

Evaluation of a new visual aid for the identification of oral mucosal diseases and conditions

S. Abati¹, P. Castellarin¹, C. Moreschi², G. Gastaldi²

¹Unit of Oral Diagnosis, Dental Clinic, Department of Health Sciences and WHO Collaborating Centre of Milan for Epidemiology and Community Dentistry, University of Milan, Italy; ²Vita Salute, San Raffaele University, Milan, Italy

Introduction and Scope. Various methods have been proposed to enhance the ability of clinicians in detecting oral mucosal lesions with visual oral examination. The loss of auto-fluorescence of tissues can be useful for cancer screening for a number of anatomic sites including the oral cavity; different devices have been developed and tested with inconsistent results. The aim of this preliminary study was to evaluate a new filtered eyeglasses device to reveal the loss of tissue autofluorescence, in a case series of patients with mucosal diseases and conditions.

Methods. The Goccles® eyeglasses has been tested in 15 patients and evaluated as a method to improve the detection of oral mucosal inflammation, precancer and cancer during the standard oral visual examination performed by four untrained dentists and dental hygienists assisted by two trained experts in oral medicine and pathology (Goccles®, Univet srl, Italy). The autofluorescence has been disclosed lighting the oral mucosa with a standard dental curing light and the loss of fluorescence classified in relation to the darkness of the involved areas; the relevant fields were recorded with a Panasonic-Leica LX-100 digital camera set for oral macro pictures with and without the custom photographic filter supplied by the manufacturer. The visual and photographic findings were immediately related and compared.

Results and Conclusions. 15 patients were examined: 3 affected by oral cancer, 5 with potentially malignant conditions (PVL, FL, OLP), 5 with inflammatory benign conditions (mucosal trauma, MG, candidiasis), 2 with BRONJ. In all patients the diagnosis have been clinically and/or histologically confirmed. The Goccles® device has been able to detect mucosal alteration due to inflammation or malignancy with different levels of dark visual appearance; a relevant finding in the study was the self-evidence of areas with bone exposure due to BRONJ that showed a vivid fluorescent appearance.

References

- Ingrams DR, et al. Autofluorescence characteristics of oral mucosa. *Head Neck*. 1997;19(1):27-32.
- Lingen MW, et al. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol*. 2008 Jan;44(1):10-22.
- Moyer VA. Screening for oral cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2014;160(1):55-60.

Sleep disturbance in patients with Burning Mouth Syndrome: a multicentric italian study

D. Adamo¹, A. Sardella², E. Varoni², C. Lajolo³, M. Biasotto⁴, G. Ottaviani⁴, P. Vescovi⁵, T. Simonazzi⁵, M. Pentenero⁶, M. Ardore⁶, F. Spadari⁷, G. Bombeccari⁷, L. Montebugnoli⁸, D. B. Gissi⁸, G. Campisi⁹, V. Panzarella⁹, M. Carbone¹⁰, L. Valpreda¹⁰, M. Giuliani¹¹, L. Lo Muzio¹¹, M. Aria¹², M. D. Mignogna¹

¹Oral Medicine Complex Unit, Department of Neurosciences, Reproductive and Odontostomatological Sciences Head & Neck Clinical Section "Federico II" University of Naples, Italy; ²UO Dentistry, AO San Paolo Hospital of Milan, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy; ³Oral Pathology and Medicine, Catholic University of Rome, School of Dentistry, Italy; ⁴Department of Medical, Surgical and Health Sciences Division of Oral Medicine and Pathology, Dental School, University of Trieste, Italy; ⁵Unity of Oral Pathology, Medicine and Laser Surgery, Department of Biomedical, Biotechnological and Translational Sciences, University of Parma, Italy; ⁶Dept. of Oncology Oral Medicine and Oral Oncology Unit, University of Turin, Oral Medicine Section Dental School Lingotto, University of Turin, Italy; ⁷Unit of Oral Pathology and Medicine, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Ospedale Maggiore Policlinico IRCCS Ca Granda Foundation, Italy; ⁸Unit of Maxillo-Facial Surgery, Department of Oral Sciences, University of Bologna, Bellaria Hospital, Italy; ⁹Department

of Surgical, Oncological, and oral Sciences, Sector of Oral Medicine "Valerio Margiotta", University of Palermo, Italy; ¹⁰Department of Surgical Sciences, Oral Medicine Section, CIR Dental School, University of Turin, Italy; ¹¹Department of Clinical and Experimental Medicine, University of Foggia, Italy; ¹²Laboratory and Research Group STAD Statistics, Technology, Data Analysis Department of Economics and Statistics, University of Naples "Federico II", Italy

Background. Sleep disturbance (SD) and mood disorders (MD) are common among Burning Mouth Syndrome (BMS) patients.

Objectives. The aim of this study was to investigate sleep complaints in a large cohort of Italian patients with Burning Mouth Syndrome (BMS) and the relationships between these disturbances, negative mood and pain.

Study design, subjects and methods design. 200 patients with BMS *versus* an equal number of age and sex-matched healthy controls, recruited in 10 centers, were enrolled. The study was based on the statistical evaluation of questionnaires examining insomnia symptoms and excessive daytime sleepiness (EDS) [Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS)], depression and anxiety [the Hamilton rating scale for depression (HAM-D) and the Hamilton rating scale for anxiety (HAM-A)] and pain [Numeric Pain Intensity Scale (NRS) and Short Form of Mc Gill Pain Questionnaire (SF-MPQ)].

Measurements and Results. Poor sleep quality (PSQI \geq 5) was present in 78.8% (160) patients with BMS. The patients with BMS had statistically higher scores in all items of the PSQI and ESS than the healthy controls. The median and inter-quartile range of the PSQI was 9 [6-12] and of the ESS 5 [2-9] for the BMS patients and 4 [2-5] and 3 [2-4] respectively for the healthy controls (P <0.001). The median and inter-quartile range of the NRS was 7 [4-8] and of the SF-MPQ was 9 [5-14] in the BMS patients. A depressed mood and anxiety correlated positively with sleep disturbance in the cases. The Pearson correlations were 0.570 for PSQI vs HAM-D (P <0.001) and 0.549 for PSQI vs HAM-A (P <0.001).

Conclusions. The BMS patients reported a greater degree of sleep disorders, anxiety, and depression as compared with the controls, highlighting the interrelation between sleep and mood. Sleep disturbance could influence the quality of life of BMS patients and could be a possible treatment target.

References

- Adamo D, Schiavone V, Aria M, Leuci S, Ruoppo E, Dell'Aversana G, Mignogna MD. Sleep disturbance in patients with burning mouth syndrome: a case-control study. *J Orofac Pain.* 2013 Fall;27(4):304-13.
- Chainani-Wu N, Madden E, Silverman S Jr. A case-control study of burning mouth syndrome and sleep dysfunction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2011 Aug;112(2):203-8.
- Lopez-Jornet P, Lucero-Berdugo M, Castillo-Felipe C, Zamora Lavella C, Ferrandez-Pujante A, Pons-Fuster A. Assessment of self-reported sleep disturbance and psychological status in patients with burning mouth syndrome. *J Eur Acad Dermatol Venerol.* 2015 Jul;29(7):1285-90.

Experimental Study

Detection of neural tropism on oral squamous cell carcinoma biopsies, a powerful tool in terms of early disease risk assessment

F. Pinto, A. Tarsitano, M. P. Foschini, L. Montebugnoli, C. Marchetti

University of Bologna, DIBINEM, Italy

Objectives. PNI is a form of metastatic tumor that spreads to the nerve bundles in surrounding tissues, this way neoplastic cells can travel far from the primary lesion along nerve tracts and are often missed during surgery. In OSCC, histologic evidence of PNI is a poor prognostic factor because it increases the risk of the presence of occult neck node metastasis and regional recurrence along with poor overall and disease specific survival. For this reason it may indicate the need for adjuvant therapy in PNI positive patients. The understanding of PNI related prognostic factors in preoperative biopsies would bring valuable and innovative informations to the scientific community.

Materials and Methods. Many molecules have been tested through the years to better understand the neurotropic spread mechanism, but to the best of our knowledge none have been tested on preoperative biopsies. IMP3 and Laminin-5 are markers associated with perineural invasion and the assessment of the immunohistochemical expression of these two in biopsies of 64 OSCCs was undertaken in order to determine whether a correlation between those and the presence of PNI in subsequent surgical specimens could be found.

Results. Relationship between the expression of IMP3 and laminin-5 in the preoperative biopsy and PNI identified in surgical specimens was found to be statistically significant (P < 0.001). All 33 PNI-positive patients (100%) showed a

positive expression of laminin-5 on pre-operative biopsy, 32 patients out of 64 (50%) expressed strong and diffuse staining (defined as involving more than 50% of the lesion). Twenty-eight out of 33 PNI-positive patients (84.8%) showed positive expression of IMP3, furthermore results of multivariate analyses showed the IMP3 status ($P = 0.001$) being an independent predictor of death.

Conclusion. The present study demonstrates that laminin-5 and IMP3 markers correlate well with PNI status and may allow for risk stratification in patients affected by OSCC.

References

- Larsen SR, Johansen J, Sorensen JA, Krogdahl A. The prognostic significance of histologic features in oral squamous cell carcinoma. *J Oral Pathol Med.* 2009;38:657-662.
- Overholt SM, Eicher SA, Wolf P, et al. Prognostic factors affecting outcome in lower gingival carcinoma. *Laryngoscope.* 1996;106:1335-1339.
- Tai SK, Li WY, Yang MH, et al. Treatment for T1e2 Oral squamous cell carcinoma with or without perineural invasion: neck dissection and post-operative adjuvant therapy. *Ann Surg Oncol.* 2012;19:1995-2002.

Experimental Study

Surgical protocol in patients taking oral or intramuscular bisphosphonates

R. Parrulli¹, F. Natalini², G. Pelliccioni³, L. Montebugnoli⁴, C. Marchetti⁵

¹Researcher Scholar, Department of Biomedical and Neuromotor Sciences, Section of Maxillofacial Surgery, University of Bologna, Bologna, Italy; ²Scholarship, Department of Biomedical and Neuromotor Sciences, Section of Maxillofacial Surgery, University of Bologna, Bologna, Italy; ³Researcher, Department of Biomedical and Neuromotor Sciences, Section of Maxillofacial Surgery, University of Bologna, Bologna, Italy; ⁴Professor, Department of Biomedical and Neuromotor Sciences, Section of Oral Science, University of Bologna, Italy; ⁵Professor, Department of Biomedical and Neuromotor Sciences, Section of Maxillofacial Surgery, University of Bologna, Bologna, Italy

Introduction. Oral and intramuscular bisphosphonates administration are approved to treat osteoporosis, osteopenia, Paget disease and osteogenesis imperfecta of childhood because of their action of osteoclastic activity inhibition. The main adverse effect related to bisphosphonates is the Medication Related Osteonecrosis of the Jaw (MRONJ). This pathology is triggered by a continuity solution of the mucosa. A principal cause of MRONJ is tooth extraction, that is the most frequent surgical treatment in dentistry. Patients may be considered affected by MRONJ if all the following characteristics are present: current or previous treatment with antiresorptive or antiangiogenic agents; exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that has persisted for longer than 8 weeks; no history of radiation therapy to the jaws or obvious metastatic disease to the jaws¹. The incidence of MRONJ in patients treated with oral and intramuscular bisphosphonates ranges from 0.5 to 1.2%².

Material and methods. 69 consecutive patients with a history of oral or intramuscular bisphosphonates administration undergone teeth extractions were considered. 165 teeth extractions were performed according to our protocol: discontinuation of bisphosphonate therapy for at least one month before and request of CTX evaluation, atraumatic extraction, prolonged perioperative antibiotic prophylaxis.

Results. The postoperative course was uneventful and complete healing of the soft tissues was achieved in the all treated sites.

Conclusions. This study seems to show the efficacy of our protocol in preventing the development of MRONJ in our 165 post-extraction sites.

References

1. Ruggiero S, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, O’Ryan F. American Association of Oral and Maxillofacial Surgeons Position Paper on Medication-Related Osteonecrosis of the Jaw. 2014 Update
2. Huang YF, Chang CT, Muo CH, Tsai CH, Shen YF, Wu CZ. Impact of bisphosphonate-related osteonecrosis of the jaw on osteoporotic patients after dental extraction: a population-based cohort study. *PlosOne.* 2015.

ONJ neither related to systemic nor local risk factors: how frequent is in a subset of 81 pharmacovigilance reports?

V. Panzarella¹, N. D'Alessandro², L. Lo Russo³, L. Lo Muzio³, O. Di Fede¹

¹Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy; ²Dep. of Health Sciences and Mother and Child Care "G. D'Alessandro", University of Palermo, Palermo, Italy; ³Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Aim. ONJ is defined as osteonecrosis of the jaw only related to anti-resorptive/angiogenic administration (i.e. bisphosphonates, denosumab). In absence of known systemic and/or local precipitating factors (i.e. diabetes/dento-alveolar trauma), ONJ is defined "spontaneous"¹: it is a very rare condition². Aim of this preliminary investigation was to explore the frequency and characteristics of ONJ not related to systemic and/or local risk factors during two years of pharmacovigilance study.

Patients and methods. From 2012 to 2014, during evaluation of 81 pharmacovigilance reports for ONJ, we detected different data: clinic, imaging, drug-related, systemic and/or local precipitating factors.

Results. On 81 ONJ (F/M=60/21; mean age=72,28 y), 73 (90,1%) were triggered by systemic/local factors (precipitated ONJ): dento-alveolar trauma was the most frequent local risk factor (77.8% extractions and 22.2% traumatic prosthesis), alone (79.2%) or in association with diabetes (20.8%) or corticosteroid treatments (36.1%). In precipitated ONJs, bisphosphonates were the most frequent administered drug, alone (58.3%) or in association (41.7%) with other anti-resorptive/angiogenic agents (denosumab, bevacizumab, bortezomib, thalidomide). The mean time of exposure was 25,4 and 71,7 months, respectively for oncologic (41/73, 56.2%) and osteoporotic (32/73, 43.8%) treatments.

When no systemic or local risk factors were recognized, spontaneous ONJ were registered (8/81,9.9%): 4 reports were related to intravenous administration (zoledronate) and 4 to *per os* (alendronate); the mean time of administration was 60,2 vs 39,5 months respectively.

Both for spontaneous and precipitated ONJ, the mandible was the most affected site (62.5 and 76.1% respectively).

Conclusions. Almost 10% of ONJ reported was spontaneous. This datum appears extremely noteworthy and further researches, especially investigating the genomic patterns, are necessary to better understand the pathogenesis of this condition.

