

ORIGINAL ARTICLE

PERFORMANCE STATUS AND QUALITY OF LIFE AFTER RECONSTRUCTIONS OF BUCCAL MUCOSAL AND RETROMOLAR TRIGONE DEFECTS BY SKIN AND FASCIAL FLAPS IN ONCOLOGICAL PATIENTS

DOI: 10.36740/WLek202007137

Oleg V. Kravets¹, Igor P. Fedzhaga^{2,3}, Vladimir I. Pivtorak³, Oleg P. Fedzhaga^{2,3}, Iryna V. Bulko³¹NATIONAL CANCER INSTITUTE, KYIV, UKRAINE²PODILLA REGIONAL ONCOLOGY CENTER, VINNYTSIA, UKRAINE³NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim: of the study is to compare surgical and functional results as well as quality of life after use of skin flaps – platysma myocutaneous flap, supraclavicular artery flap and fascial flaps – temporoparietal fascial flap, buccal fat pad for plastic closure of the buccal and retromolar trigone mucous surgical defects in cancer patients.

Material and methods: A retrospective comparative analysis of the results of surgical treatment of buccal and retromolar trigone mucosal cancer in 56 patients operated from 2009 to 2014 was made. The patients were divided into two groups: the first included 26 patients, with reconstruction of fascial flaps, the second group included 30 patients, with reconstruction of postoperative defects with skin flaps. Surgical results: the average duration of surgery, the average duration of inpatient treatment and the presence of complications were compared in the both groups. Functional results in both groups were compared in 6 and 12 months after surgery with the Performance Status Scale for Head and Neck Cancer Patients (PSS-HN), and quality of life was assessed by the University of Washington Quality of Life questionnaire, version 4.

Results: Average duration of surgery, average time of inpatient treatment, and postoperative complications did not differ significantly in both groups ($P > 0.05$). Data of performance status and quality of life in patients with reconstruction of mucosal defects and retromolar trigone with skin flaps were significantly better ($P > 0.05$) in 6 and 12 months after surgery, compared with the group of patients who underwent defect reconstruction with fascial flaps.

Conclusions: The method of choice in reconstruction of medium and large defects of the buccal and the retromolar trigone mucous is usage of skin flaps: the platysma myocutaneous flap and the flap of the supraclavicular artery.

KEY WORDS: buccal mucosal cancer, reconstruction of mucous defects, skin and fascial flaps, performance status, quality of life

Wiad Lek. 2020;73(7):1510-1515

INTRODUCTION

Oral cancer is the sixth most common malignancy worldwide [1]. Three hundred thousand patients (2.1% of the total cancer cases) were afflicted with cancer of the oral cavity and lip in 2012 [2]. Cancer of the buccal mucous and retromolar trigone in the oriental countries accounts up to 20% of all malignant neoplasms of the oral cavity and is characterized by an aggressive course and a high frequency of recurrence [3]. Treatment of patients with locally advanced mucosal cancer involves surgery with single-stage reconstruction and adjuvant radiotherapy or concomitant chemoradiotherapy [4]. Having removed a malignant tumor of the buccal mucous, a buccal defect appears, which involves several layers of the buccal, rarely – all layers, including the skin. Buccal defects can be combined with marginal or segmental defect of the mandible or partial defect of the

upper jaw [5]. Ensuring good functional and aesthetic results are crucial in the reconstruction of the buccal after oncologic resection [6].

Depending on postoperative defects of the buccal mucous size after removal of the primary tumor, they are divided into three types: small (up to 3 cm in the largest dimension); medium (3 to 6 cm in the largest dimension); large (larger than 6 cm in the largest dimension). Small defects can be closed with local tissues, while medium and large defects require plastic replacement using local, regional, or free flaps.

