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Bacteria and oral cavity: a new story to tell

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Periodontal diseases include a mild and reversible form named gingivitis (GI), and periodontitis (PD) that is the main cause of tooth loss in adults. GI, that affects gums and coronal junctional epithelium, as well as periodontitis, that is characterized by loss of connective tissue attachment, are caused by a persistent inflammatory response promoted by alteration of periodontal biofilm. The aim of the study was to test whether the prevalence of each species was associated with a particular clinical condition. Periodontal evaluation of 756 unrelated patients was performed by the Periodontal Screening and Recording (PSR) system. Subgingival samples were obtained from the site with the worst PSR score. A selection of eleven bacterial species was evaluated by quantitative real time PCR. Quantitative and qualitative analyses help to better understand the microbial changes associated with different stages of periodontal disease.

Periodontitis is a disease of bacterial etiology, related to an immune response that leads, if untreated, to the loss of teeth (1). The objective of non-surgical periodontal therapy consists of the elimination of infection and prevention of disease progression. It is now well established that prevention consists of the control of bacterial plaque, in supportive periodontal therapy (SRP) and that the maintenance of oral health is related to proper prevention care programs. Although the preventive protocols are demonstrated to be widely effective, periodontal disease may present relapse caused by poor oral hygiene. The present investigation compared the microbial colonization in different periodontal conditions. The oral cavity is populated by a large number of bacteria species that form polymicrobial communities attached to biotic or abiotic surfaces. The biofilm formed by the oral microbiota includes both symbiotic and potentially pathogenic species, similar to endodontic biofilm in which there is *Enterococcus Faecalis* (2-6). The advent of periodontal diseases appears to be associated with a microbial shift, more commonly

known as dysbiosis, that could be considered either a decrease in the number of beneficial symbionts and/or an increase in the number of pathogens (7). While the classic infection diseases are caused by a single exogenous species, periodontal diseases are considered to be the result of biofilm community changes involving a set of species that could either be endogenous and/or exogenous. In this complex scenario, the identification of the pathogenic species becomes a hard task. Indeed, as no single bacterial species was able to meet Kock's postulates, alternative and more flexible criteria of causality were suggested to unravel the etiology of periodontal diseases (8). Specifically, Hill's criteria that include biological plausibility, dose response, strength of association, specificity of association, consistency, and temporality, should be all equally considered.

MATERIALS AND METHODS

Sampling

A total of 539 patients participated in the study.

Key words: gingivitis, periodontitis, oral microbiota, biofilm, red complex

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Patients were randomly selected over a period of 8 months according to the following criteria. A lower age limit of 20 years was fixed. All individuals had good general health; exclusion criteria included diabetes, hypertension, rheumatoid arthritis, depression-anxiety, and obesity; as well as noticeable exposure to risk factors, such as alcohol consumption and smoking. Patients had not taken antimicrobial or anti-inflammatory drugs or had periodontal treatment in the previous 2 months (9-14).

Case definition was performed according to the Periodontal Screening and Recording (PSR) system. Gingival bleeding and pocket probing depth were monitored by a specific WHO probe and scored using the basic method indicated by the WHO oral health survey. Basically, the mouth was divided into sextants for the PSR examination. The probe is walked circumferentially around each tooth in the sextant being examined. The clinician observed only the position of the color-coded reference marking in relation to the gingival margin and the presence of furcation invasion, mobility, mucogingival problems, or recession (15-23). Only the highest code obtained was recorded for each sextant in the mouth.

The American Dental Association and the American Academy of Periodontology suggest that all routine dental examinations include a screening examination using PSR to identify patients who need a comprehensive periodontal assessment (24-33). The results of this screening examination are used to separate patients into two broad categories: those who have periodontal health or gingivitis, and those who have periodontitis. These two categories were the main subject of the investigation, the control group of patients with low PSR in all sextants (PSR code 0, 1, or 2), and a second group of patients with evidence of periodontitis in one or more sextants (code 3, 4, or *). In addition, healthy patients with no periodontal disease (code 0) were compared to patients with plaque-associated gingivitis (code 1 or 2).

After the removal of supragingival biofilm (34-41), a sample of the periodontal pocket microbiota was taken from a single site using sterile paper probes, among sites having the highest score, or randomly in the case of healthy patients. Specimens were processed to extract and purify DNA using a method that includes two consecutive incubations with lysozyme and proteinase K, in order to ensure an indiscriminate Gram positive and negative bacterial lysis. Once extracted, DNA was purified through

a silica spin- column (Sigma-Aldrich, St. Louis, MO, USA). Quantification of 16S rRNA genes, the PCR technique, has been described in previous papers (42-46).

Statistical analysis

Data from quantitative PCR included the amount of each of the 11 investigated bacterial species and a measurement of the bacterial load from up to 539 patients. These amounts matched the number of DNA molecules detected in the real time PCR tubes that was directly related with the number of bacteria in the specimens. In order to enhance data analysis with noise removal, statistical analysis was performed on relative amounts, calculated as ratios between the amount of each species and the total bacterial load. This was able to reduce variability due to random factors such as specimen conservation, DNA extraction efficiency, and purification yield, as well as variability due to systematic factors, for instance the higher number of bacteria expected in specimens from deeper pockets characterizing periodontitis.

It was verified that observed data, specifically the relative amounts and the absolute amounts of bacterial species, did not fit the Gaussian distribution, either as is, or after common transformations of data. For this reason, quantitative data analysis was performed with the non-parametric Mann-Whitney statistics.

RESULTS

Occurrence and amount of red complex bacteria from crevicular fluid were evaluated in 539 individuals. A single specimen from each patient was analyzed by quantitative real time PCR, obtaining measures of total bacteria load and of three species involved in periodontitis, i.e., *P. gingivalis*, *T. forsythia*, and *T. denticola*. Here we report a preliminary study focused mainly on prevalence of these three species among groups of patients with different diagnosis - regardless of different clinical aspects that may describe severity of the disease – in order to understand whether the presence of the red complex species and their relative amount may be considered predictive factors of periodontitis.

Each specie was common among healthy patients; however, the prevalence was roughly double in periodontitis group. Intermediate values, but closer

to healthy individuals were observed among patients affected by gingivitis.

The Freeman-Halton extension of Fisher's exact test indicated that the prevalence of each red complex species is different among groups of patients with high degree of statistical significance, *P. gingivalis* P value = 2×10^{-8} , *T. forsythia* P value = 1×10^{-8} , and *T. denticola* P value = 2×10^{-4} . The higher level of association with periodontitis was observed for *T. forsythia*, indeed the observed odds ratio was 6.1 (95% C.I. 3.1-11.9) when healthy individuals were compared to periodontitis patients, and 4.6 (95% C.I. 2.6-7.9) when healthy and gingivitis groups were combined and compared to periodontitis patients.

Results of quantitative data indicated that the normalized amount of *P. gingivalis* significantly differs among patient groups $F(2, 304) = 7.77$, P value = 0.001; as well as for *T. denticola* $F(2, 304) = 7.47$, P value = 0.001. On the contrary did not vary for *T. forsythia* $F(2, 304) = 1.41$, P value = 0.25.

DISCUSSION

The polymerase chain reaction (PCR) is the most sensitive and rapid method to detect microbial pathogens in clinical specimens. In particular, the diagnostic value of PCR is significantly higher when specific pathogens that are difficult to culture in vitro or require a long cultivation period such as for anaerobic bacteria species involved in periodontitis onset (47-50). A recent improvement of this technique is the real-time PCR that allow for quantitation of DNA target using fluorogenic probes in a close setup. Beside the opportunity to quantify target, the advantage to perform the assay is a closed system, in which the reaction tube is never opened after amplification, is of great value to prevent laboratory contamination and false positive results; the need of a probe in addition to the two PCR primers, further increase the specificity of the reaction.

In the present investigation we designed and tested the performance of a real-time PCR based assay to detect and quantify the red complex bacteria involved in periodontal disease. In particular, we found that *P. gingivalis*, *T. forsythia*, and *T. denticola* were strongly related to periodontitis because their prevalence was

higher among periodontitis patients. The presence of these bacterial species can significantly increase the risk to develop periodontitis, being the OR comprised between 6.1 (*T. forsythia*) and 3.4 (*T. denticola*). The results of quantitative data analysis indicated that the relative amount of *P. gingivalis* and *T. denticola* in periodontal pocket was sensibly higher in affected patients. This indicated that both the presence and relative amount of red complex bacteria is relevant data in periodontal disease diagnosis.

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Hormones and dentistry: a two-way relationship

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Stem cells of dental pulp (SCDPs) are multipotent stem cells with the potential to differentiate into various cell types. For this reason, they have been proposed as an alternative source for mesenchymal stem cells. Somatostatin (ST) is a peptide hormone with an inhibitory effect on several endogenous hormones. The aim of our study is to investigate whether somatostatin can promote or inhibit differentiation of SCDPs in osteoblasts and bone tissue. SCDPs were extracted from third molars of healthy subjects and were treated with ST at the concentration of 100 ng/ml for 24 and 48 h. Gene expression in treated SCDPs was compared with untreated cells (control) to check the effect of somatostatin on stem cell differentiation. After 24 h of treatment many genes investigated were downregulated in treated SCDPs vs untreated SCDPs. Significantly up-regulated gene (Fold change >2) was the Bone Morphogenetic Protein BMP4. On the contrary ST induced the over-expression of bone related genes after 48 h of treatment. TGFB family genes and their receptors were also significantly upregulated after 48 h of treatment. ST demonstrated to promote the self-renewal of SCDPs: in our experiments somatostatin mainly acted on TGFB family genes. Further studies are needed to explore this new way of creating bone tissue.

Somatostatin is a peptide hormone with cyclic structure, formed by 14 amino acids, synthesized by hypothalamus, pancreas, gastroduodenal mucosa, thyroid and, to a lesser extent, by other glandular formations such as the salivary glands. In 2003, some authors isolated stem cells from human exfoliated deciduous teeth (SHED). After isolation from deciduous teeth, cells showed the ability to form *in vivo* a substantial amount of bone. Dental pulp from exfoliated teeth could be a good source of postnatal stem cells (1-7).

To our knowledge, the only study on somatostatin and stem cells reported that somatostatin receptor type 2 contributes to the self-renewal of murine embryonic stem cells, and not one study on somatostatin and SCDPs was found. We aimed to investigate whether somatostatin can influence differentiation of SCDPs in osteoblasts and bone tissue (8-13). We propose a

working model that explains the possible interactions between somatostatin and SCDPs at the molecular level and describes the cellular consequences of these interactions. This model may be used as to stimulate research on the clinical applications of SCDPs in cellular therapy and tissue regeneration (14-19).

MATERIALS AND METHODS

Dental pulp stem cell isolation

After collection of informed consent, dental germ pulp was extracted from third molars of healthy young patients with an average age of 18 years. Pulp digestion was performed at 37°C for 1 h in a solution containing 3 mg/ml type I collagenase and 4 mg/ml dispase in 4 ml phosphate-buffered saline (PBS) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 500 µg/ml claritromycin.

Key words: cell differentiation, dental pulp stem cells, osteoblasts, osteogenesis, hormones

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Fibroblasts were removed from the lysate by filtration through a 70 μ m Falcon strainer (Sigma Aldrich, Inc., St Louis, MO, USA). Filtered cells were then put into culture with α -MEM culture medium (Sigma Aldrich, Inc., St Louis, MO, USA) added with 20% FCS, 100 μ M 2P-ascorbic acid, 2 mM L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin and placed in 75 ml flasks. Cultures were maintained in a 5% CO₂ humidified atmosphere at 37°C and medium was changed twice a week. Treatments were carried out for 24 and 48 h.

Flow cytometric analyses

To assess the stemness of the isolated mesenchymal stem cells, different antigens after staining with fluorochrome (FITC- or PE-) conjugated mAbs anti- human CD14-FITC, CD14-PE, CD34-FITC, CD45-FITC, CD90-PE, CD105-PE (Immunotech, Marseille, France) were analyzed by FACScan. Non-specific mouse IgG was used as isotype control to better differentiate non-specific background signal from specific one (Immunotech).

Cell treatment

For the assay, SCDPs were seeded at a density of 1×10^5 cells/ml into 9 cm² wells. Part of the wells were added with somatostatin (Sigma Aldrich, Inc., St Louis, MO, USA) at the final concentration of 100 ng/ml. Untreated cells were used as controls.

RNA processing

To isolate and purify total RNA from cells, the GenEluteTM Mammalian Total RNA Miniprep Kit (Sigma Aldrich, Inc., St Louis, MO, USA) was used following the manufacturer's instructions. After extraction, total RNA purity and quantity were evaluated with the spectrophotometer NanoDrop (Thermo Scientific Inc., MA, USA). cDNA was synthesized starting from 500 ng of each RNA sample (treated and control, at 24 and 48 h) using SuperScript[®] VILOTM cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA).

Real time PCR

Complementary DNA was amplified by Real Time Quantitative PCR using the Power SYBR[®] Green

Master MixTM (Life Technologies, Foster City, CA, USA) and the specific amplification profile for each of the investigated genes. In greater detail, 4 μ l of 10-fold diluted cDNA were amplified in 20 μ l of final volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 μ l of 2x Power SYBR[®] Green Master MixTM and adapted concentration of each primer. Gene expression quantification was conducted with the delta/delta Ct calculation method and glucuronidase beta (GUSB) was used as reference gene to normalize the gene expression levels.

RESULTS

Cell cultures were phenotypically characterized by flow cytometric analyses. Cell preparations derived from dental pulp were homogeneously CD105+, CD90+, CD34-, CD45- and CD14- which is a typical mesenchymal stem cell surface antigen profile. SCDPs were treated with insulin at the concentration of 100 ng/ml for 24 and 48 h. Gene expression in treated SCDPs was compared with untreated cells (control) to check the effect of somatostatin on stem cell differentiation.

Genes related to ossification (BMP1, BMP2 BMP3, BMP4, BMP5 BMP6), osteoblast differentiation (BMPR1A, BMPR1B, BMPR2), bone mineralization (TGF β 1, TGF β 2, TGF β 3) and skeletal development (TGF β R1 and TGF β R2) were investigated to study the potential effect of insulin in osteoblast differentiation and proliferation.

After 24 h of treatment many of the genes investigated were downregulated in treated SCDPs vs untreated SCDPs. Significantly up-regulated genes (Fold change >2) were the Bone Morphogenetic Proteins BMP3 and BMP4. Significantly up-regulated genes were Bone Morphogenetic Protein receptor BMPR1A. After 48 h, insulin induced the over-expression of bone related gene BMP1.

DISCUSSION

In our study, BMP3 and BMP4 after 24 h and BMP1 genes after 48 h were up-regulated,

inducing proliferation and differentiation of human SCDPs. Our study confirms the assumption that somatostatin promotes markers of bone formation, providing support of the view that insulin influences differentiation of SCDPs in an osteoblastic sense. Bone morphogenetic proteins (BMPs) have been shown to induce SCDPs to proliferate and then differentiate into osteoblastic cells. Somatostatin can modulate the expression of various osteogenic markers. Thus, the effects of insulin on SCDPs must be examined in light of their apparent maturity in terms of osteogenic potential. Previous studies showing that insulin promotes bone growth, suggest that insulin facilitates differentiation as the cell population reaches a more mature, post-proliferative stage. Also, some materials employed in endodontics, such as Portland and oxide zinc eugenol sealers, promote bone growth and root elongation (20-23).

SCDPs showed differentiation profiles similar to those of bone differentiation and this event makes them very interesting as a model to study the osteogenesis (24-28). Dental pulp is a good source of stem cells that differentiate into osteoblasts, as well as other phenotypes, for hard tissue engineering. During life, bone tissue needs renewal, and dental pulp represents an additional source of mesenchymal cells to bone marrow (29). Selection and expansion of stem cells are complex procedures commonly used to obtain cells for potential therapy; thus, being able to obtain sorted cells quickly after collection and to transplant them directly is an advantage that can lead to easier application of bone-engineering protocols (30-43).

There is a considerable body of work showing osteogenic actions of insulin in cortical bone in several species (44-55). In our study we demonstrated that insulin could increase SCDPs and influence differentiation and proliferation of SCDPs in osteoblasts.

Our study provides evidence that stem cells selected from human permanent dental pulp represent a population of postnatal stem cells able to extensively proliferate and differentiate

into mesenchymal cells and mainly osteoblasts. Therefore, these cells constitute a large, ideal source of cells for tissue regeneration, tissue-based clinical therapies, and transplantation.

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Hyaluronic acid and its use in dentistry

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The purpose of this paper is to evaluate the role of hyaluronic acid in bio-revitalization (HABR) by testing several extracellular matrix biological parameters in cultured dermal fibroblasts. To this aim, fibroblastic expressed genes after exposition to three HABR medical devices were evaluated. Cells were seeded on a layer of three different medical devices containing 6.2, 10 and 20 mg/ml of HABR for 24 h. Real Time Polymerase Chain Reaction was performed to investigate gene expressions. Genes encoding HABR synthesis and degradation, Metalloproteinases 2 and 3 and Desmoplakin production as well as GDF6, and IGF1 were activated by hyaluronic acid products. The in vitro study showed similar effects on tested genes despite a different concentration of hyaluronic acid contained in the medical devices and the simultaneous presence of other additives. Based on the reported data, gene activations are an aspect of metabolic modulation of signalling pathways rather than the proportional production of a specific connective tissue molecule. Indeed, different HABR concentration and the presence of other additives did not change the overall effect on the studied genes. We believe that the optimization of extracellular matrix micro-environment, obtained by enhanced structural support with HABR, leads to functional and metabolic improvement.

Hyaluronic acid in bio-revitalization (HABR), a natural polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, is synthesized by the serosa glands of the submucosa and it is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body (1-6).

HABR is used in different concentrations in bio-revitalization procedures and in different dermal fillers after crosslinking to reduce its hyaluronidase sensitivity (7-12). HABR is a polymer of dimeric units of N-acetyl-glucosamine and glucuronic acid synthesized by HABR synthetase on the inner surface of plasma membrane, and then secreted in extracellular space.

The purpose of this study is to investigate the role of HABR in bio-revitalization. To this aim, some fibroblastic expressed genes after exposition to three medical devices were evaluated. These products contain different concentrations of HA and are commonly used in aesthetic medicine.

MATERIALS AND METHODS

Primary human dermal fibroblast cell (HFb) culture

Fragments of dermal tissue of healthy volunteers were collected during surgery. The pieces were transferred to 75 cm² culture flasks containing DMEM medium (Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20% foetal calf serum and antibiotics, i.e., penicillin 100 U/ml and streptomycin 100 µg/ml (Sigma Aldrich, Inc.).

Cells were incubated in a humidified atmosphere of

Key words: bio-revitalization, fibroblast, gene expression, hyaluronic acid, skin

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5% CO₂ at 37°C. Medium was changed the next day and twice a week. After 15 days, the pieces of dermal tissue were removed from the culture flask. Cells were harvested after additional 24 h of incubation.

Cell cultures

Human dermal fibroblasts at the second passage were seeded on a layer of three different medical devices:

1 - solution of 6.2 mg/ml of non-reticulated HABR of biotechnological origin with a buffered medium containing ammonium molybdate, ammonium metavanadate, calcium chloride, iron sulphate, potassium chloride, copper sulphate, magnesium chloride, manganese sulphate, sodium acetate, sodium hydrogen carbonate, sodium chloride, sodium hydrogen phosphate, sodium metasilicate, sodium selenite, nickel chloride, tin chloride, zinc sulphate, alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, adenine (Vit. B4), biotin (Vit. B8), calcium pantothenate (Vit. B5), choline chloride, folic acid (Vit. B9), inositol (Vit. B7), nicotinamide (Vit. B3), pyridoxine (Vit. B6), riboflavin (Vit. B2), thiamine (Vit. B1), vitamin B12, deoxythymidine, glucose, putrescine, sodium pyruvate, lipoic acid, (Viscoderm Skinko E Ibsa// Revitacare, Saint Ouen d'Aumone, France);

2 - HABR sodium salt 10 mg/ml with a buffered medium containing polynucleotides 10 mg/ml, mannitol, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dehydrate (Newest Mastelli, Sanremo, Italy);

3 - Stabilized HABR gel 20 mg/ml in saline solution phosphate buffered (Restylane Vital, Uppsala, Sweden).

A set of untreated cells was used as control. The cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C for 24 h. After the end of the exposure time, cells were trypsinized and lysed for RNA extraction.

RNA processing and Real Time Polymerase Chain Reaction

Reverse transcription to cDNA was performed directly from cultured cell lysate using the TaqMan Gene Expression Cells-to-Ct Kit (Ambion Inc., Austin, TX), following the manufacturer's instructions. Briefly, cultured cells were lysed with lysis buffer and RNA released in this solution. Cell lysate were reverse-transcribed to cDNA using the RT Enzyme Mix and appropriate RT buffer

(Ambion Inc.).

Finally, the cDNA was amplified by Real Time Polymerase Chain Reaction (PCR). The amplification was performed by using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and the specific assay was designed for the investigated genes. SYBER assay reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems). Each reaction contained 10 µl 2' Power SYBR Green PCR Master Mix (Applied Biosystems), 400 nM concentration of each primer and cDNA.

All experiments performed included non-template controls to exclude contamination of reagents. PCR was performed with two biological replicates.

Expression was quantified using Real Time PCR. The gene expression levels were normalized to the expression of the housekeeping gene Homo sapiens transferrin receptor protein 1 (TFRC).

RESULTS

The effects of the three different medical devices on the primer HAS1 and HYAL1 show an up-regulation of both genes.

DISCUSSION

HABR is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body. It is a polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, which is synthesized by the serosa glands of the submucosa (13-19).

Low-molecular-weight hyaluronic acid (<300 kD) promotes the stimulation of cell proliferation and has an anti-inflammatory action, while increasing evidence suggests that high-molecular-weight hyaluronic acid (1000 kD), due to its inherent immunosuppressive effect, also helps to regulate exacerbation of symptoms caused by excessive inflammation. HABR is widely used in aesthetic medicine to improve aesthetic surgery results. Firstly, it is non-antigenic, yet it is antioxidant, anti-oedematous and biocompatible. Moreover, it

is bacteriostatic and anti-inflammatory, and thus it prevents the selection and formation of resistant bacterial strains, even when used over an extended period (20-33), which is one of the most significant side effects that occur in the chronic use of antibiotics, making it especially useful in the management of chronic diseases, particularly in respiratory disorders.

HABR plays a key role in wound healing, interacting with type I and II toll-like receptors, it regulates the body's innate immunity against bacteria and viruses by preventing penetration, promoting angiogenesis and by modulating the proliferation, migration and differentiation of the cells responsible for tissue repair (34-43).

In conclusion, the in vitro testing of the three bio-revitalization commercial products determined an up-regulation of genes responsible for HABR synthesis and degradation, for Metalloproteinases 2 and 3 activations, for Desmoplakin production and for metabolic signaling (GDF 6, IGF1) (44-50).

Bio-revitalization procedures have a similar effect despite different HABR concentrations and the concomitant presence of other molecules (i.e., amino acids, vitamins, polynucleotides) which are different in quality and amount (51-56). Only HABRS1 gene shows a different modulation by using the 3 products.

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Patient's perception of intraoral scanning: a comparison between traditional and digital dental impression

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The purpose of this work was to assess the discomfort perceived by the patient during the intraoral scan procedure using the iTero Scanner. A sample of 33 patients was selected on the basis of parameters that identify a complete dentition. All treated patients underwent a traditional polyvinylsiloxane impression with double procedure and an intraoral scan in the same session. Subsequently they were given a questionnaire concerning the discomfort perceived by both procedures. Once the completed questionnaires were obtained, preliminary statistical tests were carried out to check if the distribution of scores assigned by patients to the two procedures differed significantly from a Gaussian distribution. Furthermore, the discomfort/preference indexes for the two procedures were compared using the Wilcoxon test for paired data. Finally, the Spearman correlation test was used. From the results of the preliminary normality tests, it was decided to use non-parametric type tests that gives the intraoral scan procedure more favorable scores relative to a minor discomfort. The use of intraoral scanning and more specifically of the iTero 2.9 scanner (despite a not small wand), represents an option largely preferred by patients in terms of reduction of discomfort and classic discomfort related to relief systems traditional imprint.

The introduction of digital models has undoubtedly changed important aspects of orthodontic diagnosis in terms of providing more practical tools for information storage, information exchange, consultation, monitoring, and assessment of diagnostic data (1-12).

The different measurements that can be taken, as well as the assessments that can be carried out using digital models, such as the assessment of malocclusion severity, crowding, and prosthetic rehabilitation, have been the subject of several papers in the literature and most of them agree that digital models have the same level of reliability and repeatability as traditional models (13-17).

As reported in a recent paper (18), dental practice quality should be assessed based on three key criteria:

- clinical efficiency
- patient safety

- patient's point of view

Recently, several studies have been published on the assessment of different kinds of intraoral scanners, particularly on their precision compared to that of traditional polyvinyl siloxane (PVS) impression approaches, and on the possibility of using the scanners to produce prosthetic manufactures or digital models for orthodontic assessment. Few papers have focused on patients' experience with the different impression approaches, as pointed out by Mitchell et al. (18-21). For example, in one study, 11 patients were asked to evaluate their comfort during the intraoral scanning procedure compared to that during a traditional alginate impression technique. However, the study was based on patients' responses to a single question and not to a thorough questionnaire. In another very recent paper (22), a larger sample of patients is taken, and a statistical

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survey is conducted that is certainly more relevant and valid than the previous one, but the action of the intraoral scanner with alginate impressions is always compared (23-31).

Moreover, it is important to note that intraoral scanners, due to their high precision, should not be compared to traditional alginate impressions but rather to PVS impressions, which represent the current golden standard in terms of precision, although they are more laborious in terms of time and discomfort (32-35).

The aim of our study was to evaluate the patients' opinions about the use of both intraoral scanners and PVS impressions in terms of injury, discomfort and preference using an appropriate and detailed questionnaire. The latter was used to collect data on patients' perceptions regarding all features relevant to invasive oral procedures (36-40).

MATERIALS AND METHODS

To perform a comparison between digital and traditional models, a sample of 33 patients was selected according to the following inclusion criteria:

- No dental shape anomalies;
- No anomalies in the number of teeth;
- No decayed teeth;
- No prosthetic bridges (only single crowns accepted);
- Full dentition from second molar to second molar on both arches;
- No differences in age;
- No differences in degree of crowding;
- No differences in malocclusion.

Each participant underwent two dental impression procedures during a single appointment: 1) double impression in PVS material (Aquasil Putty heavy + light) and 2) an intraoral scan using iTero 2.9 (Cadent). At 24 h after the appointment, a 10-point questionnaire was emailed to each patient to assess their degree of discomfort/preference during both dental impression procedures. For each question, the patients were asked to respond with a score ranging from 1 to 10 for each procedure.

The ten questions were as follows:

- 1) What was the degree of general discomfort perceived during the dental impression procedure? (1=nothing, 10=very much)

- 2) What was the degree of emesis (vomiting and nausea) perceived during the dental impression procedure? (1=nothing, 10=very much)
- 3) What was the degree of xerostomia (dry mouth) perceived during the dental impression procedure? (1=nothing, 10=very much)
- 4) What was the degree of soft tissue pain perceived during the dental impression procedure? (1=nothing, 10=very much)
- 5) What was the degree of muscular effort required to maintain mouth opening during the dental impression procedure? (1=nothing, 10=very much)
- 6) What was the degree of oral cavity obstruction perceived during the dental impression procedure? (1=nothing, 10=very much)
- 7) What was the degree of tongue discomfort perceived during the dental impression procedure? (1=nothing, 10=very much)
- 8) With respect to the time spent sitting on dental chair, how do you evaluate the dental impression procedure? (1=poor, 10=excellent)
- 9) Simply in terms of preference, please score the dental impression procedure (1=poor, 10=excellent)
- 10) Looking at your general experience, please score each dental impression procedure (1=poor, 10=excellent)

Based on the patient responses to the questionnaire, for each question, we constructed two statistical variables which expressed the scores assigned by the patients to the respective dental impression procedure. In other words, each variable represented a measure of the degree of discomfort/preference perceived by the patients during the respective dental impression procedure. Patients were considered as independent data points in all the statistical analyses carried out. All the analyses conducted in this study were carried out using "Statistic release 8" software (StatSoft Inc., Tulsa, OK, USA).

First, preliminary tests were conducted to verify whether the distribution of the scores assigned by the patients to each procedure was significantly different from a normal (Gaussian) distribution. The statistical tests used for this purpose were the Kolmogorov-Smirnov (K-S) test, the Lilliefors test and the Shapiro-Wilk's W test. This preliminary analysis allowed us to select the appropriate statistical tests for achieving the main objectives of the research (see below).

To test the patients' preference for one dental

impression procedure over the other, the scores assigned by the patients to the two dental impression procedures were compared using the Wilcoxon matched-pairs test, as each patient underwent both procedures. Thus, ten tests were conducted, one for each question of the questionnaire. For each comparison, the null hypothesis was that the median values for both procedures did not differ. The probability value required to refuse this hypothesis was set at $\alpha = 0.05$. All tests were two-tailed tests, with no biased assumption that one procedure was better than the other.

