,**	8.40± ±0.23°,**	6.49± ±0.16°,**	4.00± ±0.13°,**	3.15± ±0.11°
Group C	6.90± ±0.20°	6.00± ±0,17°.*,Δ	4,82± ±0,13°.*,Δ	2,80± ±0,10°.*,Δ	2,10± ±0.09*,Δ

Notes:
1.°p<0.01 – significant difference in values compared to the control group.
2.*p1<0.01; **p1<0.05 – significant difference in values relative to the data of group A.
3.Δp2<0.01 – significant difference in values relative to the data of group B.

Table V. Dynamics of TNF-α content in the oral fluid of patients after cystectomy at different follow-up periods

Research groups	1st day	5th day	7th day	14th day	30th day
Control group			89.70±13.40 pg/ml		
Group A	109.90± ±14.20	180.00± ±15.43°	166.55± ±14.82°	100.36± ±14.10	93.26± ±23.10
Group B	99.80± ±12.65	160.63± ±14.80°	140.80± ±14.20°°	92.82± ±13.25	89.75± ±22.80
Group C	104.85± ±14.17	144.95± ±14.47°°	90.22± ±14.00*,ΔΔ	89.50± ±12.48	89.50± ±22.76

Notes:
1.°p<0.01; °p<0.05 – significant difference in values compared to the control group.
2.*p1<0.01 – significant difference in values compared to the data in group A.
3.ΔΔp2<0.05 – significant difference in values relative to the data in group B.

Table VI. Dynamics of IL-8 content in the oral fluid of patients after cystectomy in different periods of observation

Research groups	1st day	5th day	7th day	14th day	30th day
Control group			5.77±0.67 pg/ml		
Group A	16.28± ±0.82°	14.00± ±0.75°	13.80± ±0.73°	10.50± ±0.48°	8.25± ±0.30°
Group B	15.24± ±0.77°	13.14± ±0.73°	12.26± ±0.69°	9.15± ±0.46°,**	8.36± ±0.31°
Group C	16.00± ±0.80°	13.80± ±0.71°	9.45± ±0.54°,*	6.28± ±0.31*,Δ	5.72± ±0.29*,Δ

Notes:
1.°p<0.01 – significant difference in values compared to the control group.
2.*p1<0.01; **p1<0.05 – significant difference in values relative to the data of group A.
3.Δp2<0.01 – significant difference in values relative to the data of group B.

Table VII. Dynamics of NO synthase in the oral fluid of patients of the research groups at different observation periods

Observation periods	Research groups	NO2, μmol/l	NO3, μmol/l	NO2 + NO3, μmol/l
1 day	Control group	13.08±2.18	22.50±3.25	35.58±3.81
	Group A	10.15±1.26	17.46±2.18	27.61±3.44
	Group B	9.24±1.15	16.35±2.00	25.59±3.15°°
	Group C	9.70±1.18	16.91±2.15	26.61±3.33
5 day	Group A	7.95±1.14°°	8.73±1.36°	16.68±2.50°
	Group B	5.94±0.92°	8.18±1.30°	14.12±2.22°
	Group C	6.95±0.98°°	11.27±1.82°	18.22±2.80°
7 day	Group A	9.05±1.10	9.84±1.50°	18.89±2.60°
	Group B	7.14±0.99°°	9.29±1.48°	16.43±2.47°
	Group C	9.15±1.10	13.50±1.95°°	22.65±3.05°°
14 day	Group A	10.20±1.24	14.89±2.00°°	25.09±3.24°°
	Group B	9.36±1.12	15.34±2.13	24.70±3.25°°
	Group C	12.48±1.20	18.03±2.62	30.51±3.82
30 day	Group A	12.00±1.21	18.92±2.66	30.92±3.87
	Group B	11.58±1.19	17.67±2.47	29.25±3.66
	Group C	13.62±1.22	21.36±2.92	34.98±4.14

Notes. °p<0.01; °p<0.05 – significant difference in values relative to the control data.

Table VIII. Dynamics of MMP-9 content in the oral fluid of patients of the research groups at different times of the postoperative period

Research groups	Observation periods				
	1st day	5th day	7th day	14th day	30th day
Group A	4.780± ±0.956°	10.523± ±2.104°	3.507± ±0.701°	2.406± ±0.481°	1.394± ±0.278°°
Group B	6.200± ±1.240°	11.634± ±2.326°	5.817± ±1.163°	4.706± ±0.941°,ΔΔ	2.582± ±0.516°,ΔΔ
Group C	3.990± ±0.798°	9.745± ±1.949°	2.784± ±0.556°,**	1.673± ±0.334°°,*	0.773± ±0.154*
Control group	0.764±0.130 pg/ml				

Notes:
1.°p<0.01; °p<0.05 – significant difference in values relative to the control group.
2.ΔΔp1<0.05 – significant difference in values relative to the data of group A.
3.*p2<0.01; **p2<0.05 – significant difference in values compared to the data of group B.

In practically healthy subjects of the control group, the content of TNF-α in the oral fluid was at a low level (89.70±13.40 ng/ml), which probably provides only the physiological value of direct and feedback signals in the cytokine system, maintaining normal regulation of immunoreactivity (Table V). The increase in this indicator occurs under the influence of antigens of viruses or bacteria in response to the inflammatory process [22, 23].

On the 1st postoperative day after cystectomy, an increase in the level of TNF-α in the oral fluid was observed in patients of the research groups, on the 5th day of observation – a significant increase in this parameter in the research groups relative to the values in the control: by 100.66 % in group A, by 79.07 % in group B, p<0.01, p1>0.05; and by 61.59 % in group C, p<0.05, p1, p2>0.05.