There are no clear recommendations in the literature regarding the choice of reconstructive technique for replacement of buccal mucosal defects. For plastic replacement of medium defects of the buccal they use a buccal fat pad (BFP) and platysma myocutaneous flap (PMF) [7,8]. Major defects are closed with regional supraclavicular

Table I. Characteristics of the studied patients

Characteristics	Abs.	FF group (BFP, TPF)F (n=26)		SF group (SAF, PMF) (n=30)		p-value
		%	Abs.	%	Abs.	
Sex	female	5	19,2	6	20,0	0,79
	male	21	80,8	24	80,0	
Subsite of oral cavity	Retromolar trigone	10	38,5	8	26,7	0,51
	Buccal mucosa	16	61,5	22	73,3	
T staging	T2	6	23,1	3	10,0	0,39
	T3	17	65,4	22	73,3	
	T4	3	11,5	5	16,7	
N staging	N0	9	34,6	13	43,3	0,75
	N1	9	34,6	10	33,3	
	N2	8	30,8	7	23,4	
M staging	M0	26	100	30	100	>0,99
Clinical stage	III	16	61,5	20	66,7	0,91
	IV	10	38,5	10	33,3	

Note. FF – fascial flaps, SF – skin flaps, BFP – buccal fat pad, TPF – temporoparietal fascial flap, SAF – supraclavicular artery flap, PMF – platysma myocutaneus flap. Comparison of groups in accordance to Chi-square.

artery flap (SAF) and the temporoparietal fascial flap (TPFF) [9,10].

One of the key factors determining the functional results of plastic replacement of medium and large mucous defects in cancer patients is the preservation of the possibility of mouth opening, which is greatly affected by scarring and the formation of coarse connective tissue at the site of plastic closure. It depends on the presence of epithelial skin lining on the part of the flap that directly closes the buccal mucous defect.

To close the buccal mucous defect may be used fascial flap without epithelial skin lining – temporoparietal fascial flap (TPFF) and buccal fat pad (BFP), as well as flap with epithelial skin lining – platysma myocutaneus flap (PMF) and supraclavicular artery flap (SAF).

In the available literature we did not find any data according to the significance of the presence of skin epithelial lining in regional and local flaps used to close the middle and large defects of the buccal mucous for functional results and quality of life in the postoperative period.

THE AIM

The aim of the work is to compare surgical and performance status and quality of life after use of skin flaps (SAF and PMF) and fascial flaps (BFP and TPF) in cancer patients for plastic closure of mucosal defects.

MATERIALS AND METHODS

A retrospective comparative analysis of the surgery results was made in 56 patients with cancer of the buccal mucous

and retromolar trigone, who were treated at the *Head and Neck Oncology Department* in the National Cancer Institute during the period since 2009 to 2014. Patients were divided into two groups: the first included 26 patients who underwent reconstruction of postoperative defects with fascial flaps: BFP – 10 patients and TPF – 16 patients; second group included 30 patients who underwent reconstruction of postoperative defects with skin flaps: SAF – 19 patients and PMF – 11 patients.

There were 11 (19.6%) women and 45 (80.4%) men among all patients, the average age of patients was 56.0 ± 8.1 years (from 38 years to 76 years). The average age of patients with fascial flaps was 54.8 ± 7.8 years, the average age of those with skin flaps was 57.1 ± 8.4 years (the difference was not statistically significant, $p = 0.29$).

A squamous cell carcinoma of low grade (G1) returned in 15 patients, moderate grade (G2) in 30 patients and high grade (G3) in 11 patients. It was histologically confirmed prior to treatment.

In accordance to process spread stage III (T2N1M0, T3N0-1M0) was diagnosed in 36 patients (61.5% and 66.7% respectively), stage IV (T2-3N2M0, T4N1-2M0) was diagnosed in 20 patients (38.5% and 33, 3% respectively). The characteristics of the studied patients are presented in Table 1.

Having made the analysis no differences were found between the groups neither by sex, localization, stage, or TNM ($p > 0.05$).