- 11) We were interested in assessing which factors can cause discomfort in patients during dental impression procedures, and/or lead patients to prefer a given procedure over the other. To achieve this goal, we assessed the statistical association between two groups of variables:
- 12) dependent variables, i.e. those measuring the degree of general discomfort and the preference for dental impression procedures, which were based on the scores assigned by patients to the procedures in response to questions 1, 9, 10 of the questionnaire respectively; 2) independent variables, i.e. those measuring specific symptoms of discomfort, which were based on the

scores assigned by patients to procedures in response to questions 2-8 of the questionnaire. Specifically, we used Spearman correlation tests to assess the linear association between each dependent variable and each independent variable for each dental impressive procedure separately. In the correlation tests, $P < 0.05$ indicated a statistically significant linear association between variables.

RESULTS

The sets of three normality test results indicate that the distributions of scores for intraoral scans were significantly different from the normal distribution ($P < 0.05$) for all 20 variables, while the distribution of scores for the traditional procedure were often not significantly different from the normal distribution ($P > 0.05$). This pattern depended on the fact that most patients tended to systematically assign either very low or very high scores (depending on the question) to the intraoral scan, while the scores assigned to the traditional procedure were distributed much more evenly across the range of possible values ± 10 . Since many of the variables considered did not exhibit

Table I. Descriptive statistics concerning the variables included in the study.

	Valid N	Mean	Median	Mode	Freq of mode	Min	Max	Lower quartile	Upper quartile	Var	Standard deviation	Coef of variation	Standard error
General discomfort level - scan	33	2.212	2	2	13	1	7	1	3	1.672	1.293	58.459	0.225
General discomfort level – trad.	33	6.455	7	Multiple	6	2	10	5	8	5.193	2.279	35.306	0.397
Emesis - scan	33	1.424	1	1	24	1	4	1	2	0.627	0.792	55.592	0.138
Emesis – traditional	33	5.455	6	6	7	1	10	3	7	7.818	2.796	51.262	0.487
Xerostomia - scan	33	3.061	2	1	14	1	10	1	5	6.121	2.474	80.837	0.431
Xerostomia – traditional	33	4.848	4	Multiple	6	1	10	3	6	8.758	2.959	61.036	0.515
Soft tissue indolensation level - scan	33	1.818	1	1	21	1	7	1	2	1.966	1.402	77.116	0.244
Soft tissue indolensation level – trad.	33	4.182	4	5	7	1	9	2	6	4.528	2.128	50.887	0.370
Gain muscular effort to keep open mouth - scan	33	2.818	2	1	10	1	7	1	4	3.466	1.862	66.060	0.324
Gain muscular effort to keep open mouth – traditional	33	5.606	5	5	9	1	10	4	8	7.434	2.726	48.635	0.475
Oral cavity obstruction level - scan	33	2.879	2	2	13	1	7	2	4	2.985	1.728	60.014	0.301
Oral cavity obstruction level – trad.	33	6.182	7	Multiple	5	2	10	4	8	6.841	2.616	42.310	0.455
Complaint of the tongue level - scan	33	1.909	1	1	18	1	7	1	2	2.210	1.487	77.874	0.259
Complaint of the tongue level – trad.	33	4.667	4	3	7	1	10	3	7	7.917	2.814	60.293	0.490
Time spent on dental unit - scan	33	6.545	8	Multiple	8	1	10	4	9	8.756	2.959	45.207	0.515
Time spent on dental unit – trad.	33	4.758	5	5	7	1	9	2	7	5.939	2.437	51.225	0.424
Preference vote - scan	33	8.455	9	10	11	4	10	8	10	2.381	1.543	18.250	0.269
Preference vote - traditional	33	4.485	4	6	6	1	9	3	6	5.633	2.373	52.918	0.413
General experience vote - scan	33	8.667	9	10	13	4	10	8	10	2.354	1.534	17.704	0.267
General experience vote - traditional	33	4.697	5	6	8	1	9	3	6	5.530	2.352	50.068	0.409

a normal distribution, for the subsequent analyses we decided to use a non-parametric statistical approach, which would not assume variables to be normally distributed (41-43).

Table I reports descriptive statistics for all 20 variables included in the study, based on the scores provided by patients in response to the questionnaire, including both measures of central tendency (mean, median, and mode) and of variability (interquartile range variance, standard deviation, coefficient of variation, and standard error). The table shows that the mean and median scores measuring the degree of discomfort perceived by the patients (responses to questions 1-7 of the questionnaire) were much higher for the traditional procedure than for the intraoral scan. By contrast, the mean and median scores measuring patients' preference (responses to questions 8-10 of the questionnaire) were much higher for the intraoral scan than for the traditional procedure. Moreover, as mentioned above, the scores for the traditional procedure usually showed a greater variability than those for the scan.

The next step of the analysis consisted of

testing whether the differences between the scores assigned by patients to the two dental impression procedures were statistically significant. The results of the Wilcoxon matched-pairs tests showed that the median scores measuring the degree of discomfort perceived by patients (based on responses to questions 1-7 of the questionnaire) were, in all cases, significantly higher for the traditional procedure than for the intraoral scan; conversely, the median scores measuring patients' preferences (based on responses to questions 8-10 of the questionnaire) were, in all cases, significantly higher for the intraoral scan than for the traditional procedure. Therefore, all null hypotheses were rejected (Table II).

The results of Spearman correlation tests concerning the intraoral scan showed that the degree of general discomfort perceived by patients (patients' responses to question 1 of the questionnaire) was significantly and positively related to all independent variables measuring specific symptoms of discomfort (based on patients' responses to questions 2-7) , while it was significantly and negatively related to the evaluation of time spent sitting on the dental

Table II. *Results of Wilcoxon matched pairs test.*

Pair of variables	Valid N	T	Z	p-level
General discomfort level - scan & General discomfort level - traditional	33	6.0	4.824	0.000001
Emesis level - scan & Emesis level - traditional	33	0.0	4.782	0.000002
Xerostomia level - scan & Xerostomia level - traditional	33	108.0	2.368	0.017898
Soft tissue indolensation level - scan & Soft tissue indolensation level - traditional	33	28.5	4.087	0.000044
Gain muscular effort to keep open mouth - scan & Gain muscular effort to keep open mouth - traditional	33	13.5	3.787	0.000153
Oral cavity obstruction level - scan & Oral cavity obstruction level - traditional	33	16.0	4.258	0.000021
Complaint of the tongue level - scan & Complaint of the tongue level - traditional	33	23.0	4.206	0.000026
Time spent on dental unit - scan & Time spent on dental unit - traditional	33	111.0	2.095	0.036174
Preference vote - scan & Preference vote - traditional	33	24.5	4.380	0.000012
General experience vote - scan & General experience vote - traditional	33	14.0	4.400	0.000011

chair (responses to question 8). The independent variables that were more strongly correlated to the degree of general discomfort (based on the values of the correlation coefficients were the “degree of oral cavity obstruction”, the “degree of xerostomia” and the “degree of muscular effort required to maintain mouth opening”. As regards to the “preference score” and the “general experience score” for the intraoral scan (responses to questions 9-10), both dependent

variables were significantly and negatively correlated to all independent variables measuring specific symptoms of discomfort, with the exception of the “degree of muscular effort to maintain mouth opening” (for which the correlation was just marginally significant). Moreover, the two dependent variables were positively and significantly correlated to the evaluation of time spent sitting on the dental chair. Overall, the values of the Spearman correlation

Table III. *Results of Spearman correlation tests concerning the intraoral scan.*

Pair of variables	Valid N	Spearman R	p-level
General discomfort level - scan & Emesis level - scan	33	0.429	0.012707
General discomfort level - scan & Xerostomia level - scan	33	0.703	0.000005
General discomfort level - scan & Soft tissue indolensation level - scan	33	0.510	0.002426
General discomfort level - scan & Gain muscular effort to keep open mouth - scan	33	0.609	0.000167
General discomfort level - scan & Oral cavity obstruction level - scan	33	0.759	0.000000
General discomfort level - scan & Complaint of the tongue level - scan	33	0.547	0.000980
General discomfort level - scan & Time spent on dental unit - scan	33	-0.507	0.002585
Preference vote - scan & Emesis level - scan	33	-0.533	0.001400
Preference vote - scan & Xerostomia level - scan	33	-0.512	0.002303
Preference vote - scan & Soft tissue indolensation level - scan	33	-0.546	0.001012
Preference vote - scan & Gain muscular effort to keep open mouth - scan	33	-0.312	0.077030
Preference vote - scan & Oral cavity obstruction level - scan	33	-0.539	0.001199
Preference vote - scan & Complaint of the tongue level - scan	33	-0.450	0.008610
Preference vote - scan & Time spent on dental unit - scan	33	0.356	0.042219
General experience vote - scan & Emesis level - scan	33	-0.579	0.000420
General experience vote - scan & Xerostomia level - scan	33	-0.404	0.019810
General experience vote - scan & Soft tissue indolensation level - scan	33	-0.544	0.001062
General experience vote - scan & Gain muscular effort level to keep open mouth - scan	33	-0.307	0.082651
General experience vote - scan & Oral cavity obstruction level - scan	33	-0.540	0.001189
General experience vote - scan & Complaint of the tongue level - scan	33	-0.415	0.016400
General experience vote - scan & Time spent on dental unit - scan	33	0.459	0.007155

coefficient indicated a moderate association between dependent and independent variables (Table III).

The results of Spearman correlation tests concerning the traditional procedure showed that the degree of general discomfort perceived by patients (responses to question 1 of the questionnaire) was significantly and positively correlated to all the independent variables measuring specific symptoms of discomfort (based on patients' responses to questions 2-7), while it was not significantly

correlated to the evaluation of time spent sitting on the dental chair (response to question 8). The independent variables that were more strongly related to the degree of general discomfort (based on the value of the correlation coefficients) were the "degree of muscular effort required to maintain mouth opening", the "degree of emesis" and the "degree of xerostomia". Regarding the preference score and the general experience score expressed by the patients for the traditional

Table IV. *Results of the Spearman correlation tests concerning the traditional procedure.*

Pair of variables	Valid N	Spearman R	p-level
General discomfort level - traditional & Emesis level - traditional	33	0.631	0.000082
General discomfort level - traditional & Xerostomia level - traditional	33	0.625	0.000102
General discomfort level - traditional & Soft tissue indolensation level - traditional	33	0.459	0.007267
General discomfort level – trad. & Gain muscular effort to keep open mouth – trad.	33	0.674	0.000017
General discomfort level - traditional & Oral cavity obstruction level - traditional	33	0.556	0.000786
General discomfort level - traditional & Complaint of the tongue level - traditional	33	0.576	0.000448
General discomfort level - traditional & Time spent on dental unit - traditional	33	-0.184	0.304585
Preference vote - traditional & Emesis level - traditional	33	-0.497	0.003284
Preference vote - traditional & Xerostomia level - traditional	33	-0.649	0.000044
Preference vote - traditional & Soft tissue indolensation level - traditional	33	-0.460	0.007040
Preference vote - traditional & Gain muscular effort to keep open mouth - traditional	33	-0.585	0.000350
Preference vote - traditional & Oral cavity obstruction level - traditional	33	-0.498	0.003171
Preference vote - traditional & Complaint of the tongue level - traditional	33	-0.606	0.000188
Preference vote - traditional & Time spent on dental unit - traditional	33	0.452	0.008283
General experience vote - traditional & Emesis level - traditional	33	-0.509	0.002463
General experience vote - traditional & Xerostomia level - traditional	33	-0.584	0.000364
General experience vote - traditional & Indolensation level of soft tissue - traditional	33	-0.365	0.036798
General experience vote- trad. & Gain muscular effort to keep open mouth – trad.	33	-0.608	0.000174
General experience vote - traditional & Oral cavity obstruction level - traditional	33	-0.581	0.000389
General experience vote - traditional & Complaint of the tongue level - traditional	33	-0.582	0.000380
General experience vote - traditional & Time spent on dental unit - traditional	33	0.501	0.002972

procedure (responses to questions 9-10), they were significantly, moderately, and negatively correlated to all independent variables measuring the specific symptoms of discomfort, while they were positively correlated to the evaluation of time spent sitting on the dental chair (response to question 8) (Table IV).

DISCUSSION

The traditional method was associated with a significantly higher discomfort level compared to the digital procedure for all the considered symptoms (Table I, II). Moreover, high mean and median scores in measures of discomfort were observed only for the traditional procedure (Table I) (44-48). Furthermore, the results of our study indicate that all specific symptoms of discomfort, measured based on the patients' responses to the questionnaire items, influenced the degree of general discomfort perceived by patients during the impression procedures and their preference score for them. This can be partly inferred from the results of the direct comparisons between the two procedures (which were statistically significant for all items of the questionnaire; (Table II) and, mainly, from the results of the Spearman correlation tests (Table III, IV) (49-51). The latter indicate that the degree of general discomfort perceived by patients during the intraoral scan was likely influenced by all specific symptoms of discomfort considered, although the degree of oral cavity obstruction, the degree of xerostomia and the muscular effort to maintain mouth opening could contribute to a higher extent to causing the general discomfort (52-54). Moreover, they suggest that all specific symptoms of discomfort affected the general degree of discomfort perceived by patients during the traditional procedure, although the "degree of muscular effort required to maintain mouth opening", the "degree of emesis", and the "degree of xerostomia" appear to be more influential than others. Based on the results of Spearman correlation tests, it also turns out that the patients' preference scores, as well as "general experience scores", for a given impression procedure were inversely related to the degree of discomfort perceived for all symptoms (Tables III, IV).

Regarding the time spent sitting on the dental chair during both procedures, this was rated by the patients more positively for the intraoral scan than for the traditional procedure, which indicates that the patients felt that they spent less time sitting on the chair during the scan than during the traditional procedure. However, this variable was not significantly related to the general degree of discomfort perceived during the traditional procedure, probably because, in that case but not during the intraoral scan, the duration of the whole procedure was less important compared to other discomfort symptoms.

From the results of the Wilcoxon matched pairs tests, we can conclude that the patients clearly preferred the intraoral scan procedure than the traditional method due to the reductions in all the discomfort symptoms during the scan (Table II).

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Upper-lip laser frenectomy with a diode laser in a pediatric patient: a case report

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The case report aims to describe the parameters of performing upper labial frenectomy with the use of diode laser beams without infiltrated local anaesthesia. A 6-year-old patient was referred by the orthodontist for assessment of the upper anterior labial frenum. The dental treatment plan reported only the presence of caries on deciduous teeth and seals on the first permanent molars. The clinical examination reported the presence of a high attachments of labial frenum with a pathologically attachment and the presence of a diastema supports this theory. The laser used to remove the frenulum was a diode laser used with a wavelength of 980 nm with 320 microns of fiber in contact with a power of 2.0 W in continuous wave mode. The clinical examination showed an acceptable healing by secondary intention of the wound and the initial functional recovery of a physiological upper lip movements. The patient reported that the procedure was well tolerated. The diode laser can be used with good result for the removal of pathological frenum. The diode laser can be used in pediatric dentistry because of its application, adequate coagulation, no need for sutures and less inflammation and pain.

The labial frenum is a fibrous tissue covered with mucosa that comes from the lip and cheek to the alveolar periosteum (1). The main function of the labial frenum is to limit and stabilize labial movements, preventing the excessive exposition of gingival mucosa. In the upper lip frenum, high adhesion to the alveolar portion causes diastema of the dentition and eruption site abnormalities in the central incisors, accumulation of biofilm, gingivitis, as well as a decrease in keratinized gingival tissue (2-4). Placek et al. (5) classified labial frenulum attachments in four types: I-mucosal attachment; II- gingival attachment; III- papillary attachment and IV- papilla penetrating attachment (6). Mucosal and gingival types are the most popular describes as normal but papillary and papilla penetrating attachment as pathological (5, 7). The indications for surgical removal of the upper lip frenum are

prevention of median diastema formation, facilitation of oral hygiene procedures, and prevention of gingival recession (7-8). Frenectomy is a surgical procedure that aims to eliminate excessive interdental tissue and reduce the tension of marginal gingiva tissues (9). Frenectomy provide total removal of the frenum (excisional technique) and frenotomy provide the partially removed of the frenum (repositioning technique) (10). Once the need for a lip frenectomy is determined, there are multiple surgical techniques that the clinician can used to perform it. The most used technique actually is frenum excision by scalpel, and laser-assisted frenectomy (11-22). There are several types of lasers that have been used in dentistry. The most commonly used lasers in oral surgery are high power lasers as CO₂ laser, the Erbium family lasers, the Nd:YAG laser and the diode laser. The function of diode laser is based on its photothermal effect, it

Key words: frenectomy, laser, diode laser, labial frenum, pediatric dentistry

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is used for the removal of small lesions of the oral mucosa by excision or vaporization procedures (23-35). Application of diode laser provides many clinical benefits, including control of bleeding, reduction in the requirement of infiltration anesthesia (32-33), and minimization of the postoperative pain and swelling. The diode laser can be used for labial and lingual frenectomy. The objective of this case presentation is to describe the parameters of performing upper labial frenectomy with the use of diode laser beams without infiltrated local anaesthesia.

MATERIALS AND METHODS

A 6-year-old patient was referred by the orthodontist for assessment of the upper anterior labial frenum. Clinical and pathological history is negative for diseases and allergies. The dental treatment plan reported only the presence of caries on deciduous teeth and seals on the first permanent molars. The clinical examination reported the presence of a high attachments of labial frenum and the presence of a diastema (Fig. 1a-b). The laser used to remove the frenulum was a diode laser [Doctor Smile, Lambda Spa, Brendola (VI) Italy] used with a wavelength of 980 nm with 320 microns of fiber in contact with a power of 2.0 W in continuous wave mode. Since the patient was very collaborative it was possible to proceed without the use of conscious sedation with nitrous oxide and without the aid of anesthetic for infiltration. Nevertheless a 15% Lidocaine-based anesthetic spray was used.

At the beginning of the surgery the frenulum

was exposed by grasping the upper lip gently with a sterile gauze. Then the frenulum was removed using the laser hand piece according to the longitudinal and perpendicular planes with respect to the insertion of the frenulum (Fig. 2). Tip should be inclined 45 degrees to the frenum. The resulting incision and consequently wound, of lozenge shape, will be healing by secondary intention. No stitches were necessary due to the hemostasis effect of the laser. The use of diode laser has allowed to obtain an optimal and immediate hemostasis and a good visibility of the surgical area to ensure accuracy during the cutting phase. The absence of stitches has also remarkably reduced the post-surgery pain with a consequently reduction of healing time. The patient was prescribed topical application of chlorhexidine-based gel 0.2% for one week twice a day. The patient was advising to perform adequate tension home gymnastics with upper lip traction with forefinger and thumb of both hands, to avoid relapse. He was examined one (Fig. 3) and two weeks after surgery. The laser equipment was defined by the manufacturer Lambda Spa as described in Table I.

RESULTS

One month after surgery, during the follow-up, the patient and her mother reported no discomfort in the post-surgery period and in the following days. The clinical examination showed an acceptable healing by secondary intention of the wound and the initial recovery of a physiological upper lip movement. (Fig. 4). The patient reported that the procedure was well tolerated.

Table I. *The laser equipment defined by Lambda Spa.*

Model	Doctor Smile Wiser
Code	LA8D0 001.3
Wavelength	980 nm
Power spike	16 W
Pulse mode	Continued, Pulsed, Super-pulsed
Frequency	Up to 25 kHz
Size (LxHxP)	161x208x176 mm
Weight	1,85 kg
Medical class/ Laser	II B/4

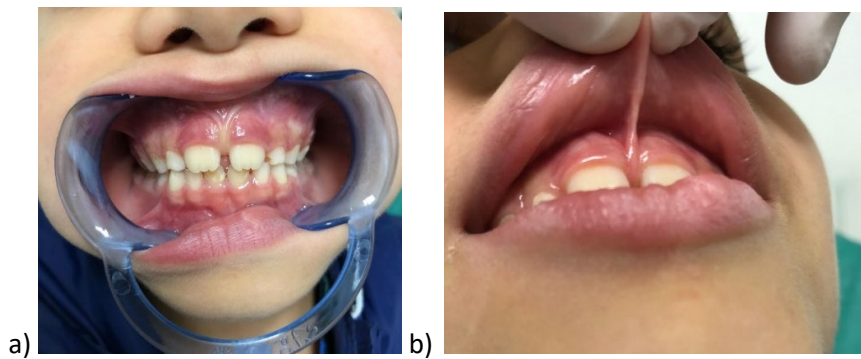


Fig. 1. *a) A preoperative view of upper-lip attachment. b) Another preoperative view of upper-lip attachment.*



Fig. 2. *The final laser cut does not require sutures or periodontal dressing.*

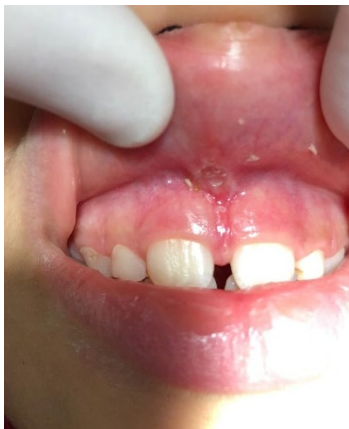


Fig. 3. *Two weeks after surgery.*

DISCUSSION

Frenectomy is a common procedure in the field of oral and maxillofacial surgery. Labial frenectomy

is often considered an over-treatment when the intervention is performed in mixed dentition. At age of 8-10 years, midline diastema is a physiological condition. However, when it is associated with anatomical anomalies and abnormalities of labial frenum, it is important to predict the probability of high labial frenum persistence after the eruption of permanent canines is completed. (34-38), have described indications for surgical removal of pathological frenum: in anomalous maxillary labial frenum associated with inflamed gingiva and gingival recession, in anomalous and abnormal maxillary labial frenum, associated to median diastema during mixed dentition, in patients needing orthodontic therapy, in anomalous maxillary labial frenum, associated to median diastema after complete eruption of the permanent canines (39-46). The advantage of laser surgery includes higher precision when compared to the conventional scalpel, less pain, bleeding, less swelling (47-52). The procedure is easy to perform, and no sutures are required, which decreases the risk of post-operative infection. The variables measured included surgical time, pain, discomfort while chewing and discomfort during speech on post-operative days (53-63). The great advantage of diode laser frenectomy in pediatrics should be the avoidance of needle infiltrated anesthesia. This case report described use of laser surgery for upper lip frenectomy.

The diode laser can be used with a satisfactory result for the removal of pathological frenum, being used in a pediatric patient due to their easy application, adequate coagulation, no need to suture



Fig. 4. *Upper-lip view one month later.*

and the slightest inflammation and pain. The use of diode laser has allowed to obtain an optimal and immediate hemostasis and a good visibility of the surgical area to ensure accuracy during the cutting phase.

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The role of periodontal bacteria, *Porphyromonas Gingivalis*, in Alzheimer's disease pathogenesis and aggravation: a review

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The aim of this study was to establish the role of *Porphyromonas gingivalis* in Alzheimer's disease. An electronic search of publications was established from three electronic databases: Cochrane, PubMed and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; in vitro analysis; in vivo on animals and post-mortem biopsies on humans; studies analyzing the correlation between periodontal disease and Alzheimer. The search resulted in 262 titles. Only 9 articles were included in the quantitative analysis. An inflammatory status in the oral cavity might be connect to a brain degeneration syndrome such as dementia and AD. However, a strictly connection is still not evincible. More trials are recommended in order to investigate the role of periodontal bacteria and *Porphyromonas gingivalis* in AD pathogenesis and aggravation.

Alzheimer's disease (AD) is the most common acquired cerebral degeneration and the main cause of dementia in Western countries (50%-66%). It is characterized by a progressive decline in thinking, memory, language and learning capacity. Studies have suggested that AD is caused by accumulation of amyloid β peptide (A β), leading to tauopathy and synaptic dysfunction. The abnormal formation of A β plaques is associated with its low levels in the cerebrospinal fluid (CSF) (1) and an aberrant hyperphosphorylation of the microtubule-associated tau protein which forms neurofibrillary tangles leading to destruction of the neurons (2). Therefore, the "gold standard" for the diagnosis of AD is the formation of amyloid plaques and neurofibrillary

tangles in the frontal neocortex and limbic systems of the brain (3).

The A β deposits cause intracerebral inflammation and some inflammatory biochemical markers such as interleukin 6 (IL-6) and the cerebral levels of IL-6, interleukin 1 Beta (IL-1) and tumor necrosis factor alpha (TNF- α) seem to be elevated in patients with AD when compared with healthy subjects of the same age. In healthy person and in normal physiological conditions, the blood-brain barrier (BBB) prevents exposure of the brain to constant oscillations in the concentration of blood and keeps the immune cell migration in low concentrations (4). This kind of surveillance fails during normal aging and in several diseases of the central nervous system (CNS) which

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facilitate the passage of peripheral immune cells and inflammatory mediators through it (5). Current studies suggest that an infection elsewhere in the body might aggravate these inflammatory processes in the brain (6).

Dental infections, especially periodontitis, are associated with dementia and are risk factors for the onset or the aggravation of AD. Periodontitis is a chronic inflammatory status in the oral cavity associated with anaerobic, Gram-negative bacteria that leads to a progressive loss of the supportive tissues of teeth and induces and amplifies low-grade systemic inflammation resulting from the relevant bacteria and their components entering the body through the systemic circulation (7-18). The dental plaque is a biofilm that forms on the teeth, in subgingival spaces and in root canals, and it evokes chronic inflammation on periodontal tissues (19-25). It is proposed that the inflammation due to chronic periodontitis might add to the inflammatory pool generating several pro-inflammatory mediators.

In chronic periodontitis there are a raised serum pro-inflammatory state as shown by increases in C Reactive Protein (CRP) and pro-inflammatory cytokines with a reduction in anti-inflammatory markers (26-35). The evidence for the association between chronic periodontitis and AD has been established through several studies but it was not well elucidated the exact mechanism of this linkage. It was suggested that a bidirectional relationship between the two pathologies. The poor oral hygiene in AD can be possible because of a lack of manual dexterity or the inability of the patients to receive professional care resulting in chronic periodontitis and finally in tooth loss. Afterwards, the development of nutritional deficits reduces brain stimulation from chewing and the numbers of pyramidal cells in the brain decrease resulting in a minor acetylcholine levels in the hippocampus and a diminished memory. On the other hand, a reverse causal association is possible (36-45). It is feasible that a poor oral hygiene causes chronic periodontitis and indirectly increases the risk for AD. Several biochemical and microbiological studies proposed that elevated levels of antibodies against some periodontal pathogens (*P. gingivalis*, *A. actinomycetemcomitans*, *Tannerella forsythia*)

were identified in AD patients but not in cognitive normal individuals (46-50). *P. gingivalis* (PG) was especially detected in the postmortem brain tissue of AD patients. These could represent some markers for the future possible development of AD (11). PG, a gram-negative anaerobe bacterium, is one of the keystone pathogens among the bacteria associated with periodontal disease (51-54). It possesses many virulence factors including lipopolysaccharide, capsular material, fimbriae, and proteases.

Among different proteolytic enzymes secreted by PG, the arginine- and lysine- specific cysteine proteases, the so-called gingipains, are the most important for the bacterium pathogenicity. Gingipains are highly potent modulators of host immune response, e.g., they degrade antimicrobial peptides, destroy the bactericidal activity of complement, and deregulate the cytokine network.

MATERIALS AND METHODS

Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; *in vitro* analysis; *in vivo* on animals and post-mortem biopsies on humans; studies analyzing the correlation between periodontal disease and Alzheimer. The exclusion criteria were: publications that reported the same data as later publications by the same authors; commentaries and letters to the editor; case reports; case series; reviews and works that correlated Alzheimer's disease to systemic syndromes or pathologies.

Types of interventions

All the studies examined compared the possible association between chronic periodontitis and AD. Early studies investigated if the presence of IL-6 and TNF- α in bloodstream could be an indication of association between chronic periodontitis and AD. In addition, recent studies tried to understand if *P. gingivalis* plays a main role in induction and/or aggravation of AD.

Outcome measures

Each study evaluated the possible association between chronic periodontitis and AD by the measure of cytokines level. The serum level of cytokines (IL-6, IL-1, and

TNF- α) were investigated by venous blood samples of the subjects, acquired concomitant to clinical exam.

Research question

Pico question. We have formulated a specific question based on the PICO format where P stands for participants, I for intervention, C for comparison and O for the objective. The question was: “Is there a correlation (O) between periodontal disease (I) and Alzheimer’s (C) in studies published in the last five years (P)?” An electronic search of publications was established from three electronic databases: Cochrane, PubMed, Cochrane and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. The detailed search design and strategies, including keywords, developed for each database are presented below.