References

1. de Molon RS, Cheong S, et al. Spontaneous osteonecrosis of the jaws in the maxilla of mice on antiresorptive treatment: a novel ONJ mouse model. *Bone*. 2014 Nov;68:11-9.
2. Yazdi PM, Schiodt M. Dentoalveolar trauma and minor trauma as precipitating factors for medication-related osteonecrosis of the jaw (ONJ): a retrospective study of 149 consecutive patients from the Copenhagen ONJ Cohort. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015 Apr;119(4):416-22.

Analysis of the macrophages phenotype in oral tongue squamous cell carcinomas

M. Mascitti¹, S. Agarbati², E. Paolucci², A. Santarelli¹, F. Fazioli²

¹Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy; ²Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy

Tumor associated macrophages (TAMs) comprises an important part of the tumor microenvironment and their mobilization into tumor tissues is a critical event in malignant progression. Like other immune effectors cells, TAMs can have multiple subtypes and take on various phenotypes depending on the context of the molecular stimuli: each phenotype displays a differential expression profile of cytokines, enzymes and cell-surface specific markers. However, in

general, they are divided broadly into two categories: classical pro-inflammatory M1 macrophages (commonly identified by staining the CD11c antigen) and alternative anti-inflammatory M2 macrophages (expressing the highly-specific CD163 antigen). Although TAMs have been detected in oral squamous cell carcinomas, little is known about their phenotype in the context of the tongue tumor lesions, which have the highest incidence in oral maxillofacial malignant tumors. Therefore, in the present study, we performed an immunohistochemical analysis to characterize the macrophage polarization in surgically resected specimens from 71 patients with tongue carcinomas (36 graded as G1 and 35 graded as G3) and to examine the importance of their relative localization (tumor stroma, inflammation area or tumor nest) on tumor-promoting capabilities and tumor-prognostic relevance. Our results have shown that infiltration of CD163⁺ or CD11c⁺ macrophages in the tumor nest was not associated with any clinicopathological features. On the contrary, CD163⁺ macrophages in the inflammation area surrounding the tumor positively correlated with higher grade. Tumor stroma lacked CD11c expression in all the analysed samples, while dense infiltration of CD163⁺ macrophages was often observed in the same areas. In addition, the presence of CD163⁺ macrophages in the stromal area was associated with a more favorable prognosis. These findings highlights the importance of analyzing the location rather merely the presence of TAMs as a prognostic marker.

References

- Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anti-cancer therapies. *J Pathol.* 2002 Mar;196(3):254-65.

Experimental Study

Oral brushing as a useful method to study epigenetic alterations in oral mucosa from OSCC patients

L. Luccarini¹, L. Morandi², V. Monti², A. Tarsitano³, D. B. Gissi¹

¹Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy; ²Department of Biomedical and Neuro-motor Sciences, Section of Anatomical Pathology "M. Malpighi" at Bellaria Hospital, University of Bologna, Italy; ³Department of Biomedical and Neuromotor Sciences, University of Bologna, Section of Maxillo-facial Surgery at Policlinico S. Orsola-Malpighi, Bologna, Italy

Objectives. A non invasive method for the early diagnosis in Oral Oncology may have a major impact on survival and quality of life. Cancer-related genetic alterations have been widely demonstrated in Oral Squamous Cell Carcinoma (OSCC) but recently epigenetic alterations, such DNA methylation alterations, have gained importance and seem to be stable and easily detectable from body fluids as saliva and exfoliated cells ¹. Aim of the present study was to evaluate the feasibility of a new non-invasive assay, based on brushing of oral mucosa and DNA methylation analysis using quantitative Bisulfite-Next Generation Sequencing (NGS).

Methods. Oral brushing was carried out by two operators and applied on 73 consecutive patients, with suspected OSCC, potentially malignant lesions and healthy controls. A total of 684 differentially methylated genes in OSCC was investigated by NGS: a set of 8 genes was tested in 48 patients and remaining 25 patients was tested for 12 genes. In each sample, fluorimeter quantus was used for a quantitative DNA evaluation, and DNA amount >1 ng/ul was considered adequate for DNA methylation analysis. PAP smear evaluation was done on 10 selected samples for a qualitative cell evaluation. Site, clinical appearance and diagnosis of the lesions, smoking habit and operator were considered as confounding variables. Multiple ANOVA was used for the statistical analysis.

Results. PAP smear evaluation showed presence of keratinocytes of the upper and medium layer within the brush. 66/73 samples showed a DNA amount >1 ng/ul (90,4%), without any significant difference among the considered variables. Informative sequences about DNA-methylation pattern were obtained in 663/684 genes investigated (96,9%).

Conclusions. Oral brushing seems to be a reliable procedure to obtain adequate DNA amounts from keratinocytes, and it may be used as a not invasive, easy to-perform method to study epigenetic alterations, such DNA methylation pattern, in oral mucosa.

References

1. Jithesh PV, Risk JM, Schache AG, Dhanda J, Lane B, Liloglou T, Shaw RJ. The epigenetic landscape of oral squamous cell carcinoma. *Br J Cancer.* 2013;108:370-9.

Accuracy of staging of infiltration depth in early oral cancer by high resolution intraoral ultrasonography: clinico-pathological analysis

L. Limongelli¹, A. Tempesta¹, E. Maiorano³, G. Angelelli², G. Favia¹

¹Complex Operating Unit of Odontostomatology, Department of Interdisciplinary Medicine, "Aldo Moro" University of Bari, Italy; ²Complex Operating Unit of Radiology, Department of Interdisciplinary Medicine, "Aldo Moro" University of Bari, Italy; ³Complex Operating Unit of Pathological Anatomy, Department of Emergency and Organ transplantation, "Aldo Moro" University of Bari, Italy

Background. The depth of invasion is an important histological prognostic factor in early oral squamous cell carcinoma (T1-T2cN0), especially as a predictor of cervical lymph node metastasis. Generally, the depth invasion is measured on the slide. In order to perform a tridimensional staging and excision it is necessary to achieve this measurement pre-operatively. To date, the only radiological exam able to give the depth of invasion measurement is the high quality intraoral ultrasonography (IU). The aim of this study is to evaluate the usefulness and the accuracy of IU comparing IU measurement with those achieved from the slides.

Materials and methods. We report on the cases of 20 patients affected by T1-T2cN0 squamous cell carcinoma, which referred to Complex Operating Unit of Odontostomatology, Policlinic of Bari. Each patient underwent to IU executed using L8-18i transducer with bandwidth of 18 MHz. Each patient's slide was analysed and measured using Confocal Laser Scanning Microscope. The IU measurements were compared with histologic ones.

Results. High-quality ultrasonic images were obtained. There was a significant correlation ($p < 0,001$) between measurements obtained with IU and ones from histological slides.

Conclusions. The high quality intraoral ultrasonography is a useful and accurate diagnostic tool for early oral squamous cell carcinoma necessary to achieve the depth of invasion and to perform a tridimensional staging and surgical excision.

References

- Luongo de Matos L, Manfro G, Vieira dos Santos R, Stabenow E, Sobroza de Mello E, Alves VAF, et al. Tumor thickness as a predictive factor of lymph node metastasis and disease recurrence in T1N0 and T2N0 squamous cell carcinoma of the oral tongue. *Oral and Maxillofacial Pathology*. 2014;18:209-217.
- Salmon B, Le Denmat D. Intraoral ultrasonography: development of a specific high-frequency probe and clinical pilot study. *Clin Oral Investig*. 2012.

Periodontal status in oncologic patients with MRONJ: a case control study

E. C. Bodea, E. Lerda, A. Gambino, F. Erovigni, A. Dell'Acqua

Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy

Introduction. Poor oral health is generally discussed in the "medication-related osteonecrosis of the jaw" (MRONJ) literature; however, there is a deficit of well-conducted studies concerning the real influence of local factors (e.g., dental plaque, caries, or periodontitis) on the development of MRONJ.

Aim. To evaluate the periodontal status of MRONJ patients and compare it with that of healthy controls.

Materials and methods. A prospective case-control study was undertaken on 20 patients treated with *i.v.* bisphosphonates for bone metastases (10 with clinical MRONJ, and 10 with no signs of jaws disease). Parameters evaluated were the Oral Hygiene Index, Full Mouth Plaque Score (FMPS), Full Mouth Bleeding upon probing Score (FMBS), Probing Depth (PD), DMFT index and presence of removable prosthesis.

Results and discussion. Thirteen of the 20 patients were women and 7 men, with an average age of 68 years. Among MRONJ patients, 5 were affected by multiple myeloma, 3 by breast cancer and 2 by prostate cancer. Intra-

venous infusions of 4 mg zoledronate were administered every 3 to 4 weeks in all patients analysed. In this study, patients with MRONJ had poorer oral hygiene, showed more complications of caries, and had worse periodontal status if compared with those without MRONJ. However, to date, it is not possible to ascribe an accompany ingrole to concerning these features. Improvement of dental hygiene was shown to reduce the incidence of MRONJ in patients with multiple myeloma and metastatic cancer.

Conclusions. Our preliminary results showed that periodontal status is worse in MRONJ patients if compared with controls. Oncologic patients treated with bisphosphonates should be offered preventive care to reduce dental plaque, calculus, dental caries, and periodontal disease to prevent the onset of jaws necrosis.

References

- Carmagnola D, Celestino S, Abati S. Dental and periodontal history of oncologic patients on parenteral bisphosphonates with or without osteonecrosis of the jaws: a pilot study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2008;106:10-5.
- Kos M. Association of dental and periodontal status with bisphosphonate-related osteonecrosis of the jaws. A retrospective case controlled study. *Arch Med Sci.* 2014;10:117-23.
- Oteri G, Bramanti E, Nigrone V, Ciccù M. Decayed, missing, and filled teeth index and periodontal health in osteoporotic patients affected by bronj: an observational study. *J Osteoporos.* 2013;2013:231289.

Experimental Study

A database-based analysis of dentistry literature to evaluate clinical research

A. Khalil, E. Scarabelli, E. M. Varoni, S. Rizzi, G. Lodi

Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi di Milano, Italy

Randomized controlled trials are the core of the clinical medical research and, since first study on tuberculosis patients in 1948, they represent the best way to assess efficacy of treatments. The analysis of available scientific literature allows summarizing the past of dental research at a glance and analyzing the quality of research¹. Here, we focused on published randomized clinical trials in dentistry and oral health, in order to describe the evolution of clinical research and to evaluate the impact of certain studies over some others. We retrieved the most relevant works by means of the currently available web-based resources (Web of Sciences, PubMed, Cochrane websites). In addition to the literature analysis on generic dentistry, specific dental areas, such as oral surgery, mouth disease, caries and periodontics, were further considered and compared each other. The results obtained from the three databases were mostly in accordance, showing that the first randomized studies dated back to the late 60s and early 70s. The commonly faced topics concerned caries and its prevention. This analysis allows a full overview of dentistry literature, contributing to better understand the evolution and the overall quality of clinical research in this field of medicine.

References

1. Adetugbo K, Williams H. How well are randomized controlled trials reported in the dermatology literature? *Arch Dermatol.* 2000;136(3):381-5.

Experimental Study

Preliminary results on residual bisphosphonate amount in alveolar bone sequestra

C. Intra, C. Soru, M. Minoli, F. Lo Faro, E. M. Varoni

Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi di Milano, Italy

Aim. The aim of the study is to identify the amount of two commonly used bisphosphonates (BPs), i.e. zoledronate and alendronate, in bone sequestra from patients affected by Bisphosphonate (BP)-Related Osteonecrosis of the Jaw (BRONJ). BPs possess a strong chemical interaction with bone tissue, lasting even months after therapy suspension^{1,2}.

Methods. In April 2008, a pilot observational study began at the Unità di Odontostomatologia II A.O. "San Paolo" (Università degli Studi di Milano) and Unità di Odontoiatria Ospedale Papa Giovanni XXIII di Bergamo. All BRONJ patients, who experienced spontaneous or surgical removal of bone sequestra, were included, thus bone sequestra were collected and stored at -80 C° until further analysis. Personal and clinical information were recorded. BP content was, then, analysed via High Performance Liquid Chromatography (HPLC) coupled to spectrometry mass (AB-SCIEX) at Sezione di Tossicologia (Università degli Studi di Milano).

Results. Zolendronate and alendronate were successfully quantified in human necrotic bone. 33 bone samples from patients under current or previous treatment with zolendronate were obtained, and 27 of them analyzed. The average duration of therapy was about 3 years. We found a mean amount of the drug equal to 6 ng/mg. Regarding alendronate, specimens from 21 patients were collected, and 19 of them quantified; the average duration of the therapy was around 7 years. In this case, the mean amount of drug was of 73 ng/mg. Notably, each drug was still detectable in samples from patients who discontinued the drug, even several years before bone sequestra removal.

Conclusion. We observed residual amount of both alendronate and zolendronate in bone sequestra, even after drug suspension, confirming their strong chemical interaction with bone tissue. Although preliminary, these data may contribute to better elucidate the pathogenesis of BRONJ. Nonetheless, the set-up of experimental protocols for the detection of further BPs is also required.

References

1. Badel T, Pavicin IS, Carek AJ, Rosin-Grget K, Grbesa D. Pathophysiology of osteonecrosis of the jaw in patients treated with bisphosphonate. *Coll Antropol.* 2013;37(2):645-51.
2. Paulo S, Abrantes AM, Laranjo M, Carvalho L, Serra A, Botelho MF, Ferreira MM. Bisphosphonate-related osteonecrosis of the jaw: specificities. *Oncol Rev.* 2014;8(2):254.

Experimental Study

An early surgical approach based on Er: YAG laser and LLLT in medication related osteonecrosis of the jaw. Experience with 248 patients

G. Ghidini, E. Merigo, M. Meleti, M. Manfredi, P. Vescovi

Department of Biomedical, Biotechnological and Translational Sciences, Center of Oral Pathology and Laser Surgery, University of Parma, Italy

Background. Medication related osteonecrosis of the jaw (MRONJ) is a potential side effect associated to the use of several drugs including bisphosphonates and antiresorptive drugs.

The aim is to highlight possible differences of the outcome of 5 therapeutical options in a group of patients affected by MRONJ.

Methods. Two hundred eighty one (81 Male and 200 Female, M/F:1/2,5) patients affected by MRONJ were treated between 2003 and 2015 at the Academic Hospital of Parma, Italy. Thirty-three sites of MRONJ (Group 1-G1) were treated with a medical approach (amoxicillin 2 gr/day and metronidazole 1gr/day); 63 sites (Group 2-G2) were treated with medical approach and low level laser therapy (LLLT) applications (Nd:YAG 1064 nm Fidelis Plus®, Fotona-Slovenia 1,25 W-15 Hz); 16 sites (Group 3-G 3) were treated with medical therapy and traditional surgery; 45 sites (Group 4-G 4) were treated with medical therapy, traditional rotary surgery and LLLT and 91 sites (Group 5-G 5) were treated antibiotic, surgery with Er: YAG laser (2940 nm FidelisPlus®, Fotona-Slovenia 250 mJ, 20Hz VSP-Fluence 50J/cm²/300 mJ 30Hz VSP-Fluence60J/cm²) and LLLT. Patients were furtherly subclassified into cancer (i.e. multiple myeloma and osteolytic bone metastasis) and noncancer patients (i.e. osteoporosis and other bone metabolism's diseases). Stages of MRONJ (I,II or III) were also considered. Outcomes were assessed using the scoring system proposed by Vescovi et al.¹ Fisher's tests and X² tests were used for statistical evaluation.