All patients were underwent surgical treatment in the volume of resection of the buccal and/or retromolar trigone, cervical lymph node dissection and simultaneous

Table II. Post-operative complications after reconstruction of defects of the buccal mucosa and retromolar trigone with regional skin and fascial flaps

Complication	FF group (BFP, TPDF), (n=26)	SF group (SAF, PMF), (n=30)	p-value
Flap complication			
Flap failure	0	1 (3,3 %)	>0,99
Partial flap necrosis	0	2 (6,7 %)	
Marginal flap necrosis	2 (7,7 %)	0	
Total	2 (7,7 %)	2 (10 %)	
Recipient site complication			
Salivary fistula	0	2 (6,7 %)	>0,99
Orostoma	0	0	
Haematoma	2 (7,7 %)	0	
Infection complication	1 (3,8 %)	2 (6,7 %)	
Wound dehiscence	0	0	
Total	3 (11,5 %)	4 (13,3 %)	
Donor site complication			
Focal alopecia	8 (30,7 %)	NA	0,047
Damage to the frontal branch of the facial nerve	0	0	
Damage to the marginal branch of the facial nerve	0	0	
Cheek deformity	1 (3,9%)	0	
Wound dehiscence	0	3 (10 %)	
Impairment of shoulder function	0	0	
Total	9 (34,6 %)	3 (10 %)	

Note. NA – not applicable

Group comparisons were performed by Fisher's exact test.

plastic replacement of the defect. Buccal reconstruction was performed simultaneously with the removal of the primary tumor and regional lymph nodes.

The patients were included in the study by the criteria: locally advanced mucosal cancer without prior radiotherapy or chemotherapy; plastic replacement of the middle or large defects of the buccal alone or combined with the mandibular or maxillary defects after the removal of the primary tumor.

Criteria not to include the patients in the study were: defects of the buccal mucous combined with a segmental defect of the mandible; perforated buccal defects; decompensated heart failure; diabetes with unstable hyperglycemia; the period of 3 months after a heart attack or stroke; when the patient's condition was defined as a contraindication to reconstructive surgery.

In both groups, comparison of the early results of flaps use for the reconstruction of the buccal with and without the skin pad, the features of surgical technique, the average duration of surgery, the average duration of inpatient treatment and the presence of complications was made.

Late functional results of using flaps with and without the skin pad were compared in 6 and 12 months after surgery, basing on the Performance Status Scale for Head and Neck Cancer Patients (PSS-HN). Quality of life was assessed by the University of Washington Quality of Life questionnaire, version 4 (UW-QOL v4).

Statistical analysis of the study results was carried out in the package MedCalc v. 18.11 (MedCalc Software bvba, Belgium, 1993-2018).

The mean value (\bar{X}) and its standard deviation (\pm SD) were calculated to represent the quantitative traits, and the trait frequency (%) for the qualitative traits. When comparing quantitative traits, the Student's criterion (in the case of a normal distribution law) or the W-Wilcoxon criterion (in the case of a distribution law other than the normal one) were used, and the normality distribution test was performed using the Shapiro-Wilk test. The Chi-square test (with Yeats' correction) was used to compare qualitative indicators. To evaluate the clinical effect, its magnitude and 95% probable interval (95% CI) were calculated. The critical level of significance is assumed to be $\alpha = 0.05$.

RESULTS

The average duration of the reconstructive surgery with skin flaps was 4.3 ± 0.7 h, it didn't significantly differ ($P = 0.88$) from the reconstructive surgery with fascial flaps, which lasted 4.2 ± 0.8 h. The mean duration of inpatient treatment in the group of patients with fascial flaps was 10.3 ± 2.1 days, it didn't significantly differ ($P = 0.48$) from the group of patients with skin flaps, in which the average length of stay in hospital was $10.7 \pm 2,1$ days.

Table III. Performance status of Head and Neck (PSS-HN) after 6 and 12 months after surgery in patients with reconstruction of defects of the buccal mucosa and retromolar trigone by regional skin and fascial flaps

PSS-HN	6 month after surgery			12 month after surgery		
	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Normalcy of diet	58,8±15,8	68,3±14,6	0,004	61,7 ±15,0	74,1 ±14,2	0,007
Eating in public	70,2±17,3	77,5±7,6	0,04	69,6±18,3	80,5±11,2	0,06
Understandability of speech	86,5±12,7	87,5±12,7	0,78	89,1 ±12,7	91,7 ±12,0	0,81

Note. W-Wilcoxon test was used for comparison: ehe significant difference is in bold.