Search strategy

The search strategy involved searching electronic databases (MEDLINE, EMBASE, and COCHRANE LIBRARY) and was supplemented by cross-checking the bibliographies of relevant review articles. We used two filters to follow data for the research: publication dates about 5 years, and publications in English. The following combination of words was used: (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR “alzheimer”[All Fields]) AND (“periodontal diseases”[MeSH Terms] OR (“periodontal”[All Fields] AND “diseases”[All Fields]) OR “periodontal diseases”[All Fields] OR (“periodontal”[All Fields] AND “disease”[All Fields]) OR “periodontal disease”[All Fields])) AND (“2015/01/18”[PDat] : “2020/01/16”[PDat]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR “alzheimer”[All Fields]) AND periodontal[All Fields] AND (“Pathogens”[Journal] OR “pathogens”[All Fields]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR (“alzheimer’s”[All Fields] AND “disease”[All Fields]) OR “alzheimer’s disease”[All Fields]) AND (“*Porphyromonas gingivalis*”[MeSH Terms] OR (“*porphyromonas*”[All Fields] AND “gingivalis”[All Fields]) OR “*Porphyromonas gingivalis*”[All

Fields]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR (“alzheimer’s”[All Fields] AND “disease”[All Fields]) OR “alzheimer’s disease”[All Fields]) AND (“*Treponema denticola*”[MeSH Terms] OR (“treponema”[All Fields] AND “denticola”[All Fields]) OR “*Treponema denticola*”[All Fields]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR (“alzheimer’s”[All Fields] AND “disease”[All Fields]) OR “alzheimer’s disease”[All Fields]) AND (“*Fusobacterium nucleatum*”[MeSH Terms] OR (“fusobacterium”[All Fields] AND “nucleatum”[All Fields]) OR “*Fusobacterium nucleatum*”[All Fields]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR (“alzheimer’s”[All Fields] AND “disease”[All Fields]) OR “alzheimer’s disease”[All Fields]) AND (“*Prevotella intermedia*”[MeSH Terms] OR (“prevotella”[All Fields] AND “intermedia”[All Fields]) OR “*Prevotella intermedia*”[All Fields]) OR (“alzheimer disease”[MeSH Terms] OR (“alzheimer”[All Fields] AND “disease”[All Fields]) OR “alzheimer disease”[All Fields] OR (“alzheimer’s”[All Fields] AND “disease”[All Fields]) OR “alzheimer’s disease”[All Fields]) AND bacterial[All Fields] AND (“plaque, amyloid”[MeSH Terms] OR (“plaque”[All Fields] AND “amyloid”[All Fields]) OR “amyloid plaque”[All Fields] OR “plaque”[All Fields] OR “dental plaque”[MeSH Terms] OR (“dental”[All Fields] AND “plaque”[All Fields]) OR “dental plaque”[All Fields]) AND (“mouth”[MeSH Terms] OR “mouth”[All Fields] OR (“oral”[All Fields] AND “cavity”[All Fields]) OR “oral cavity”[All Fields])).

RESULTS

The search resulted in 262 titles. Following the first stage of screening, after the records identification through database manual searching, 100 potentially relevant studies were identified. After the second stage screening, 40 full text publications were obtained and analyzed and 20 were excluded. Afterwards, 20 articles resulted eligible after full text

reading and a cross search of the articles references was accomplished; 9 articles were consequently added. In the end only 9 articles were included in the quantitative analysis (Fig. 1).

DISCUSSION

This review assessed to research a possible link between chronic periodontitis and AD and to evaluate the contribution of *P. gingivalis* in the aggravation

of these pathologies. An inflammatory status in the oral cavity might be connect to a brain degeneration syndrome such as dementia and AD. In 2008 Kramer et al. proposed that chronic periodontitis with significant bacterial and inflammatory burden may enhance the presence of inflammatory cells in the brain. In fact, Cestari et al. in 2016 showed that patients with AD and chronic periodontitis had an elevated concentration of IL-6 and TNF-alfa. This could contribute to the initiation or the progression

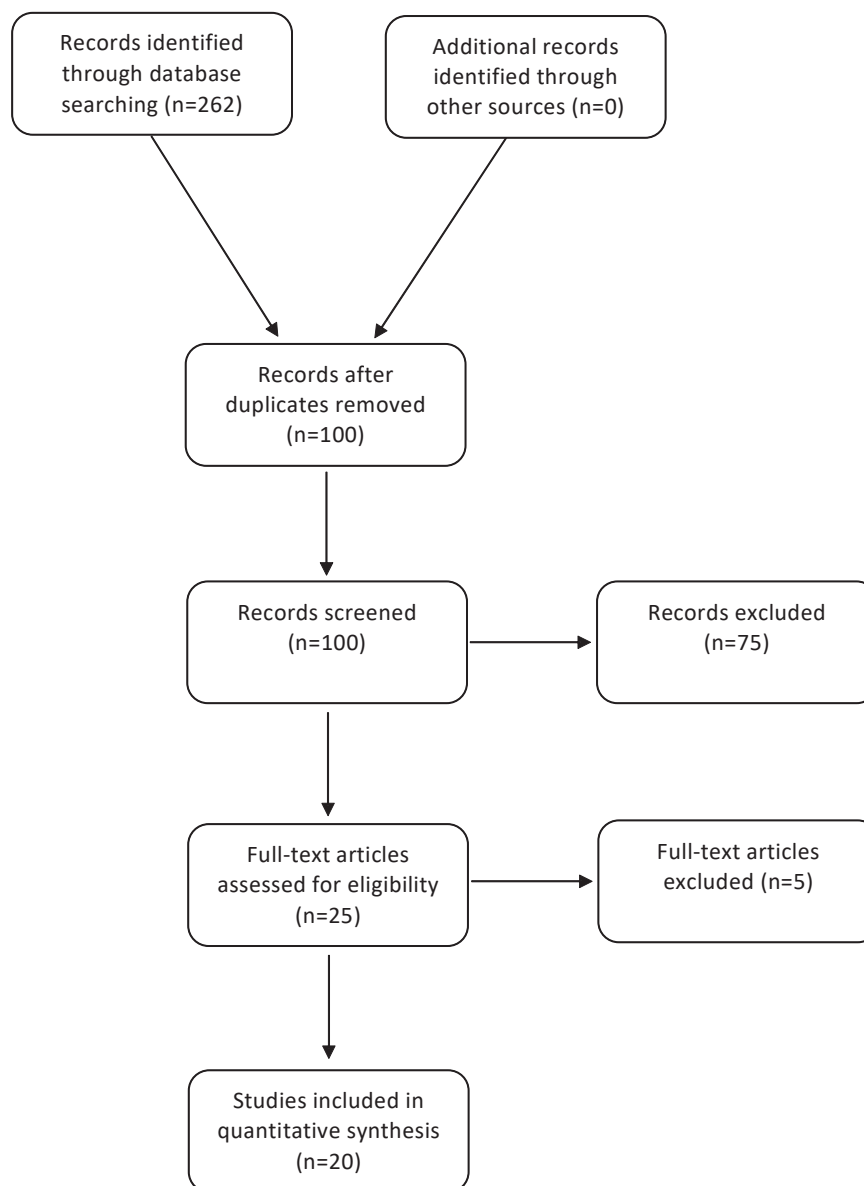


Fig. 1. *Studies' flow diagram*

of AD. In 2016 Ide et al. reported that there is not a clear relationship between severity of brain diseases and degree of periodontitis. However, they also showed that patients who had AD and chronic periodontitis had a marked increase in cognitive decline over the follow-up period (55-58). Chen et al in 2013 in a retrospective matched-cohort study demonstrated that 10-year of chronic periodontitis exposure was associated with a 1.707-fold increase in the risk of developing AD. However, there were no direct evidence that chronic periodontitis and AD are strictly connected (59-67).

A review from 2014 by Gaur et al. reported that status of chronic peripheral inflammation could increase the permeability of the BBB to peripheral mediators. In addition it had been proposed that these mediators could diffuse into the brain through vascular or sensory pathways. However, these factors require further investigation.

In the last years several studies investigated if there is a possible connection between AD and intraoral infection with *P. gingivalis*.

Sparks et al. retrospectively compared sera of individuals developing AD with those who did not. In the patients who developed AD, antibody levels were higher for *Prevotella intermedia*, *T. denticola*, and *Fusobacterium nucleatum* already at a baseline (without cognitive impairment) and increased further after onset of AD, which was accompanied with increased levels of antibodies against PG.

In 2018 Singhrao et al. reported that the lipopolysaccharide (LPS) and the microbullets of *P. gingivalis* contribute to the development of AD. They suggested that neurodegeneration can result from direct invasion of oral and other microbes to the brain.

Another study by Dominy et al hypothesized that AD patients could produce antibodies in CSF against *P. gingivalis*. They showed in 2019 how *P. gingivalis* causes infection in the brain of AD patients by matching saliva samples and cerebrospinal fluid (CSF) samples. They demonstrated the presence of *P. gingivalis* DNA in AD brains and proposed the evidence that *P. gingivalis* and gingipains in the brain play a central role in the pathogenesis of AD. On the other hand, in 2018 Laugisch et al. suggest that a local production of antibodies in CSF directed against *P.*

gingivalis and other related periodontal pathogens may occur in patients with dementia, but there was no association with AD. In fact, despite a high prevalence of periodontal disease, the periodontal pathogens selected in the study were not detected in serum or CSF. The difference could be linked to the different age of the participants in the study, i.e., younger than 70 years, compared to the overwhelming majority of the participants of the previous studies who were all around 80-years-old (late-onset AD).

The relationship between brain diseases and periodontitis is still not evincible. However, an inflammatory status in the oral cavity might be connected to a brain degeneration syndrome such as dementia and AD. In some studies patients with AD and chronic periodontitis reported elevated concentration of IL-6 and TNF- α which could be a connection between the two pathologies. More clinical trials are recommended to investigate the role of periodontal bacteria, such as *Porphyromonas Gingivalis*, in Alzheimer's disease pathogenesis and aggravation.

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Guided endodontic microsurgery in apicoectomy: a review

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The purpose of this review was twofold. The first aim was to show predictable protocols of guided endodontic microsurgery (EMS) and to describe its clinical outcomes. The second aim was to verify the accuracy of this technique compared to the traditional pathway. An electronic search of publications was established from two electronic databases, Cochrane and PubMed, by two independent researchers. The search strategy used a combination of controlled vocabulary and free-text words. Inclusion and exclusion criteria were defined by the authors before the start of the study. Inclusion criteria were: all studies published in English language; studies in vivo on humans; human cadaver studies; studies analyzing guided surgery in apicoectomy. The initial search yielded 67 citations, of which 10 were included. The studies included in this review analysis have shown two different technique both for digitization of dental arch and for surgical procedures too. Guided endodontic micro-surgery is a more predictable technique with less intra-operative and post-operative complications compared to the "freehand" technique and this procedure has shown a greater accuracy.

The goals of endodontic treatment are to prevent or resolve root canal infections (1-10). In the case of periradicular pathosis, potential pathogens are usually eliminated by non-surgical root canal treatment with subsequent obturation and coronal restoration (11-15). However, treatments can fail due to anatomical difficulties, iatrogenesis and lack of bacterial removal. Therefore, when conventional endodontic techniques are exhausted, endodontic surgery becomes an alternative treatment to remove unreachable infected areas and seal the root canal, allowing for healthy periapical tissue recovery (16-18)

The success rate of conventional endodontic surgery is relatively low, between 43.5% and 74%. However, by applying contemporary techniques, including high-power magnification and illumination, microsurgical instruments, and modern filling

materials, success rates of surgery have significantly increased, and, in turn, surgery has become a more effective treatment. Success rates for endodontic microsurgery (EMS) have been reported to be between 88.9% and 100%. EMS is, in its broadest sense, defined as the treatment performed on the root apices of an infected tooth, which was unresolved with conventional root canal therapy.

EMS was introduced in the 1990s and has been continuously developing over the years (19-22). Generally, procedure for EMS encompasses the removal of the buccal bone in order to accurately locate the root apices of an infected tooth, which may include the removal of intact bone. It also includes three critical steps to eliminate persistent endodontic pathogens (23-31): 1) surgical debridement of pathological periradicular tissue, 2) root end

Key words: guided endodontic surgery, guided root resection, endodontic microsurgery, apicoectomy, surgical guide, digital planning

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resection (apicoectomy), and 3) retrograde root canal obturation (root-end filling) (2) with Mineral Trioxide Aggregate (MTA) or Biodentine as a new way (32-35). Lastly the surgical site is sutured. Nowadays the use of Cone Beam Computer Tomography (CBCT) is mandatory to perform an exact diagnosis of type, and extension of the periapical lesion, shape and anatomy of the roots and of the channels system, and its use have contributed to increase the success rate.

Recently, CBCT combined to intraoral scan has been recognized as means to build an endodontic surgical guide to perform a facilitate access to the apex, similar to implant dentistry where, in the last decade, CBCT imaging was extensively used to plan and perform accurate guided implant placement by surgical guides (36-44). Surgical templates are particularly helpful in difficult cases involving teeth close to potentially problematic anatomic structures. Structures such as adjacent root tips, the inferior alveolar nerve, the mental foramen, and the maxillary sinus contribute to the difficulty in surgical approaches to the root apex (45-55).

Therefore, virtual three-dimensional (3D) planning in endodontics and the surgery guidance represents new important aspects for the treatment of complex cases. The first aim of this review was to show predictable protocols of guided EMS and to describe its clinical outcomes. The second aim was to verify the accuracy of this technique compared to the traditional pathway.

MATERIALS AND METHODS

Inclusion and exclusion criteria were defined by the authors before the start of the study. Inclusion criteria were: all studies published in English language; studies in vivo on humans; human cadaver studies; studies analyzing guided surgery in apicoectomy.

The exclusion criteria were: in vitro and in vivo on animal analysis; publications that reported the same data as later publications by the same authors; commentaries and letters to the editor and reviews of literature.

Types of interventions

All studies that presented new endodontic surgical guided techniques, thanks to the use of customized guides

for the individual patient obtained through CBCT 3D planning. Besides, studies that analyzed the accuracy of these techniques compared to traditional ones.

Outcome measures

Each study that showed a new micro-surgical endodontic guided technique and evaluated clinical outcomes and precision of guided surgery compared with the traditional approach in apicoectomy.

Research question

Pico question. We have formulated a specific question based on the PICO format where P stands for participants, I for intervention, C for comparison and O for the objective. The question was: “Does exist an improvement of clinical outcomes and accuracy (O) using guided surgery (I) respect to traditionally surgery (C) in the treatment of patients (P) affected by periapical/periradicular lesion requiring apicectomy?”. An electronic search of publications was established from two electronic databases: Cochrane and PubMed. The search strategy used a combination of controlled vocabulary and free-text words. The detailed search design and strategies, including keywords, developed for each database are presented below.

Search strategy

(guided[All Fields] AND (“surgery”[Subheading] OR “surgery”[All Fields] OR “surgical procedures, operative”[MeSH Terms] OR (“surgical”[All Fields] AND “procedures”[All Fields] AND “operative”[All Fields]) OR “operative surgical procedures”[All Fields] OR “surgery”[All Fields] OR “general surgery”[MeSH Terms] OR (“general”[All Fields] AND “surgery”[All Fields]) OR “general surgery”[All Fields])) AND (“endodontics”[MeSH Terms] OR “endodontics”[All Fields]) OR (guided[All Fields] AND (“surgery”[Subheading] OR “surgery”[All Fields] OR “surgical procedures, operative”[MeSH Terms] OR (“surgical”[All Fields] AND “procedures”[All Fields] AND “operative”[All Fields]) OR “operative surgical procedures”[All Fields] OR “surgery”[All Fields] OR “general surgery”[MeSH Terms] OR (“general”[All Fields] AND “surgery”[All Fields]) OR “general surgery”[All Fields])) AND (“apicoectomy”[MeSH Terms] OR “apicoectomy”[All Fields])

RESULTS

The search from electronic databases resulted in 55 titles, for an amount of 67 title by 12 additional records identified by manual searching. Following the first stage of screening, setting the filter at last 10 years, 30 potentially relevant studies were identified.

After the second stage screening 14 articles were eliminated by reading the titles, 4 further articles by reading the abstract. Afterwards, after the full text reading of the 12 articles eligible, only 10 articles were included in the quantitative analysis (Fig. 1).

DISCUSSION

Apicoectomy consists in the resection of the dental apex associated to periapical lesion and in the retrograde seal of the root canal. The approach to apicoectomy has significantly changed over the years; the advent of new technologies, in fact, has allowed the transition from “freehand” surgery to

guided surgery. Traditional approach consisted: mucoperiosteal flap preparation, ostectomy if necessary, surgical debridement of the periradicular tissue, resection of the end of the root and retrograde obturation of the canal. The freehand technique showed, by the literature, numerous disadvantages: removal of a greater quantity of bone, longer intervention time, post-operative problems, such as pain and swelling (56-62). Less experienced clinicians could mistakenly perform excessive osteotomy in difficult anatomical cases. The diameter of a bony defect influences healing outcomes after endodontic surgery. Extensive bone destruction tends to result in uncertain or unsuccessful healing, delays in the healing process, and risk for postoperative complications. However, one of the most critical disadvantages of conventional root-end resection included the damage to anatomically vital structures such as inferior dental nerve, mental nerve, adjacent root and maxillary sinus. The guided EMS involves the creation of a

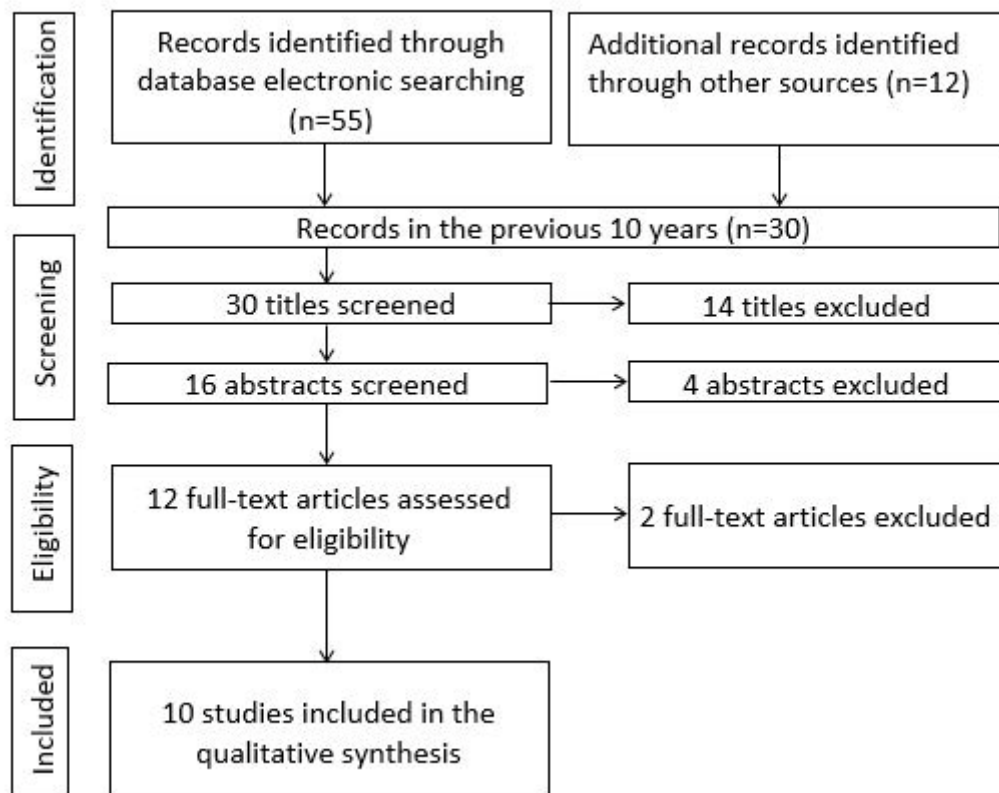


Fig. 1. Diagram of article selection process–Search strategy.

surgical guide through a dedicated software. This procedure is similar to the one used for the creation of a template that guides implant placement. The 3D planning to produce the surgical templates is certainly costly and still time-consuming compared with conventional procedures and requires technical expertise, equipment, and software for merging files to designing and printing 3D surgical guides (63-67). The template allows a correct inclination of the handpiece and of the dedicated drills for a correct resection surgery of the root apex. In this way, access to the root apex is planned and performed correctly, safely and minimally invasively by minimizing the risks of damaging vital structures and by

decreasing the extent of the osteotomy. Decreasing the extent of the osteotomy, by means of 3D printed surgical templates, could also help reduce surgical complications and promote healing. Compared to the conventional approach, EMS using the guide template has shown many advantages such as below:

1. root apex can be more accurately located through the provision of drilling hole, which will result in the minimal invasive preparation;
2. Surgical time and the volume of bone preparation can be significantly reduced;
3. Post-operative healing is more favorable and reduced risk of infection leads to better prognosis;
4. more predictable and less-technique sensitive

Table I. Case reports included in the review analysis.

Author	Year	Digitization technique*	Surgical guided procedure	Clinical Outcome(s)
Liu Y et al.	2014	Indirect	Guided osteotomy, apex localization and root resection	This case report illustrates that with additive manufacturing (AM) and digital design methods, optimal operational plans can be designed and realized for apicoectomy, and the quality and efficiency of clinical surgery are greatly improved compared with conventional methods.
Strbac GD et al.	2017	Direct	Guided osteotomy, apex localization and root resection	There were no post-operative complications, and clinical and radiologic assessments verified complete healing of the teeth. The guided microsurgical endodontic treatment presented appears to be a viable technique that allows for predefined osteotomies and root resections.
Giacomino CM et al.	2018	Indirect	Guided osteotomy, apex localization and root resection	Targeted EMS could prove to be an important breakthrough allowing precision-guided surgery in anatomically complex areas for teeth that may have otherwise required extraction. Surgical phase simplification is achieved and time is reduced.
Ahn SY et al.	2018	Indirect	Guided bone perforation and apex localization	A computer-aided design/computer-aided manufacturing (CAD/CAM)-guided surgical template minimized the extent of osteotomy and enabled precise targeting of the apex. There were no post-operative complications. A CAD/CAM-guided surgical template is useful in endodontic surgery for complicated cases.
Kim JE et al.	2019	Indirect	Guided bone perforation and apex localization	Endodontic microsurgery (EMS) using the guide template could be useful method in calcified canal which was untreatable with conventional root canal therapy and unable to track the position of the apex due to the absence of fistula.
Tavares WLF et al.	2019	Direct	Guided osteotomy, apex localization and root resection	The method employed was demonstrated to be very straightforward and reliable. This method allowed the patient to be treated expeditiously with very precise tissue removal. The patient remained asymptomatic, and the tissue healed normally.
Sutter E et al.	2019	Direct	Guided osteotomy, apex localization and root resection	Guided apicoectomy using a patient specific CAD-CAM drilling template is a precise, time-effective surgical intervention with high precision and predictability. This innovative treatment method is primarily indicated for apicoectomy in complex anatomical areas with proximity to vulnerable structures.
Avantaggiato P et al.	2020	Direct	Guided bone perforation and apex localization	Further investigation are needed to test and improve the effectiveness of the treatment but this technique seems very promising because it is less invasive for the patient and simplifies the work for the dentist who can perform micro-surgery in an easier and faster way.

*Indirect: arch digitalization from traditional impression with extra-oral scanner; Direct: arch digitalization with intra-oral scanner.

results can be expected regardless of clinician's experience.

The case reports included in this review article demonstrated the presence of two different techniques described in literature both regarding the planning of 3D surgical guides and surgical procedures (Table I.).

The surgical strategies documented to date provide the use of guides only for bone perforation and apex localization or contrary to obtain a micro invasive osteotomy and the programmed apical resection with dedicated tips. Regarding the surgical guides we can show an indirect and a direct method of dental arch digitization.

In the indirect technique, a preliminary impression of the dental arch is detected and subsequently a stereolithography (STL) file of the plaster cast is generated using an extra-oral scanner. In the direct technique the STL file is obtained through an intraoral scanner (IOS) directly. Consequently, the STL of the dental arch and the digital imaging and communication in medicine (DICOM) CBCT files are imported into the planning software. Since the superimposition, virtual planning is performed to determine the exact localization for root resection. Subsequently, a tooth-supported drilling template is designed, and 3D printed. Accuracy of the impression process may have a significant effect on the accuracy of guide template (6). Although all case reports have come to the same conclusions previously reported; therefore, the use of a 3D printed surgical guide is a more predictable method with less intra-operative and post-operative complications compared to the "freehand" technique, further investigation is needed to compare the accuracy of the two described techniques (45-50).

According to the clinical outcomes of the case reports showed in this review, Ackerman et al. (17) have assessed the accuracy of surgical guides designed by CBCT for the use during endodontic surgery in a cadaveric model. In the experimental group, surgical guides were designed and printed using 3D printer. In the control group, surgical access was completed "freehand" by visually approximating the measurements only from the CBCT scan. The deviation of the surgical access point in the experimental CBCT-guided group versus

the approximate freehand group with CBCT control were compared. The mean deviation for the guided group was 1,473 (± 0.751 mm), significantly less than that of the approximate freehand group where the mean deviation was 2,638 mm (± 1.387) ($P 0.0009$).

Also Fan et al. (18) have conducted a human cadaver study and have compared CBCT imaging and prefabricated grids to guide apical access during EMS to the non-guided method. The mean deviation of the drill paths from the target points was 0.66 mm (± 0.54 mm) for grid-based drilling and 1.92 mm (± 1.05 mm) for non-guided drilling ($P < .001$). Grid-based drilling was on average 1.27 mm (95% confidence interval, 0.81–1.72 mm) closer to the target point than non-guided drilling.

Both studies reached the same conclusion: the use of a surgical guide is a more accurate method for accessing the apical portion of the root during surgical endodontics than an approximate non guided method.

Within the limitations of this review study, the guided EMS, in the field of apicoectomy, appears to represent a predictable technique to manage this surgical procedure. Moreover, it has shown better results compared to the traditional "freehand" technique, in terms of accuracy, surgical time, volume of bone preparation, control of post-operative pain and swelling. Lastly, guided EMS showed less-technique sensitive results that can be expected regardless of the clinician experience.

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The health of soft tissues around four dental implants loaded immediately supporting a 4-year-old fixed screw-retained prosthesis

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The aim of this study was to assess the soft tissues health around the implant/abutment interfaces of fixed screw-retained prosthesis supported by four dental implants after at least 1-year in function. All the implants were placed between December 1, 2015 and April 30, 2019. Digital implant surgical planning was performed for all the complete-arch rehabilitations and then full-guided surgery was performed. The fixed-interim prostheses were delivered the day of the surgery and replaced by definitive prostheses after the healing period. Patients were followed-up to determinate peri-implant scores, such as Plaque Score (PS) and Bleeding on Probing (BoP). A total of 160 implants were placed in 37 patients, whereas 3 patients received both arches rehabilitated. A total of 40 complete-arch rehabilitations were performed, 26 in the maxilla and 14 in the mandible. Only 5 implants failed resulting in an overall implant survival rate of 96.9%. BoP was detected around 6 implants (3.7%) and 16 implants showed a superficial amount of plaque resulting a Plaque Score of 10%. Within the limitation of this study, it seems that the use of a fixed screw-retained prostheses supported by four dental implants to rehabilitate edentulous jaws could be a valid treatment option in the short and medium term without critical peri-implant issues. However, several perspective studies with longer follow-up are needed to achieve more predictable results.

Nowadays, treatment of edentulous patients aims to restore both masticatory and aesthetic function by means of prostheses that are functionally stable and identical from the natural dentition over time (1-9). Fixed prostheses supported by dental implants have become more and more required by the patients. These prostheses are a well-documented method to treat from a single tooth gap to full mouth rehabilitation (10-19). The main limitation of full-arch rehabilitation is the possible reabsorption of posterior jaws due to the presence of hopeless teeth or long-time edentulism.

Bone reabsorption is often related to a pneumatization of maxillary sinus in the maxillary jaws and a surfacing of the inferior alveolar nerve. In the beginning of 2000, it was proposed to insert implants with >12 mm length in tilted position of 30° in order to avoid the maxillary sinus or the mental foramen and to get in contact with native bone (20-27). The technique provided the use of four implants with two straights in frontal area and two tilted in the posterior region (28-33). The four implants had to be placed in regular and symmetric manner to provide an inter-implant distance as large as possible.

Key words: computer guided surgery, digital workflow, soft tissue, full arch

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Traditionally, a mucoperiosteal flap had to be raised to visualize the implant recipient site. This can be unnecessary when computer-guided surgery is performed by means of surgical templates that give guidance to the surgeon because the accuracy that 3D study gives to the clinician provides to perform a minimally invasive flapless implant surgery causing less pain and discomfort to the patients and improving healing process due to the preservation of blood circulation in the soft tissues.

Immediate loading protocol for edentulous complete arches have been developed and have been largely accepted by clinicians and patients. The immediate loading of dental implants to restore complete arch is performed by means of an interim screw-retained prosthesis solidarized onto the implants. However, oral hygiene maintenance can be a hard challenge for the patients (34-39). A considerable accumulation of plaque can be encountered around the implants/abutments interfaces and plaque deposits have been reported to cause mucositis.