Results. Taking into account the clinical improvement and complete healing, significant results were highlighted comparing G1 vs G2 (p<0.0001, p=0.1533), G1+G2vsG3+G4+G5 (p<0.0001) and G3 vs G4+G5 (p=0.0003,p=0.0141). Significant results comparing an early approach (stage I) vs therapies in stage II and III (p<0.0001) were also observed.

Conclusions. The use of laser significantly improves the outcome of treatments, both for cancer and noncancer patients. An early surgical approach leads in almost every cases to a complete healing (95,74%).

References

1. Vescovi P, Manfredi M, Merigo E, Guidotti R, Meleti M, Pedrazzi G, Fornaini C, Bonanini M, Ferri T, Nammour S. Early surgical laser-assisted management of bisphosphonate-related osteonecrosis of the jaws (BRONJ): a retrospective analysis of 101 treated sites with long-term follow-up. *Photomed Laser Surg.* 2012 Jan;30(1):5-13. doi: 10.1089/pho.2010.2955. Epub 2011 Nov 4.
2. Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B; American Association of Oral and Maxillofacial Surgeons. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws-2009 update. *J Oral Maxillofac Surg.* 2009 May; 67(5 Suppl):2-12. doi: 10.1016/j.joms.2009.01.009.

Experimental Study

Periodontal therapy in patients with primary and secondary Sjögren's syndrome: a double blind randomized clinical trial

A. Gambino, A. Cafaro, P. G. Arduino, P. Carcieri, R. Broccoletti

Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy

Introduction. Patients with Sjögren's Syndrome (SS) generally have a significantly higher plaque index score compared to healthy patients. Moreover, a higher frequency of oral symptoms is observed in patients with primary (p)SS respect to patients with secondary (s)SS.

Aim. The aim of the study was to evaluate the difference of oral health status before and after non-surgical periodontal therapy in patients with pSS than those with sSS.

Materials and methods. We undertook a 3-months, double blind, randomized trial involving 24 patients with SS, on the basis of AECG criteria. An operator performed basal and stimulated salivary flow, salivary pH, VAS, DMFT index, presence of mucosal infection (T0). A second operator performed the causal therapy with follow-up after 4 weeks (T1). After 3 months, the first operator measured again the initial indicators (T2). A third operator compared the values and the results recorded by dividing the population into subgroups: A =pSS and B = sSS.

Results and discussion. The patients with pSS were 14 (average age 65,2) while patients with sSS were 10 (average age 63,7); in both group an improvement in salivary flow, pH and VAS was noticed, without a statistically significant difference. Significant differences were observed between t0 and t2 in the baseline flow, pH (rs 0.008 p<0,05), VAS (rs 0.016 p<0,05) in group A. Nine patients developed new carious lesions: 3 in group A and 6 in group B. In accordance with previous studies, we found that the DMFT score is inversely correlated to salivary flow rates. However, it has not yet been possible to identify a cut-off value for salivary flow rate that can predict the risk of developing dental caries, as caries is a multifactorial disease.

Conclusion. SS has a large impact on oral health. Education in oral health is imperative for subjects with this pathology and more frequent check-ups may be useful in decreasing the levels of oral diseases.

References

- Hernández-Molina G, Avila-Casado C, Cárdenas-Velázquez F, Hernández-Hernández C, Calderillo MI, Marroquín V, Soto-Abraham V, Recillas-Gispert C, Sánchez-Guerrero J. Similarities And Differences Between Primary And Secondary Sjögren's Syndrome. *J Rheumatol.* 2010;37:800-8.
- Meijer J, Meiners P, Huddleston Slater J, Spijkervet F, Kallenberg C, Vissink A, Bootsma H. Health-Related Quality Of Life, Employment And Disability In Patients With Sjögren's Syndrome. *Rheumatology.* 2009;48:1077-82.
- Olate S, Muñoz D, Neumann S, Pozzer L, Cavalieri-Pereira L, De Moraes M. A Descriptive Study Of The Oral Status In Subjects With Sjögren's Syndrome . *Int J Clin Exp Med.* 2014;7:1140-44.

Activity of the CIR-Dental School, University of Turin, for patients at risk of MRONJ: a five-year hospital based analysis

F. Erovigni, E. Lerda, V. Arata, A. Dell'Acqua, A. Gambino

Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy

Introduction. The number of patients taking drugs at risk of “medication-related osteonecrosis of the jaw” (MRONJ) is estimated to increase in the next years, since the introduction of new antiresorptive and antiangiogenetic agents being more used in order to better contrast bone metastases.

Aim. The aim of the study was to describe the clinical activity of the MRONJ clinic, CIR-Dental School, University of Turin (Prof. Stefano Carossa), in order to compare these data with the international literature.

Materials and methods. The clinical data of the patients referred to Dental School of Turin from 2009 to 2014 were reviewed in order to describe the prevalence and the incidence of MRONJ regarding gender, age, kind of disease, kind of medication and timing of the event from the beginning of the antiresorptive/antiangiogenetic therapy, site, type of therapy (surgical vs. non surgical) and actual condition.

Results and discussion. Among 1.197 selected cases, 93 MRONJ cases (7.7%) were diagnosed [26% male and 74% female, average age 73 years (SD± 2.07)]; the average time of occurrence was 10.67 months (SD± 5.0) after the beginning of the antiresorptive therapy. Among 93 cases, 40 (43.01%) were affected from breast cancer, 9 (9.68%) prostatic cancer, 21 (22.58%) multiple myeloma, 14 (15.05%) other cancers and 9 (9.68%) osteoporosis; 82 positive patients (88.17%) had taken zoledronate, 9 patients (9.68%) intra-muscle or oral bisphosphonate, 1 (1.08%) denosumab and only 1 (1.08%) had an antiangiogenetic therapy (bevacizumab) associated with zoledronate. In 64 patients (68.82%), MRONJ occurred in the lower jaw, in 26 (27.96%) in the upper jaw and only in 3 patients (3.23%) involved both jaws; 76 patients (81.72%) were treated with surgery and only 17 (18.28%) were treated only with conservative therapy. After treatment, 73 subjects (78.49%) improved or recovered; the other patients worsened or died before an improvement of the MRONJ. Actually 53.76% are still alive.

References

- Lopes RN, Rabelo GD, Rocha AC, Carvalho PA, Alves FA. Surgical therapy for bisphosphonate-related osteonecrosis of the jaw: six-Year Experience of a Single Institution. *J Oral Maxillofac Surg.* 2015;73:1288-1295.
- Ristow O, Otto S, Troeltzsch M, Hohlweg-Majert B, Pautke C. Treatment perspectives for medication-related osteonecrosis of the jaw (MRONJ). *J Craniomaxillofac Surg.* 2015;43:290-3.
- Napeñas JJ, Kujan O, Arduino PG, Sukumar S, Galvin S, Baričević M, Costella J, Czerninski R, Peterson DE, Lockhart PB. World Workshop on Oral Medicine VI: Controversies regarding dental management of medically complex patients: assessment of current recommendations. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2015;120:207-226.

Cancer stem cells from head and neck squamous cell carcinoma

M. Dioguardi¹, D. Sartini², A. Santarelli^{2,3}, L. Lo Muzio¹, M. Emanuelli²

¹Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; ²Department of Clinical Sciences, Polytechnic University of Marche, Ancona, Italy; ³I.R.C.C.S. I.N.R.C.A., Ancona, Italy

Introduction. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, and its incidence is still increasing. According to cancer stem cell (CSC) theory, a distinct subpopulation of tumor cells, termed cancer stem cells (CSCs), possesses the ability to self-renew, differentiate, initiate and maintain tumorigenesis. Therefore, analyses focused on CSC-enriched populations, rather than the entire tumor, could provide im-

portant and reliable information about cancer biology. The aim of this project was to identify and characterize CSCs obtained from Hep-2 cell line.

Methods. CSC enrichment from Hep-2 cell line and HNSCC primary cultures was obtained through sphere formation, by cultivating cell line in defined serum-free medium. Real-Time PCR and immunocytochemical analysis were performed to characterize CSCs. The expression levels of the enzyme Nicotinamide N-methyltransferase (NNMT), known to be upregulated in several neoplasms, were also explored. In addition, Hep-2 cells and CSC-enriched populations were injected into immunocompromised mice.

Results. CSC-enriched populations showed an increased expression of stem cell markers and a strong capability to form tumor *in vivo*. Interestingly, NNMT levels were significantly higher in CSC-enriched populations compared with parental cells.

Discussion and conclusions. Our study provides an useful procedure to identify and enrich CSCs from Hep-2 cell line, allowing their molecular and phenotypic characterization. Therefore, our work may help to explore the etiopathogenic role of CSCs in HNSCC and may contribute to the development of new strategies for head and neck cancer therapy.

Experimental Study

MiR-200c is inversely related to DPAGT1 and EMT-related transcription factors in oral cancer cells

G. Del Corso¹, T. Packer¹, A. Villa², X. Varelas³, M. A. Kukuruzinska¹

¹Department of Molecular and Cell Biology, Boston University School of Dental Medicine, USA; ²Division of Oral Medicine and Dentistry, Brigham and Women's Hospital, and Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, USA; ³Department of Biochemistry, Boston University School of Medicine, USA

Objectives. The aim of the present study is to identify which miRNA is related to the expression of N-glycosylation gene *DPAGT1* and Epithelial Mesenchymal Transition (EMT)-associated transcription factors in oral cancer cell lines.

Methods. Using gene arrays in SCC2 cells, with siRNAs-mediated knockdown of *DPAGT1*, we identified miRNAs with altered expression using a cut-off of -1.7 fold change (FC) and a p-value<0.05. Six miRNAs were selected for functional characterization in non-metastatic OSCC CAL27 cells transduced with lentiviral *DPAGT1*, and metastatic SCC2 cells with lentiviral knockdown of *DPAGT1*. MiRNA and total RNA were extracted using Roche high pure miRNA isolation kit and Qiagen RNeasy RNA isolation kit. MiRNA and total RNA were evaluated by qPCR to determine their relationship to changes in *DPAGT1* expression. Statistical analyses were performed paired student T-test.

Results. Of the 25 miRNAs related to *DPAGT1* knockdown found in the arrays, six miRNAs were selected based on their reported roles in EMT. They included: miR-21, miR-181b, miR-200c, miR-205, miR-221, miR-222. In SCC2 cells with *DPAGT1* knockdown, miR-200c was upregulated (FC=11.5; p=0.04) while in CAL27 cells overexpressing *DPAGT1*, miR-200c was reduced (FC=0.47; p=0.01). EMT-associated transcription factors, in particular ZEB1, expression were found down-regulated in SCC2 cells with *DPAGT1* knockdown and up-regulated in CAL27 cells transduced with *DPAGT1*.

Conclusions. miR-200c is inversely related to *DPAGT1* and ZEB1 expression; *DPAGT1* gene impacts ZEB1 through the inhibition of miR-200c; miR-200c may serve as predictive marker for treatment efficacy in the era of personalized medicine.

Head and neck lymphadenopathy in childhood and in adults: a bidepartmental review

R. De Luca R, A. Moscariello, D. Di Pinto, E. Pota, G. Colella

Dipartimento Multidisciplinare di Specialità Medico-chirurgiche ed Odontoiatriche, Seconda Università degli Studi di Napoli, Italy

Introduction. Lymphadenopathy, an abnormality in size or character of the lymph nodes, is caused by invasion or propagation of either inflammatory or neoplastic cells in the node. Up to 90% of children between the ages of 4-8 years have palpable cervical lymphadenopathy¹. The rate of malignant lymphadenopathy etiologies is very low in childhood, but increase with the age. The aim of our bidepartmental study is to analyze contemporary approaches to the diagnosis and management of childhood and adults cervical lymphadenitis.

Patients and methods. A retrospective review identified 170 pediatric patients and 182 adult patients with cervical lymphadenitis carried out at the Pediatric Oncology Service and Maxillofacial Surgery Unit of Second University of Naples, between January 1991 and December 2009. In the study were included all cases with head and neck lymphadenopathy of ≥ 1 cm in diameter lasting more than one week of presentation. Final diagnoses were based on the basis of laboratory testing, according to the patients' clinical picture and US evaluation, carrying out fine-needle biopsy or excision biopsy when needed.

Results. A total of 352 patients with head or neck lymphadenopathies were carried out in our retrospective review: 170 pediatric patients with mean age of 6,5 years and 182 adult patients with mean age of 49.8 years. The lymphadenopathy was considered as single in 33 (9.4%) pediatric and 89 (25.3%) adult cases, and as multiple in 137 (38.9%) pediatric and 93 (26.4%) adult cases, respectively. At presentation, no signs or symptoms could be found in 80 (47%) pediatric and 148 (81.3%) adult patients, respectively. Conversely, in 90 (52.9%) pediatric and 34 (18.6%) adult patients the lymphadenopathy was associated with signs and/or symptoms. Instead, 54 pediatric and 5 adult patients several signs or symptoms at diagnosis, respectively. Fever was the most common general symptom accompanying lymphadenopathy and it was observed in 26 cases (16 pediatric and 10 adult patients). Final diagnoses were established on the basis of clinical findings or laboratory testing or histology as follows: in 58 pediatric and 13 adult patients by serologic tests, in 40 pediatric and 122 adult patients by excisional biopsy. Reactive inflammatory changes of non specific origin were observed in 78 (45.8%) children and 45 (24.7%) adult patients, respectively. Infections were diagnosed in 58 (34.1%) of children and 23 (12.6%) of adult patients. In 31 pediatric cases (18.2%) and 108 (59.3%) adult patients a diagnosis of neoplasia was performed, respectively. Moreover, cervical lymph nodes involvement was a manifestation of non infectious origin or of a systemic disease with an inflammatory component in 2 (1.1%) children and 6 (3.2%) adults, respectively.

References

1. Srouji IA, Okpala N, Nilssen E, Birch S, Monnery P. Diagnostic cervical lymphadenectomy in children: a case for multidisciplinary assessment and formal management guidelines. *Int J Pediatr Otorhinolaryngol.* 2004 May;68(5):551-6.

Endoscopic management of salivary gland diseases in patients with Sjögren's syndrome

R. De Luca, A. Vicidomini, G. Tartaro, G. Colella

Dipartimento Multidisciplinare di Specialità Medico-chirurgiche ed Odontoiatriche, Seconda Università degli Studi di Napoli, Italy

Objective. To evaluate on the basis of our clinical experience, the reliability of an endoscopic approach in the management of obstructive salivary diseases related to Sjögren's syndrome.