On the 7th day, in patients of group C, the concentration of TNF-α in the oral fluid did not differ statistically from that in the control group, p>0.05, and was lower than in patients of groups A and B by 84.60 % and 56.06 %, respectively.

The content of IL-8, which is produced under the influence of bacterial endotoxins and cytokines, mainly TNF-α and IL-1 [24, 25], increased in the oral fluid of the subjects on the 1st postoperative day compared to the control data: in 2.8 times in patients of group A, in 2.6 times in patients of group B, and 2.7 times in patients of group C, p<0.01; p1, p2>0.05. On the 5th day of the research, a gradual decrease in the content of IL-8 was observed, on the 7th day of postoperative observation, the minimum values were studied in patients of group C – 9.45±0.54 ng/ml, p, p1, p2<0.01, which were 1.5 times and 1.3 times, p1>0.05, lower compared with those

in patients of groups A and B, respectively, p<0.01. On the 14th day of the postoperative research in patients of group C, the concentration of IL-8 in the oral fluid did not differ significantly from that in the control group, p>0.05 (Table VI).

On the 1st day of postoperative intervention in patients of the research groups, after cystectomy, the decrease in the content of NO synthase in the oral fluid was studied in relation to the data in the control group. At the same time, the maximum decrease in these parameters was determined in patients of groups B and C, on average, by the level of NO_2 – by 22.60 %, NO_3 – by 26.10 %, and $NO_2 + NO_3$ – by 26.64 %, p>0.05, p1>0.05. On the 5th postoperative day, a further decrease in the studied levels of indicators in the oral fluid was determined. On the 7th day of the postoperative period, positive dynamics of NO_2 content in the oral fluid of subjects of groups A and C was noted, relative to the data in the control group, p>0.05 (Table VII).

After 2 weeks of observation (14 days) in patients of group C, the data on the content of NO synthase in the oral fluid were normalized and equal to the values in the control group, p>0.05.

It should be added that during the study at all periods of observation, there was no significant difference in the values of NO synthase in the oral fluid in the intergroup comparison, p1, p2>0.05.

It was found that in the control group, the content of MMP-9 in the oral fluid (Table VIII) was 0.764±0.130 pg/ml. At the same time, in patients of the research groups on the 1st postoperative day, an increase in the values of the parameter was established relative to the data in the control group. On the 5th day of postoperative observation, the maximum increase in the level of MMP-9 in the oral fluid was studied.

At the same time, on the 7th-14th days of the investigation, the content of MMP-9 in the oral fluid decreased, but remained significantly higher than in the control group after two weeks of observation. At the end of the research (the 30th day), in patients of group C, who underwent electric welding of the edges of the postoperative wound, the content of MMP-9 in the oral fluid was equal to that of the control group, p>0.05, p2<0.01.

DISCUSSION

One of the most common interventions in the clinic of surgical dentistry, which ranks second after tooth extraction and accounts for more than 40 %, is the removal of odontogenic cysts [26]. The share of radicular cysts among the total number of maxillofacial cysts accounts for 52 % [27]. Modern advances in medicine allow the use of fundamentally new methods of treatment of odontogenic cysts in order to improve the results of treatment and subsequent rehabilitation [28, 29]. The method we propose is based on the use of tissue electric welding technology to approximate the wound edges after cystectomy.

Analysis of the effectiveness of different methods of approximation of the oral mucosa wound on inflammatory and immunological parameters showed that on the first postoperative day the content of leukocytes in the oral fluid increased in patients of all research groups compared to the data in the control group, p<0.05; 0.01. At the same time, the values of the obtained indicators did not differ in statistical significance in the intergroup comparison. On the 5th postoperative day, the quantitative content of leukocytes in the oral fluid continued to increase and was significantly higher than in the control group in all research groups, p<0.01. However, the minimum values of the analyzed parameter were determined in the patients of groups C and A – 523.90±61.80 x 10^9/L and 554.20±60.15 x 10^9/L, respectively, p<0.01, p1>0.05. The maximum values of this parameter were studied in the patients of group B – 763.90±62.18 x 10^9/L, which was, on average, by 29.43 % higher than in the patients of groups A and C, p1<0.05, p2<0.05. In 1 month after cystectomy in the patients of group A, where the edges of the postoperative wound were sutured with «Vicryl», and in patients of group C, when using the device ECVZ-300 «Patonmed», the quantitative content of leukocytes in the oral fluid was equal to that in the control group, p>0.05. At the same time, when approximating the edges of the oral mucosa with a laser, in patients of group B, the leukocyte content was by 36.26 % higher than in patients of the other groups, on average, p, p1, p2>0.05. Similar data were obtained by analyzing other indicators.

In a previous research [30] related to this problem, we studied the dynamics of clinical parameters when using different methods of approximating the edges of the oral mucosa. The results showed the effectiveness of the proposed method using electric welding of tissues.

CONCLUSION

When comparing the methods of approximation of postoperative oral mucosa wounds by different methods, based on the results of these inflammatory and immunological parameters, the best results were obtained using electric welding of tissues. In the surgical treatment of odontogenic cysts by the proposed method, a decrease in inflammatory and immunological reactions in the oral fluid of the subjects was found, which was confirmed by a decrease in the levels of IL-1 β, p – p1>0.05, p2<0.05, TNF – L, p>0.05, p1 – p2<0.01, on the 7th day of observation; quantitative content of leukocytes, p – p1>0.05, p2<0.05, levels of IL – 8, p>0.05, p1 – p2<0.01, activity of NO – synthase, p – p2>0.05 on the 14th day of research; IL concentrations – 6, p>0.05, p1 – p2<0.01, MMP activity – 9, p – p2>0.05 on the 30th day after treatment and ESR parameters on the 7th day of observation, p – p2>0.05.

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