The aim of this study was to assess the soft tissues health around the implant/abutment interfaces of fixed screw-retained prosthesis supported by four dental implants after at least 1-year in function. This research is reported according to the STROBE statement.

MATERIALS AND METHODS

This retrospective study included all patients treated in a private dental clinic with fixed full arch screw retained prosthesis supported by four dental implants loaded immediately in the maxilla and/or mandible between December 1, 2015 and April 30, 2019 to have at least 1 year of follow-up after definitive prosthesis delivery.

All patients signed an informed consent for data collection. The medical status of patients regarding any diseases was documented. The patients included in this study needed to present complete edentulous jaws or hopeless teeth requiring extraction refusing invasive grafting procedures. In addition, the patients needed to suite the following inclusion criteria: absence of oral mucosal disease; suitable bone dimension for the insertion of 4 implants of the following minimum dimensions: Ø

≥ 4.1mm x length 8mm or Ø3.3mm x length 10mm. Furthermore, patients with one of these conditions were excluded: heavy smokers (more than 5 cigarettes per day); drug or alcohol abuse; poor oral hygiene; radiotherapy in the head and neck region; patients undergoing antitlastic chemotherapy. The procedures accomplished in this study were in accordance with the Helsinki Declaration of 1964 and revised in 2013 about ethical principles regarding human experimentation.

For each rehabilitation, a case study was digitally performed by means of implant planning software (coDiagnostix, Dental Wings, Montreal, Canada) (Fig. 1).

The digital data from the patients were merged together in order to perform a full guided static-template assisted surgery. On the day of the surgery, all patients received antibiotic prophylactic therapy: 2 g of amoxicillin (or clindamycin 600 mf if allergic to penicillin) 1 hour before the surgery and rinsed with chlorhexidine mouthwash 0.2% for 1 minute before the intervention. Local anesthesia was performed using articaine with adrenaline 1:100000 (40-41). All hopeless teeth were extracted the day of the implant surgery as gently as possible if they cannot be useful to support the surgical template. The precise fit of surgical templates was checked before the surgery.

To stabilize the templates a surgical silicon index was used, and two or three pre-planned anchor pins were inserted. All implants were inserted using a full guided surgery. Implants sites were prepared using a dedicated drill set (Straumann, Straumann Holding AG, Basel, Switzerland) following manufacturer's guidelines. Two types of dental implants (Straumann Bone Level and Straumann Bone Level Tapered, Straumann) could be used. Mucoperiosteal flaps were raised if any implant site needed guided bone regeneration (GBR). Care was taken to not overextend the flaps to maintain mininvasivity. The implant insertion torque was measured after the removal of surgical template to avoid any interferences. Following implant placement, screw retained abutments (SRA, Straumann) were placed to correct angle compensation for tilted posterior implants and to correct implant angulation in the anterior zone when placed in the residual alveolar ridge. Soft tissue closure was performed using Polyglycolic Acid (PGA) sutures (Vicryl, Ethicon, Somerville, NJ, USA).

The interim prosthesis based on the digital plan was designed and milled by the dental technician from a block

of polymethyl methacrylate (PMMA, dima Mill temp, Kulzer) and blocked-out to the titanium copings. The prosthesis, without cantilever, was attached to the SRAs and it was screwed to 15 Ncm of torque. Patients were instructed to rinse with 0.2% chlorhexidine mouthwash for one minute twice a day for 15 days, to have a soft diet for one month and to avoid any trauma on the surgical sites. If an implant demonstrated mobility, pain or soft tissue irritation it had to be removed, recorded in the database as a failure, and then replaced after 3 months. The definitive prosthesis consisted of a titanium infrastructure around which acrylic resin denture base material with denture teeth (Fig. 2).

The occlusal scheme was adapted to the opposing dentition. In addition, patients were instructed to wear a nightguard to protect the acrylic teeth during the night. Patients were recalled every 4 months for professional oral hygiene and every 12 months for the annual radiographic and clinical examination. Panoramic radiographs were performed by an independent operator in order to assess radiographic peri-implant bone quality following the European Association for Osseointegration guidelines¹⁰ (Fig. 3). Periodontal



Fig. 2. *Definitive prosthesis.*

parameters bleeding on probing (BoP) and plaque score (PS) were recorded at each planned visit.

Outcomes

Soft tissue parameters around the implant/abutment interfaces (Fig. 4) were assessed by means of a periodontal probe (Hu-Friedy PGF-GFS, Hu-Friedy, Chicago, IL., USA). The BoP was recorded at four sites around each implant (mesial, distal, buccal and lingual) according to the Mombelli Index. The probe was carefully inserted 1 mm into the sulcus and the provoked bleeding within 20 s was assessed (0 = no bleeding; 1 = spot bleeding, 2 =

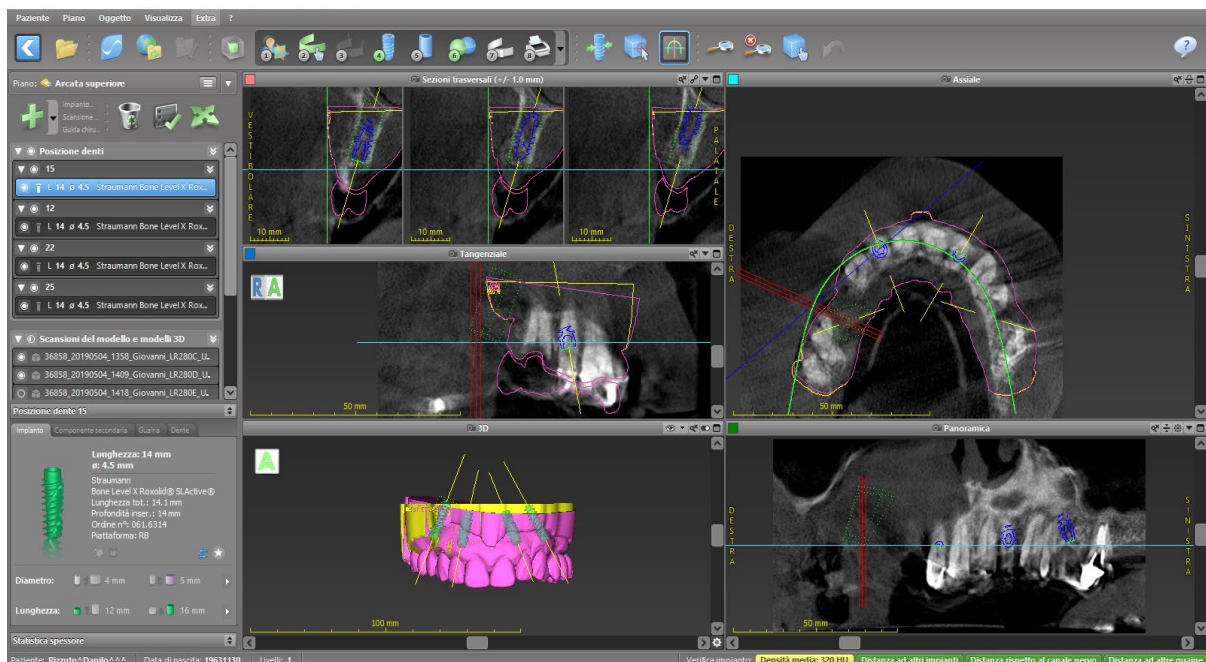


Fig. 1. *Digital planning of the rehabilitation.*



Fig. 3. *Implant/abutment interface.*

linear bleeding, and 3 = spontaneous bleeding).

The PS was measured by running the periodontal probe parallel to the abutment surfaces and scored at one site for implants as the presence of plaque (yes/no) on the abutment/restoration complex. An independent blinded dental hygienist who was not involved in the study recorded all the periodontal measurements.

Statistical analysis

All the data were imported onto a master spreadsheet and the name of the patients were codified to be de-

identified. Descriptive analysis was performed using mean and standard deviation. The implant was used as unit in the statistical analysis.

RESULTS

Clinical data from 37 patients of both sexes (20 men and 17 women), mean age, 68.3 years ranged from 46 to 97 years, rehabilitated with a fixed screw-retained prosthesis supported by four dental implants inserted using computer guided surgery, and followed-up with a minimum period of 1 year were collected and analyzed in the statistical analysis (Table I).

A total of 160 dental implants were placed and all of them were loaded immediately receiving a fixed screw-retained interim complete-arch prostheses without cantilevers (Table II). A total of 26 maxillary arches and 14 mandibular arches were rehabilitated. Three out of 40 patients received both arches rehabilitated. One-hundred four implants were placed in the maxilla and fifty-six were placed in the mandible. BoP was detected around 6 implants (3.7%) and 16 implants showed a superficial amount of plaque resulting a Plaque Score of 10%.

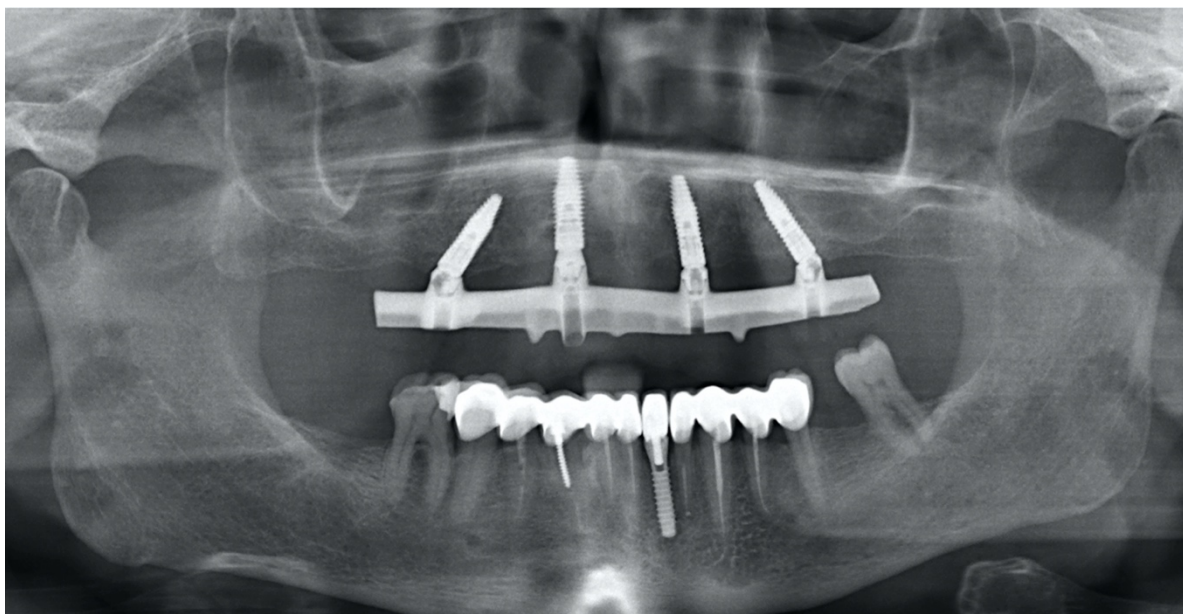


Fig. 4. *Panoramic X-Ray of the definitive prosthesis.*

Eighty-three implants were placed in healed sites (51.9%) while seventy-seven implants were placed in post-extraction sockets (48.1%). One-hundred six implants were placed flapless (66.2%) while fifty-four implants were placed raising a mucoperiosteal

flap (33.8%). Forty-eight implant sites needed GBR (30%). Two different types of dental implants were used: 12 Bone Level (BL, Straumann, Straumann Institute AG) and 148 Bone Level Tapered (BLT, Straumann, Straumann Institute AG). A total of

Table I. *Main characteristics of the patients.*

Patients (Female, Male)	37 (17, 20)
Age	68.3 (46-97)
Smokers ≤ 5 cigarettes per day	9
Complete arches rehabilitated	40
Maxilla	26
Mandible	14
Opposite dentition	
Natural teeth and ceramic crowns	17
Removable dentures	13
Natural teeth	4
Both arches treated	3

Table II. *Main characteristics of the implants.*

Number of implants	160
Failed implants	5
Type of implants	
Bone Level Tapered RegularCrossfit®	108
Bone Level Tapered NarrowCrossfit®	40
Bone Level RegularCrossfit®	12
Implants in the maxilla	104
Implants in the mandible	56
Implants length:	
14 mm	87 (54.4%)
12 mm	58 (36.2%)
10 mm	12 (7.5%)
8 mm	3 (1.9%)

120 4.1 mm Ø RegularCrossfit® implants (RC, Straumann, Straumann Institute) were used, while 40 3.3 mm Ø NarrowCrossfit® implants (NC, Straumann, Straumann Institute) were used. The most used implant length was 14 mm 87 times (54.4%), followed by 12 mm 58 times (36.2%), 10 mm 12 times (7.5%) and 8 mm 3 times (1.9%). Forty definitive screw-retained prostheses were delivered. Concerning the opposite dentition, 17 patients presented natural teeth and some fixed ceramic crowns, 13 patients had removable dentures, 4 patients presented all natural teeth and 3 patients had both arches rehabilitated. No dropouts occurred during the entire follow-up. After the healing period, a total of 40 definitive prostheses were delivered (Fig. 5). All the 37 patients were followed up for at least 1 year. All the data collected were included in the statistical analysis. No deviations occurred and all the 37 patients were treated following the original protocol. At the last follow-up.

DISCUSSION

This retrospective study aimed to assess the soft tissues health around the implant/abutment interfaces of fixed screw-retained prosthesis supported by four dental implants after at least 1-year in function (42-51). The main limitation of this study is in its intrinsic retrospective nature. The rehabilitation of complete arch by means of four dental implants supporting a fixed screw-retained prosthesis had



Fig. 6. *Prosthetic scalloped design.*

become a validated therapeutic option³. Through the last years, several studies investigated how long only four dental implants can support fixed-complete-arch prosthesis over short- and long-term follow-up.

The peri-implant tissues healthy is strictly connected also to the prosthetic design. (Fig. 6). The interim prostheses had no cantilevers while the definitive prostheses were designed in order to have 12 mm or 15 mm maximum of cantilevers in the maxilla and in the mandible respectively to minimize biomechanical risks. In addition, several studies investigated the amount of plaque and the presence of BoP around the implant/abutment interfaces. The results are not similar with this study. As a matter of fact, a study by Menini et al reported a BoP of 21.1% . The type of dental implants used in this study can have influenced the peri-implant values recorded. All the implants used had core made of titanium-zirconium alloy (Roxolid®) and a chemically



Fig. 5. *Definitive prosthesis in situ.*

modified sandblasted, large-grit and acid-etched surface (SLActive®). The described characteristics of the type of dental implants used may be crucial when immediate loading is performed. Frequently, implant failures occur in the early healing phase of osseointegration. The period between the 2nd and the 4th week after implant placement is the turning point from the primary mechanical stability to the secondary biological stability.

The possibility to achieve a secondary stability by means of a hydrophilic modified surface faster reduces the risks in the early phase and eliminates the stability dip between the primary and secondary stability (52-59). The prosthesis realization has to be carefully made in order to achieve a high prosthesis success rate over the medium and long period. Constant recall to perform professional oral hygiene and occlusion and radiographic checks in combination with nightguard wearing are mandatory to achieve a successful rehabilitation through the years.

If any implant site required GBR a mucoperiosteal flap was raised only above the requiring site to not compromise surgical template stability and maintaining an acceptable implant surgery accuracy (60-63). Finally, it is mandatory to remind that diagnosis, treatment planning and surgical procedures have to be performed carefully and a learning curve is required to better understand and use the computer-assisted implant planning software to achieve successful and free of issues rehabilitations.

Complete arch rehabilitations are always a hard challenge to face for every clinician and the maintenance is as hard as the clinical situations. However, within the limitation of this study, it seems that the use of a fixed screw-retained prostheses supported by four dental implants to rehabilitate edentulous jaws could be a valid treatment option in the short and medium term without critical peri-implant issues. However, several perspectives studies with longer follow-up period are required to assess the reliability of this treatment protocol.

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Microbial leakage evaluation of warm gutta – percha techniques

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The aim of this study is to compare bacterial leakage of MicroHeat and continuous wave with and without endodontic sealer. Thirty-eight single-rooted extracted mandibular premolars were selected and randomly divided into four experimental groups (n=8) and two control groups (n=3). Teeth were prepared with Mtwo NiTi files and obturated with MicroHeat or System B with or without endodontic sealer. Three teeth were used as positive controls (Ct+) and three intact teeth served as negative controls (Ct-). All samples were tested for bacterial infiltration every day for 60 days. On day 32 overall contamination value was 62.5% for Mseal, 75% for Mnoseal, 75% for SBseal and 37.8% for SBnoseal; after 60 days, the final contamination result was 100% for Mseal, Mnoseal and SBseal and 87.5% for SBnoseal. At the end of the observation period, groups showed no statistically significant differences.

The aim of root filling is to create a bacterial-tight seal, thus minimizing the risk of infection or reinfection of the root canal system (1) and preventing periradicular pathosis. Obturation of the root canal involves the use of gutta – percha in combination with root canal sealer to provide an adequate seal. Without a sealer, canal obturations exhibit greater leakage. In contrast to gutta-percha, which is chemically and dimensionally stable, the areas filled by sealer are more vulnerable because it can dissolve over time in contact with tissue fluids. Therefore, the amount of sealer should be kept at the lowest, whereas the amount of gutta-percha placed into the canal must be maximized (2). Various root canal filling methods have been developed to increase the success of root canal treatment. Penetration of bacteria and their products from the oral cavity into the obturated root canals put at risk the endodontic treatment success. Therefore, evaluating the quality of root canal obturation as the final stage of root canal treatment is essential. Coronal leakage may occur

due to voids or loss of restorative material, allowing the root filling material to come into contact with oral fluids.

Among several methods of evaluation of the sealing ability of endodontic materials, bacterial leakage experiments provide biologically and clinically relevant information (3-10). Studies that use a bacterial tracer derived from specific culture or from human saliva are considered to be more reliable than tests with dye. However, bacterial leakage studies are limited statistic model that do not simulate any condition found in the oral cavity such as temperature changes, dietary influences and salivary flow.

There is a wide range of bacteria found to be responsible for secondary infection of endodontically treated teeth, but one of the most commonly found is *Enterococcus faecalis* (11-25). For this reason, we selected it for our infiltration test. The null hypothesis was that there is no difference in coronal sealing abilities of two different root warm gutta-percha canal filling

Key words: bacterial leakage, continuous wave, endodontic sealer, filling method, MicroHeat

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techniques with and without endodontic sealer. Our two main purposes were to compare the MicroHeat and continuous wave techniques and to compare the same techniques with and without sealer to evaluate seal ability using a bacterial invasion *ex vivo* test.

MATERIAL AND METHODS

Thirty-eight human mandibular premolars extracted for orthodontic reasons were collected with verbal consent from patients to be used for in vitro studies. No specific consent from Ethical Committee was needed for the present in vitro study. Each tooth was examined by buccal and proximal radiographs and only teeth with single straight canals and a single apical foramen were chosen.

Thirty-five teeth were sectioned to remove the crown and expose the canal leaving root portions of 15 mm in length. The remaining three samples were left intact to be used as negative control. The length of the root canal was established using a #10 K – file (Dentsply/Maillefer, Baillaigues, Switzerland) up to the apical foramen and subtracting 0,5 mm from this measurement. Each tooth was instrumented with the Mtwo technique (Sweden & Martina, Italy) (5) following basic sequence: 10.04/15.05/20.06/25.06 or 25.07 for System B samples. During the preparation, each canal was irrigated using syringes with 2 mL 5% NaOCL (Niclor Ogna, Italy) and 10 mL 10% EDTA (Tubuliclean Ogna, Italy). Apical gauging was verified using a #25 K-file. An additional step of shaping using a rotating apical 25/40 0.02 taper (Sweden & Martina, Italy) was performed to provide apical stop and complete instrumentation necessary for all 2 techniques. After preparation, a final prolonged irrigation with NaOCL and EDTA was performed to remove the smear layer. During all procedures throughout the experiment, the teeth were kept moist. In order to remove any bacteria or contaminants, every tooth was singularly sterilized through autoclaving for 60 min at 134°C and 2 atm.

After sterilization, the samples were randomly divided as follows:

- **Group 1 (Mseal):** 8 root canals were obturated with MicroHeat technique and endodontic sealer. After the canal drying procedure, a .02 taper size 40 master gutta-percha point (Dentsply Maillefer) was introduced at 1 mm from the WL. Zinc oxide eugenol-based Pulp Canal Sealer (Kerr, Salerno, Italy) was applied on the

tip of the master cone. A 25.04 MicroHeat spreader (Sweden & Martina) was set at 300 rpm up to 2 mm from the WL. The Pac – Mac condenser (Sweden & Martina) was coated with warm gutta-percha using a microflow cartridge (EIE Analytic Technology). The Pac – Mac was inserted to 2 mm from the WL and rotated at 6000 rpm. The procedure was repeated at least 3 times per canal to obtain a sufficient filling.

- **Group 2 (Mnoseal):** 8 root canals were obturated with MicroHeat technique using the same procedures in group 1 without endodontic sealer.
- **Group 3 (SBseal):** 8 root canals were obturated with System B and endodontic sealer. All samples were instrumented as in group 1 and 2 with the only difference of using an Mtwo 25.07 as the last shaping instrument. The root canal was thinly coated with Pulp Canal Sealer. For the root filling using System B 25.06 MtwoGutta gutta-percha points (Sweden & Martina, Italy). It was set at 0.35 mm diameter with a caliper. Tug back adaptation was checked. The sealer-coated cone was placed to 0.5 mm of the WL. For the continuous wave of condensation, a Fine Medium System B (EIE Analytic Technology) was set 4 mm of the working length and heated up to 300°C to fill the apical third of the root canal. Once at the proper depth, heat was removed, and the apical pressure was maintained for 10 s. Backfill of the canal was accomplished by condensing the additional gutta-percha cones.
- **Group 4 (SBnoseal):** 8 root canals were obturated with System B without endodontic sealer.

The three specimens in the positive control group were instrumented without performing any root canal filling and leaving the canal empty. Another three teeth with intact crowns served as the negative control group. The filled roots were stored at 37°C and 100% humidity for 15 days to guarantee setting of the sealer. The apparatus used to evaluate bacterial leakage consisted of an upper chamber and a lower chamber. The upper chamber was formed by a glass pipe, obtained by a local anesthetic vial emptied of its content and sterilized. The lower chamber was constituted by a Beta counter tube. The two chambers were assembled through cold-polymerization resin, in order to obtain a hermetic system and to prevent any external access. The testing apparatus was sterilized in autoclave (134°C, 1 h to 2 atm). The external surface of the

bottom of the upper chamber was roughened with a dental burr. The cyanoacrylate (SuperAttack®) was applied on the glass surface and EE-bond (Tokuyama Dental, Italy) was also applied on the coronal dental surface to foster the bond between tooth and glass. A mass of dental composite was used to connect the dental element to the bottom of the upper chamber. Two coats of nail varnish were applied on the external surface of all teeth, except for 1 mm around the apical foramen, in order to prevent bacterial leakage through lateral canals or other discontinuities in the cementum. A standard strain of *E. faecalis* (ATCC 29212) was used and its initial concentration was 1.5 McFarland. The medium culture used during the test was the Brain Heart Infusion (BHI). One mL of solution containing *E. faecalis* was transferred to the upper chamber contacting the coronal portion of the filling material. The lower chamber was then filled with 5 mL of sterile broth so that about 2 mm of the root apex was immersed in the broth. The whole apparatus was incubated at 37°C and checked

daily for the appearance of turbidity in the BHI broth for 60 days. To guarantee the vitality of the bacteria, the refresh of contaminated samples in the upper chamber was performed every 4 days. When turbidity of the medium was observed, confirmation of cell morphology was carried out by Petri dishes with Agar. To estimate the time and probability of coronal infiltration Kaplan-Meier curves were used.

RESULTS

No growth was observed when checking the sterilization of the whole apparatus. All specimens of the positive control group showed broth turbidity within 1 day of incubation. There was no evidence of broth turbidity in the negative control group.

The first positive (Mseal) sample was observed on the fourth day. The other samples showed positive results on the ninth and fifty – seventh day with the

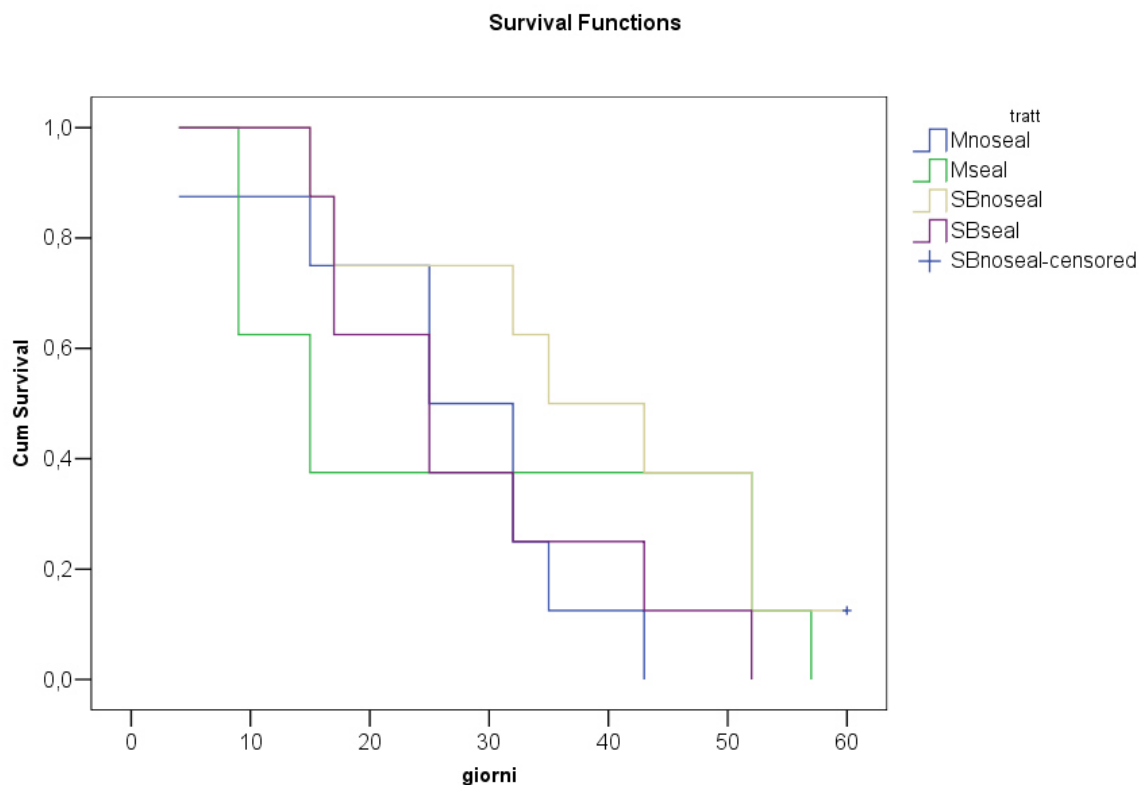


Fig. 1. Bacterial infiltration curves based on observation time.

exception of one element (SBnoseal) that did not show signs of contamination (Table I). On day 32, 62.5% of the Mseal samples were contaminated, 75% of the Mnoseal and SBseal samples were contaminated and 37.8% of SBnoseal samples were contaminated. On day 60, 100% of Mseal, Mnoseal, SBseal were contaminated and 87.5% of SBnoseal were contaminated (Table II, Fig. 1).

DISCUSSION

The use of sealer with gutta-percha is necessary to fill the voids and gaps between the filling material and the root walls. Without a sealer, canal obturation exhibit greater leakage (26-33).

Previous study evaluated sealing ability of two warm gutta-percha systems that used injected gutta-

percha, Obtura II system, and vertical condensation with and without the use of sealers, because warm gutta-percha has the ability to conform to canal irregularities and radiographically appear dense without sealer. Results showed that obturation groups without sealer demonstrated the highest amount of leakage (34-39). However, Obtura II system was unable to obtain a great sealing root canal filling. Obtura II must be used as backfill in combination with more performing obturation systems (40-49). Although the study did not describe clearly vertical condensation steps, but it is very likely that manual spreader and plugger were used. They did not reach at the apical level the same temperatures as electrical instruments of the System B technique (50-57).

This study investigated the sealing ability of the thermoplastic gutta-percha alone using two

Table I. Bacterial infiltration test results.

Days		GROUP																																						
		Mseal								Mnoseal								SBseal								SBnoseal								Ct+			Ct-			
TIME		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	1	2	3	
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	32	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	35	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	43	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	52	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
57	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Table II. Distribution of teeth exhibiting bacterial leakage after 60 days of evaluation.

GROUP	TOTAL (n)	NO LEAKAGE	LEAKAGE	%	RANGE DAYS
Mseal	8	0	8	100	9 – 57
Mnoseal	8	0	8	100	4 – 43
SBseal	8	0	8	100	15 – 52
SBnoseal	8	1	7	87,5	15 – 52
Ct +	3	0	3	100	1
Ct -	3	3	0	0	-

modern obturation methods: System B, development of vertical condensation of warm gutta-percha technique, and MicroHeat, development of MicroSeal technique. Our study uses a variant of monomicrobial bacterial leakage (58-61) with *E. faecalis* as infiltrating agent in a two-chamber system to evaluate the microbial leakage through filling material. All root canals of endodontically treated, with were not coronally restored teeth, were recontaminated within 19 and in less than 30 days, respectively (62-65).