Material and methods. Retrospective review of all the patients affected by Sjögren's syndrome followed up at the Maxillo-Facial Unit of the Second University of Naples Hospital and referred from Rheumatology Unit of the same

Hospital from September 2007 to July 2013 complaining for chronic obstructive sialadenitis unresponsive to medical therapy.

Interventions. After the detection of the impaired gland, under local anesthesia to the orifice region and a gradual dilatation of the duct orifice, the diagnostic unit was introduced into the duct and was advanced forward, until reaching the ductal system and thanks to continuous lavage with isotonic saline solution. The plaques were washed out, and any strictures were dilated. Mucous plugs and debris were removed with irrigation or with a forceps if necessary.

Results. Our cohort included 29 female and 5 male patients, with a mean age of 51,76 years. A total of 60 parotid glands and 25 submandibular glands were explored and treated. Strictures were founded in 38 glands (38/85-45%), mucous plugs in 47 glands (47/85-55%), sialectasis in 20 glands (20/85-23%), strictures and sialectasis together in 16 glands (16/85-19%), mucous plugs and strictures together in 3 glands (3/85-4%), kinks in 2 glands (2/85-2%). In 32 parotid glands (32/60-53%) Stensen's duct was affected, in 2 (2/60-3%) only secondary ducts, in 18 (18/60-30%) both. In submandibular glands explored strictures and mucous plugs were mainly observed in Wharton duct. We reached symptomatic improvement in 29 patients (29/34-85%), in a follow-up period ranging from 5 months to 6 years.

Conclusions. Interventional sialoendoscopy is a viable technique to treat acute symptomatology in patients with obstructive salivary gland diseases related to Sjögren's Syndrome and refractive to conventional management.

References

- Capaccio P, Torretta S, Ottaviani F, Sambataro G, Pignataro L. Modern management of obstructive salivary diseases. *Acta Otorhinolaryngol Ital.* 2007;27:161-172.
- Shacham R, Puterman M, Ohana N, Nahlieli O. Endoscopic Treatment of Salivary Glands Affected by Autoimmune Diseases. *J Oral Maxillofac Surg.* 2011. 69:476-481.
- Strychowsky JE, Sommer D, Gupta M K, Cohen N, Nahlieli O. Sialendoscopy for the Management of Obstructive Salivary Gland Disease. *Arch Otolaryngol Head Neck Surg.* 2012;138(6):541-547.

Experimental Study

Histological analysis of oral lesions in pediatric patients: a retrospective observational study

F. D'Amore, R. Franchini, E. M. Varoni, A. Carrassi, G. Lodi

Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi di Milano, Italy

Background/Purpose. Database's analysis of histological findings can provide important insights to investigate the frequency and the impact of a specific disease on a certain population¹⁻³. The aim of this study was to retrospectively analyze histological data from a hospital register (AO San Paolo, UO Odontostomatologia II, University of Milan), which included oral lesions with bioptical analysis, over the last twenty years. We focused on pediatric patients and the objective was to examine categories and incidence of recorded oral lesions.

Methods. The database used for this study was created by the unit of Oral Medicine and Pathology (University of Milan) in 1994 and updated up to 2014 and includes 6,058 records. Biopsies records concerning pediatric patients, i.e. from 0 to 14 years, were retrieved. Then, patients were divided into three age groups (0-5, 6-10, and 11-14 years) and the oral lesions were classified into 4 main categories: traumatic, reactive, infective, and "other" lesions.

Results. One hundred and forty-two biopsies, out of a total of 6058, referred to pediatric patients. The most common histological findings belonged to the category of "traumatic lesions", followed by reactive, infective, and "other" lesions. The majority of oral biopsies were taken from patients who were 11-14 years old. The top five oral lesions in pediatric patients were mucoceles (49 cases), epulides (14 cases), papillomas (12 cases), cysts (7 cases), and granulomas (7 cases).

Conclusion. A relatively high incidence of inflammatory and reactive lesions in pediatric patients is, by this retrospective analysis on Italian population, confirmed. Particularly, mucoceles were the most common finding.

References

1. Wang YL, Chang HH, Chang JY, Huang GF, Guo MK. Retrospective survey of biopsied oral lesions in pediatric patients. *J Formos Med Assoc.* 2009;108(11):862-71.
2. Das S, Das AK. A review of pediatric oral biopsies from a surgical pathology service in a dental school. *Pediatr Dent.* 1993;15(3):208-11.
3. Sousa FB, Etges A, Corrêa L, Mesquita RA, de Araújo NS. Pediatric oral lesions: a 15-year review from São Paulo, Brazil. *J Clin Pediatr Dent.* 2002;26(4):413-8.

Epidemiological and clinico-pathological correlation of several types of HPV and oral potentially malignant disorders (OPMD)

L. Crocamo, L. Corcione, M. Manfredi, P. Vescovi, M. Meleti

Department of Biomedical, Biotechnological and Translational Sciences, Center of Oral Pathology and Laser Surgery, University of Parma, Italy

Introduction. The role of some HPV subtypes in the etiopathogenesis of oral potentially malignant disorders (OPMD) and oral carcinoma is controversial. The aim is to evaluate the possible correlation between HPV infection and some OPMD with emphasis on the carcinogenesis.

Methods. One hundred thirty-eight patients treated at the Academic Hospital of Parma, Italy, between 1997 and the 2009 were included. Patients were subclassified as follows: 29 patients with oral lichen planus (OLP), 21 patients with oral lichenoid lesions (OLL), 24 patients with lichenoid dysplasia, 51 patients with oral leukoplakia (OL) and 13 patients with proliferative verrucous leukoplakia (PVL). The diagnosis was based on clinical and histopathological criteria. Fluorescence *in situ* hybridization (FISH) was used for research and typing HPV DNA. Viral genotypes were divided into High Risk HPV (HR-HPV) and Low Risk HPV (LR-HPV). The mean follow-up was 114 months (ranging from 24 to 204). Statistical analysis was performed through Chi-squared and Fisher's exact test.

Results. 55.1% of OPMD were HPV positive. It was found a significant association between viral infection and male gender (X^2 Yates correction=0.0001). In lichenoid dysplasia it was found similar prevalence of HR genotypes in lesion with mild and severe dysplasia. In OL group HR-HPV prevalence was higher in non dysplastic lesions than in severe dysplasia. Statistical analysis showed a significant associations between HR genotypes and epithelial dysplasia in lichenoid lesion ($X^2=0.0266$; $p=0.0261$). Epithelial dysplasia in PVL was associated both with HR genotypes ($X^2=0.03375$) and LR ($X^2=0.0028$; $p=0.0022$).

During follow-up 11 malignant lesions in the context of OPMD were diagnosed. 63% of these were HPV negative and 36,3% were HPV positive (9,1% HR-HPV; 27,2% LR-HPV).

Conclusion. These results show an epidemiological association between HPV and OPMD. Further studies are needed to confirm or excluded the role of HPV in OPMD malignant transformation.

References

- Termine N, Panzarella V, Falaschini S, et al. HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988–2007). *Ann Oncol.* 2008;19:1681-1690.
- Syrjanen K, Syrjanen S, Lamberg M, Pyrhonen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg.* 1983;12:418-424.

Reduced salivary flow rate and hematopoietic stem cell transplantation: an emerging problem

D. Conrotto¹, L. Giaccone², M. Suria¹, R. Broccoletti¹, P. G. Arduino¹

¹Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy; ²Division of Haematology, Azienda Ospedaliera Città della Salute e della Scienza, University of Turin, Turin, Italy

Introduction. Patients who undergo to hematopoietic stem cell transplantation (HSCT) can develop many oral complications. Saliva is fundamental for oral health: it prevents caries, periodontal diseases and helps wounds healing. Nevertheless, few studies considered the changes of salivary parameters. The aim of this study was to evaluate the unstimulated salivary flow (USF) and salivary pH in a cohort of patients treated with HSCT.

Material and methods. A cross-sectional study was designed. Patients referred to the Oral Medicine Unit of the CIR-Dental School, University of Turin, who were previously subjected to HSCT, were evaluated for oral and dental

examination. All subject had their UWS measured at mid morning, at least two hours after the last food intake. Saliva was collected for a period of 15 minutes and the flow expressed in ml/min. Levels of pH were measured using an Oakton pH5/6® pH meter. The χ^2 test, Fisher's exact test, and the linear trend chi-squared test were used to evaluate the association between the reduced USF and variables related to HSCT.

Results. 32 (14 females, 18 males) patients were unrolled on this study. 13 patients (40.6%) had an UWS ≤ 1.5 ml in 15 minutes, 28 patients (81.25%) were ≤ 4.5 ml in 15 minutes (mean value in the healthy population), pH was 7.41 ± 0.61 . In the group of patients with erosive lesions pH was 6.97 ± 0.58 ; 10 patients (32.5%) had disgeusia; 19 patients (70%) had clinical signs of chronic graft versus host disease (cGVHD). We did not find any statistical correlation between hyposalivation and type of conditioning, presence of mucous cGVHD, type of medication used after the transplant, and time occurred after HSCT.

Discussion. In our cohort, hyposalivation was very frequent. The causes of this condition are unknown; the study failed in demonstrating a correlation between hyposalivation and variables related to HSCT. The most accredited hypothesis is an immune-mediated damage of salivary glands related to GVHD.

References

- Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness: association with medication, anxiety, depression, and stress. *J Dent Res.* 2000;79:1652-58.
- Imanguli MM, Atkinson JC, Mitchell SA, Avila DN, Bishop RJ, Cowen EW, Datiles MB, Hakim FT, Kleiner DE, Krumlauf MC, Pavletic SZ. Salivary gland involvement in chronic graft-versus-host disease: prevalence, clinical significance, and recommendations for evaluation. *Biol Blood Marrow Transplant.* 2010;16:1362-9.
- Mays JW, Fassil H, Edwards DA, Pavletic SZ, Bassim CW. Oral chronic graft-versus-host disease: current pathogenesis, therapy, and research *Oral Dis.* 2013;19:327-46.

Experimental Study

Detecting presence of Human Papillomavirus (HPV) in the oral cavity using brush biopsy: preliminary study

C. Ciolfi¹, C. Russo¹, A. Pierangeli², G. Palaia¹, U. Romeo¹

¹Department of Head and Neck, "Sapienza" University of Rome, Italy; ²Department of Molecular Medicine, "Sapienza" University of Rome, Italy

Human papillomavirus (HPV) is involved in the genesis of oral squamous cell carcinoma (OSCC), having both an epidemiologic and prognostic relevance. Because of this, it is important to individuate a screening test in order to identify and, in second stand, to prevent the progression of potentially malignant disorders into malignancy. A total of 63 individuals (29 males, 34 females), aged between 18 and 75 years, affected by suspected HPV lesions, potentially oral malignant disorders (POMD) and without clinical alteration, have been enrolled at Odontostomatologic Clinics of Policlinic Umberto I of Rome divided in two groups, to detect, through a cytobrush, HPV-DNA, HPV positivity and viral loads. The quantitative real-time PCR (qPCR) was used for the common low-risk (LR) HPV 6, 11 and high-risk (HR) HPV16, 18, 31, 33, 53, 58. In patients affected by oral lesions respect to those ones apparently healthy, HPV detection rate was 70.2 vs 48.5% and the percentage of HR was significantly higher (60.6 vs 33.2%). The median number of HPV-DNA copies in sample cases resulted to be significantly higher ($p=0.004$). The highest positivity has been found in lichen planus (75%) and HPV-related lesions (54.5%). High HPV positivity rates were higher in oral lesions infected by HPV16 and 18 in case group than control group. The utility of qPCR in POMD screening can be suggested. Despite this, prospective studies and a higher number of cases are needed to understand if elevated viral loads might be useful markers for the risk of malignant progression of POMD.

References

- Huang SF, Li HF, Liao CT, Wang HM, Chen IH, Chang JT, Chen YJ, Cheng AJ. Association of HPV infections with second primary tumors in early-staged oral cavity cancer. *Oral Dis.* 2012 Nov;18(8):809-15.
- Prabhu SR, Wilson DF. Human papillomavirus and oral disease-emerging evidence: a review. *Aust Dent J.* 2013 Mar;58(1):2-1.

BP180, BP230, DSg1, DSg3 circulating autoantibodies: a possible false positive scenario in immune-mediated oral diseases

C. Cinquegrana, R. Ninivaggi, A. M. Di Palma, M. Tampoia, M. Petruzzi

Università di Bari "Aldo Moro" – DIM (Dipartimento Interdisciplinare di Medicina Sezione di Malattie Odontostomatologiche), Italy

Background. Mucocutaneous *Pemphigus Vulgaris* (PV) and Mucous Membrane Pemphigoid (MMP) are clinical Subsets of Autoimmune Bullous Diseases (AIBDs). The current diagnostic gold standard of AIBDs is the detection of autoantibodies by direct immunofluorescence (DIF), directed to desmosome and basement membrane zone in PV and MMP, respectively. ELISA anti-DSg3 and anti-BP180 autoantibodies allow a high diagnostic accuracy in the diagnosis of skin AIBDs.

Aim. To estimate the presence of circulating anti-DSg1, anti-DSg3, anti-BP180 and anti-BP230 autoantibodies in patients with oral immune diseases, unaffected by PV and MMP.

Methods. Patients with oral lesions clinically mimicking PV and MMP were enrolled. Two oral biopsies for histological analysis and DIF were collected. The cut-off values for anti-DSg1 and DSg3 ELISA were 20 U/mL and for anti-BP180-NC16A and anti-BP230-Ct and Nt ELISA employed a cut-off values of 8.2 U/mL and 8.7 U/mL, respectively. The sensitivity, the sensibility, both the positive and negative predictive value and the diagnostic accuracy were calculated. The histological data and DIF were the diagnostic gold standard.

Results. Eighty patients were enrolled (M:F=1:4) with a median age of 62 years with the following diagnosis: 37 MMP, 17 PV, 21 Oral atrophic/erosive Lichen Planus (OLP), 3 Lichenoid Dysplasia (LD), 1 erythema multiforme and 1 systemic lupus erythematosus. ELISAs data were: 51% sensitive, 69% specificity, 74% PPV, 44% NPV and 57% diagnostic accuracy. There were 9 false positive of which 5/21 (24%) (2 BP180, 2 BP230, 1 DSg1/BP180) in OLP, 1/3 (33%) (DSg1/DSg3) in LD and 3/17 (18%) (3 BP180) in PV.

Conclusions. The autoantibodies in OLP and LD patients may be generated by an "epitope spreading" mechanism or they could be an epiphenomenon of the inflammatory infiltrate. In PV patients detection of anti-BP180 autoantibodies, may be due to MMP slatentization or to polyautoimmunity. The role of autoantibodies in pathogenesis remains still elusive.