Table IV. Quality of life according to the UW-QOL v4 questionnaire 6 and 12 months after surgery in patients with reconstruction of buccal mucosa and retromolar trigone with regional skin and fascial flaps

UW-QOL v4	6 month after surgery			12 month after surgery		
	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value
	$\bar{X} \pm SD$	$\bar{X} \pm SD$		$\bar{X} \pm SD$	$\bar{X} \pm SD$	
Pain	75,0±20,0	81,7±18,5	0,21	76,1±19,2	84,3±18,5	0,12
Appearance	71,2±26,2	80,8±10,8	0,24	72,8±27,1	82,4±11,6	0,37
Activity	78,8±18,3	81,7±17,3	0,57	83,7±16,2	85,2±15,9	0,74
Recreation	76,0±16,6	77,5±17,8	0,72	79,3±19,4	81,5±16,4	0,75
Swallowing	95,2±10,0	97,5±7,6	0,33	97,8±7,2	97,2±8	0,78
Chewing	53,8±28,0	70,0±24,9	0,03	52,2±31,9	72,2±25,3	0,03
Speech	91,9±13,6	92,0±13,5	0,98	96,1±10,3	93,3±12,7	0,4
Shoulder	83,8±15,3	77,0±12,9	0,07	80±18,3	75,6±11,9	0,2
Taste	57,7±18,8	51,3±20,3	0,23	67,8±13,5	68,1±12,4*	0,95
Saliva	46,9±20,2	47,3±20,2	0,94	66,5±11,5*	65,6±12,8*	0,78
Mood	66,3±17,2	72,5±15,2	0,13	66,3±14,3	73,1±16,9	0,15
Anxiety	50,8±24,2	68,3±20,7	0,006	55,2±24,1	75,9±19,1	0,002
General 1	50,0±15,8	56,7±17,3	0,13	51,1±17,6	57,4±11,6	0,19
General 2	53,1±13,8	58,7±12,8	0,14	53,9±15,3	58,5±12,3	0,26
General 3	55,4±13,0	62,0±16,1	0,12	56,5±15,6	63±15,4	0,16

Note. W-Wilcoxon test was used for comparison: whehe significant difference is in bold.

* - the difference from the corresponding indicator in 6 and 12 months. after surgery, statistically significant, p <0.05.

The analysis of postoperative complications in both groups included the assessment of complications in the transplanted flap, in the postoperative wound and in the donor site (Table 2). We did not observe significant differences in the transplanted flap complications in both groups (7.7% vs 10%, p > 0.99) and in the postoperative wound (11.5% vs 13.3%, p > 0.99). At the same time, complications in the donor site in the group of patients with flap reconstruction without skin pad were significantly greater comparing with the skin flaps group (34.6% vs 10%, p = 0.047), due to Focal alopecia observed in 8 patients, representing 30.7% of the RFF group after using TPFF.

Significantly better data of diet completeness (58,8 ± 15,8% vs 68,3 ± 14,6%, p = 0,004) and nutrition in public

(70.2 ± 17.3% vs 77.5 ± 7.6%, p = 0.04) in accordance to the PSS-HN scale were observed in the group of patients with skin flaps reconstruction after 6 months. In 12 months the Normalcy of diet in accordance to the PSS-HN scale was significantly better (p = 0.007) in the RSF group. Understandability of speech in both groups did not differ significantly (Table 3) in 6 months (p = 0.78) and 12 months after surgery (p = 0.81).

In both groups comparing the quality of life data (Table 4) in accordance to the scale UW-QOL v4 it was found that in the RSF group Chewing was significantly better than the RFF group after 6 months (70.0 ± 24.9% vs 53.8 ± 28.0%, p = 0.03) and 12 months (72.2 ± 25.3% vs 52.2 ± 31.9%, p = 0.03) after surgery.

In 6 and 12 months after surgery in both groups comparing the quality of life data (Table 4) in accordance to the UW-QOL v4 scale it was found that after 6 months ($70.0 \pm 24.9\%$ vs $53.8 \pm 28.0\%$, $p = 0.03$) and 12 months ($72.2 \pm 25.3\%$ vs $52.2 \pm 1.9\%$, $p = 0.03$). After surgery in the RSF group Chewing was significantly better than in the RFF group. Anxiety, like Chewing, significantly differed ($p < 0.05$) in both groups after 6 and 12 months after surgery.