By asking the question about the evaluation of short- and long-term root canal filling, the observation has been subdivided in two time periods: from 0 to 30 days, according to other study, and from 30 to 60 days because the results of longer evaluation times can produce more precise data.

Taking into account the results of the System B technique with and without sealer, the samples showed a consistent and uniform pattern of behavior having both an infiltration time between day 15 and 52. Moreover, the behavior of System B without sealer was in accord with the results of previous study where System B without sealer samples were a control group, because it was assumed that techniques without sealer were unreliable. The results of System B with sealer are different from those of tests that evaluate microbial leakage.

In a reference work one half of the samples survived during a period of observation between day 7 and 62, while the results of a test that evaluated saliva leakage showed that 2/3 of samples survived between day 3 and 52. In another coronal infiltration study (8) the samples were contaminated between day 24 and 54. However, by comparing the data of different works it is possible to say that samples always have a different behavior like they are strongly dependent on the kind of experiment.

In a study that evaluated coronal leakage of MicroSeal, Thermafil and System B (8), less than half of the filled roots through MicroSeal and System B techniques resulted positive after a period of observation of 32 days, therefore showing that the techniques are more efficient over time. In our study, Mseal and Mnoseal groups have maintained a uniform behavior to themselves but different to

previous work and a similar trend with SBseal in the first 32 days.

A previous study that investigated apical leakage of the System B and MicroSeal (Analytic Endodontics, Orange, CA) obturation techniques showed that there was no significant difference between the apical leakage of the System B obturation and the MicroSeal obturation method.

As already pointed out in other studies, comparing MicroSeal with and without sealer, it is possible to say that the choice to use sealer did not influence the outcomes of this high – pressure technique around the apical control zone. Gutta – percha alone showed better filling at both 3 mm and 1 mm in the MicroSeal and System B techniques. However, there are no other works in the literature investigating coronal leakage of MicroSeal without sealer. Discordant behavior of MicroHeat technique compared to MicroSeal could be associated with different physical and chemical characteristics of gutta – percha. Improvement from earlier works, in this study MicroHeat gutta – percha was used and it was less adhesive, with a higher softening time and with higher viscosity compared to MicroSeal gutta – percha (SybronEndo, CA, USA).

Our study also underlined the bacterial permeability of the barrier gutta – percha/ seal over a period of 60 days, despite the techniques used, confirming the necessity of a coronal restoration to protect the filled endodontic system.

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Radiographic and electronic working length comparison. An *in vivo* study of three Electronic Apex Locator devices

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The aim of this study is to compare the working length measured with Root ZX (EAL1), Propex II (EAL2) and Endo Analyzer Model 8005 (EAL3) with radiographic measurements during endodontic treatment. Fifty single canal teeth scheduled for endodontic treatment were selected for the study. After endodontic access preparation, pulp chamber was irrigated with 5mL of 5% sodium hypochlorite (NaOCl). The canal was scouted with a size 10 K-file introduced slightly over the apex to verify the canal patency. The working length (WL) was measured using all three EAL devices and radiographically. All measured working lengths were recorded and compared using ANOVA for repeated measures considering the multiple comparison of paired data Least Significant Difference (LSD). The WL mean values for EAL1 were 19.1 ± 1.7 mm; for EAL2 were 19.4 ± 1.7 ; for EAL3 were 19.4 ± 1.6 and for RG were 19.2 ± 1.7 . The Pearson correlation coefficients indicated that the following correlation between electronic devices and radiographic WL: EAL1- 0.986, EAL2- 0.953 and EAL3- 0.931. Considering the mean values compared to radiographic measures, Root ZX gave better results than the other tested EAL devices. Endo Analyzer Model 8005 showed the worst performance.

The goal of endodontic therapy is to remove infected pulp tissue, necrotic material, and microorganism from the root canal system (1). Failure to measure working length compromise the clinical outcome of the root canal treatment (2). Many authors considered the apical constriction as the anatomical reference point where the root canal instrumentation and obturation should end (3). Variations in root morphology and radiographic distortion might cause a difference between the radiographic apex and the anatomical one. To determine the working length (WL), several techniques, including tactile sensation, radiographs and electronic apex locators, are routinely used by clinicians (4.). Radiography (RG) is the most common technique to determine WL, giving information about the root canal anatomy and periapical tissues. A frequently used

method for determining WL is based on radiographic visualization and interpretation of an instrument placed in the root canal, this is known as the Ingle technique (5). However, the accurate determination of root canal length radiographically can be hampered by anatomical changes, interference from anatomical structures or errors in projection (6). Bone structures interferences, shortening and elongation distortion, interpretation variability, and lack of three-dimensional representation can negatively affect the correct interpretation of the images (7-15). Ideally the working length should end at the dentine-cemental junction which is shorter than the root apex and can coincide with the apical constriction. The difference between endodontic apex and apical foramen or anatomical apex can vary due to anatomy or pathological resorption of the root end. In teeth

Key words: radiographic working length, endodontic working length, apex locator, root canal system, apical foramen

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with vital pulp and no periapical pathologies many authors reported a higher success rate for endodontic therapies ending 1mm shorter than the radiographic length (16-27).

Electronic root canal working length determination and digital radiology are becoming increasingly common, to overcome the limits set by traditional methods. The electronic method is more accurate, easy and fast, requires no adjunctive X-ray exposures and increases patient comfort. EALs are claimed to precisely locate the position of the minor foramen, reducing the error during WL determination; some studies have demonstrated that EALs are more reliable than radiographic measurements (28). Numerous electronic apex locating devices have been introduced since the 1960s. In 1962, Sunada demonstrated that the electrical resistance between the periodontal ligament and oral mucosa has a constant value that can be measured (29). As a result, some devices were developed for use as clinical help in apex location. However, many first-generation devices often performed unpredictably from patient to patient. Propex II (Dentsply Maillefer, Ballaigues, Switzerland) is fourth generation EAL. Propex II is based on multiple frequencies to determine the root canal length (8). The Root ZX (J Morita Corp. Kyoto, Japan) is a third generation EAL that measures the root length by calculating the ratio of two different AC frequencies. The Endo Analyzer Model 8005 (Analytic, Sybron Dental, Orange, CA) is a second-generation device and measures the phase-angle values of impedance in the canal over five signal frequencies (0.5, 1, 2, 4, and 8 kHz). The aim of the present in vivo study is to investigate the accuracy of these EAL devices comparing them to radiographic measurements during endodontic treatment.

MATERIAL AND METHODS

The study was held in the Department of Conservative Dentistry and Endodontics of University of Rome "Tor Vergata". Ethical clearance was obtained from the Ethical Committee. Informed consent of each patient was obtained after explaining the clinical procedure. Fifty single canal teeth scheduled for endodontic treatment were selected for the study (20 mandibular premolars, 20 incisors and

10 canines). The sample size was based on the level of significance and the power and effect size of the previous studies. Thus, a total of 50 root canals were available for the study. A standardized preoperative periapical radiograph was taken for each tooth. The inclusion criteria were teeth responding positively to a cold sensibility test and root canals being clearly visible in the radiograph without any evidence of calcifications. The exclusion criteria were periapical radiolucency in the radiograph, curved roots canals, and evidence of root resorption in the radiograph. Patients that used heart pacemakers were excluded.

After administering local anesthesia, teeth were isolated with rubber dam. Decayed tissue was removed, and a pulpal chamber access cavity was performed using a high-speed round diamond bur. The pulp chamber was irrigated with 5ml of 5% sodium hypochlorite (NaOCl), and a size 10 K-file (Dentsply, Maillefer) was used to scout the canal slightly long over the apex to verify the canal patency. Excess fluid was removed from the pulp chamber, but no attempt was made to dry the canal. The WL was determined using all three EALs devices and radiographically. The EAL to be used first was selected on a random basis.

All the working length recordings were made using the largest instrument that would pass to the minor diameter (10 or 15 K-file) (Dentsply Maillefer). For the Root ZX (EAL1) the lip clip was attached to the patient's lip and the electrode connected to the file. A K-file was advanced apically into the canal until the screen showed apex. A flashing bar between APEX and 1, and a flashing tooth on the LCD display, indicates the minor diameter. For the Propex II (EAL2), the file was advanced into the root canal until the EAL displayed the flashing word "APEX" and "0.0" according to the manufacturer's instructions. For the Endo Analyzer Model 8005 (EAL3) the file was advanced until the numeric display read 0.0 and flashed. The LCD display also had a flashing word, apex, and an audible sound was heard. This indicates the location of the foramen according to the manufacturer's instructions. After recording the first working length, the same file was reinserted to determine the working length using the second and third EAL and measured in the same mode. Measurements were considered valid if the device remained stable for at least 5s. Subsequently, radiographic measurements (RG) using Ingle's technique were obtained to determine the fourth measurement.

Table I. Descriptive result values.

	EAL1	EAL2	EAL3	RG
N	50	50	50	50
Mean	19,1	19,4	19,4	19,2
sd	1,7	1,7	1,6	1,7
Median	19,0	19,0	19,3	19,0
Min	16,0	17,0	17,0	16,0
Max	23,0	23,0	23,0	23,0

Based on the preoperative X-ray, a radiograph was taken with a size 15 K-file 2 mm short of the radiographic apex. The difference between the tip of the file and the radiographic apex was added and a new radiograph was taken; the results were recorded. The distance from the file tip to the base of the rubber stop was measure for all the techniques using an endodontic ruler with an accuracy of 0.5 mm. The classification was made as “correct (1)” measurements when $\text{errorEALx} \leq 0.5$; “discrete (2)” measurements when $0.5 < \text{errorEALx} < 1.0$; “incorrect (3)” measurements when $\text{errorEALx} > 1$. After testing measurements all root canals treatments were completed with mechanical instrumentation and warm gutta-percha obturation in the same appointment.

Statistical analysis

The descriptive statistical analysis consisted of the mean $\pm$ standard deviation per parameter with Normal distributions (after confirmation with histograms and the Kolgomorov-Smirnov test), median and interval

(minimum) for variables with non-Normal distributions or percentages for categorical variables. The analysis of comparisons between methods was made using the ANOVA for repeated measures considering the multiple comparison of paired data Least Significant Difference (LSD). Pearson’s correlation analysis was conducted to evaluate the best linear best relationship between method measurements and the RG measurement by determining the Pearson coefficient. The Bland & Altman method have been conducted to define limits of confidences and verify possible data drifts. The Chi-Square test or the Fisher exact test was conducted to highlight any significant occurrences of error between methods. A p-value of 0.05 was considered statistically significant.

RESULTS

The mean values of WL measurements for EAL1 (RootZX) were 19.1 ± 1.7 mm; for EAL2 (Propex II) were 19.4 ± 1.7 ; for EAL3 (Endo Analyzer model 8005) were 19.4 ± 1.6 and for RG (Radiographic Length) were 19.2 ± 1.7 (Table I). The results of the ANOVA test for comparison between methods are shown in Table II.

Statistically significant differences were found between RG and EAL1 ($P = 0.002$) and EAL2 ($P = 0.017$). The Pearson correlation coefficients indicated that the electronic devices correlation with the RG is EAL1 with 0.986 followed by EAL2 with 0.953 and then EAL3 with 0.931 (Table III). The Confidence limits of vs RG show that $d\text{EAL1} = -0.1$, $d\text{EAL2} = 0.2$ and $d\text{EAL3} = 0.1$ (Table IV). EAL1 shows 96% of error 1, 4% of 2 “discrete” and 0% of error 3; EAL2 shows 80% of error 1, 18% of error 2 and 2% of error 3. EAL3 shows 80% of error 1, 14% of error 2 and 6% of error 3 (Table V).

Table II. ANOVA: Tests of Within-Subjects Contrasts factor = EAL1:EAL2; EAL3:RG4. Pairwise Comparisons Adjustment for multiple comparisons: Least Significant Difference.

						Source	Sum of Squares	df	Mean Square	F	Sig.
						factor	,306	1	,306	4,910	,031
						Error(factor)	3,056	49	,062		
I)factor	J)factor	Sig.	I)factor	J)factor	Sig.	I)factor	J)factor	Sig.	I)factor	J)factor	Sig.
EAL 1	EAL 2	,000	EAL 2	EAL 1	,000	EAL 3	EAL 1	,004	RG 4	EAL 1	,002
	EAL 3	,004		EAL 3	,647		EAL 2	,647		EAL 2	,017
	RG 4	,002		RG 4	,017		RG 4	,109		EAL 3	,109

Table III. *Pearson correlation coefficients.*

		EAL1	EAL2	EAL3
RG	R di Pearson	0,986(**)	0,953(**)	0,931(**)
	N	50	50	50

(**) Correlation is significant at the 0.01 level (2-tailed).

Table IV. *Limits of confidence.*

limits		dEAL1	dEAL2	dEAL3
CI 95%	mean diff(EALx)	-0,1	0,2	0,1
	L inferior	-0,7	-0,8	-1,0
	L superior	0,4	1,2	1,3
AI 95%	mean diff(EALx)	-0,1	0,2	0,1
	A inferior	-1,2	-1,7	-2,0
	A superior	0,9	2,0	2,3

Table V. *Evaluation of the error between methods.*

		Precision			Total
		 EALx <=0,5	0,5< EALx <1,0	EALx >1	
		CORRECT (1)	DISCRETE (2)	INCORRECT (3)	
EAL1	Count	48	2	0	50
	% within EAL	96,0%	4,0%	,0%	100,0%
	% within prec	37,5%	11,1%	,0%	33,3%
EAL2	Count	40	9	1	50
	% within EAL	80,0%	18,0%	2,0%	100,0%
	% within prec	31,3%	50,0%	25,0%	33,3%
EAL3	Count	40	7	3	50
	% within EAL	80,0%	14,0%	6,0%	100,0%
	% within prec	31,3%	38,9%	75,0%	33,3%
Total	Count	128	18	4	150
	% within EAL	85,3%	12,0%	2,7%	100,0%
	% within prec	100,0%	100,0%	100,0%	100,0%

DISCUSSION

The establishing of a correct working length is crucial for cleaning, shaping and final obturation of the root canal (30-38). For a successful endodontic treatment, all procedures should be limited within the root canal without overextensions (39-43). An

overestimation of WL may result in no control of instrumentation and overfilling obturation, whereas underestimated WL may cause persistence of bacteria, necrotic tissue, and lead to underfilling of the root canal. The radiographic measure was traditionally used for the WL, but it can be affected by image distortion and external radicular

resorption. X-ray images can also be hard to read in case of other anatomical structures superimposed to the root canals (44-49). Electronic apex locators were introduced in endodontics since 1962 as a new method to determine the working length. Since then, many new generations of EAL were proposed. Modern devices are less prone to adverse external factors like presence of irrigates, exudates or pulpal tissues. The reliability and clinical accuracy of EALs in *in vitro* studies are controversial (50-53).

An *in vivo* method was selected for this study to compare the accuracy of Root ZX, Propex II and Endo Analyzer Model 8005 to the radiographic method of WL determination. To reduce variables, only one experienced operator performed the electronic and radiographic WL measurements. These differences were significant for Root ZX and Propex II ($p = 0.002$ and $P = 0.017$) and no significant for Endo Analyzer ($P = 0.109$). Pearson's correlation analysis resulted in the following ranking of correlation: EAL1 (correlation coefficient = 0.986) followed by EAL2 (0.953) and EAL3 (0.931). According to the 95% confidence limits (95% CI) and with limits of agreements of Bland & Altman (AI 95%), the EAL1 method gave best results even if it involves a slight underestimation of the apex value ($dEAL1 = -0.1$) vs RG. On the other hand, the other 2 methods gave an overestimation of the WL ($dEAL2 = 0.2$ and $dEAL3 = 0.1$). All errors were within the predicted accuracy (<0.5 mm). The assessment of the error class confirmed the Root ZX's best accuracy, with 96% of "correct" values (± 0.5). The results demonstrated that there were differences between the EALs devices in terms of accuracy. Under the conditions of this *in vivo* study the Root ZX resulted more accurate than other EALs when compared to RG.

In establishing the WL, some authors declared that ± 0.5 mm is acceptable, and others declared that ± 1 mm is acceptable (13). A literature search revealed no *in vivo* study comparing the accuracy of this three EALs devices to compare the results of this study. However, our results are in agreement with some studies where same EALs were used in comparison. An *in vitro* comparative evaluation of conventional radiography and Root ZX by Javidi et al showed an almost perfect correlation with the radiographic

method (correlation coefficient = 0.983).

Our results were different from Cianconi et al study where the accuracy in locating the apical foramen of the Endex, Propex II, and Root ZX was 86.1%, 83.2%, and 65.3%, respectively. In this *ex vivo* study Propex II resulted more accurate than Root ZX in determining the WL (54-61).

Another two *in vitro* studies found no significant differences between the measurements when compared to visual method (93% for Root ZXII and 90% for Propex II) (12), (Root-ZX 89.7% and Propex II 82.1%). Finally, Vasconcelos et al. found no differences in accuracy between Root ZX and Propex II with both EALs displaying similar results in their *in vivo* study (62-66).

There are only few papers in literature on the accuracy of the Endo Analyzer Model 8005. The paper from Welk et al. shows that the ability to locate the minor diameter (± 0.5 mm) was 90.7% for the Root ZX and 34.4% for the Endo Analyzer Model 8005. Welk et al. considered that the Endo Analyzer Model 8005 results can be particularly less precise in case of apical resorption. In our study the teeth have not been extracted thus this finding could not be confirmed, but Endo Analyzer Model 8005 resulted as the less precise.

This study confirms the accuracy of electronic measurement in endodontics, as an essential tool for achieving the correct working length. Although with some differences, the electronic measurement technologies compared in this study have been shown to have clinical reliability. The comparative study however considered that, the mean values compared to radiographic measurement, Root ZX has better performance than the other EALs devices examined, whereas the Endo Analyzer Model 8005 show the worst performance.

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Reduction of radiations exposure in endodontics: comparative analysis of direct (GX S-700, Gendex) and semidirect (VistaScan Mini View, Dürer) digital systems

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The importance of intraoral periapical radiography is crucial as it represents a fundamental mean of evaluation and diagnosis for supporting bone and teeth hard tissues diseases and pathologies. Even though conventional film technology is still widespread, presently there are many digital radiography alternatives. Direct digital systems are devices with an intraoral sensor wired or paired wireless with a computer. These devices do generate an image immediately during exposure and acquisition. The semi direct digital systems instead are based on phosphor image plates. Digital radiography offers many chances to improve our patient's healthcare standards reducing exposition to X-rays and the related stochastic radiation risk. Aim of this study is to compare the quality of images for endodontic purposes comparing three systems: conventional films, direct digital radiography and semidirect digital radiography.

The importance of intraoral periapical radiography is crucial as it represents a fundamental mean of evaluation and diagnosis for supporting bone and teeth hard tissues diseases (1) and pathologies. In endodontics, a periapical preoperative radiograph is always needed to address periapical pathologies, evaluate the endodontic anatomy and more generally make a correct diagnosis (2-9). Even though conventional film technology is still widespread, presently there are many digital radiography alternatives. Direct digital systems are devices with an intraoral sensor wired or paired wireless with a computer. These devices do generate an image immediately during exposure and acquisition. The semi direct digital systems (10) instead are based on phosphor image plates; the exposed image plate needs to be read from a digital scanner to obtain the desired radiographic image (11-15).

An advantage for semidirect systems is that the image plates are very thin (same thickness of conventional films) and are available in several different dimensions to meet different clinical

situations. The conventional dimension allows for use of conventional intraoral radiography holders (16-25).

Direct systems, being based on a physical intraoral sensor, are available in few dimensions, varying from brand to brand, and are always rigid and thick. These sensors do need specific holders and can be more difficult to be used in not optimal situations such as lack of space or small mouths. Despite these drawbacks they can generate the images immediately without the need for processing in a separate dedicated scanner (26-32).

The great advantage of digital over traditional radiology is reduced radiation dose needed to generate image, speed of image generation, easy recording and handling of the image, easy image sharing, and finally possibility of image editing in dedicated software to increase contrast, lightness sharpness (33-36). Overall, digital radiography offers many chances to improve our patient's healthcare standards reducing exposition to x-rays and the related stochastic radiation risk.

Some authors found that direct digital systems

Key words: intraoral radiography, semi direct digital system, direct digital system, X-rays, stochastic radiation risk

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have same accuracy of traditional film technology in diagnosing periapical lesions; comparing different digital radiology devices, some authors did not find any significative difference in the generated images (37-41).

On the other hand, comparing traditional radiology with digital radiology, many other authors claim that the old traditional film still is slightly superior to any digital technology. In the comparison between direct and semidirect digital systems there is no agreement in literature to assess if one system is superior to the other (42, 43). Presently, there is no definitive word on what the best radiographic technique is to evaluate periapical lesions. In the last years, the diffusion of digital radiography systems increased due to reduction of radiation exposure and ease of record managing (44-53) but there is still a lack of knowledge in what system is better than the other. Aim of this study is to compare the quality of images for endodontic purposes comparing three systems: conventional films, direct digital radiography and semidirect digital radiography.

MATERIALS AND METHODS

Inclusion and exclusion criteria and sample size

All included patients for the study were in good health and between 20 and 60 years of age. The patients were enrolled in the UOSD-Emergency Dental Department and Unit of Conservative, Restorative and Endodontic Dentistry of the Tor Vergata Policlinic, Rome, Italy. All patients with cognitive deficit or in treatment with mind-altering drugs, pregnant women, patients professionally exposed to X-rays or recently undergone to high radiation doses for diagnostic or treatment reasons, were excluded from the study.

The study was approved from Tor Vergata Ethical Committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The sample size was calculated using the necessary parameters (mean, effect size, standard deviation) and resulted in 150 patients (Power 80% and Significance 0.05%).

Radiographic images

All radiographic images were taken with the same X-ray generator unit: X70PP (Castellini, Imola-Italy).

This unit is fully compatible with all imaging technologies used in the study varying the exposure time according to producers' indications.

1. Conventional films used: Carestream Ultra-speed DF-58 (Carestream Dental, Italy).
2. Semidirect digital system used: Image Plates Plus size 2 3x4 and digital scanner VistaScan Mini View (Durr Dental, Germany).
3. Direct digital system used: GX S-700 Intraoral Digital Sensor (Kavo Gendex, Germany).

To reduce variability all radiographic films were processed with a Durr automatic developer using dedicated developer and fixer solutions. All processed films were stored and evaluated on the same white light X-ray film viewer with 4.5x optical magnification (10) in a controlled light ambient.

All digital radiographs were stored and evaluated with the same laptop PC with an integrated high-definition monitor and in a controlled light ambient. All radiographs were taken with appropriate intraoral holders following indications of the producers.

Anamnestic data, informed consent and operative procedure

During first visit appointment a complete anamnestic interview and an informed consent was obtained from each enrolled patient. According to inclusion and exclusion criteria, 150 patients were enrolled for the study, 68 males (45.3%) with age 44.56 ± 16.39 years and 82 females (54.7%) with age 48.65 ± 17.45 years.

Each patient received only one kind of radiography out of: radiographic film, direct digital and semidirect digital, from one expert endodontist (more than 10 years of practice). The sample was randomly divided into three groups:

- Group 1 (n=50): radiographic film
- Group 2 (n=50): semidirect digital imaging with Image Plates Plus size 2, 3x4cm
- Group 3 (n=50): direct digital imaging with intraoral sensor

Images, analysis and evaluation

Images were analyzed by six examiners: three expert endodontists (with more than 10 years of experience) and three limited experience operators (young dentists with less than one year of practice). All six examiners

participated to a preliminary calibration session to the evaluation of the different radiographic images and the scoring procedure. Each examiner individually evaluated the 150 radiographic images giving a score to these parameters (Table I):

- 1) ease of visualization of the periodontal ligament at the cervical and middle third of the root (score 0-1-2) (LPCM);
- 2) ease of visualization of the periodontal ligament at the apical third of the root (score 0-1-2) (LPA);
- 3) ease of visualization of endodontic canal/s at the cervical and middle third of the root (score 0-1-2) (RCCM);
- 4) ease of visualization of endodontic canal/s at the apical third of the root (score 0-1-2) (RCA).

Score: 0- not visible; 1- partially visible; 2- perfectly visible.

Statistical Analysis

All data were initially entered into an EXCEL database (Microsoft, Redmond, Washington – United States) and the analysis was performed using the Statistical Package for the Social Sciences Windows, version 15.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics consisted of the mean $\pm$ standard deviation for parameter with normal distributions (after confirmation with histograms and the Kolgomorov-Smirnov test), median and range (min/Max.) for variables with non-normal distributions and for frequencies percent. Comparison among groups was performed *Chi-Square* test or Fisher's exact test (if cells<5) for categorical variables. A p value of <0.05 was considered statistically significant.

RESULTS

The three expert examiners gave similar scores.

Results of the two groups of examiners and experts vs novices, gave slightly different scores. Comparing the three expert examiners with each other for each single parameter, no statistically significant differences emerged in the assessments given, while for novices this applies to the LPCM and RCCM parameters, while the assessments of LPA and RCA are less uniform. By assuming the values expressed by the group of experts as a reference parameter, it is possible to construct an assessment of agreement by parameter with respect to the method.

For the LPCM parameter, the test performed is significant. Method 3 determines more concordances vs method 2 vs method 1. Method 1 therefore determines a greater discrepancy. For the LPA parameter the test is not significant. Methods 2 and 3 appear to have similar agreement, method 1 less. For the RCCM parameter the test is not significant, and the 3 methods are quite concordant. Finally, for the RCA parameter the test is significant. Method 2 determines more concordances vs method 1 and 3. Conversely, method 1 determines a greater discrepancy.

DISCUSSION

Digital radiography has substantial advantages such as reduction in X-ray dosage and digital image acquisition without the use of a dark room and liquids for development and fixing. The two types of intraoral digital radiographs mainly used are semi-direct and direct (phosphors). Some authors (54-58) report that the direct system (phosphors) is better than the semi-direct system, but the limit of this *ex vivo* study is that of having radiographs performed in the absence of saliva and without the presence of

Table I. Legend of parameters.

Parameter	Legend
LPCM	Periodontal ligament of the cervical and middle third
LPA	Periodontal ligament of the apical third
RCCM	Root canal of the cervical and middle third
RCA	Root canal of the apical third

soft tissues. Furthermore, according to the authors, the semi-direct system is more difficult to handle and to place in the oral cavity than the direct one.

In our study we conducted an analysis of the results obtained for the group of experts and for the group of novices. In the group of experts, for the LPCM parameter, it seems that there is no disproportion in the values obtained and therefore there is no statistically significant difference. As regards the intergroup analysis between methodologies, this is highly significant for the group of expert operators for the LPCM parameter. In fact, there is a difference in the results, as the response to the two digital technologies is discordant with respect to the conventional methodology (59-62).

As regards the LPA parameter, the results obtained are not statistically significant. It should be emphasized that, however, a more consistent trend was seen in the judgment of operators 2 and 3 compared to operator 1 among the expert. The results between the 3 methodologies reported that in methodologies 2 and 3 no operator had doubts on the detection of LPA (no 0-value reported), and therefore it is more amplified for LPA than LPCM. This makes us assume that the expertise of expert is certainly more similar to the conventional methodology as it is still easily interpretable for operators. On the contrary, digital methodologies are more difficult to interpret by expert operators, probably due to less experience with the digital system.

Analyzing the RCCM parameter, the operators are in agreement with the results and there is no statistically significant difference. In the analysis between the methods there are no concordant results and therefore there appears to be a highly significant difference, even if there is uniformity. If we consider the conventional method as a reference, parameter 0 is the most represented. There is no statistically significant difference between the examiners for the RCA parameter. For the methodologies there are no values of 0 (possible maximum errors), probably associated with the fact that the direct system is the one most subject to distortion.

The analysis of the group of novices showed less uniformity in the results compared to the expert group.

Examining the LPCM parameter, it emerged that operator 2 provided more 0 results than the others, although this is not statistically significant. Also, for the methodologies examined, there are no statistically significant results. This consistency of results with respect to 0 shows that these operators have more doubts than the group of experts analyzed previously. This indicates that non-expert traders are less in agreement on the evaluation of the analog system than experienced traders.

As for the LPA parameter, in the analysis between operators there are limit values and therefore there is a statistically significant difference. The value 0 is what determines the most relevant values. The analysis, on the other hand, between methodologies, there are percentage inversions between methodology 1 and 2 (less among the experts) and therefore there is a statistically significant difference.

The RCCM parameter is even more relevant since in the analysis between operators there is no statistically significant difference even if operators 1 and 3 report clearly different values for the values of 0; while for the methodologies there is a highly significant difference, even if the methodologies 2 and 3 report almost overlapping values. Finally, for the RCA parameter, discordant results emerged among the operators, while for the methodologies the values are uniform, but this does not express statistical significance.