References

- Kinjyo C, et al. Oral lichen planus with antibodies to desmoglein 1 and 3. *J Dermatol.* 2014;41:1-2.
- Kobayasi K, et al. Simultaneous occurrence of anti-BP180 mucous membrane pemphigoid and mucosal dominant pemphigus vulgaris. *Clinical and Experimental Dermatol.* 2009;34:e785-e788.
- Tampoia M, et al. Diagnostic accuracy of enzyme-linked immunosorbent assay (ELISA) to detect anti-skin autoantibodies in autoimmune blistering skin diseases: A systematic review and meta-analysis. *Autoimmun Rev.* 12(2):121-6.

Oral burning and psychological profile in patients with keratotic forms of Oral Lichen Planus: a case control clinical study

M. Cascone, A. Celentano, E. Ruoppo, S. Leuci, D. Adamo

Oral Medicine Complex Unit, Department of Neurosciences, Reproductive and Odontostomatological Sciences, Head & Neck Clinical Section "Federico II" University of Naples, Italy

Aim. To analyze oral burning, intraoral, extraoral somatic symptoms and psychological profiles in symptomatic patients with Reticular Keratotic (RK) forms of Oral Lichen Planus (OLP).

Methods. Thirty symptomatic RK-OLP (sRK-OLP) patients were compared with an equal number of non-symptomatic RK-OLP (nsRK-OLP) patients, burning mouth syndrome (BMS) patients and healthy subjects matched for age, sex and educational level. The Numeric Rating Scale (NRS), the short form of the McGill Pain Questionnaire (SF-MPQ), and the Hamilton rating scales for Depression (HAM-D) and Anxiety (HAM-A) were administered. Descriptive statistics, non-parametric ANOVA procedure by Kruskal-Wallis, exact Fisher test and multiple comparison test by Mann-Whitney U test were performed.

Results. The sRK-OLP and BMS patients had statistically higher scores in HAM-D, HAM-A, NRS and SF-MPQ than the nsRK-OLP and healthy controls. The median and inter-quartile range of HAM-D and HAM-A were 11.7-24.0 and 13.7-27.2 for BMS and 12.0-15.0 and 10.7-18.0 for sRK-OLP patients respectively. The median and inter-quartile range of HAM-D and HAM-A were 2.0-3.2 and 2.0-4.0 for nsRK-OLP and 2.0-4.0 and 2.0-4.0 for the control group respectively. The median and inter-quartile range of NRS and SF-MPQ were 7.7-10.0 and 9.0-12.2 for BMS and 7.7-10.0 and 7.0-13.0 for sRK-OLP patients respectively. The BMS and sRK-OLP patients reported oral burning in 96.7% and in 90% of cases, respectively, while no nsRK-OLP patients or healthy subjects reported oral burning and only 10% of each group showed extraoral somatic symptoms.

Conclusion. Oral burning and extraoral somatic symptoms are significantly correlated to higher levels of anxiety and depression in patients with sRK-OLP. These findings strongly suggest screening for depression and anxiety in sRK-OLP patients because pain perception could be deeply modulated by mood disorders.

References

- Adamo D, Ruoppo E, Leuci S, Aria M, Amato M, Mignogna MD. Sleep disturbances, anxiety and depression in patients with oral lichen planus: a case-control study. *J Eur Acad Dermatol Venereol.* 2015 Feb;29(2):291-7.
- Alves MG, do Carmo Carvalho BF, Balducci I, Cabral LA, Nicodemo D, Almeida JD. Emotional assessment of patients with oral lichen planus. *Int J Dermatol.* 2015 Jan;54(1):29-32.
- Balasubramaniam R, Klasser GD, Delcacho R. Separating oral burning from burning mouth syndrome: Unravelling a diagnostic enigma. *Aust Dent J* 2009;54:293-9.

Experimental Study

Conscious sedation with anxiolytic benzodiazepines in oral surgery according to the *Manani* protocol: a case series

M. Carbone¹, G. Manani², D. Conrotto¹, P. G. Arduino¹, R. Broccoletti¹

¹Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy; ²Professor of Anesthesiology in dentistry, University of Padua, Italy

Aim. The aim of this study was to evaluate the safety and the efficacy of conscious sedation with benzodiazepines performed by general dentists in patients undergoing oral surgery procedures.

Methods. A group of consecutive patients undergoing oral surgery, between March and June 2015, was evaluated. The *Manani* protocol was performed in all cases; subjects were pre-sedated with chlordemetyldiazepam (EN[®]) 1-2 mg, while induction of maximum subjective tranquillity was obtained with fractionated doses of intravenous diazepam (VALIUM[®]). No additional dose of *i.v.* diazepam was administered during the operation. Preoperative anxiety level was evaluated with Corah Dental Anxiety Scale and Visual Analogue Score for anxiety (VAS-A). Heart rate (HR), arterial pressure (AP) and arterial oxygen saturation (SpO₂), were monitored automatically before, during and after surgery by a three-way electrocardiography. Data were recorded at baseline and at 15, 30, 45, 60, 75 and 90 minutes. Newman test was applied to evaluate the post-operative psychomotor state of the patient and to authorise the discharge.

Results. Fifteen consecutive Caucasian patients (10 women and 5 men, mean age 56.8, range 35-80) were enrolled. The dose of chlordemetyldiazepam was 1 mg for 4 patients and 2 mg for 11, according VAS-A and Corah test. The mean dose of *i.v.* diazepam necessary to obtain maximal preoperative subjective tranquillity was 5.8 mg (range 4-10). Two patients with a history of drug abuse required an additional dosage of 2 mg *i.v.* chlordemetyldiazepam before starting surgery. The AP and HR values respectively increased and decreased during intervention. SpO₂ remained stable. A normal psychomotor recovery was found in all patients.

Conclusions. This preliminary study indicated that the conscious sedation according to the *Manani* protocol could be safe and easily performed also by general dentists. Further controlled studies, with larger series, are however needed.

References

- Corah NL, Gale EN, Illing SJ. Assessment of a dental anxiety scale. *J Am Dent Assoc.* 1978;97:816-819.
 - Facco E, Zanette MD, Favero L, Bacci C, Sivolella S, Cavallin F, Manani G. Toward the validation of Visual Analogue Score for Anxiety. *Anesth Prog.* 2011;58:8-13.
 - Manani G, Alberton L, Bazzato MF, et al. Analysis of an anxiolytic technique applied in 1179 patients undergoing oral surgery. *Minerva Stomatol.* 2005;54:551-68.
-

Experimental Study

Placebo *versus* low level laser therapy in the management of burning mouth syndrome: a randomized controlled single-blind study

A. Cafaro, A. Gambino, R. Broccoletti, M. Garrone, P. G. Arduino

Department of Surgical Sciences, CIR-Dental School, University of Turin, Turin, Italy

Introduction. The placebo effect is one of the most easily seen phenomena in medical practice and clinical research, but at the same time, is one of the most mysterious and controversial.

Aim of the study. To evaluate the placebo effect *versus* Low Level Laser Therapy (LLLT) in the management of the Burning Mouth Syndrome (BMS).

Materials and methods. The study included 29 BMS patients, randomized into two groups: group A (17 patients) underwent LLLT, group B (12 patients) underwent “false” LLLT. The sessions were performed twice weekly for 5 weeks, using a diode laser 980 nm, having a 0.6 cm spot diameter, with point to point technique. In the group receiving LLLT, the device was set to get a fluence of 10 J / cm² (0.3 W output power, CW, Power Density 1W / cm², spot area 0.28 cm²); in the group receiving false LLLT, gated emission mode with T-on zero was selected. The psychological and emotional state of patients (OHIP-49, HADS, GDS), the intensity and characteristics of the symptoms experienced (VAS, McGill Pain Questionnaire and PPI) were evaluated, with a follow up of 3 months after the end of treatment.

Results. A reduction of VAS was achieved in all patients, but this was not maintained over time in placebo group; significantly better results of McGill and the PPI were observed in the laser group, while results of OHIP-49, HADS and GDS were similar.

Discussion and conclusions. Regarding the average VAS, OHIP-49, HADS and GDS, the results obtained showed a similar pattern in both groups, while VAS values after each therapy session, McGill and PPI indicated lower values in the laser group, albeit not statistically significant. Symptoms improvement encountered, although of different magnitude, in both groups was probably due to the strong suggestion effect that a 10-sessions therapy arouses in patients with a clinical history full of therapeutic failures. Being placed in an “innovative” treatment may have contributed to strengthen the placebo effect.

References

- Coculescu EC, Radu A, Coculescu BI. Burning mouth syndrome: a review on diagnosis and treatment. *J Med Life.* 2014;7:512-515.
 - dos Santos Lde F, Carvalho Ade A, Leão JC, Cruz Perez DE, Castro JF. Effect of low-level laser therapy in the treatment of burning mouth syndrome: a case series. *Photomed Laser Surg.* 2011;29:793-796.
 - Nitzan U, Chalamish Y, Krieger I, Erez HB, Braw Y, Lichtenberg P. Suggestibility as a predictor of response to antidepressants: A preliminary prospective trial. *J Affect Disord.* 2015;185:8-11.
-

Experimental Study

Laser treatments and burning mouth syndrome

M. Bosotti, M. Porrini, G. P. Bombeccari, M. Rossi, F. Spadari

Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Unit of Oral Pathology and Medicine, Maxillo-Facial and Odontostomatology Unit, Ospedale Maggiore Policlinico, Fondazione IRCCS Ca'Granda, Italy

Background. Burning Mouth Syndrome (BMS) manifests as a burning sensation of the tongue, lips or entire oral cavity, in the absence of any objective lesions or laboratory test findings capable of accounting for the discomfort. The revised chronic pain classification of the International Headache Society (Headache Classification 2004) defines BMS as a burning sensation for which no medical or dental cause can be established, which is present for at least 4-6 weeks. BMS can be classified as primary type, when it is not associated to other local and/or systemic diseases. BMS has been associated to a neuropathic pathogenesis, affecting peripheral or central levels of the nervous system. At present, most of the studies for BMS treatment are lacking of controls, the measurements are not reproducible, and the sample sizes are limited. This case-control study aims to evaluate the change of symptoms after Low Level Laser Therapy (LLLT) in patients with primary type of BMS.

Material and methods. Fifty-two patients with diagnose of primary BMS, who were examined at the Oral Medicine Unit, Maxillo-Facial and Odontostomatology Unit, University of Milan, were randomly divided into 2 groups: a) test group, receiving LLLT local therapies; b) control group, receiving inactive/placebo laser. Six sessions were planned (T0-T6). Numeric Rating Scale (NRS), Visual Analogic Scale (VAS), Verbal Rating Scale (VRS), and McGill Pain Questionnaire (MGPPQ-RF) were evaluated according to subjective pain reporting.

Results. From T0 to T3 sessions were found no differences between both groups. In LLLT group (n= 24, mean age: 61.38 SD±10.14 years), based on the Student-t test, significant score differences have been found from T3 to T6 sessions (P= 0.012-0.00011), in all pain scales, *versus* control group (n=28, mean age: 60,18; SD±9,55).

Conclusions. LLLT for the treatment of the BMS discomfort can be considered an alternative tool to the relief of oral burning.

References

- Dos Santos Lde F, Carvalho Ade A, Leão JC, Cruz Perez DE, Castro JF. Effect of low-level laser therapy in the treatment of burning mouth syndrome: a case series. *Photomed Laser Surg.* 2011 Dec;29(12):793-6. doi: 10.1089/pho.2011.3016.
- Pezelj-Ribarić S, Kqiku L, Brumini G, Urek MM, Antonić R, Kuis D, Glazar I, Städtler P. Proinflammatory cytokine levels in saliva in patients with burning mouth syndrome before and after treatment with low-level laser therapy. *Lasers Med Sci.* 2013 Jan;28(1):297-301. doi: 10.1007/s10103-012-1149-5. Epub 2012 Jul 8.

Experimental Study

Prevalence of oral mucosal lesions in 13 to 18 year-old children

E. Bardellini, F. Amadori, N. Pedrini, F. Gorla, A. Majorana

University of Brescia, Department of Oral Pathology, Italy

Objective. The aim of this study was to determine the prevalence of oral lesions in 13 to 18-year-old adolescents.

Methods. A retrospective cross-sectional study was carried from January 2009 to December 2013 on children aged 13 to 18-year-old. Clinical charts were examined to collect data including age, gender, detection of pathological mucosal lesions and medical information.

Results. A total of 4873 adolescents without systemic diseases was enrolled. Out of the total sample, 1544 (31.7%) children showed one or more oral mucosal lesion. The correlation between occurrence of mucosal lesions and sex was not statistically significant ($p > 0.05$). The most frequent oral lesions detected were: linea alba (19.5%), ulcerative lesions (15.5%), herpes simplex virus (12.9%), candidiasis (10.7%), traumatic lesions (6.1%), geographic tongue (6.1%) and piercing related mucosal lesions (5.9%). Lesions related to papilloma virus, multiform erythema, oral lichen planus and Abrikosoff's tumour were also detected.

Conclusion. Even if data of our study correspond to those reported in Literature, "new" and rare types of lesions as piercing alterations and papilloma virus lesions were recorded; the knowledge of their frequency could lead to act on risk factors.

References

- Castellanos JL, Diaz-Guzman L, Guanajuato L. Lesions of the oral mucosa: an epidemiological study of 23785 Mexican patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:79-85.
- Majorana A, et al. Oral mucosal lesions in children from 0 to 12 years old: ten years' experience. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010;110:e13-e18.
- Parlak AH, et al. Prevalence of oral lesions in 13- to 16-year-old students in Duzce, Turkey. *Oral Diseases.* 2006;12:553-558.

Efficacy of tocopherol topical treatment of lichen planus oral reticular: randomized controlled double-blind, cross-over vs placebo

C. Bacci, V. Vanzo, L. Sbricoli, S. Sivoiella, M. Valente

Department of Neurosciences (Head D D'Avella), Section of Clinical Dentistry (Head Prof E Stellini), Unit of Oral Pathology and Medicine, University of Padova, Italy

This controlled, randomized, crossover, double-blind study was performed to evaluate the efficacy of tocopherol acetate in terms of improvement of the clinical aspect and the discomfort level in patients with oral reticular Oral Lichen Planus comparing the results a Placebo. We recruited 26 patients with clinical and histological diagnosis of OLP. Patients were divided in two groups (A and B) according to a randomization list generated by a specific software (SAS 9.2 for Windows, SAS Institute Inc., Cary, NC, USA). Both groups were assigned to one of two treatments, each lasting one month, then, after two weeks of wash-out (cross-over), the second treatment. Treatment consisted of tocopherol acetate in oily formulation, placebo was Paraffinum Liquidum. The primary variable was measured in discomfort, as measured by a visual analogue scale (VAS) with range of valid values from 0 to 10. Secondary variable was evaluated measuring the extension of the reticular lesions. This was assessed by measuring the extent of the lesions with a caliber and even take a picture at the time of each visit. The photos were then compared with each other using the program ImageJ, calibrating each photograph with measurements performed during clinical visits. Finally it has been applied a scale of scores to 6°, according to clinical criteria elaborated by Thongprasom. This study exploits the role of antioxidant tocopherol as a factor in preventing the oxidation of polyunsaturated fatty acids, in the development of the process of lipid peroxidation. Tocopherol has proven to be a valuable support in the management of OLP reticular, although asymptomatic. Surprisingly, we noticed significant differences ($P < 0.05$) between two treatment sequences for two periods, limited the extent of lesions and the scale of Thongprasom; while there has been no statistical significance ($P > 0.05$) in the comparison of VAS, nor between the lengths.