Analyzing the dynamics of changes of the Saliva index in the RFF group (BFP, TPF) an increase was revealed ($p < 0.05$) in 12 months ($66.5 \pm 1.5\%$) comparing with its value in 6 months ($46.9 \pm 20.2\%$) after surgery. Similar changes in the Saliva index were estimated in the RSF group in 6 and 12 months (SAF, PMCF), $p < 0.05$. Within 12 months, a significant improvement in the Taste index ($68.1 \pm 12.4\%$ vs $51.3 \pm 20.3\%$, $p < 0.05$) was found after 12 months compared to 6 months. In the RFF group, the change in the Taste index after 6 and 12 months was insignificant ($67.8 \pm 13.5\%$ vs $57.7 \pm 18.8\%$, $p > 0.05$). Comparing other data of quality of life, no significant difference was found in both groups in 6 and 12 months after surgery ($p > 0.05$).

DISCUSSIONS

The experience of plastic replacement of postoperative mucosal defects as well as the analysis of the oncological results of treatment of patients with squamous cell carcinoma of the buccal mucous are based on studies with a small amount of clinical observations [11]. We hope, that our study will complete the data of literature, including the study of the performance status and quality of life in patients with skin and facial flaps to replace the defects of the buccal mucous and retromolar trigone in cancer patients.

The morbidity of squamous cell carcinoma of the buccal mucosa in Europe and North America is only 10% of all malignant neoplasms of the oral cavity. In Taiwan [1], mucosal cancer is a much more common disease, but a little research has been done to investigate various reconstructive approaches to correct mucosal defects.

We obtained the results of replacement of medium and large defects of the buccal mucous with regional or local flaps and related data: features of surgical technique, the average duration of surgery, the average length of stay in the hospital, the presence of complications in the transplanted flaps and in the recipient wound, which showed no differences between groups.

A small number of complications in the transplanted flap, in particular the supraclavicular artery flap (we did not observe cases of total necrosis of SAF), are associated with the refinement of surgical technique, in particular the flap marking was performed according to the course of the supraclavicular artery, which was determined by Doppler ultrasound. Described by Padiyar and co-authors [9], total flap failure of SAF in 28% of cases is associated with flap marking by anatomical landmarks. Therefore, in our opinion, it is the performance status and quality of life of the patients that are the key criteria for the choice of reconstruction method for the replacement of medium and large defects of the buccal mucous in cancer patients.

In the group of patients who underwent reconstruction with fascial flaps, our data confirm data of D. Hwang [7] that use of BFP may cause development of post-operative trismus and related functional disorders as well as deterioration of quality of life after plastic closure of small size defects, less than 6 cm. In the closure of small defects in the group of patients with skin flaps, among which we most often used PMF the data obtained by us are similar to the results obtained by L. Huong.

In closure of large defects of the buccal mucous (more than 6 cm) in the group of patients with fascial flaps we most often used TPF, and in the group of patients with a skin pad flaps we most often used SAF. We found that the use of SAF in comparison with TPF to replace major mucosal defects provides significantly higher normalcy of diet and nutrition in public in accordance to the PSS-HN functioning scale as well as data of chewing, anxiety in accordance to the UW-QOL quality of life questionnaire. In our opinion, lower values of Chewing, Normalcy of diet and nutrition in public in the case of TPF, as well as in the case of BFP are conditioned by fibrotic buccal changes and trismus, which led to impaired chewing function. Patients are less concerned in case of SAF due to the fact that postoperative scar on the shoulder is more easily perceived by patients than the scar in the temporal and parietal region, which is often accompanied by Focal alopecia.

A promising direction for further research is to compare the results of replacement of medium and large mucosal defects with the regional, local and free flaps.

CONCLUSIONS

1. There were no statistically significant differences in the duration of surgery, inpatient treatment, the incidence of complications after flap transplantation, postoperative complications between groups of patients whose medium and large defects of the buccal mucous and the retromolar trigone were replaced by skin and fascial flaps.
2. The use of skin flaps to replace the medium and large defects of the buccal mucous significantly improves the performance status of the diet normalcy and nutrition in public and the quality of life of patients in terms of chewing, anxiety compared with the use of fascial flaps without skin pad.
3. In the reconstruction of medium and large defects of the buccal mucous the method of choice is the use of skin flaps – platysma myocutaneu flap (PMF) and regional flap of the supraclavicular artery (SAF).