The comparison of the three studied types of radiographic images does not give the supremacy of one technique on another. All three radiographic imaging techniques demonstrated the same accuracy in visualizing correctly small structures such as the periodontal ligament and the endodontic canal. The results confirm that all three techniques are valid diagnostic media for endodontic diagnosis and treatment planning. The direct and semidirect digital techniques offer the advantage of a highly reduced radiation dose for the patient maintaining a very high quality of the image (63-69). Between direct and semidirect systems, the semidirect is often less invasive in the oral cavity due to the much lower thickness similar to conventional films. Digital systems offer the great advantage of an immediate image, no chemicals for processing that need to

be disposed of ease of image enhancing if needed and measuring software tools. A digital image is also very useful for patient data storage and referral purposes. Overall, the most important factor to digital radiography is the reduction of X-ray exposure which means less risks for the patient (70-72). As for almost every other application in radiology, the advance of technology made the conventional films a thing of the past. The present and the future of radiology is digital imaging.

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Craniofacial and occlusal features of individuals with Turner Syndrome: A cephalometric study

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Craniofacial features of 18 individuals with Turner Syndrome (TS) were compared with age and gender matched healthy individuals. Dental history, panoramic radiograph, dental casts and cephalometric measurements were assessed. The dental casts analysis showed a significantly higher PH/PW ratio in individuals with TS under GH therapy compared to healthy individuals ($p=0.004$; paired t-test). This data objectively supported the definition of a high-narrow palate. The ANB angle and the Wits index were similar in the two group, showing a skeletal class I malocclusion. The vertical characteristics did not differ between the two groups, showing a mesofacial growth pattern. Our results showed similar cephalometric characteristics in individuals with TS treated with GH and healthy controls.

The Turner Syndrome (TS) was first described in 1938 as “A syndrome of infantilism. congenital webbed neck and cubitus valgus” by Henry Turner, who reported the presence of short stature, *Pterygium colli*, *Cubitus valgus*, primary amenorrhea and sexual infantilism, due to hypopituitarism, in 7 adult women. TS is a chromosomal disease associated with the partial deletion or monosomy of the X chromosome. The X chromosome monosomy is responsible for less than half of the TS diagnosis; many of them are mosaicism or related to abnormal X or Y chromosome (deletion, isochromosome X, dicentric chromosome). The TS shows different somatic manifestations, but some phenotypic features are common in many TS individuals including short stature, gonadal dysgenesis, lymphedema and congenital heart diseases. The TS is characterized by skeletal dysplasia, mild epiphyseal dysplasia and typical bony alterations (1). Other features include *Cubitus valgus*, webbed neck, widely spaced nipples

and developmental delay (2). The 10–20% of girls with TS develop scoliosis; kyphosis and/or vertebral wedging are also common (3-10).

Short stature is probably the most common, readily recognizable clinical feature of TS. Therefore, the treatment of girls with TS with growth hormone (GH) has become a common therapy in many countries to promote the growth during the childhood and increase the adult stature (4).

Congenital heart diseases are the first cause of death for girls with TS (11). In fact, 65-70% of patients show aortic structural abnormalities, leading to aortic dissection in patients with Turner syndrome. Moreover, bicuspid aortic valve, ectasia of the transverse aortic arch, aortic coarctation, aberrant right subclavian artery are frequent findings in girls or women with Turner syndrome (12).

In the medical literature, most of data regard the oro-dental features of TS girls or women with no GH therapy (13-20). Few studies evaluate cranio-

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facial characteristics of TS girls or women under GH therapy (21-30). Giving the lack of data on this topic, the aim of this case series was to describe the craniofacial characteristics of 18 Italian girls with TS in comparison with 18 Italian healthy girls.

MATERIALS AND METHODS

Study population

This study was conducted on outpatients attending the Unit of Special Needs Dentistry and Pediatric Dentistry, Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy. The dental records of the individuals with NS attending the Unit of Special Needs Dentistry between January 2015 and January 2019 were recruited. Individuals with NS were referred from the Unit of Rare Disease, Syndromology and Auxology of the St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Italy. The following inclusion criteria were applied: documented genetic diagnosis of TS, growth hormone (GH) therapy for at least two years, Italian origin and nationality of both parents, availability of good quality orthopantomogram, lateral cephalogram and dental casts. Individuals with a previous orthodontic treatment were excluded. As a control group, for each child with TS, one age and gender matched healthy subject was found. The following inclusion criteria were applied: Italian origin and nationality of both parents, normal, or only minor deviations from normal occlusion; availability of good quality orthopantomogram, lateral cephalogram and dental casts. The exclusion criteria were the following: chronic diseases except for allergy; medical condition associated with oral diseases; history of surgical/medical treatments that might have affected the craniofacial development (e.g. cancer therapies); craniofacial anomalies; dental agenesis; supernumerary teeth; extracted permanent teeth; history of orthodontic treatment; bad quality radiographs.

The investigators were two dentists trained in special needs dentistry and pediatric dentistry. The research protocol of this study was approved by the local Ethics Committee of the Bologna University Hospital Authority St. Orsola-Malpighi Polyclinic (PG. N 0019293). In full accordance with the ethical principles of the Helsinki Declaration, the written informed consent for participation and publication was obtained from the adults responsible for each individual.

Dental casts evaluation

The relationship between the upper and lower dental arch in the three planes of space (sagittal, vertical, transverse) was assessed. The Palatal Width (PW) (the distance between the upper first molars at the gingival margin) and the Palatal Height (PH) (distance between deepest point of palatal surface and line of palatal width) were measured. The palatal height index (PHI), which compares PH and PW, was calculated according to Premkumar (2011): $PHI = [(PH/PW) \times 100]$.

Cephalometric assessment

Standardized lateral cephalograms were obtained using the digital radiographic equipment Planmeca Promax© (Planmeca Oy, Helsinki, Finland) with the patient in an upright position, with natural head posture and centric occlusion. The analysis of the lateral cephalograms was performed using a software for cephalometric analysis (Delta Dent) by the same investigator on the same monitor with the aid of magnification. To avoid examination bias, each radiograph was coded by another investigator with a numerical ID and the name and the date of birth of the child were not visible on the image. The calibration of distances was performed storing a millimeter scale with the images. The investigator, trained in digital cephalometric analysis, identified standard skeletal landmarks for each lateral cephalogram. These landmarks were used to define a series of linear and angular cephalometric measurements (Table Ia-Ic).

Reproducibility

Aiming to evaluate the reproducibility of the method, two independent observers performed cephalometric analysis and analysis on casts belonging to 10 patients. One of the two observers repeated the tests at day 7, following the same modalities. Intraclass correlation coefficient (ICC) measured reliability of the quantitative data. A high intra observer reproducibility was evidenced (90,4% of parameters $ICC \geq 0.8$). A high inter observer reproducibility was evidenced ((73% of parameters $ICC \geq 0.8$) even if lower than intra observer reproducibility).

Statistical analysis

Descriptive statistical analyses were first performed. Continuous variables were tested for distribution using the Shapiro-Wilk test. If data were normally distributed,

Table 1a. *Reference points, lines and angles in lateral cephalometric radiographic analysis: Hard and soft tissue points.*

Symbol Point and Name	Definition
Hard tissue points	
S (Sella)	The centre of the sella turcica in the pituitary fossa.
N (Nasion)	The most anterior point of the frontonasal suture.
Ba (Basion)	The lowermost point of the anterior margin of the occipital foramen.
Or (Orbitale)	The lowermost point in the lower margin of the bony orbit.
Pr (Porion)	The most superior point at the upper margin of external auditory meatus.
A (Subsupinale)	The point of the deepest concavity on the anterior outer contour of the maxilla.
B (Submentale)	The point of the deepest concavity on the outer contour of the mandibular symphysis surface.
APOcc (Anterior Point Occlusal)	The middle point of the Overjet, in occlusion.
PPOcc (Posterior Point Occlusal)	The most distal point of the occlusion, in the molar region.
Co (Condylion)	The most posterior superior point on the outline of the mandibular condyle.
Gn (Gnathion)	The most anterior and inferior point on the mandibular symphysis.
Go (Gonion)	The intersection point of the tangent to the posterior border of the ramus and the lower border of the mandible.
Me (Menton)	The most inferior point of the mandibular symphysis.
Ar (Articulare)	The intersection point between the posterior border of the mandibular condyle and the lower margin of the occipital bone.
Pg (Pogonion)	The most anterior point on the mandibular symphysis.
Pns (posterior nasal spine)	Meeting point of the hard palate with the anterior margin of the pterigo-maxillary fissure and with the soft palate.
Ans (anterior nasal spine)	The most anterior point of the anterior nasal spine.
Lia (Lower incisor apex)	The apical point of the most prominent central lower incisor.
Lie (Lower incisor edge)	The incisal margin of the most prominent central lower incisor.
Uia (Upper incisor apex)	The apical point of the most prominent central upper incisor.
Uie (Upper incisor edge)	The incisal margin of the most prominent central upper incisor.
Soft tissue points	
Pn (Pronasal)	The most anterior point on the soft tissue tip of the nose.
Col (Columella)	The lower margin of the nose.
Sn (Subnasale)	The point in the junction between the lower border of the nose and beginning of the upper lip in the mid-sagittal plane.
UL (Upper Lip)	The most anterior soft tissue point of upper lip.
Sn (Subnasale)	The point in the junction between the lower border of the nose and beginning of the upper lip in the mid-sagittal plane.
LL (Lower Lip)	The most anterior soft tissue point of the lower lip.
Pg' (Pogonion)	Soft tissue Pogonion point, the most anterior point on the chin soft tissue.

Table Ib. *Reference points, lines and angles in lateral cephalometric radiographic analysis: Lines or planes.*

Name and symbol	Definition
Lines or planes	
Sella – nasion line (S-N)	The anterior cranial base, the line extending from sella to nasion.
Sella – basion line (S-Ba)	The posterior cranial base, the line extending from sella to basion.
Frankfort horizontal line (FH)	The line between porion point and orbitale point.
Wits	The distance between the perpendiculars from points A and B on the maxilla and mandible, respectively, onto the occlusal plane. Sagittal relation of maxilla to mandible.
Condylion – point A line (Co-A)	The maxilla length, in the sagittal plane. The line extending from condylion to point A
Condylion – Gnathion line (Co-Gn)	The mandible length, in the sagittal plane. The line extending from condylion to gnathion.
Articulare – Gonion line (Ar-Go)	The length of the mandibular ramus. The line extending from articulare to gonion.
Gonion – Pogonion line (Go-Pg)	The length of the mandibular corpus. The line extending from gonion to pogonion.
Sella – Gonion line (S-Go)	The posterior facial height. The line extending from sella to gonion.
Nasion – Menton lines (N-Me)	The anterior facial height. The line extending from nasion to menton.
Ans-Pns	The palatale plane. The line extending from the anterior to the posterior nasal spine.
Aesthetic Line (E)	The line connecting soft tissue pogonion' to pronasal.
Overjet (OVJ)	The amount of horizontal overlap between the maxillary and mandibular incisors.
Overbite (OVB)	The amount of vertical overlap between the maxillary and mandibular incisors.
Jaraback's ratio	The relation between the anterior and posterior facial heights.
U1	The line between the upper central incisor apex and its edge.
L1	The line between the lower central incisor apex and its edge.

mean value and standard deviation (SD) were used to describe the data; if data were not normally distributed, median and interquartile range (IQR) were used. For the inferential analysis (significance tests) the unit of study was the couple of subjects. Between-groups comparisons would be performed with paired t-test if data were normally distributed and with Wilcoxon test if data were not normally distributed. The level of significance was set at 0.05. SPSS for Windows (23.0, SPSS Inc, Chicago, IL, USA) was used.

RESULTS

Eighteen Italian individuals with TS were included in the study group. The mean age was 12.8 ± 5.4 years (range: 6-23). Eighteen Italian age and gender matched healthy individuals were included in the control group. When the comparison of the PH was done between the study group (median: 13 mm; IQR: 11-17) and the control group (median: 11 mm; IQR: 11-12), no statistically

Table Ic. Reference points, lines and angles in lateral cephalometric radiographic analysis: Hard and soft tissue angles.

Symbol	Definition
Hard tissue angles	
NSBa	The angle between nasion - sella - basion. The inclination of the cranial base.
SNA	The angle between sella - nasion line and A point. Sagittal relation of the maxilla to anterior cranial base.
SNB	The angle between sella - nasion line to B point. Sagittal relation of the mandible to anterior cranial base.
ANB	The angle of A point - nasion - B point, it measures the positions of the maxilla to mandible relative N point. Sagittal relation of maxilla to mandible.
NSAr	The sella angle. The angle between nasion – sella – articulare point. Sagittal relation of the mandible to posterior cranial base.
SArGo	The articulare angle. The angle between sella – articulare point – gonion.vIt measures the vertical growth pattern.
ArGoMe	The gonial angle. The angle between articulare – gonion – menton. It measures the vertical growth pattern.
ArGoN	The upper gonial angle. The angle between articulare – gonion – nasion. The vertical growth of the mandibular ramus.
NGoMe	The lower gonial angle. The angle between nasion – gonion – menton. The vertical growth of the mandibular corpus.
Sum (NSAr+SArGo+ArGoMe)	The sum angle, it is the sum of the sella, articulare and gonial angles. It measures the vertical growth pattern.
SN-GoGn	The divergent angle. The angle between sella – nasion line and gonion – gnathion line. It measures the vertical growth pattern.
U1-AnsPns	The angle between the upper incisor line and palatal plane.
L1-GoMe	The angle between the lower incisor line and gonion - menton line.
U1-L1	The interincisal angle. The angle between the upper and lower central incisor.
Soft tissue angle	
ColSnUL	The naso-labial angle. The angle between the columella and subnasale – upper lip line.

significant difference was found ($p=0.051$; Wilcoxon test). The mean PW was 28.22 ± 3.31 mm in the study group and 30.22 ± 3.38 mm in the control group, without a statistically significant difference between the groups ($p=0.223$; paired t-test). The PH/PW ratio was significantly higher in the study group (48.11 ± 7.66) compared to the control group (37.17 ± 6.19) ($p=0.004$; paired t-test).

Cephalometric assessment

Cranial base characteristics:

The median SN was significantly higher in the

study group compared to the control group ($p=0.038$; Wilcoxon test).

Sagittal characteristics:

The mean SNA and SNB were significantly lower in the study group compared to the control group (respectively $p=0.018$ and $p=0.012$; paired t-test).

When the comparisons of the parameters ANB, Wits, Co-A, Co-Gn, Ar-Go, Go-Me were done between the two groups, no statistically significant differences were found ($p>0.005$; paired t-test and Wilcoxon test).

Vertical characteristics:

When the comparisons of the vertical parameters

(NSAr, SArGo, ArGoMe, the sum angle, ArGoN, NGoMe, SN-GoMe, Jarabak's ratio) were done between the two groups, no statistically significant differences were found ($p>0.005$; paired t-test and Wilcoxon test).

Dental-basal characteristics:

The mean U1-L1 was significantly higher in the study group compared to the control group ($p=0.008$; paired t-test).

When the comparisons of U1-SN, L1-GoMe,

OVJ and OVB were done between the two groups, no statistically significant differences were found ($p>0.005$; paired t-test and Wilcoxon test).

Soft-tissue characteristics:

When the comparisons of UL-E, LL-E and naso-labial angle were done between the two groups, no statistically significant differences were found ($p>0.005$; paired t-test and Wilcoxon test). Data in detail are shown in Table II and Table III.

Table II. Cephalometric parameters, comparison across the study groups.

Cephalometric parameters	Mean $\pm$ SD		p-value
	study group	control group	
Cranial base characteristics			
NSBa (degree)	133.95 $\pm$ 6.48	132.36 $\pm$ 4.95	0.414
Sagittal characteristics			
SNA (degree)	77.07 $\pm$ 5.51	80.93 $\pm$ 2.45	0.012*
SNB (degree)	75.27 $\pm$ 3.52	77.33 $\pm$ 2.27	0.018*
Ar-Go (mm)	41.15 $\pm$ 4.67	39.12 $\pm$ 6.08	0.268
Go-Pg (mm)	67.38 $\pm$ 6.36	67.20 $\pm$ 7.04	0.935
Vertical characteristics			
NSAr (degree)	125.78 $\pm$ 4.98	123.68 $\pm$ 4.38	0.187
SArGo (degree)	145.51 $\pm$ 9.21	147.16 $\pm$ 6.84	0.546
ArGoMe (degree)	123.27 $\pm$ 5.63	122.15 $\pm$ 6.88	0.596
Sum (NSAr+ SArGo+ ArgoMe) (degree)	394.57 $\pm$ 6.65	392.99 $\pm$ 4.49	0.320
ArGoN (degree)	50.21 $\pm$ 7.12	50.34 $\pm$ 5.34	0.952
NGoMe (degree)	73.06 $\pm$ 3.96	71.80 $\pm$ 3.14	0.300
SN-GoGn (degree)	34.50 $\pm$ 5.69	32.77 $\pm$ 3.45	0.227
Jarabak's ratio (%)	62.98 $\pm$ 4.44	63.17 $\pm$ 3.67	0.887
Dento-basal characteristics			
U1-AnsPns (degree)	111.12 $\pm$ 7.48	115.05 $\pm$ 7.03	0.113
L1-GoMe (degree)	91.80 $\pm$ 6.96	95.52 $\pm$ 5.85	0.091
Interincisal angle (degree)	133.93 $\pm$ 10.32	124.8 $\pm$ 9.12	0.008*
Soft-tissue characteristics			
UL-E (mm)	-2.64 $\pm$ 3.04	-1.23 $\pm$ 1.24	0.878
LL-E (mm)	-1.31 $\pm$ 3.09	-0.99 $\pm$ 1.90	0.714
Naso-labial angle (degree)	122.57 $\pm$ 9.32	106.99 $\pm$ 10.99	p<0.001*

* statistically significant; paired t-test.

Table III. *Cephalometric parameters, comparison across the study groups.*

Cephalometric parameters	Median (IQR)		p-value
	study group	control group	
Cranial base characteristics			
S-N (mm)	64.45 (63.35; 67.97)	62.30 (59.95; 66.20)	0.038*
S-Ba (mm)	39.45 (37.30; 42.77)	39.95 (35.17; 45.23)	0,95
Sagittal characteristics			
ANB (degree)	2.45 (-0.17; 4.42)	3.5 (2.3; 3.9)	0,319
Wits (mm)	-1.70 (-5.35; 0.95)	-1.45 (-1.72; -0.50)	0,465
Co-A (mm)	78.60(73.32; 81.42)	76.25 (73.05; 80.9)	0,776
Co-Gn (mm)	102.10 (98.60; 111.10)	99.00 (93.55; 107.67)	0,133
Dento-basal characteristics			
OVJ (mm)	4.3 (3.47; 5.27)	3.95 (3.07; 5.55)	0,486
OVb (mm)	1.05 (-2.92; 2.62)	2.05 (0.67; 3.50)	0,179

* statistically significant; Wilcoxon test.

DISCUSSION

This study included 18 individuals with TS under growth hormone therapy and a control group of age and gender matched healthy individuals. The craniofacial features were objectively described using dental casts and lateral cephalograms (31-44).

Previous studies, focused on the cranio-facial features of TS untreated with GH therapy, showed common characteristics. Mitbø et al. (1996) in two studies on 33 children with TS, reported a short posterior cranial base, retrognathic and posteriorly rotated maxilla and mandible. While the corpus and the total length of the mandible were found to be reduced, the length of the maxilla was normal. Moreover, they showed an increased anterior and lateral open-bite, a lateral cross-bite and Angle class II malocclusion. Svanberg et al. (2016) found similar results, studying 10 women with TS without GH: bimaxillary retrusion, posterior-rotation of the maxilla and a skeletal class II malocclusion. One-hundred-eight patients with TS were studied by Rizell et al. (2013): bimaxillary retrusion, bimaxillary posterior-rotation and short posterior cranial base and posterior facial height were described according to Jensen (1985) and to Rongen-Westerlaken et al. (1992) (45-50).

To the best of our knowledge, only two studies evaluated the occlusal features of TS without GH therapy. Szilágyi et al. (2000) studied 29 women with TS and reported Angle class II malocclusion, later cross-bite, anterior open and deep bite and a high narrow palate. Lopez et al. (2002) clinically assessed a high narrow palate in 23 women from Argentina. In the present study, the dental casts analysis showed a significantly higher PH/PW ratio in individuals with TS under GH therapy compared to healthy individuals. This data objectively supported the definition of a high-narrow palate. Oral habits, as oral breathing and non-nutritive sucking can interfere with the palatal growth. No data regarding oral habits were collected in the present study (45-52). This limitation may be taken into consideration in the interpretation of the results.

The GH therapy in individuals with TS is worldwide approved and its efficiency in increasing adult stature is demonstrated. However, few studies described the craniofacial features of individuals with TS treated with GH. Thirteen girls with TS under GH therapy, compared to 13 girls with TS without GH therapy, showed higher anterior and posterior facial heights, and longer mandibular rami and body and maxilla length. While the linear measurements were increased, the angular measurements and facial

height ratio did not show statistically significant difference. Davidopoulou and Chatzigianni (2017) showed that GH therapy had a major effect on the vertical growth of mandible with an anterior rotation, while the anterior and posterior cranial bases were not influenced. Rongen-Westerlaken et al. (1993) examined 19 children with TS before and after 2 years of GH therapy and demonstrated a significant growth of the mandibular rami and the anterior rotation of the mandible. Simmons et al. (1999) examined 19 girls with TS after 1 year of GH therapy and showed significant increased linear measurements of the mandible (53-60).

Hass et al. (2001) analyzed the effect of GH in 28 females with TS during their growth. The authors concluded that the results are minimal on the craniofacial growth, but their sample included: ten patients who were naive to GH at the initiation of the study and were treated with GH after 12 months of enrollment, three patients who never did the therapy and fifteen girls who had initiated GH treatment prior to enrolling in the study. A recent review by Wojcik and Ben-Skowronek (2020), reported that the exact amount and pattern of growth after GH administration is unpredictable; however, the facial convexity decreases and both the mandibular length and the posterior facial height increase (61-65).

In the present study, to assess the anteroposterior positioning of maxilla and mandible in relation to the anterior cranial base, the SNA (mean value $82^{\circ} \pm 2^{\circ}$) and SNB (mean value $80^{\circ} \pm 2^{\circ}$) **angles were calculated according to Steiner analysis (1953). In TS group, the mean SNA (77.07°) and SNB (75.27°) were significantly lower compared to the control group (mean SNA= 80.93° and SNB= 77.73°), indicating a retruded position of the jaws. The cranial base measurements showed a significantly higher NS in the study group, influencing the sagittal position of the N point thus reducing the SNA and SNB angles.**

The ANB (mean value $2^{\circ} \pm 2^{\circ}$) and WITS index (0 ± 2 mm) exhibit the anteroposterior relationship between the maxilla and the mandible. In the present study, the ANB angle and the Wits index were similar in the two groups, showing a skeletal class I malocclusion.

To evaluate the growth pattern, vertical characteristics were measured. All the parameters did not differ between the two groups, showing a mesofacial growth pattern.

The dento-basal characteristics, the OVJ and the OVB were similar in the two groups. The interincisal angle was significantly higher in the study group compared to the control group ($p=0.008$), but the mean value in the study group (133.93 ± 10.32) fell in the range of normality ($130^{\circ} \pm 5^{\circ}$) showing a correct relationship between the upper and lower incisors. The soft-tissue evaluation showed an increased naso-labial angle in the study group compared to the control group.

The results obtained from the present study added data to the literature regarding the craniofacial characteristics of individuals with TS under GH therapy. Our results showed similar cephalometric characteristics in individuals with TS treated with GH and healthy controls. Due to the limited number of individuals included in this case series, to test this hypothesis, an analytic study with sample size calculation is needed to assure an adequate power to detect a statistically significant difference between groups if a difference is truly present (66-72).

According to the results of all the studies on TS girls untreated with GH, the growth pattern showed a tendency to a posterior rotation and retrusion of both the maxilla and the mandible, with a reduced length of the mandible, a vertical growth and a skeletal class II malocclusion. Moreover, regarding the occlusion, an anterior open bite, lateral cross bite and high narrow palate are reported.

The data, collected from the studies that evaluated the craniofacial characteristics of TS subjects under GH therapy and the results obtained from the present study, highlighted the tendency towards a normal mandibular length, a mesofacial growth pattern and a skeletal class I malocclusion. Regarding the occlusion, the palatal dimension is shown to be reduced.

It is known that growth hormone increases the expression of insulin-like growth factors (IGFs) in cartilage, and it influences skeletal growth primarily by stimulating the growth of cartilage in areas of endochondral ossification. Although growth hormone

therapy in Turner syndrome increases statural height toward or within the normal population range, the role of GH on the the craniofacial growth is unclear. It is likely that due to the timing of the growth pattern of the cranial synchondroses and endochondral ossification and the beginning of the GH therapy, the major effect of the therapy is on the mandibular growth. In fact, the tendency to a skeletal class I characteristics and a mesofacial growth pattern in girls with TS treated with GH are the results of an increased mandibular length, due to the response of the mandibular condyle cartilage to the therapy.

An early orthodontic diagnosis of narrow palatal is necessary to establish the proper treatment plan and timing of intervention. Future studies should test whether functional appliances used for skeletal class II malocclusion could increase the effect of the GH therapy in individuals with TS.

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Impact of self-ligating orthodontic brackets on dental biofilm and periodontal pathogens in adolescents

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The aim of the present prospective study was to evaluate periodontal health and subgingival microbiological alterations in adolescents treated with fixed self ligating orthodontic brackets in comparison to subject without any orthodontic appliance. A total of 40 adolescents (23 females and 17 males; mean age: 13.2 ± 3.2 years) were included: 30 subjects with self ligating brackets (test group) and 10 patients without orthodontic appliances (control group). Follow-ups were as follows: T1 (1 month), T2 (3 months), T3 (6 months) from the beginning of the orthodontic therapy. Clinical parameters (plaque index, gingival index and clinical attachment level) were measured for every patient and a microbiological analysis was performed. Mann Whitney test was performed to evaluate clinical parameters between test and control group and Friedman test and Fisher test were adopted to evaluate intra group differences at different follow-ups. Student *T*-test was performed to compare clinical attachment level between the two groups. Significance level was set at $p < 0.05$. No periodontal pathogens and no clinical attachment loss were found in the whole sample. A slightly higher plaque index and gingival inflammation were recorded in the test group in comparison to the controls.

Orthodontic brackets are often associated with gingival inflammation and higher dental plaque accumulation (1). Conventional brackets need elastomeric ligations or stainless-steel ligature wires while self ligating brackets utilize a permanently installed, moveable component to entrap the orthodontic archwire that may allow a lower dental biofilm accumulation (2). The Russel lock was a self ligating bracket introduced in 1935 and nowadays self ligating brackets are used worldwide because of several advantages: provide a full orthodontic wire engagement, lower time to remove and replace the archwires in comparison to conventional brackets, a rapid tooth movement because of the low friction forces and an easier dental hygiene because of the fewer retentive

sites for microbial accumulation; however, there is insufficient high-quality evidence to support the use of self-ligating brackets over conventional appliances (3).

Active self ligating brackets have a metallic slide that create an active labial surface invading the orthodontic slot while passive brackets have an in-built metal face that did not invade the slot (4). The risk of gingival inflammation in orthodontic patients treated with fixed brackets is increased in subjects with high levels of bacterial plaque and poor oral hygiene and the presence of gingival inflammation over time may facilitate the onset of periodontal disease in adult age; therefore, an early diagnosis and timely treatment of gingival inflammation in orthodontic patients would avoid the onset of more severe periodontal issues over time

Key words: orthodontics, microbiology, dental plaque, gingival inflammation

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(5). Furthermore, orthodontist may decide to debond orthodontic brackets earlier in patients that show a poor oral hygiene. Patients that neglect oral hygiene during fixed orthodontic treatment are associated with a higher incidence of white and brown spots, dental caries and gingival inflammation. Orthodontic tooth movements did not cause bone resorption; however, patients with bad oral hygiene and severe gingival inflammation may show some bone defects (6).

Moreover, microbiological changes in subgingival dental biofilm may be found in orthodontic patients and a higher prevalence of: *Porphyromonas gingivalis*, *A. actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella intermedia*, *Prevotella nigrescens*, *Campylobacter rectus*, *Peptostreptococcus micron*, *Fusobacterium nucleatum* are often observed during orthodontic therapy (7).

It was stated that fixed orthodontic treatment may cause a periodontal attachment loss because of the orthodontic forces; however, in orthodontic patients with good dental hygiene no periodontal attachment loss was observed (8).

It was found that metallic ligatures allow a lower dental plaque accumulation in comparison to elastic ligatures that exhibit significant volumetric and structural changes during the treatment (9). As regards self ligating system few studies have analysed dental biofilm accumulation and microbiological changes during the orthodontic treatment: Pandis et al. evaluated conventional brackets in comparison to self ligating system and did not found statistical differences in salivary streptococcus mutans levels in patients with conventional and self-ligating brackets (10). Pellegrini et al. observed a lower association of periodontal species and self ligating brackets in comparison to conventional elastic ligatures (11-20).