References

- Lodi G, Carrozzo M, Furness S, Thongprasom K. Interventions for treating oral lichen planus: a systematic review. *Br J Dermatol*. 2012 May;166(5):938-47.
- Thongprasom K, Carrozzo M, Furness S, Lodi G. Interventions for treating oral lichen planus. *Cochrane Database Syst Rev*. 2011 Jul 6;(7):CD001168.
- Keenan AV, Ferraiolo D. Insufficient evidence for effectiveness of any treatment for oral lichen planus. *Evid Based Dent*. 2011;12(3):85-6.

Polydatin: study of its effects on head and neck cancer

L. Lo Russo¹, F. Ardito¹, G. Capocasale², D. Perrone¹, L. Lo Muzio¹

¹Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; ²Department of Oral Sciences, Section of Oral Medicine, University of Palermo, Palermo, Italy

Objectives. Polydatin is a natural compound from *Polygonum cuspidatum*. A large number of studies have suggested that polydatin may have anticarcinogenic effects¹. In the present study, we evaluated the inhibitory effect of polydatin on cell proliferation in order to find one concentration able to act effectively on the head neck cancer. In addition, we also evaluated the action of polydatin on survivin; this inhibits programmed cell death and acts as a weapon of survival of the diseased cells. In fact, it is known that the inhibition of the protein survivin induces suicide in cancer cells.

Methods. *Cell cultures.* MG63, PE/CA-PJ15, PE/CA-PJ15D cells were cultured in DMEM with 10% FBS, 1% penicillin/streptomycin at 37 °C in a 5% CO₂.

MTT viability assay and xCELLigence system. After 24 hours of cellular growth, it was added polydatin, at the following concentrations: 5 μ M, 10 μ M, 20 μ M, 50 μ M and 100 μ M. To evaluate the cytotoxic effects of polydatin we used Vybrant MTT cell proliferation assay kit. With xCELLigence, the electronic readout of cell-sensor impedance is displayed in real-time as CI, a value directly influenced by the cell proliferation.

Western blotting analysis. The cells treated and not treated with polydatin were lysed after 24 hours. To assess the activity of polydatin we evaluated the expression of survivin.

Conclusions. MTT viability assay showed a lowering of cell viability proportional to the concentration of compound. The MG63 cells are more resistant and only in PE/CA-PJ15D there was a 50% reduction of vitality for the maximum concentration of 100 μ M. xCELLigence shows that by the addition of polydatin, after 24h there was a 50% reduction of cell viability at the concentration of polydatin of 50 μ M. Instead, there was a reduction of survivin in cells treated with polydatin. Therefore, the results suggest that polydatin has potential therapeutic applications in the treatment of head and neck cancer.

References

1. Soleas GJ, Goldberg DM, Grass L, Levesque M, Diamandis EP. Do wine polyphenols modulate p53 gene expression in human cancer cell lines? *Clin Biochem.* 2001;34:415-420.

Experimental Study

Prognostic value of clonality analysis in patients with secondary oral squamous cell carcinoma

C. Amadasi¹, L. Morandi², A. Tarsitano³, A. Gabusi¹, D. B. Gissi¹

¹Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy; ²Department of Biomedical and Neuro-motor Sciences, Section of Anatomic Pathology "M. Malpighi" at Bellaria Hospital, University of Bologna, Italy; ³Department of Biomedical and Neuromotor Sciences, University of Bologna, Section of Maxillo-facial Surgery at Policlinico S. Orsola-Malpighi, Bologna, Italy

Introduction. mtDNA (D-loop) sequence analysis was proposed as a reliable method for establishing the clonal relationship of a second tumor lesion with the index tumor^{1,2}, because this is not a simply problem of classification but may influence prognosis and the choice of treatment. In the present study, mtDNA D-loop analysis was utilized in patients experiencing a second loco-regional neoplastic manifestation after surgical resection of a primary Oral Squamous Cell Carcinoma (OSCC); the purpose was to evaluate differences in terms of survival rate related to the clonal relationship between primary and second manifestation.

Material and Methods. 29 patients treated for a primary OSCC showed a second loco-regional neoplastic manifestation: 21/29 (72,4%) were limited to the oral cavity whereas 8/29 (27,6%) presented a neck nodal metastasis (LNM) as second event. mtDNA D-loop analysis was performed by deep sequencing and phylogenetic clusterization in all samples. Disease-free survival endpoints was defined as the duration between surgical resection and dead of disease or last follow-up visit.

Results. mtDNA analysis showed 9/29 cases (31,1%) phylogenetically related to index OSCC and 20/29 cases (68,9%) phylogenetically independent. The presence of a clonal relationship between primary and second tumor resulted a variable significantly related with a lower survival rate ($p < .04$); indeed 7/9 events phylogenetically related to index OSCC (77.7%) failed as compared to 6/20 second events phylogenetically independent (30%). No differences in terms of survival rate was found between second events presenting as LNM or limited to the oral cavity.

Conclusions. The presence of a clonal relationship between primary and secondary manifestations in patients with multiple OSCCs is a variable that influences the prognosis and mtDNA analysis may be considered a useful tool to discriminate secondary neoplastic lesions that have developed along independent or similar genetic pathways.

References

1. Morandi L, Tarsitano A, Gissi D, Leonardi E, Balbi T, Marchetti C, Montebugnoli L, Foschini MP. Clonality analysis in primary oral squamous cell carcinoma and related lymph-node metastasis revealed by TP53 and mitochondrial DNA next generation sequencing analysis. *J Craniomaxillofac Surg.* 2015.
2. Montebugnoli L, Leonardi E, Morandi L, Farnedi A, Gissi DB, Marchetti C, Tarsitano A, Balbi T, Gentile L, Cocchi R, Foschini MP. Genetic relationship between multiple squamous cell carcinomas arising in the oral cavity. *Head Neck.* 2013 Apr 30.

Prognostic value of Ki67 from “normal” distant mucosa in patients surgically treated for oral squamous cell carcinoma

L. Alessi¹, A. Tarsitano², E. Dioto³, A. Gabusi¹, D. B. Gissi¹

¹Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy; ²Department of Biomedical and Neuromotor Sciences, University of Bologna, Section of Maxillo-facial Surgery at Policlinico S. Orsola-Malpighi, Bologna, Italy; ³Department of Biomedical and Neuromotor Sciences, Section of Anatomic Pathology at Bellaria Hospital, University of Bologna, Italy

Introduction. The aggressive behavior and long-term prognosis of Oral Squamous Cell Carcinoma (OSCC) have recently been related to the mucosa surrounding the primary mass, consisting of genetically altered cells that might be responsible for cancer progression. In two previous studies Ki-67 index in oral “non-neoplastic” mucosa distant from primary mass was used as a surrogate for presence of an abnormal proliferative status and proposed as prognostic marker^{1,2}. Aim of the present study was to evaluate in a group of patients treated for OSCC with long-term follow-up whether Ki67 in distant mucosa is associated with a poor prognosis in terms of Local Recurrence (LR), Second Primary Tumor (SPT) or Lymph Node Metastasis (LNM).

Material and Methods. 55 patients underwent surgery for OSCC from 2004 to 2009. Disease-free survival endpoints were defined as the duration between surgical resection and the diagnosis of LR, SPT or LNM or last follow-up visit. Proliferative status in distant areas (opposite cheek) was evaluated by immunohistochemical expression of Ki67. A Ki67 value >20% was considered “abnormal” as reported in previous studies.

Results. All disease-free patients had a minimum of 12-month-follow-up (mean 51,46 ±33,23 months; range 12-110 months). 23/55 (41,8%) patients developed a second loco/regional manifestation. 17/55 (30,9%) patients presented an “abnormally high” Ki67 value in the distant mucosa at the time of surgery. Multivariate analysis showed that Ki67 value in distant mucosa was a powerful independent prognostic factor for the disease-free survival rate, greater than tumor differentiation or lymph node positive at diagnosis. Furthermore Ki67 resulted the only variable statistically related with poor prognosis in T1-2N0 OSCCs.

Conclusions. The present study confirmed Ki67 in distant area as an important prognostic factor for OSCC patients and it could be included in the list of the clinic-pathological markers utilized for therapeutic protocol.

References

1. Montebugnoli L, Badiali G, Marchetti C, Cervellati F, Farnedi A, Foschini MP. Prognostic value of Ki67 from clinically and histologically “normal” distant mucosa in patients surgically treated for oral squamous cell carcinoma: a prospective study. *Int J Oral Maxillofac Surg.* 2009 Nov;38(11):1165-72.
2. Montebugnoli L, Gissi DB, Badiali G, Marchetti C, Cervellati F, Farnedi A, Foschini MP. Ki67 from clinically and histologically “normal” distant mucosa as prognostic marker in early stage (T1-T2N0) oral squamous cell carcinoma: a prospective study. *J Oral Maxillofac Surg.* 2011 Oct;69(10):2579-84.

Comparison of CBCT images with Dental Scan and Maxillofacial CT in diagnosis of ONJ lesions

G. Lorenzini¹, A. Addamo¹, M. Viviano²

¹Siena University, Medical Biotechnologies Department, Odontostomatology Section, Italy; ²Azienda Ospedaliera Universitaria Senese - U.O.C. Odontostomatology, Italy

Objective. Aim of this study was to evaluate the quality of radiographic images taken with different techniques (CBCT, Dental Scan and Maxillofacial CT) as tool for diagnosis of MRONJ.

Study design. Inside the people attended in our Department for ONJ, we selected 22 patients showing clinical or radiologic signs of ONJ where, at the same time, Maxillofacial CT or Dental Scan were unable to pinpoint diagnosis or lesion extension and, therefore, the correct therapy.

After a sharp clinical examination of the suspected area this was subjected to a small FOV CBCT, and these images were compared with other images owned by the patient (Maxillofacial CT or Dental Scan).

Single CBCT-image was compared, respectively, with Maxillofacial CT- or Dental Scan-image of the same area; in the X-ray two skilled referee (expert Radiologist and Dentist) had to examine 4 different structures (tooth, cancellous bone, lamina dura and soft tissue) and to state a judgment about its quality (1 = poor, 5 = excellent).

Results. Statistical evaluation of collected elements highlighted a very better quality of CBCT images *versus* Maxillofacial CT/Dental Scan ones. This technique allowed the identification of bone lesions when others were uncertain or, even, negative. The better definition of CBCT images may be able to diagnose early lesions and to show sharp particular and real extension of bone lesions.

At the least the ionizing radiation lower rate for the patient, make this kind of investigation preferable to others for ONJ study but, probably, for many others dental pathologies.

References

- Assaf At, Zrnc Ta, Remus Cc, Adam G, Zustin J, Heiland M, Friedrich Re, Derlin T. Intraindividual comparison of preoperative ^{99m}Tc-MDP spect/ct and intraoperative and histopathological findings in patients with bisphosphonate- or denosumab-related osteonecrosis of the jaw. *J craniomaxillofac surg.* 2015 Jun 27.
- Miyashita H, Shiba H, Kawana H, Nakahara T. Clinical utility of three-dimensional spect/ct imaging as a guide for the resection of medication-related osteonecrosis of the jaw. *Int j oral maxillofac surg.* 2015.

Experimental Study

Synergic effects of curcumin and polydatin on MG63-osteosarcoma cells proliferation are mediated through down-regulation of Survivin

G. Troiano¹, F. Ardito¹, D. Perrone¹, N. Termine², L. Lo Muzio¹

¹Department of Clinical and Experimental Medicine, University of Foggia, Italy; ²Department of Surgical, Oncologic and Stomatologic Sciences, University of Palermo, Italy

Nutraceuticals agents are among the most promising chemoprevention and treatment options for the management of cancer. Curcumin (diferuloylmethane), commonly known as turmeric, is a polyphenol derived from the curcuma longa plant. Curcumin has been found to possess anticancer activities via its effect on a variety of biological pathways involved in mutagenesis, apoptosis, tumorigenesis, cell cycle regulation and metastasis¹. More recently, it has been demonstrated that curcumin possesses antioxidant, anti-inflammatory and anticancer properties. Polydatin is a phytoalexin, molecules produced by spermatophytes plants to protect germinal centers, fruits, and roots, by attack by fungi, bacteria or free radicals². Both these molecules have demonstrated anticancer activity in many types of pre-clinical models. Curcumin activity seems to be mediated through the inhibition of NF-κB and MAPK; while Polydatin acts as an antioxidant agent. Up today, nobody have evaluated a possible effect of combination of these two agent on cancer preclinical models. In this work, we evaluated a combination schedule of curcumin and polydatin against MG63 (doxorubicin resistant) osteosarcoma cells. Cells viability has been evaluated through MTT assay and xCELLigence system³; we found a synergic effect of this two agent against MG63 proliferation. In addition, after Real-time PCR and Western Blot analysis, we found a strong downregulation of survivin expression mediated by the combination therapy.

References

1. Zlotogorski A, Dayan A, Dayan D, Chaushu G, Salo T, Vered M. Nutraceuticals as new treatment approaches for oral cancer-I. Curcumin. *Oral oncology.* 2013;49:187-91.
2. De Maria S, Scognamiglio I, Lombardi A, et al. Polydatin, a natural precursor of resveratrol, induces cell cycle arrest and differentiation of human colorectal Caco-2 cell. *Journal of translational medicine.* 2013;11:264.
3. Dickhuth J, Koerd S, Kriegebaum U, et al. In vitro study on proliferation kinetics of oral mucosal keratinocytes. *Oral surgery, oral medicine, oral pathology and oral radiology.* 2015.

Orofacial granulomatosis: a case series

S. Tovu¹, I. Parlatescu¹, C. Gheorghe¹, L. Neacsu¹, M. Tovu²

¹Oral Medicine/Oral Pathology Discipline, Faculty of Dental Medicine, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania; ²Dermatology Discipline, Faculty of Dental Medicine, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

Orofacial granulomatosis (OFG) is a rare disease characterised by non-caseating granulomatous inflammation in the absence of any recognized systemic condition¹. It presents a variety of clinical features such as persistent enlargement of the soft tissues of lips, face and mucosa, fissured tongue, mucosal tags, linear oral ulceration and a cobblestone appearance of buccal mucosa^{2,3}.