REFERENCES

1. Warnakulasuriya S. Causes of oral cancer – an appraisal of controversies. *Br Dent J*. 2009 Nov 28;207(10):471–5 doi: 10.1038/sj.bdj.2009.1009.
2. Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015 Mar 1;136(5):E359–86. doi: 10.1002/ijc.29210.
3. Dhanuthai K., Rojanawatsirivej S., Thosaporn W., Kintarak S., Subarnhesaj A., Darling M., et al. Oral cancer: A multicenter study. *Med Oral Patol Oral Cir Bucal*. 2018 Jan 1;23(1):e23–e29. doi: 10.4317/medoral.21999.

4. Kim I., Myoung H. Squamous cell carcinoma of the buccal mucosa involving the masticator space: a case report. *J Kor Assoc Oral Maxillofac Surg.* 2017 Jun 28; 43(3): 191-196. doi: 10.5125/jkaoms.2017.43.3.191
5. Patel S.Y., Meram A.T., Kim D.D. Soft tissue reconstruction for head and neck ablative defects. *Oral Maxillofac Surg Clin North Am.* 2019 Feb; 31(1): 39-68. doi: 10.1016/j.coms.2018.08.004.
6. Fang Q.G., Li Z., Zhang X., Liu F., Xu Z., Sun C. Clinical reliability of radial forearm free flap in repair buccal defects. *World J Surg Oncol.* 2013 Jan 30;11:26. doi: 10.1186/1477-7819-11-26.
7. Hwang D., Park J., Kim U., Park H., Kim G., Ryu M. Reconstruction of cheek mucosal defect with a buccal fat pad flap in a squamous cell carcinoma patient: a case report and literature review. *Maxillofac Plast Reconstr Surg.* 2018 May 26; 40(1): 11. doi: 10.1186/s40902-018-0150-8.
8. Long H., Xinchun J., Xinqun C., Tong S., Canhua J. Application of muscle pedicled platysma myocutaneous flap in the reconstruction of buccal mucosa defects. *West China J of Stomat.* 2017 Apr 1;35(2):162-6. doi: 10.7518/hxkq.2017.02.010
9. Padiyar B., Azeem Mohiyuddin S., Sagayaraj A., Merchant S. Usefulness of supraclavicular flap in reconstruction following resection of oral cancer. *World J Otorhinolaryngol Head Neck Surg.* 2017 Aug 4; 4(2): 148-152. doi: 10.1016/j.wjorl.2017.01.004.
10. Lam D., Carlson E. The temporalis muscle flap and temporoparietal fascial flap. *Oral Maxillofac Surg Clin North Am.* 2014 Aug; 26(3): 359-69. doi: 10.1016/j.coms.2014.05.004.
11. De Conde A., Miller M., Palla B., Lai C., Elashoff D., Chhetri D. et al. Squamous cell carcinoma of buccal mucosa: a 40-year review. *Am J Otolaryngol.* 2012 Nov-Dec; 33(6): 673-7. doi: 10.1016/j.amjoto.2012.04.006.

ORCID and contributionship:

Oleg V. Kravets: 0000-0002-0226-6238 ^{A,B,D}
 Igor P. Fedzhaga: 0000-0002-2760-2242 ^B
 Vladimir I. Pivtorak: 0000-0001-7234-3596 ^{E,F}
 Oleg P. Fedzhaga: 0000-0002-6424-5766 ^B
 Iryna V. Bulko: 0000-0001-9561-3384 ^C

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR**Vladimir I. Pivtorak**

National Pirogov Memorial Medical University,
 56 Pirogova st., 21018 Vinnytsia, Ukraine
 tel: +380632883015
 e-mail: pivtorakv@gmail.com

Received: 21.10.2019

Accepted: 19.05.2020

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis,
 D – Writing the article, E – Critical review, F – Final approval of the article