The aim of the present study was to evaluate periodontal health and subgingival microbiological alterations in adolescents treated with fixed self-ligating orthodontic brackets.

MATERIALS AND METHODS

In the present prospective study 40 patients (23 females and 17 males; mean age: 13.2 ± 3.2 years) were included. The test group included 30 orthodontic patients

(17 females and 13 males) treated with self ligating passive brackets (Damon Q Ormco and buccal tubes on maxillary and mandibular molars) and the control group included 10 patients (6 females and 4 males) with normal occlusion, without any orthodontic appliance and without periodontal diseases or gingival inflammation at T0 (beginning of the study).

Inclusion criteria were as follows: age between 12 and 18 years old, absence of periodontal diseases or periodontal pockets ≥ 4 mm, teeth without dental caries or extensive fillings and patients that exhibited a good compliance during orthodontic therapy and visited the orthodontist regularly.

Exclusion criteria were as follows: drugs or antibiotics that may influence microbiological environment, chronic use of antiinflammatory drugs and pre-medication within three months prior to study, systemic diseases that might have caused alterations of the periodontal status, mouthwash use, smoking.

Toothbrushing frequency was at least twice a day (Bass technique was adopted for every patient included in the study) with a duration of 2 minutes with a manual toothbrush. Furthermore, other cleaning tools were included: dental floss and proxy brushes to clean around brackets and wires at least once/day. Moreover, all the patients gave written informed consent to participate in the study. All procedures were conducted according to the principles expressed in the Declaration of Helsinki.

All patients showed similar diet habits and sugar intake at T0. Before orthodontic treatment, all patients underwent a session of oral hygiene aimed at obtaining a plaque index of zero. Pattern of scheduled follow up appointments was as follows: T1 (1 month), T2 (3 months), T3 (6 months). Daily oral hygiene methods and strategies specific to the orthodontic patient were adopted and oral hygiene evaluation was performed during follow-ups and a dental hygiene diary was used by each patient. Plaque index and gingival index were measured for every patient on every surface of two teeth: 11 and 25.

The periodontal status of each subject was examined by the same calibrated operator with a mouth mirror and UNC 15 periodontal probe. Clinical Attachment Level (CAL) was measured: the distance between the cemento-enamel junction and the bottom of the pocket or sulcus. The calibrated UNC 15 probe was inserted parallel to the long axis of each tooth at four sites of a

tooth (mesiobuccal, buccal, disto-buccal, and mid-lingual). The deepest pocket was considered among all CAL measurements.

The clinical findings were rounded up to the nearest millimeter and each examination was performed with a 1-week interval in order to evaluate the intra-examiner error (Kappa statistics: 0.78).

Microbiological samples were collected on two teeth (11 and 25) and put in a sterile tube with dimethyl sulfoxide (DMSO) that is an organosulfur compound that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water.

Bacteria can survive for a short period of time at 4°C; therefore, samples were stored in a refrigerator (-30 degrees celsius) and precaution has been taken to avoid contamination.

Polymerase chain reaction (PCR) technique was applied to detect and identify oral microorganisms: *Aggregatibacter actinomycetemcomitans* (Aa 652, Aa JP2), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythensis* (Tf).

Statistical analysis

The data were processed using the program SPSS 15.0 (Windows). Mann Whitney test was performed to evaluate clinical parameters between test and control group. Moreover, Friedman test and Fisher test were adopted to evaluate intra group differences at different follow-ups. Student T test was performed to compare clinical attachment level between the two groups. Significance level was set at: $p < 0.05$.

RESULTS

Microbiological analysis showed absence of: Aa 652 e Aa JP2, *Tannerella forsythensis*, *Prevotella intermedia*, *Porphyromonas gingivalis* in all the samples. In the test group, plaque index (tooth 11) was significantly higher at T2 ($p < 0.05$). From T2 to T3 a significant decrease ($p < 0.05$) was recorded (Fig. 1).

A significant difference was observed between test and control group at T2 ($p < 0.05$) (Fig. 2).

In the test group, plaque index (tooth 25) was

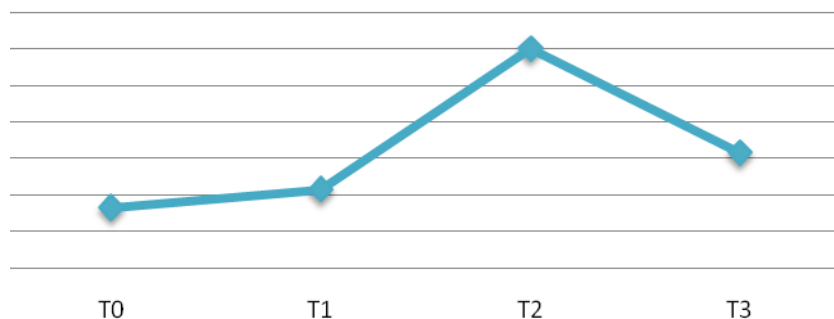


Fig. 1. PI (tooth 11) in the test group: T0 (0.3), T1 (0.4), T2 (1.2), T3 (0.6). Friedman and Fisher test: $p < 0.05$.

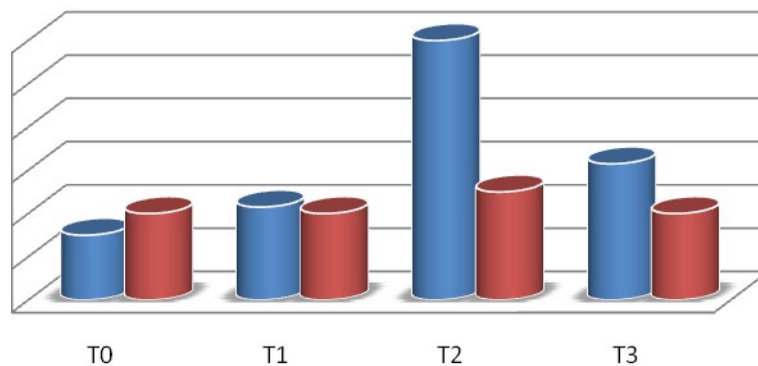


Fig. 2. Plaque index (tooth 11) inter group comparison (test group: left column and control group: right column). Test group: T0: 0.2; T1: 0.3; T2: 1.2; T3: 0.55. Control group: T0: 0.3; T1: 0.25; T2: 0.4; T3: 0.3. Mann Whitney test and Fisher test: $p < 0.05$.

significantly higher at T2 ($p < 0.05$). Moreover, a significant decrease ($p < 0.05$) was recorded at T3 (Fig. 3). The test group showed a higher value ($p < 0.05$) in comparison to the control group both at T2 and T3 (Fig. 4).

Gingival index (tooth 25) was significantly higher at T2 ($p < 0.05$) in the test group. No significant difference was observed comparing the two groups ($p > 0.05$) (Fig. 5).

We did not find any periodontal pocket ≥ 4 mm in the test group. Moreover, also in the control group no pathological periodontal pockets were recorded. Therefore, CAL did not show any significant difference ($p > 0.05$).

DISCUSSION

Fixed orthodontic treatment is often associated with dental caries, enamel demineralisation and gingival inflammation (21). Adolescents usually did

not have a good oral hygiene and they are recognized as having possible distinctive needs due to a potentially high caries and periodontal disease rate, a tendency for poor nutritional habits (soft drinks and high sugar intake), dental phobia, cigarette smoke and social and psychological aspects (22).

Gingivitis with swollen gums and gingival bleeding is generally associated with fixed orthodontic treatment in a high percentage of patients because brackets, bands and tubes increase dental biofilm accumulation, and it is difficult to maintain oral hygiene at home.

However, after brackets debonding gingival status usually improves because gingivitis is the early, reversible phase of periodontal disease. Diet, daily maintenance and regular professional care are the main factors that prevent gingival inflammation (23-35).

In the present study it was observed a slightly higher gingival inflammation and dental plaque accumulation in patients with orthodontic brackets

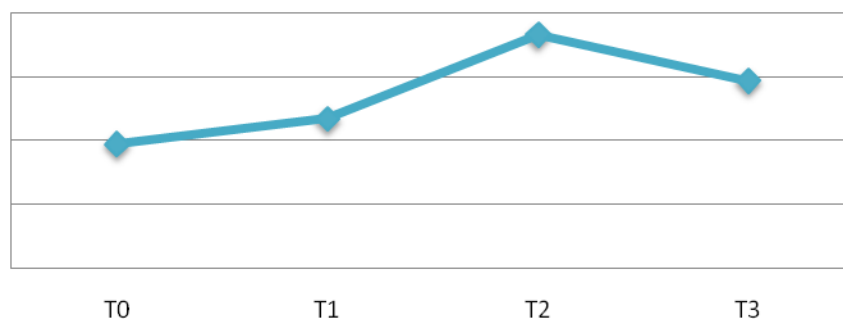


Fig. 3. Plaque index (tooth 25) in the test group (T0: 1; T1: 1.2; T2: 1.8; T3: 1.5). Friedman and Fisher test: $p < 0.05$.

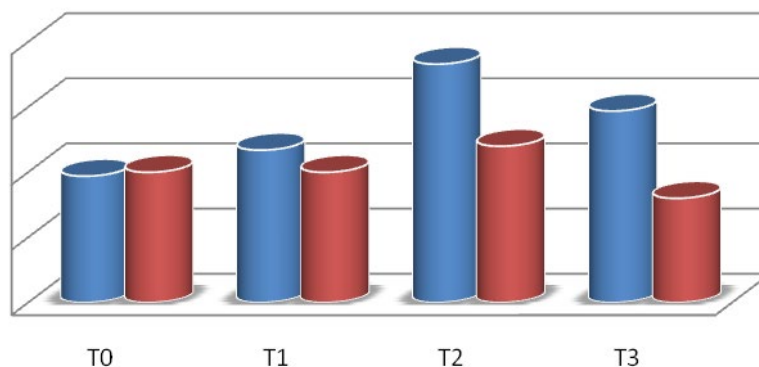


Fig. 4. Plaque index (tooth 25) in the two groups (test group: left column and control group: right column). Test group: T0: 0.8; T1: 1; T2: 1.7; T3: 1.3. Control group: T0: 0.8; T1: 0.8; T2: 1; T3: 0.7. Mann Whitney and Fisher test, $p < 0.05$.

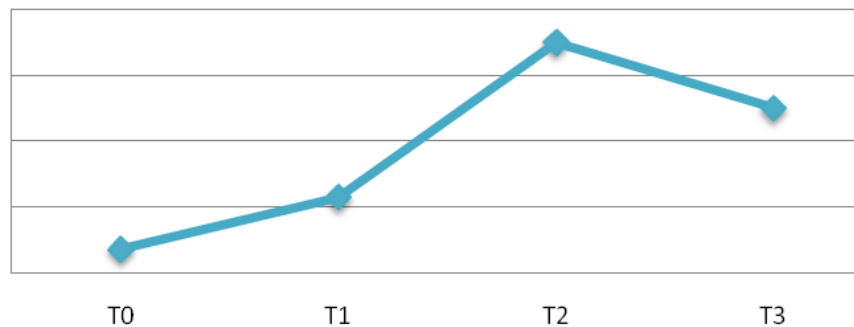


Fig. 5. *Gingival index (tooth 25) in the test group (T0: 0.1; T1: 0.2; T2: 0.7; T3: 0.5). Friedman and Fisher test: $p < 0.05$*

in comparison to subjects without any orthodontic appliance. However, after 6 months (T3) gingival status was better in the test group in comparison to T2 (3 months), probably because dental hygienist reinforced oral hygiene habits.

The upper central incisor showed a lower gingival inflammation in comparison to maxillary premolar probably because it is easier to brush than the upper second bicuspid.

Furthermore, in this study we did not find any pathological periodontal pocket; in literature it was observed a slight clinical attachment loss in some orthodontic patients. However, it should be stated that in the present study we did not used molar bands that are often associated with gingival inflammation. Moreover, self ligating brackets may have promoted a decrease dental biofilm accumulation. A limit of the present study is the absence of a group of patients treated with conventional elastic or metallic ligatures, therefore we cannot state if the self ligating system promote oral health in clinical practice. Finally, in our study we did not find any periodontal pathogens after the microbiological analysis and we should consider that we only included adolescents in the study, while adults usually exhibit a higher prevalence of periodontal pathogens (36-44).

The absence of periodontal pathogens was associated with the clinical absence of periodontal pockets higher than 4 mm. Therefore, we can conclude that fixed orthodontic treatment in the first 6 months of therapy did not cause attachment loss or bone resorption.

The results of the present study showed that only a slightly higher dental plaque accumulation and gingival inflammation is associated with fixed orthodontic treatment with self ligating brackets in comparison to subjects without any orthodontic appliance (45-55). No periodontal attachment loss was observed in the sample and no microbiological changes were observed during the first 6 months of orthodontic treatment (absence of periodontal pathogens). Upper central incisor exhibited a lower dental biofilm accumulation and gingival inflammation in comparison to maxillary second bicuspid (56-60).

After 3 months we observed a higher dental plaque accumulation and gingival inflammation in compariso to T0; however, after 6 months both plaque index and gingival index decreased.

Further studies will be necessary to compare self ligating brackets with conventional orthodontic brackets with elastic or metallic ligatures (60-66).

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Oral manifestations in paediatric patients with hepatobiliary diseases: a review

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It is well known that greenish pigmentation of the teeth is seen in children following remission of severe jaundice and clinical and serum bilirubin, a degradation product of haemoglobin, may be permanently trapped in forming dental hard tissues causing discolouration and enamel and dentine hypoplasia. Neonatal jaundice is the most common cause of hyperbilirubinemia and pigmentation of the deciduous teeth is the consequence of this condition. Various hepatobiliary pathologies may have a clinical finding in the oral cavity; furthermore, oral manifestations of hepatic pathologies are not just limited to the pigmentation of the deciduous teeth but also the permanent dentition and the mucous membranes can be affected.

Some hepatobiliary disorders might be associated with oral manifestations; Langmead (1) and Thursfield (2), first proposed, in 1912, that dental changes could be the result of jaundice. First of all, hyperbilirubinemia, defined as a serum concentration of bilirubin greater than 1.5mg/100mL (3), must be considered, as the cause of intrinsic greenish pigmentation of human teeth. Plasma bilirubin in normal subjects is virtually all in the unconjugated form and ranges from 0.3 to 1.0 mg/dL (3) but when the serum bilirubin concentration rises above normal, the clinical condition called *jaundice* occurs, with yellow-green pigmentation of the mucous membranes, skin and sclera. Bilirubin is extensively deposited throughout the body during hyperbilirubinemia, dispersing caudally as the level increases, although it disappears from soft tissue after remission (4). Nevertheless, in hard tissues bilirubin is permanently trapped, because after maturation these tissues lose metabolic activity (3).

Types of jaundice

There are different forms of jaundice: the clinical

condition in which the yellow pigmentation is visible only at the level of the sclera and lingual frenulum is defined as latent jaundice (bilirubin concentration >1.5mg/dL), while the full-blown form of jaundice (bilirubin concentration > 2mg/dL) is clinically more widespread, also affecting the skin. Jaundice can be obstructive, hepatocellular or haemolytic in origin.

Etiopathogenesis

The obstructive jaundice, characterized by an high serum concentration of conjugated bilirubin, is caused by a lack of outflow of bile from the liver, linked to an intrahepatic or extrahepatic obstruction of the bile ducts, associated with cirrhosis or cholestasis, which in young child is often caused by biliary atresia but it could be also caused by other conditions such as infections (4), cystic malformation of the biliary tree (5) and Alagille Syndrome (6).

The second form of jaundice, caused by an impaired function of liver cells (e.g., Gilbert or Crigler-Najjar or Lucey-Driscoll syndromes) is characterized by a high serum level of unconjugated bilirubin, as well as in the hemolytic form. The latter

Key words: hepatobiliary diseases, oral manifestations, hyperbilirubinemia, green teeth, jaundice

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is caused by massive destruction of erythrocytes which occurs in the following medical conditions: haemolytic anemia associated with infections or diseases such as galactosemia or favism; ineffective erythropoiesis or splenic hyperfunctionality.

Even drug-induced liver diseases can lead to hepatocellular or colestatic jaundice because of a liver failure and children can also be affected by this condition, often linked to the use of paracetamol (7).

Neonatal hyperbilirubinemia

Neonatal hyperbilirubinemia occurs in 60% of newborns during the first week and this medical condition is called neonatal jaundice. This appears to be a physiological condition linked both to the increased synthesis of indirect bilirubin and to the still ineffective activity of the liver enzymes destined for its metabolism. Indeed, at the time of birth, the onset of lung function and the increased availability of oxygen lead the spleen to dispose of many aged and superfluous erythrocytes, producing large quantities of unconjugated bilirubin. One of the main risk factors for neonatal jaundice is premature birth. This condition is physiological if it occurs from the second day of life and lasts for a maximum of two weeks. It is considered pathological jaundice if it appears in the first 24 hours of life and persists for more than two weeks, associated with comorbidities. Unconjugated hyperbilirubinemia can be associated with neonatal anemias, intoxications or infections in cases of jaundice due to increased hemolysis or associated with metabolic diseases and hypothyroidism in icteric forms due to reduced hepatic conjugation of bilirubin. The most common cause of haemolytic neonatal jaundice is maternal-fetal AB0 or Rh incompatibility. So, all the possible causes of hyperbilirubinemia listed so far must be taken into consideration in the case in which a patient with greenish pigmentation of the deciduous (or more rarely permanent) dentition comes to the dentist.

Green pigmentation of teeth due to hyperbilirubinemia

There is no estimate, but the prevalence of green teeth is low and some findings suggested a lack of gender susceptibility (8-15). From the knowledge emerged from the literature it appears that the primary dentition is most frequently affected and

that when the permanent dentition is affected, all patients are found to have had a history of biliary atresia. The explanation for this is that the degree and localization of tooth staining is linked to the severity and duration of hyperbilirubinemia in correlation with dental maturation. Knowledge of the chronologic development of teeth is essential, in conjunction with the history of total bilirubin levels, to predict the degree and pattern of greenish pigmentation that will be found in the dentition.

Further oral manifestations of hepatobiliary pathologies

Hepatobiliary diseases can also affect the oral cavity in other ways. Up-to-date publications suggest that children with liver failure are more prone to oral mucosa lesions than generally healthy children, which might result from hypoproteinaemia, coagulopathy, malnutrition or immunodeficiencies (16-17). In previous studies the prevalence of angular cheilitis, strawberry lip with erosions and geographic or smooth tongue, petechiae and yellow discolouration was statistically significant in children with liver diseases. Patients with severe liver diseases (class B/C according to the Child-Plug scale) are more frequently prone to oral candidiasis and this is also indicated by the bacterial and fungal dysbiosis typical of cirrhotic patients who, due to the overuse of antibiotics and immunosuppressants, are subject to frequent infections and characterized by an unfavourable gut microbiome, with a lower autochthonous bacterial taxa and a low fungal diversity.

Some dental defects, such as opacities and hypoplasia, have also been associated with hepatic pathology and this is presumably due to the state of malnutrition typical of these patients. Indeed, when bile passage to the intestine is decreased or completely blocked, malabsorption of fat-soluble vitamins A, D and K occurs. Bleeding due to inadequate vitamin K absorption and inadequate synthesis of clotting factors is common as are rachitic changes in bone and mineralized dental tissues due to inadequate vitamin D.

DISCUSSION

Tooth discoloration may manifest as different

colours, with green pigmentation being uncommon. Its etiology is classified into extrinsic and intrinsic causative factors. The known extrinsic causes include chromogenic bacteria, systemic antibiotics (e.g., minocycline), copper salts contained in mouth rinse, and copper and nickel metal ions (19-23). Intrinsic causes include hyperbilirubinemia and drugs such as minocycline and ciprofloxacin. There are various phenotypes of tooth pigmentation by bilirubin, ranging from a mild yellow to a darker green shade. Green is the most frequently reported, followed by brown, yellow, and gray. The degree of pigmentation is known to be proportional to the serum bilirubin level but there is no direct correlation between the degree of green discoloration and the duration of hyperbilirubinemia. It is considered that the degree and location of pigmentation is determined by the timing of hyperbilirubinemia so that the crown portion with a normal hue developed when the bilirubin level is normal and the green portion of the teeth formed during the hyperbilirubinemia period, are always clearly distinguished by lines, as it can be seen in Fig. 1.

Occasionally, green teeth are also affected by enamel hypoplasia, which could be due to the effects of osteopenia and other disturbances of calcium and phosphate metabolism encountered in chronic liver disease or due to intubation in neonatal periods, so that this clinical aspect could be not directly linked to the liver disease but related to a life-saving maneuver that may be necessary especially in premature babies (24-29).

In regard to the pigment responsible for greenish pigmentation of teeth, Shibata's study demonstrated by spectrophotometric analysis the presence of bilirubin in artificially pigmented teeth of some rats, and this has undermined the hypothesis according to which the colour of green staining was due to biliverdin, an oxidation product of bilirubin. Furthermore, the histological analysis of murin pigmented teeth showed the presence of a green stripe in the dentine running parallel to the incremental line. These observations were subsequently validated also by a histological study carried out on human teeth extracted from patients with severe liver dysfunction and hyperbilirubinemia. Also, in human teeth pigmented stripes running parallel to the dentinal



Fig. 1. Green pigmentation of deciduous and permanent teeth in a 11 years old male child diagnosed with an intrahepatic cholestasis: most of the permanent teeth were characterized by a green pigmentation and also the attached gingiva of deciduos teeth showed yellow-greenish pigmentation. Liver transplant was performed after 2 years from birth therefore teeth showed white (mineralisation >2 years old) and green (mineralisation <2 years old) enamel colour changes (18). **A:** front view; **B:** upper arch in occlusal view; **C:** view of the first and fourth quadrants; **D:** view of the second and third quadrants.

incremental lines were observed histologically, and the correlation between childhood hyperbilirubinemia and changes in dentin morphology seems to include even a decrease in the density of the dentin tubules and a reduction in the thickness of peritubular dentin in green teeth (30-33).

There are a lot of causes of green teeth reported in Literature and we have summarized the main causes of pathological hyperbilirubinemia in Table I. However, the most common cause appears to be biliary atresia that is the result of progressive bile duct destruction from an inflammatory disease of unknown cause. It is related to the persistence or

lack of remodeling of the embryonic ductal plate and consists of both intrahepatic and extrahepatic biliary atresia. The damage causes obstruction of bile flow and if left untreated, this can result in cirrhosis and hepatic failure. This is the reason why the primary diagnosis for liver transplant was mainly biliary atresia (57.5%) followed by other causes of cholestasis (22.5%) (34-41).

In Literature it is reported that children with liver disease are at high risk of developing dental caries, possibly due to the need of continuous feeding to compensate for the low intestinal absorption of nutrients or due to an inadequate

Table I. Schematization of the various pathologies causing hyperbilirubinemia.

Pathologic hyperbilirubinemia	
1) increased bilirubin load -hemolytic	a. immune mediated (e.g. rh factor incompatibility; AB0 incompatibility) b. RBC enzyme defects (e.g. G6PD deficiency; pyruvate kinase deficiency) c. RBC membrane defects (e.g. spherocytosis; elliptocytosis) d. Hemoglobinopathies (e.g. thalassemia; sickle cell disease)
2) increased bilirubin load-non hemolytic	a. extravascular blood (e.g. hematomas) b. polycythemia (e.g. delayed cord clamping; transfusion; increased intrauterine erythropoiesis caused by placental insufficiency or intrauterine hypoxia) c. exaggerated enterohepatic circulation (e.g. breast milk jaundice; cystic fibrosis)
3) impaired bilirubin conjugation	a. physiologic hyperbilirubinemia b. Crigler-Najjar syndrome c. Gilbert syndrome d. congenital hypothyroidism e. breastfeeding jaundice f. breast milk jaundice (in the second week of life)
4) impaired bilirubin excretion	a. biliary obstruction (e.g. genetic biliary atresia; Alagylle syndrome; drug-induced liver disease) b. infection (e.g. cytomegalovirus; reovirus type 3) c. chromosomal abnormalities (e.g. Turner's syndrome; trisomy 18; trisomy 21) d. metabolic disorders

absorption of macronutrients and micronutrients indispensable for dental development. As regards the differential diagnosis, the anamnesis is of fundamental importance for the clinician because teeth pigmentation could be caused even by chromogenic bacteria, medicaments, dentinogenesis imperfecta, amelogenesis imperfecta, tetracycline or congenital erythropoietic porphyria. The anamnesis reported by the parents is therefore a fundamental therapeutic moment for the management of the clinical case, when clinicians have to treat a patient with liver disease: it is important to plan the prevention of carious pathology with regular visits that allow the early identification of any oral problem caused by liver diseases, as well as aesthetically improve the appearance of damaged teeth (42-52).

It is important to underline that green dentin pigmentation is clearly visible through translucent enamel and can cause great anxiety for the families involved and significant peer problems for children. The pigmentation is not easily disguised with restorative materials, however, cosmetic improvements to these teeth may increase the chances of normal physical, psychological, and social development of the patient. In order to plan for the most appropriate treatment, it is important for clinicians to understand that the pigmentation is confined to dentin and dental bleaching is unlikely to be effective even if some authors suggested a bleaching technique with the use of transillumination with ultraviolet light to accelerate the breakdown and dissipation of bilirubin products (, 53-65).

Direct composite veneers could be placed but indirect veneers provide a more uniform opaque layer to disguise the green pigmentation. Porcelain or Belleglass could be used to mask the underlying pigmentation and the latter are recommended for a temporary restoration in children because Belleglass veneers don't require any tooth preparation prior to bonding, have a wear resistance comparable to that of human enamel and are less abrasive on the opposing natural dentition (66-72).

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Critical investigation of the pre-surgical anatomic factors that influence the aesthetic result in post-extractive implantology and immediate prosthetic.

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The aesthetic goals of implant dentistry should be similar to those of conventional prostheses. In implant-prosthetic rehabilitations of the anterior sectors, the quantity and quality of hard and soft tissue play a fundamental role in achieving the desired aesthetics and in integrating the restoration in a complete and harmonious way into the existing anterior dentition. Post-extraction implantology is a treatment option for implant therapy after the extraction of a single tooth in the anterior jaw. This article presents some key factors to analyze and follow in order to achieve a predictable and acceptable aesthetic result. The creation of a predictable peri-implant aesthetics requires adequate preservation of the bone and soft tissues around the teeth that will be extracted and a correct positioning of the 3D implant. Furthermore, aesthetic success requires the creation of a correct transmucosal path, during the provisional prosthetic phases, which must be replicated and maintained with the definitive crowns.

Implant-prosthetic rehabilitation in aesthetic areas is one of the most challenging treatments for obtaining and maintaining correct mucosal architecture as well as osseointegration. The maintenance of hard and soft tissues after tooth extraction is essential for the subsequent implant placement and above all for the final prosthetic result.

The healing of the soft tissue in a post-extraction site with keratinized tissue occurs after 25-35 days, at the end of which both horizontal and vertical loss of hard and soft tissues remain (1). Wohrle, in 1998, was the first to obtain excellent results with post-extraction implantology and immediate prosthetics with single implants in the aesthetic area in the upper jaw (2). The most fascinating result of this technique is the effectiveness of preserving both the bone and mucous architecture.

The first systematic review on post-extraction implantology was in 2003 (3rd ITI Consensus

Conference in Gstaad, Switzerland). The authors conclude that immediate and deferred implants both have a high predictability, comparable with implants placed in healed bone (3).

The initial success of post-extractive implantology is due to the belief that this innovative technique could allow the preservation of bone tissue and mucosal architecture as well as reduce the time of edentulism and of treatment in general. However, recent studies have questioned if the insertion of post-extraction implants alone can prevent bone resorption (4-10).

The criticality is in the morphology of the post-extraction socket, which if incongruous can lead to the incorrect position of the implant, in the lack of primary stability, but the greatest criticality is the difficult management of soft tissues, especially in the aesthetic sectors (11-19).

In the aesthetic sectors, the primary purpose of post-extraction implant therapy is the achievement of

Key words: post-extractive implantology, implant-prosthetic rehabilitations, peri-implant aesthetics

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an excellent aesthetic result with high predictability. To achieve this, without incurring complications that would affect the result, the anatomy of the element to be extracted must be carefully evaluated before establishing the implant treatment (20-27).

The purpose of this article is to identify and evaluate the correct pre-surgical anatomy to achieve predictable long-term aesthetic results avoiding risk factors, in immediate post-extractive implantology in aesthetic areas.

MATERIALS AND METHODS

Indications in post-extraction implantology

1. Parodontal biotype and thickness of the vestibular bone

The implant's direct placement into a post-extractive site depends on the presence of the vestibular cortical bone, that has to be thicker than 1 mm, and on the thick biotype of the mucosa. Parodontal biotype around the clinical crown of natural teeth is defined as thick and flat, thin and festooned (6), instead soft tissues around implants are defined as wide, medium and thick. When these conditions are both present there is a low risk of recession.