The aim of this study is to describe the demographical data, clinical features and laboratory findings in OFG patients.

Material and Methods. Clinical and laboratory data for 24 OFG patients who attended the Oral Medicine Department in Bucharest, Romania between 1999 to 2014 were reviewed from the medical charts.

Results. The study group consists of 24 patients: 18 women and 6 men, with median age of 49 years. 17 (71%) patients had recurrent orofacial swelling, 4 (17%) permanent swelling and 1 recurrent swelling with a stable character after the last episode. Facial nerve palsy was present in the history of 6 cases. The swelling involved only the lower lip in 8 (33%) cases, only the upper lip in 6 (25%), both lips in 7 (29%). The oral manifestations were present in all patients thus: swelling of the oral mucosa in all cases, cobblestone like appearance in 8 (33%) and fissured tongue in 3 (12%). Topographically the swelling involved the buccal mucosa in 7 cases, the inner lower lip in 5, and inner upper lip in 2 cases. The dental infectious foci present in 14 (58%) cases, were suppressed in 11 and a complete remission was subsequently detected in 1 patient. Biopsies and histological diagnosis were done in 19 (79%) cases. In 23 cases the final diagnosis was incomplete Melkersson-Rosenthal Syndrome (MRS) and 1 case had complete MRS.

Conclusions. The data of our series show a variable clinical appearance of OFG. Most of OFG cases are incomplete Melkersson-Rosenthal syndrome. The dental foci play an important role and their removal should be considered the first step in OFG management.

References

1. Grave B, McCullough M, Wiesenfeld D. Orofacial granulomatosis-a 20-year review. *Oral Dis.* 2009;15(1):46-51.
2. McCartan B, Healy C, McCreary C, Flint S, Rogers S, Toner M. Characteristics of patients with orofacial granulomatosis. *Oral Dis.* 2011;17(7):696-704.
3. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L. The multiform and variable patterns of onset of orofacial granulomatosis. *J Oral Pathol Med.* 2003;32(4):200-05.

No association between periodontal bacterial load and Alzheimer's disease: preliminary data from a rural Sicilian community

N. Termine¹, G. Campisi¹, L. Laino², L. Lo Muzio², R. Monastero³

¹Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy; ²Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; ³Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Palermo, Italy

Aim. In recent years, chronic inflammation sustained by periodontal disease (PD) has been suggested as a potential risk factor in Alzheimer's disease (AD). Some evidences suggested a possible promoting direct or indirect role sustained by periodontal bacteria. Aim of the present study was to compare the prevalence/severity of PD and the bacterial periodontal load in patients with and without AD.

Patients and methods. A case-controlled clinical trial was designed and all participant were enrolled in a Sicilian rural community (Sambuca di Sicilia, AG, Italy). The test group (T) included 46 patients with AD (M: 20; F: 26, range

age 64-98 yrs); 46 cognitively normal adult individuals were selected as control group (C), matched for age and sex (M: 20; F:26; range age 64-92 yrs). The number of teeth and measurement of the probing depth (using CPI and PSR index) were registered. All subjects, excluding totally edentulous ones, were subjected to the sampling of sub-gingival plaque and successive RT PCR analysis for the quantitative determination of six marker organisms of PD (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *F. nucleatum* and *P. intermedia*) using Carpegen® Perio Diagnostics kit. Data were analysed using univariate analysis.

Results. 18/46 AD patients (39.1%) were totally edentulous, conversely 13/46 (28.3%) of controls have the same condition ($p=0.27$). High PSR and CPI scores (≥ 3) were recorded among partially edentulous patients in both groups (T:11/28; 39% vs C:18/33;54%) ($p=0.23$). Microbiological tests did not show significant differences among both groups, with similar median average scores of periodontal bacterial load in AD and normal patients. Only one AD patient resulted positive to *A. actinomycetemcomitans*, main marker of PD.

Conclusion. In this rural Sicilian elderly community, the hypothesis of a major severity of PD and of a greater periodontal bacterial load in AD patients rather than normal individuals is not confirmed.

References

- Noble JM, et al. Poor oral health as a chronic, potentially modifiable dementia risk factor: review of the literature. *Curr Neurol Neurosci Rep.* 2013;13(10):384.
- Singhraj SK, et al. Oral inflammation, tooth loss, risk factors, and association with progression of Alzheimer's disease. *J Alzheimers Dis.* 2014;42(3):723-37.
- Stein PS, et al. Tooth loss, dementia and neuropathology in the Nun study. *J Am Dent Assoc.* 2007;138(10):1314-22.

Experimental Study

Oral cancer and precancer in bone marrow transplanted patients affected by chronic graft versus host disease

A. Tempesta¹, L. Limongelli¹, V. Zurlo¹, E. Maiorano², G. Favia¹

¹Complex Operating Unit of Odontostomatology, Department of Interdisciplinary Medicine, "Aldo Moro" University of Bari, Italy; ²Complex Operating Unit of Pathological Anatomy, Department of Emergency and Organ Transplantation, "Aldo Moro" University of Bari, Italy

Background. Chronic Graft-Versus-Host Disease (cGVHD) is an immune regulatory disorder presenting as a frequent complication of allogeneic Bone Marrow Transplantation (aBMT). The pathogenesis is probably related to the attack of the donor immune cells against the recipient tissues. Frequently, the first symptoms of the systemic cGVHD are the oral manifestations presenting as atrophic-erosive and/or hyperkeratotic lesions that clinically mimic autoimmune diseases such as Lichen Planus, with oral inflammation and erythema, atrophy, tongue depapillation, hyposalivation and pain.

This work was aimed at describing the development of oral cancer and precancer in patients affected by cGVHD, thus suggesting the importance of periodical follow-up to detect premalignant or malignant lesions at an early stage.

Methods. Forty patients who underwent aBMT from consanguineous and with a diagnosis of cGVHD came to our attention to the Complex Operating Unit of Odontostomatology, Policlinic of Bari; 19 of them showed 47 lesions on different oral sites which clinically appeared as:

- leuko-erythroplastic lesions (79%)
- ulcers (12%)
- nodules (9%).

All these lesions underwent incisional biopsy and histopathological analysis; specifically for neoplastic tissues, 6 prognostic factors were also evaluated: tumor thickness, invasion pattern (single cell, large front), vascular, neural, salivary glands ducts and muscle infiltration.

Results. All the surgical wounds healed without complications. The histopathological examination of the surgical samples highlighted that:

- 52% were oral cancers
- 31% were precancerous lesions (OIN 1 and 2)
- 17% were hyperkeratotic or lichenoid lesions.

Conclusions. Oral cancer and precancer represent a serious complication in cGVHD patients. Therefore, a periodic follow-up is recommended to detect these premalignant and malignant lesions at the initial stage with a better prognosis.

References

- de Araújo RL, Lyko Kde F, Funke VA, Torres-Pereira CC. Oral cancer after prolonged immunosuppression for multiorgan chronic graft-versus-host disease. *Rev Bras Hematol Hemoter.* 2014;36(1):65-8.
- Hull K, Kerridge I, Avery S, McCullough M, Ritchie D, Szer J. Oral chronic graft-versus-host disease in Australia: clinical features and challenges in management. *Intern Med J.* 2015;45(7):702-10.
- Mays JW, Fassil H, Edwards DA, Pavletic SZ, Bassim CW. Oral chronic graft-versus-host disease: current pathogenesis, therapy, and research. *Oral Dis.* 2013;19(4):327-46.

Experimental Study

Oral brushing as a useful method to show aberrant methylation pattern in genes from adjacent and distant mucosa in OSCC patients

L. Sozzi¹, L. Morandi², M. De Luca², V. Monti², D. B. Gissi¹

¹Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy; ²Department of Biomedical and Neuro motor Sciences, Section of Anatomic Pathology "M. Malpighi" at Bellaria Hospital, University of Bologna, Italy

Introduction. The high recurrence rate of Oral Squamous Cell Carcinoma (OSCC) may be explained by the persistence of genetically altered mucosa after surgery. It has been supposed that normal mucosa adjacent to OSCC can have epigenetic alterations, e.g. an aberrant methylation pattern¹. Aim of the present study was to analyze the DNA methylation pattern in a set of 12 genes, evaluating oral brushing specimens from OSCCs, oral mucosa in the surgical site of OSCC resection and their respective normal counterpart.

Methods. We collected oral brushing specimens from the following groups: 5 OSCC (1a) and their respective normal counterpart (1b), 5 samples from the site of surgical OSCC resection with no evidence of dysplasia on the margin of resection (2a) and their normal counterpart mucosa (2b), 4 samples from the site of surgical OSCC resection with presence of dysplasia on the margin of resection (3a) and their normal counterpart mucosa (3b) and 7 healthy controls (4). A set of previously described differentially methylated genes in OSCC were investigated by bisulfite-Target NGS using MiSEQ platform (Illumina, San Diego, CA). The statistical significance between different groups was investigated using Wilcoxon-Mann-Whitney test.

Results. All genes were altered in OSCC with respect to controls; 4 genes showed the same methylation pattern in OSCC and in the respective normal counterparts. Mucosa in surgical site also showed 11 altered genes with respect to controls and 6 genes with the same pattern with respect to their normal counterpart revealed. No differences in any of the 12 genes were found between the dysplastic and non dysplastic mucosa from surgical resection sites.

Conclusions. The analysis of gene methylation pattern in epithelial cells collected by oral brushing has permitted to demonstrate the presence of genes with an aberrant methylation pattern in adjacent and also distant areas from OSCC.

References

1. Langevin SM, Butler RA, Eliot M, Pawlita M, Maccani JZ, McClean MD, Kelsey KT. Novel DNA methylation targets in oral rinse samples predict survival of patients with oral squamous cell carcinoma. *Oral Oncol.* 2014;50(11):1072-1080.

Experimental Study

Nuclear Survivin as prognostic factor in squamous cell carcinoma of the oral cavity

A. Santarelli¹, M. Mascitti¹, C. Rubini², M. Procaccini¹, L. Lo Muzio³

¹Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy; ²Department of Biomedical Sciences and Public Health, Marche Polytechnic University, Ancona, Italy; ³Department of Clinical and Experimental Medicine, Foggia University, Foggia, Italy

Oral squamous cell carcinoma (OSCC) as well as most human tumours is characterized by an imbalance of regulatory mechanisms controlling cell process as apoptosis. Survivin, a member of inhibitor of apoptosis family, is over-expressed in most solid and haematological malignancies and correlates with reduced overall survival rate. Thus, the aim of this study was to correlate nuclear Survivin expression with clinico-pathological data and prognosis in OSCC patients. A total of 152 OSCC samples from 96 males and 56 females were immunohistochemically investigated for nuclear Survivin expression. Then, Survivin was semi-quantitatively scored by an immunoreactivity score (IRS), calculated multiplying the percentage of positive cells with the staining intensity. Through the use of digital image analysis software, OSCC patients were scored in 4 groups according to their IRS (group 1, IRS 0-2; group 2, IRS 3-4; group 3, IRS 6-8; group 4, IRS 9-12). When comparing T stage, patients with small tumor size showed lower Survivin levels than those found in patients with tumors of larger size ($p < 0.05$). These results suggest a significant relationship between tumor size and nuclear Survivin expression. Furthermore, the patients with a lower IRS score displayed a statistically significant better survival rates than patients with a higher IRS score. Since the expression of Survivin at nuclear level seems to suggest a poor prognosis in OSCC patients, the evaluation of nuclear Survivin IRS may identify patients with more aggressive and disseminated disease, influencing follow-up and therapeutic protocols.

References

- Engels K, Knauer SK, Metzler D, Simf C, Struschka O, Bier C, et al. Dynamic intracellular survivin in oral squamous cell carcinoma: underlying molecular mechanism and potential as an early prognostic marker. *J Pathol.* 2007 Apr;211(5):532-40.
- Preuss SF, Weinell A, Molitor M, Stenner M, Semrau R, Drebber U, et al. Nuclear survivin expression is associated with HPV-independent carcinogenesis and is an indicator of poor prognosis in oropharyngeal cancer. *Br J Cancer.* 2008 Feb 12;98(3):627-32.

Experimental Study

Role of podoplanin expression as predictive marker in oral leukoplakia

A. Ruggeri¹, A. Gabusi¹, E. Dioto², V. Monti², D. B. Gissi¹

¹Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy; ²Department of Biomedical and Neuromotor Sciences, Section of Anatomic Pathology at Bellaria Hospital, University of Bologna, Italy

Objectives. Recent studies have identified podoplanin, a mucin type transmembrane glycoprotein, as a biomarker for oral cancer risk in patients with oral leukoplakia (OL)¹⁻³. Podoplanin-positive cells in the epithelium may represent upward clonal expansion of stem cells during carcinogenesis, and oral potentially malignant disorders with such clonal expansion may imply higher risk of malignant transformation. The present study investigated a group of OLs, to evaluate a possible relationship between high expression of podoplanin and high grade dysplasia, and to compare the results from podoplanin with those obtained from two widely used predictive markers.

Methods. The study population consisted of 20 consecutive patients with a single white lesion clinically and histologically diagnosed as OL; 3 samples showed High Grade Squamous Intraepithelial Lesion (HG SIL), while 17 samples showed Low Grade Squamous Intraepithelial Lesion (LG SIL). Distinction between HG SIL and LG SIL was performed according to Ljubjana classification 2014. Immunohistochemical expression of podoplanin, p53 and Ki67 was analyzed in all samples. Lesions with podoplanin expression in one or more areas of the suprabasal layer of the epithelium (pattern 2 in Kawaguchi classification) were considered as positive, whereas the cut off for both p53 and Ki67 overexpression was set to 20%.

Results. Podoplanin resulted positive in 2 out of the 3 samples with HG SIL, while showed positivity in 2 out of 17 samples with LG SIL (Chi squared 4.8; $p < .02$). P53 and Ki67 were also overexpressed in 2/3 samples with HG SIL, but did also show overexpression in LG SIL samples (respectively 4 p53 positive, and 13 Ki67 positive).

Conclusions. All 3 markers have demonstrated a good sensitivity to detect samples with HG SIL; however podoplanin was the only marker that associated a good sensitivity to a very high specificity and can be a promising marker to discriminate OL at high risk of developing cancer.

References

- de Vicente JC1, Rodrigo JP, Rodriguez-Santamarta T, Lequerica-Fernández P, Allonca E, García-Pedrero JM. Podoplanin expression in oral leukoplakia: tumorigenic role. *Oral Oncol.* 2013 Jun;49(6):598-603.
- Kawaguchi H, El-Naggar AK, Papadimitrakopoulou V, Ren H, Fan YH, Feng L, Lee JJ, Kim E, Hong WK, Lippman SM, Mao L. Podoplanin: a novel marker for oral cancer risk in patients with oral premalignancy. *J Clin Oncol.* 2008 Jan 20;26(3):354-60. doi: 10.1200/JCO.2007.13.4072.

3. Kreppel M1, Kreppel B, Drebbler U, Wedemayer I, Rothamel D, Zöller JE, Scheer M. Podoplanin expression in oral leukoplakia: prognostic value and clinicopathological implications. *Oral Dis.* 2012 Oct;18(7):692-9.

Experimental Study

Oral psoriasis: an observational study

F. Rocchetti¹, G. Palaia¹, M. Caputo¹, A. Richetta², U. Romeo¹

¹Department of Oral and Maxillo-Facial Sciences, "Sapienza" University of Rome, Italy; ²Department of Internal Medicine and Medical Specialties, "Sapienza" University of Rome, Italy

Psoriasis (PSO) is a chronic immune-mediated inflammatory disease involving mainly skin and joint. It has a bimodal age distribution with the earlier peak incidence between 15 and 30 years of age (Type I) while the second one is between 50 and 60 years of age (Type II). PSO is classified clinically into several subtypes: vulgaris, erythrodermic, guttate, pustular, inverse, onychodystrophic and psoriatic arthritis.

Even though it is a common disorder, in the International Literature there are only few publications about oral PSO. Oral manifestations can be divided in two categories: the first ones are authentic psoriatic lesions confirmed by biopsy and with a parallel course with skin lesions and the second ones are non-specific lesions such as Fissured Tongue (FT), Geographic Tongue (GT), Periodontitis, Candidosis and Temporo-Mandibular Joint Disorders (TMJD).

The aim of this study is to detect the prevalence of oral mucosal lesions in 60 psoriatic patients (Study Group) enrolled at the Department of Internal Medicine and Medical Specialties, "Sapienza" University of Rome, comparing with 100 healthy patients (Control Group) enrolled at the Department of Oral and Maxillo-Facial Sciences, "Sapienza" University of Rome.

After clinical examination, further specific exams such as Panoramic XR, TMJ Magnetic Nuclear Resonance (MNR), parodontal evaluation, oropharyngeal buffer and incisional biopsies by scalpel were performed when necessary.

Psoriatic patients present FT in 48,4% of cases, GT in 10%, parodontal disease in 40% and TMJD in 21,6%. In spite of this, healthy patients were affected by FT in 18% of cases, GT in 3%, parodontal disease in 12% and TMJD in 6%. In no case was observed Candidosis.

Due to the strong association between PSO and oral lesions, a cooperation between dermatologists and oral pathologists is mandatory. The aim should be to achieve and to maintain an oral health status in order to reduce the risk of infections that may exacerbate the PSO.

References

- Yesudian PD, Chalmers RJ, Warren RB, Griffiths CE. In search of oral psoriasis. *Arch Dermatol Res.* 2012 Jan;304(1):1-5.
- Darwazeh AMG, Al-Aboosi MM, Bedair AA. Prevalence of oral mucosal lesions in psoriatic patients: A controlled study. *J Clin Exp Dent.* 2012;4(5):e286-91.

Experimental Study

A retrospective cohort study on endodontic treated teeth in patients with a history of bisphosphonate therapy

C. Prati, C. Pirani, F. Viesti, L. Montebugnoli, M. G. Gandolfi

Department of BioMedical and Neurological Sciences, (DIBINEM) Master in Endodontology, School of Dentistry, Alma Mater Studiorum, University of Bologna, Italy

Background. A number of clinical studies suggest that extraction of compromised teeth must be limited in patients which received bisphosphonate therapy to prevent the formation of so-called "Medicated related osteonecrosis of jaw (Mronj)". It would seem prudent to recommend in these patients some less-invasive alternatives to extraction. Only few data are available on the survival rate of seriously compromised teeth that were endodontically treated to prevent extraction.

Aim. The purpose of this retrospective cohort clinical study was to evaluate the survival rate and healing time of compromised teeth (group 1; n= 24) and seriously compromised teeth (group 2; n= 32) that were endodontically treated in patients which received bisphosphonate therapy for at least 12 months (mean duration 3.7±0.9 y). The complication observed during and after endodontic therapy were reported. Healing time of periapical lesions was also reported.

Materials and methods. Thirty-nine patients (mean age 74, range 49-87 years) were treated for a total of 56 teeth. Twenty-five patients were in stage 0, ten in stage 1 and four in stage 2-3. All patients in stage 2-3 received Zometa. Twenty four teeth (group 1) were rehabilitated with a conventional restoration or crown. The seriously compromised teeth (group 2) received only endodontic therapy and a free-occlusal definitive restoration to prevent occlusal load. All teeth presented moderate mobility and PD ranged from 3 to 5 mm. High mobility and PD > 8 mm were considered as contraindication to perform endodontic therapy.

Results. One patient from group 2 developed a Mronj and fast expulsion of 2 incisors 3 weeks after endodontic therapy followed by healing of the area. Another patient in group 2 developed a chronic fistula. Mean healing time of periapical lesion was approx. 9 months. No periapical lesions were developed after endodontic treatment. No root fractures were reported.

Conclusions. Endodontic treatment resulted a suitable therapy to conserve seriously compromised (and hopeless) teeth affected by deep carious lesions with/without acute/chronic periapical lesions and to avoid extraction risks. The study represents a preliminary protocol to evaluate clinical parameters and to define type of patient for a randomized clinical study to compare endodontic therapy *versus* extraction.

Experimental Study

Clinical cohort study to evaluate survival rate of endodontically treated premolars *versus* implant placement

C. Prati, F. Zamparini, C. Pirani, G. A. Pelliccioni, M. G. Gandolfi

Master in Endodontology, School of Dentistry, DIBINEM, University of Bologna, Alma Mater Studiorum, Italy

The survival rate of endodontically treated premolars is still a great clinical challenge. Anatomical conditions, occlusal stress, infection of root canals are clinical limits and may represent clinical criteria to replace teeth with osteointegrate implants. On the other hands, surgical and economic problems may force to keep premolar and preserve their function.

This longitudinal "best practice" retrospective clinical study was design to evaluate the critical steps in the decisions to treat or replace compromised premolars affected by periapical lesions. Survival rate and other critical parameters were evaluated after 6 months, 1 year, 2 years.

Forty-one patients treated from January 2010 to December 2013 in the Clinical Endodontic Section of School of Dentistry were recalled. Preoperative periapical status (radiography and clinical symptoms/signs) scored as PeriApical Index (PAI), crown condition, post presence, periodontal parameters and condition of crown tissue were obtained by Clinical Chart of each patient. The decision to replace or to treat/retreat roots was also recorded. Premolars were endodontically treated (group EnT; n=21) or extracted and replacement by implant (group ImP; n= 20). Each patient was recalled after 6 months to monitor the therapy and re-evaluated after 2 years for: survival rate of premolar/implant, bone defect around tooth/implant, periodontal parameters around tooth/implant, secondary caries. Time to complete the therapy and cost for the entire treatment (endodontic treatment/post/provisional and definitive crown versus extraction/implant placement, provisional and definite crown) were calculated by the university price list.

Results. Seven patients were excluded from the study as not able to attend the recall program. Thirty-four patients were evaluated.

One patient in group ExI lost implant after 9 months. Two patients in group EnT had root fractures after 10 and 12 months from root therapy.

The cost for the entire therapy was 2.410 Euro for group Imp *versus* 1.660 Euro for EnT. The number of appointments was 13±3 for Imp *versus* 10±3 for EnT. The mean time to complete the rehabilitation was 7±2 months for Imp group *versus* 6±8 months for EnT. The clinical and radiographic healing time for ExI group was 6 months *versus* 9 months for EnT.

Conclusions. After 2 years both treatments showed similar results and showed a reduced number of clinical problems. Implant therapy may reduce healing time, but requires a greater number of appointments and higher cost.

Low Level Laser Therapies (LLLTs) in the oral surgery

M. Porrini, G. P. Bombeccari, M. Bosotti, M. Rossi, F. Spadari

Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Unit of Oral Pathology and Medicine, Maxillo-Facial and Odontostomatology Unit, Ospedale Maggiore Policlinico, Fondazione IRCCS Ca'Granda, Italy

Background. Surgical removal of impacted mandibular third molars (IMTMs) is one of the most common outpatient procedures in oral surgery. Pain is one of the most common symptoms in the post-operative phases. Considering the local effects of the LLLTs, the aim of this prospective, randomized, double blind case-control clinical trial was to evaluate the efficacy of LLLT for decreasing post-operative pain in patients undergoing surgical removal of IMTMs.

Material and methods. Forty-four patients were enrolled to undertake the surgical removal of IMTMs, in accordance with the classification of Winter and Pell and Gregory with the values ranging from 6 to 8. Patients were randomly divided into 2 groups: a) LLLT test group, receiving real LLLT after completion of the suture; b) control group, receiving inactive laser treatments. Five sessions were planned (T0-T5). Numeric Rating Scale (NRS), McGill Pain Questionnaire Short-Form (MGPQ-SF) and Relief Scale (RS) were evaluated according to subjective pain and discomfort reported.

Results. Based on the Student-t test, both groups (test group: N=20, mean age: 29.85; SD±3.15; control group: N=24, mean age: 29.71; SD±3.04), have showed a decrease in pain and discomfort intensity by starting from T0 to T5 sessions, markedly more significant in the test group (P=0.001-0.005), compared with the control group (P=0.02-0.04). In comparison, the test group recorded a constant decreasing of the symptoms (NRS: P=0.044-0.005; MGPQ-RF: P=0.042-0.0005) *versus* control group.

Conclusions. The irradiation with LLLT in post-extractive sites of IMTMs can represent an additional procedure for better control of postoperative pain and discomfort.

References

- Paschoal MA, Santos-Pinto L. Therapeutic effects of low-level laser therapy after premolar extraction in adolescents: a randomized double-blind clinical trial. *Photomed Laser Surg.* 2012 Sep;30(9):559-64. doi: 10.1089/pho.2012.3243. Epub 2012, Aug 7.
- Vescovi P, Meleti M, Merigo E, Manfredi M, Fornaini C, Guidotti R, Nammour S. Case series of 589 tooth extractions in patients under bisphosphonates therapy. Proposal of a clinical protocol supported by Nd:YAG low-level laser therapy. *Med Oral Patol Oral Cir Bucal.* 2013 Jul 1;18(4):e680-5.

Bibliometric analysis of studies on oral medicine

R. Pluchino, C. Soru, G. Lodi, E. M. Varoni

Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Italy

Objectives. As previous literature points out^{1,2}, bibliometric analysis of published works can help to better understand the trends of research and which topic affected mostly the scientific and clinical scenarios. Our aim was to perform a bibliometric examination of the articles published in Oral Medicine, via consulting the leading databases.

Materials and methods. We searched articles on the ISI Web of Science platform considering all those journals under the category of "Dentistry, Oral surgery and Medicine", "Dermatology", "Oncology" and "Otorhinolaryngology". Other bibliographic resources, i.e. Google Scholar and Scopus, were also consulted. For each article, we retrieved number of citations, number and names of the authors, name of the journal, year of publication, the 2014 impact factor of the journal, type of study, design of study and area of research.

Results. We classified the articles considering the number of citations. Among the journals with the largest number of the most cited articles, we found the New England Journal of Medicine and the Journal of Clinical Oncology. On what concerned the type of articles, papers mainly referred to clinical observational studies and narrative reviews.

Conclusions. The analysis of the bibliometric indexes of articles published on Oral Medicine represents a useful tool for assessing topics and Authors, which mainly contributed to major advances in clinical and research settings, in this medical area.

References

1. Feijoo JF, Limeres J, Fernández-Varela M, Ramos I, Diz P. The 100 most cited articles in dentistry. *Clin Oral Investig.* 2014 18(3):699-706.
2. Fardi A, Kodonas K, Gogos C, Economides N. Top-cited articles in endodontic journals. *J Endod.* 2011 37(9):1183-90.

Experimental Study

Prevention program to reduce incidence and outcome of MRONJ in oncologic and hematologic patients: a cohort study

R. Vecchiatini^{1,3}, P. Felisatti¹, M. E. Guarnelli^{1,2}, N. Mobilio³, L. Trombelli^{1,2,3}

¹ Dental Unit, University Hospital, Ferrara, Italy; ² Research Centre for the Study of Periodontal and Peri-implant Diseases, University of Ferrara, Italy; ³ Department of Biomedical and Specialty Surgical sciences, University of Ferrara, Italy

Aim. Antiresorptive agents effectively reduce the incidence skeletal-related events in patients with metastatic bone cancer and multiple myeloma, thereby placing them at potential risk for developing medical-related osteonecrosis of the jaw (MRONJ). For these patients it is mandatory to prevent nor to reduce risk for MRONJ onset.

Material and methods. In collaboration with the Hematology and Oncology Unit of the University Hospital of Ferrara, Dental Unit developed specific patient-centred risk pamphlet and preventive program to reduce MRONJ incidence, then developed minimally invasive protocol to manage signs and symptoms in all cases of MRONJ.

Results. During 24 months observation time 98 patients, eligible for bisphosphonates and denosumab treatment (cohort 1), mean age of 67 years (range 33-92), received complete dental preventive treatments, including dental extraction; 81 patients, previously exposed to bisphosphonates and denosumab treatment (cohort 2), mean age of 67 years (range 44-87), received only non surgical treatments. On average, patients received 9.7 drug treatment cycles (range 1-48). No MRONJ was recorded in cohort 1 at 0, 3, 6 and 12 month after first cycle of bisphosphonates nor denosumab. 22 patients in cohort 2 developed MRONJ, during treatment cycles (range 9-48). Comparing cohort 1 and cohort 2 it was observed efficacy of preventive program in reducing risk for ONJ onset (relative risk reduction: 100 %, 95 % [CI] 86-100).

Conclusion. MRONJ is a clinically significant adverse effect of antiresorptive agents. A mandatory preventive program for oral health, involving a multidisciplinary team should be developed for all patients eligible for antiresorptive agents.

References

- Campisi G, Bedogni A, Di Fede O, Vescovi P, Fusco V, Lo Muzio L. Osteonecrosi dei mascellari associata a bisfosfonati, denosumab, e farmaci anti-angiogenetici nei pazienti oncologici e osteoporotici: diagnosi e terapia. *Dental Cadmos.* 2013;9:566-589.
- Henry D. Delaying skeletal-related events in a randomized phase 3 study of denosumab versus zoledronic acid in patients with advanced cancer: an analysis of data from patients with solid tumors. *Support Care Center.* 2014;22(3):697-687.
- Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, O’Ryan F; American Association of Oral and Maxillofacial Surgeons. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw—2014 update. *J Oral Maxillofac Surg.* 2014 Oct;72(10):1938-56.