In a study of Braut et al. (7) it is examined the vestibular bone thickness in different sectors of the anterior maxilla. The authors conclude sustaining that a fenotype >1mm belongs only to the 4.6% of superior incisors and to the 27.5% of premolars. It is necessary to remember that often superior central incisors have a slim biotype, that together with the presence of dehiscence and fenestrations of vestibular flat due to an infective process, vertical root fractures and endodontic complications, can all affect the presence of an intact vestibular bone (8).

2. Tridimensional implant placement

The correct implant placement in a post-extractive site is the basic step to satisfy the needed aesthetic, promote health and stability of the surrounding tissues and obtain a good implant stability, fundamental requirement for an immediate prosthetic. The insertion of a post-extraction implant is a delicate procedure that requires not only the presence of an intact buccal bone, but also the presence of 2-4 mm of apical bone to the alveolus to obtain a stable anchorage of the implant and therefore a good implant stability (torque 35 Ncm) (28-31).

In the post-extraction sites of the anterior maxilla, the implant must be inserted with a vestibular-palatal inclination, maintaining a gap of about 2mm between the implant shoulder and the vestibular margin of the residual alveolus, this allows to avoid the shoulder implant to be in a "dangerous vestibular" position with a consequent increase in the percentage of vestibular recessions.

In the event that the space between the implant body and the alveolar walls is ≤ 3 mm, no regenerative procedures are necessary since the gap is physiologically filled with new bone even if we can foresee its horizontal and vertical resorption. To prevent this, the peri-implant space can be filled with bone substitutes which will help reduce post-surgical vestibular resorption and thus ensure soft tissue stability.

The coronal-apical position of the implant shoulder should be at least 3-4mm below the amelocement line of the adjacent dental elements, since the biological width observed around the implants is approximately 4 mm (32-37). The minimum space to be maintained between the implant and the tooth is about 1.5 mm, this space is necessary for the future maturation of the papilla even if to completely fill the interpapillary space there other anatomical and prosthetic factors.

3. Factors that determine the presence of the papilla

In implant prosthetic rehabilitation between two elements, the filling of the interpapillary space is foreseeable when there is the presence of mesial and distal bone peaks in the adjacent teeth and their distance is ≤ 5 mm from the point of contact of the future implant crown.¹⁴ Another aspect to consider in the diagnostic phase is the shape of the teeth which consequently determines the periodontal biotype and the formation of the future papilla.

A thick biotype is frequently associated with square teeth having the most apical contact point; in these cases it is easier to fill the interdental space than a thin biotype with high scallop, associated with triangular teeth where the contact point is much more coronal (38-49).

4. Management of the temporary and definitive crown

A key factor in optimizing the future final aesthetic result and its maintenance over time is the prosthetic phase. The adaptation of a temporary crown immediately after the insertion of a post-extraction implant finalizes and enhances the design of the surgical act. The temporary crown therefore becomes fundamental not only for

aesthetic reasons but because thanks to its anatomy it allows correct healing of the soft tissues.

CASE REPORTS

Case 1

The 66-year-old female patient came to our observation reporting pain in the upper right anterior region and difficulty in biting. A local intraoral examination shows a distal destructive caries affecting the 1.1. An intraoral x-ray confirms the impossibility of saving the dental element. (Fig 1).

The adjacent teeth did not show caries, mobility and probing depth. Because of the bad prognosis of the dental element, it was decided to extract it and place an implant at the same time in order to create an immediate aesthetic prosthetic of 1.1. The choice of inserting the implant immediately in the post-extraction site and the immediate aesthetic is dictated by a careful pre-surgical plan of the anatomical site. The treatment was discussed and accepted by the patient.

After antibiotic therapy (Amoxicillin 1 gr 2 hours

before surgery), local infiltration is done with 2% lidocaine, the tooth is extracted in an atraumatic manner in the aim of not damaging the anatomical structures. After careful curettage of the socket (Fig. 2), the implant socket is prepared. Ideally, the implant should engage the palatine wall of the post-extraction socket extending the preparation approximately 4 mm above the apical end of the socket.

This preparation allows us to obtain that the long axis of the implant is slightly lingual to the incisal edge of the mesial and distal teeth and even to obtain a good implant stability necessary for immediate prosthetic. A 4.5 x11 implant is placed (Neoss Ltd, Harrogate.UK) and a stability of 75 ISQ was obtained, evaluated with the resonant frequency (RFA) method using Osstel ISQ Mentor Device, AB, Goteborg, Sweden). The temporary abutment is screwed to the implant and the temporary resin crown is relined. The gap between the implant and the buccal bone is filled with biomaterial (Osteobiol Gen-Os) immediately before screwing the temporary crown. (Fig. 3).



Fig. 1. a) Initial intraoral image. The destructive caries affecting 1.1 is noted. b) Lateral vision c) Periapical x-ray of element 1.1



Fig. 2. *Post-extractive socket.*



Fig. 3. *View of the temporary restoration relined immediately after implant surgery. Note the absence of mucous compression on the vestibular side and the position of the contact points to promote the formation of the papilla.*



Fig. 4. *Temporary crown. Note the concave and carefully polished emergence profile.*

It is important that the emergence profile of the temporary crown is carefully polished, and concave in shape to allow healing of the connective tissue, while the area in direct contact with the gingival margin is made flat to avoid compression of the vestibular gingival mucosa. (Fig. 4).

The occlusion is checked in centric and laterality. After 5 months of undisturbed healing (Fig. 5) the implant stability (78 ISQ) is checked and the final prosthetic phase begins. (Fig. 6-7a, b and Fig. 8.)

We can conclude that using the previously outlined guidelines, the clinician can achieve predictable and lasting functional and aesthetic results.

Case 2

The patient comes to our observation with loss of elements 1.1 and 2.1 due to a sport trauma that occurred 24 hours earlier (Fig. 1a and 1b). Two post-extraction implants were then inserted. The vestibular cortex was perfectly preserved (Fig.2a



Fig. 5. *Clinical aspect of the temporary prosthetic rehabilitation. Follow up at 5 months.*

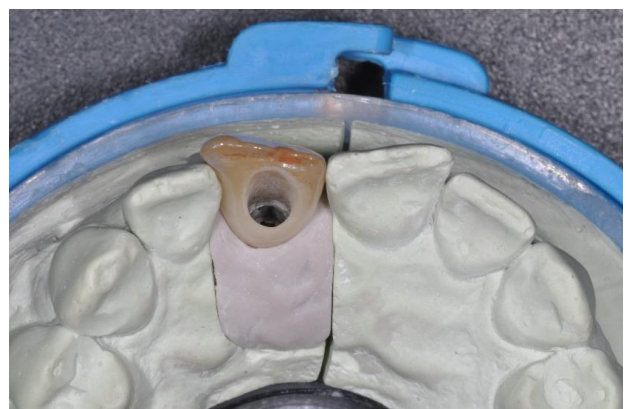


Fig. 6. *Detail of the final prosthetic crown on the laboratory model.*

and 2b). The immediate impression was then taken. (Fig 3) and two temporary elements were inserted on the same day, discarding the plaster model and perfectly sealing the socket for 3 mm from the free marginal gingiva (Fig. 4). The insertion of the temporary crowns 3 mm inside the tissues creates an ischemia that we can appreciate from Fig. 5.

After 30 days from the cementation of the temporary crowns, a tissue check was performed (Fig.6). After 4 months from the operation, the impression for the definitive crowns was taken and we can appreciate the state of health of the tissues at that time (Fig.7). A couple of weeks later the final restoration was then performed (Fig. 8a and 8b). A



A



B

Fig. 7. a): Front view one year after the insertion of the definitive implant restoration. Note the well-preserved gingival architecture; b): lateral vision.

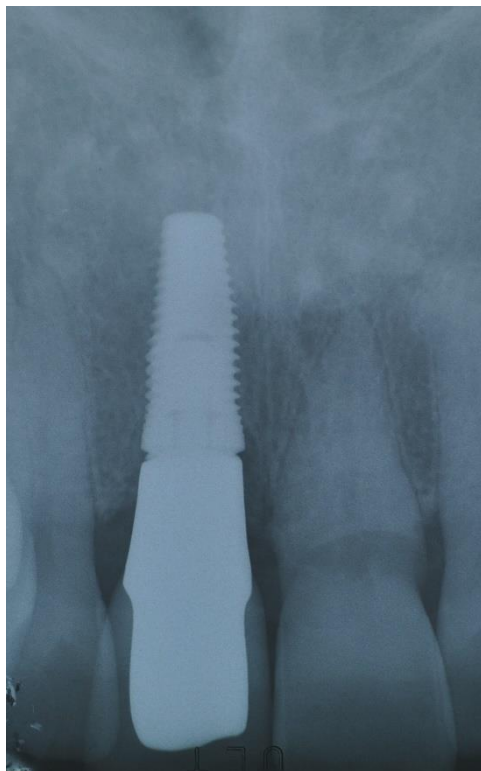


Fig. 8. Periapical x-ray at one year follow-up.



a.



b.

Fig. 9. a): front view of the tooth loss; b): periapical x-ray of elements 1.1 and 2.1.



Fig. 10. a): front view of post-extractive implants; b): periapical X-ray of the implants.



Fig. 11. Immediate impression for temporary crowns.



Fig. 12. View of the discarded plaster model used to create the temporary crowns.



Fig. 13. Tissue ischemia caused by the temporary elements.



Fig. 14. Tissues check 30 days after surgery.



Fig. 15. Tissue check at 4 months after surgery.

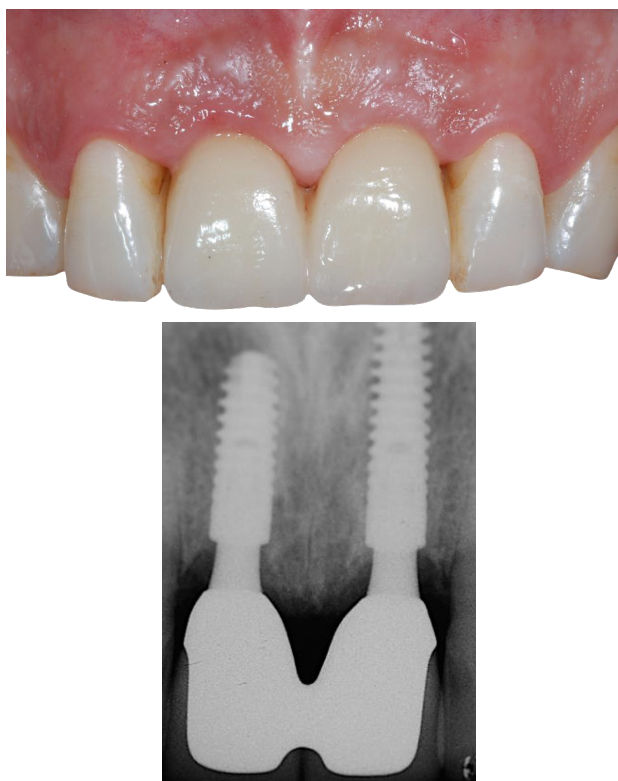


Fig. 16. a): definitive crowns; b): periapical X-ray of the definitive crowns.

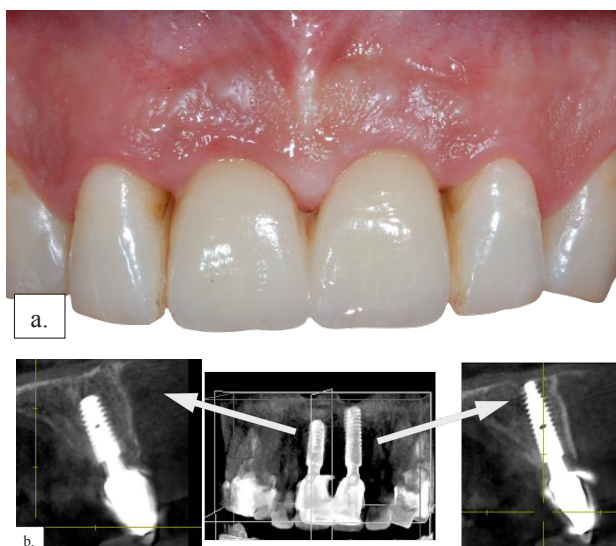


Fig. 17. a): view of definitive crowns 6 months after surgery; b): cone beam of elements 1.1 and 2.1 6 months after surgery.

clinical and radiographic control was done 6 months later, where we can appreciate that the vestibular cortex is perfectly preserved (Fig.9a, 9b).

The insertion of an implant in a post-extraction site and its immediate prosthetics requires careful pre-surgical planning (50-58). The thick periodontal biotype, an intact vestibular plate, the possibility of obtaining excellent implant stability, a square shape of the element to be replaced and an atraumatic extraction are all essential requirements for this type of treatment. This surgical-prosthetic protocol should be considered as a procedure sensitive to the technique we use, with a careful selection of the clinical case.

The absence of one of the parameters involves the evaluation of a different implant insertion timing (59-71).

DISCUSSION

Post-extraction implants and their immediate prosthetics involve risk factors that must be well studied in order to obtain long-term aesthetic results. This is particularly true for single tooth replacements in the anterior area, where the esthetic expectations of patients present a challenge for clinicians

The insertion of an implant in a post-extraction site and its immediate prosthetics requires careful pre-surgical planning; the thick periodontal biotype, an intact vestibular plate, the possibility of obtaining excellent implant stability, a square shape of the element to be replaced and an atraumatic extraction are all essential requirements for this type of treatment.

This surgical-prosthetic protocol should be considered as a procedure sensitive to the technique we use, with a careful selection of the clinical case (15). The absence of one of the parameters involves the evaluation of a different implant insertion timing.

Clinicians need a correct therapeutic plan to obtain a good aesthetic result with a high degree of predictability and a low risk of complications.

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Guided sinus lift: virtual planning of surgical templates for lateral access

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The sinus lift with lateral approach is a regenerative technique nowadays considered predictable to increase the height of bone in the atrophic posterior maxilla. Knowledge of sinus anatomy and evaluation of risk factors are the basis of regenerative and rehabilitative surgical success. The positioning and size of the lateral antrostomy represent critical factors in the execution of regenerative surgery, due to the difficulty in transferring radiological information to the lateral wall of the maxillary sinus even for skilled surgeons. The knowhow of guided implant surgery in recent years is also finding use in planning and precisely delineating the lateral access to the maxillary sinus using CBCT imaging and dimensional reconstruction software, through the realization of surgical guides with 3D printing, as shown in the presented case.

The Maxillary sinus lift, with lateral access, is a surgical technique first described in literature in 1980 by Boyne and James. The lateral approach differs from the crestal approach due to the creation of a bone window on the external wall of the maxillary sinus; after the Schneiderian membrane has been detached, the bone window is tilted inside the sinus with simultaneous lifting of the membrane itself; the space between the bone floor and Schneider's membrane is filled with regenerative material. In this way, the bone height required for contextual or delayed implant placement in the edentulous maxilla can be increased in a predictable manner (1-5).

Many reviews attest to the predictability in terms of survival rate of the implants inserted in the augmented sinuses. Crucial factors for regenerative success and implant rehabilitation are evolution of radiological imaging and the piezosurgical approach as well as proper regenerative techniques (6-13).

Despite technical and technological progress, are also frequent reviews of complications and accidents

associated with this procedure. The most frequent complications are perforation of the Schneider membrane and excessive bleeding. Both have or may have bad consequences for the success of regeneration and rehabilitation, with the lengthening of surgical time and an increase in the difficulty of the operation (14-23).

The design of lateral anthrostomy must consider both rehabilitation needs and the anatomical conditions of the sinus, characterised by the possible presence of a number of factors: Underwood bone septa, maxillary arterial branches, roots of adjacent teeth, sinus recesses, abnormal thickness of the vestibular bone wall, reduced sinus width, pathological thickening of the sinus membrane.

Correct tri-dimensional radiological knowledge and visualization is now essential and allows a reduction of perforation risk of Schneider's membrane if are properly transferred to the surgical table (14-31).

The positioning of the anthrostomy, size and

Key words: sinus lift, surgical guide, sinus augmentation, 3-D print guide

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extension must be defined on the TCCB and precisely positioned on the lateral wall, avoiding what Mandelaris and Rosenfield have called Mental Navigation. The authors in 2008 described the use of digital surgical templates for lateral access to the maxillary sinus. Their protocol included the acquisition of DICOM data via conventional CT or TCCB and on the basis of these, the required implant rehabilitation was outlined using 3D surgical planning software. With a different software, the osteotomic margins of the anarthostomy were designed. The project thus obtained was sent to a milling centre where the surgical template was made (32-40).

MATERIALS AND METHODS

In September 2019 we observed a 60-year-old male, non-smoker patient in good general health, with sufficient oral hygiene control, wearing a partial mobile prosthesis and presenting bilateral maxillary edentulousness

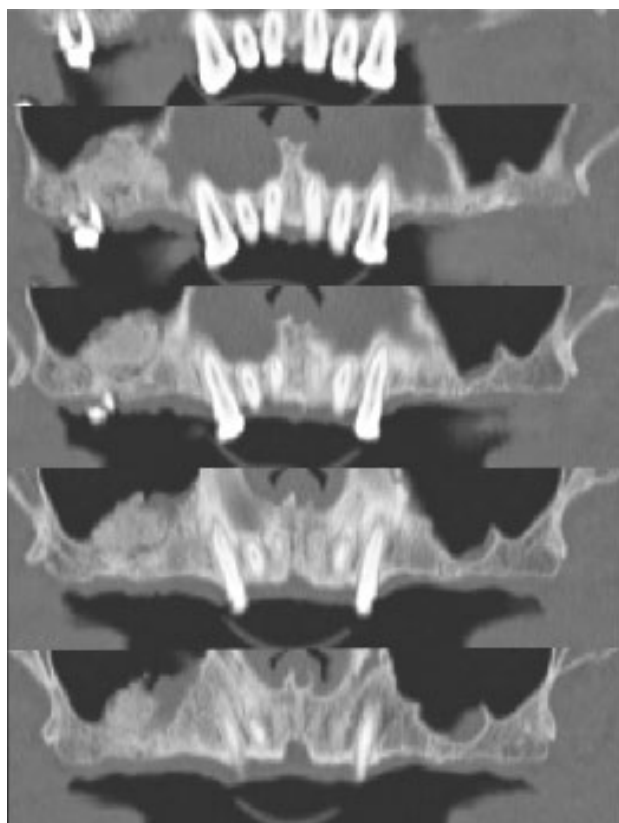


Fig. 1. TCCB - Panorex view, bone height (3.5 mm) in zone 2.6 and the presence of septum of Underwood in zone 2.7.



Fig. 2. The intraosseous course of the antral artery in zone 2.6, about 10 mm from the residual ridge.

following the loss of numerous dental elements; already treated 10 months earlier for maxillary sinus elevation with lateral approach in quadrant 1, he presents distal edentulousness in quadrant 2 at 2.3.

The residual left maxillary ridge on clinical examination was of adequate width and the interarchate relation (vertical and horizontal) was acceptable. The prosthetic implant rehabilitation proposed was an Implant supported bridge, on 2 implant fixtures 2.4 and 2.6, after radiographic evaluation of sufficient bone volume for standard implant (4.1 mm x 10 mm).

The CBCT examination of the upper jaw (NewTom VGi; NewTom Inc) showed that there was insufficient bone height (3.5 mm) in zone 2.6 for the planned implant length (10 mm). In addition, there was a septum of Underwood in zone 2.7 and the intraosseous course of the antral artery in zone 2.6, about 10 mm from the residual ridge (Fig. 1, 2).

The treatment proposed in this case involves the Sinus Augmentation with a lateral approach and delayed insertion of implants. The digital workflow for planning the surgical stent to perform the maxillary sinus augmentation was described in 2018 by B.J. Goodacre:

1. The DICOM file of CBCT is transformed into a 3D bone model using the reconstruction software (InVesalius).
2. The bone model is exported as STL file; this is superimposed on the CBCT data using the surgical planning software (Blue Sky Bio). (Fig. 3).
3. In relation to the planned position of the implant, a surgical guide is designed to cover the lateral wall of the sinus with particular attention to the septum and

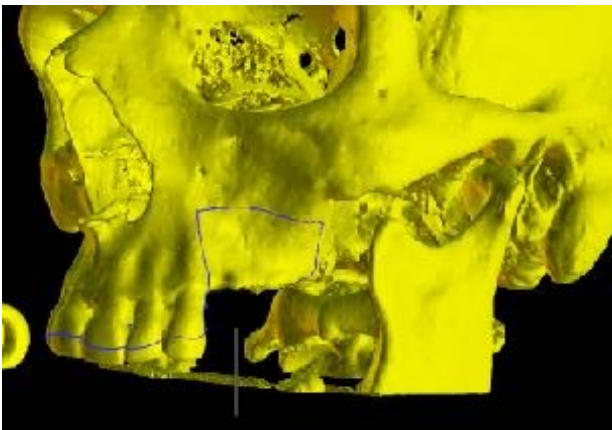


Fig. 3. Planning the layout of the template, with support and extension on the lateral wall using Blue Sky Bio software.

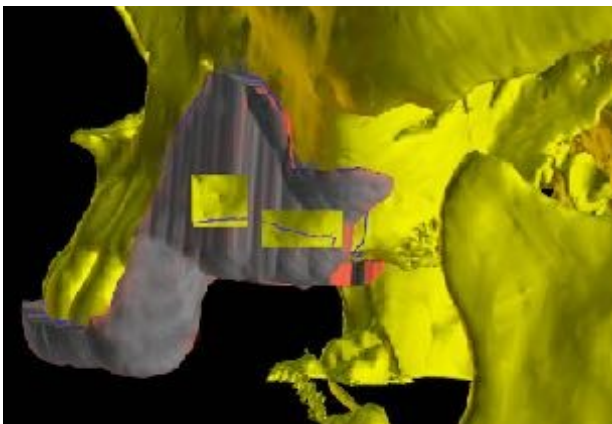


Fig. 4. Evaluate the support of the template and view the finalized stent.

the intraosseous course of the vessel.

4. The lateral window is designed with the appropriate extension and localization.
5. The surgical guide is exported as an STL file, its contours are defined using the Meshmixer software.
6. In conclusion, the STL file of the surgical guide is imported into the printing software. (Fig. 4).

RESULTS

The digital workflow for the realization of a surgical template to support sinus augmentation surgery with lateral approach and implant insertion is shown in Fig. 5. The DICOM data from CBCT together with the intraoral digital scan of the mouth are processed to obtain an STL bone/dental model. On this model, through the surgical programming software, the type

of support of the template and the implant positioning (number, position, length, inclination) are planned; on the basis of 3D sinus anatomy, the antrostomy is drawn, outlining the margins and their extensions. The STL file of the surgical template thus designed, can be printed through 3D printer or sent to milling centers. (41-46).

DISCUSSION

The maxillary sinus lift by a lateral approach has several critical issues; the localization and the realization of a lateral antrostomy that allows an effective and secure detachment of the schneiderian membrane, avoiding surgical complications related to the sinus anatomy. The regeneration of the maxillary sinus must also be of sufficient volume and size to allow the insertion of the planned implants, without the need for further intrasinus regenerative surgical procedures (47-54).

Therefore, the use of surgical supports that allow to prevent mental navigation, i.e., to prevent the

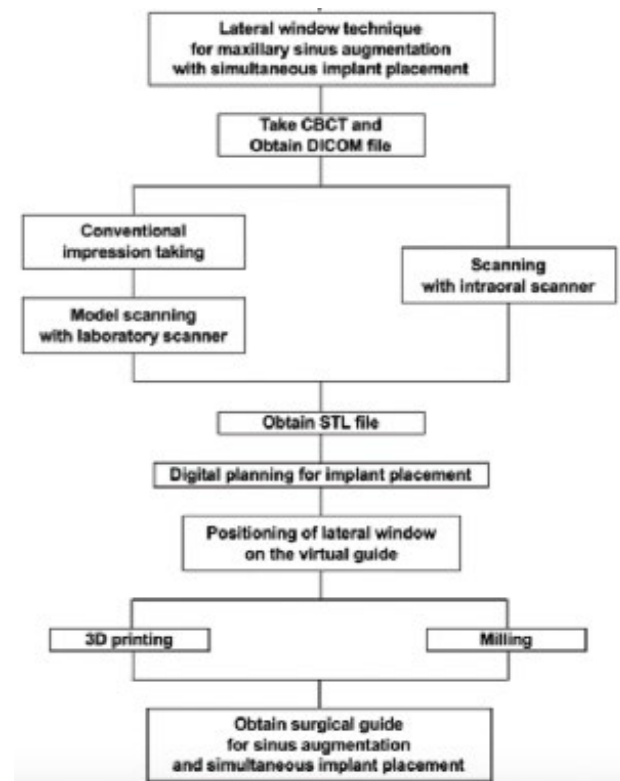


Fig. 5. Digital workflow diagram for the realization of a surgical template for the Sinus lift augmentation.

realization of the anarthrostomy in an uncontrolled way, seems desirable. This can facilitate the surgical procedure, in terms of reducing the rate of intraoperative accidents (copious bleeding and/or extensive perforations of the membrane), also helping to reduce the surgical stress of the patient and helping the surgeon allowing the execution of more procedures in less time (55-63).

There is no doubt that these devices have advantages and disadvantages, and it is up to the clinician from time to time to identify the application criteria and the cost/benefit ratio. The possibility that with the same template it is possible to carry out the anarthrostomy and guided implant insertion, is recommended especially for reducing the surgical time. The surgical template should be seen as a support aid to reduce the incidence rate of events during this procedure with a significant saving of time at the surgical table, better working ergonomics, less operator stress and greater predictability of the regenerative procedure, especially in "difficult" cases. These supports require a learning curve of the software functionality, as well as economic investments (64-69).

However, further studies are needed to evaluate on a large scale the results of the use of computer guided guides in the ability to reduce the number of complications and thus demonstrate the increase in the success rate in sinus Augmentation procedures with lateral approach.

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Endodontic retreatment and bioceramics: A case report

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The success rate in endodontic retreatment can be significantly boosted by using materials that guarantee both intimate adhesion to the canal walls and long-term stability. Bioceramic cements used in root filling show interesting properties including extraordinary sealing capacities, antimicrobial activity that stimulates periapical healing and the continuous production of hydroxyapatite for a long time. This case report deals with the application of bioceramic cement in endodontic retreatment. The Bioroot RCS (Septodont), thanks to its ability to firmly adhere both to the gutta-percha and to the walls of the canal and to definitively seal the apical third, can allow to obtain extraordinary healing in a relatively short time by exploiting its antimicrobial abilities. However, further studies are needed to assess the effectiveness of this technique over the long term.

Primary endodontic treatment does not always lead to clinical success. The causes are many and can be determined by three main consequences: incomplete cleaning, modeling or filling of root canals, the presence of radiologically visible lesions and pain during both percussion and during palpation and chewing (1-16).

Retreatment is the best therapeutic approach to solve these clinical situations and if well performed, the literature reports a success rate of 85%. The goal is to improve the initial therapy by modeling, cleaning and filling the parts that may not have been treated with the aim of drastically reducing the contamination of bacteria present in root canals, responsible for endodontic lesions (17-29).

Endodontic retreatment is recommended in the presence of radiologically visible lesions, iatrogenic damage, incongruous treatment, even in the absence of symptoms or persistent painful symptoms even in the absence of lesions (30-40).

It is mandatory to follow a systematic approach: isolation with rubber dam, removal of the previous restoration and any decomposed tissue, obtaining proper access to the root canal system, removal of materials within the endodontic space and shaping, cleaning and filling of the channels.

The success rate in endodontic retreatment is certainly lower than the primary treatment because inside the canals you can find some bacterial species, such as *Enterococcus faecalis* and *treponema denticola*, which are much more resistant to the various mechanical and irrigation phases that characterize a rework and for their ability to penetrate the dentinal tubules and reactivate after a long time. Therefore, the success rate can be significantly increased by using materials that guarantee both intimate adhesion to the canal walls and long-term stability (41-45).

The gold standard of the endodontic seal was Schilder's vertical hot condensation, whereas today

Key words: Bioroot RCS, bioceramics, dental cement, hydroxyapatite

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the trend is to believe that the single cone cold closure associated with bioceramic cement (46) can provide us with the three-dimensional stability of the long-term endodontic seal we are constantly looking for (47-62).

In fact, with hot sealing, gutta-percha contracts as it cools, creating a gap between itself and the walls of the canal, which over time will potentially allow any residual bacteria to recolonize the endodontic space; bioceramics, on the other hand, are cements that have exceptional properties. In fact, they showed no resin presence and therefore no shrinkage, no vacuum in the interface, no tubular infiltration and allowed the formation of hydroxyapatite and dentine mineralization.

Clinical case

39-year-old male Caucasian patient, MLR, reported by a colleague who could not completely remove a fiber post in the distal root; he was performing reprocessing because the root canals, under radiographic examination, seemed to be undersized and poorly prepared. The patient complained of pain during palpation and percussion, as well as the presence of an active vestibular fistula. The radiographic examination showed an extensive periradical lesion of the mesial root. Once the operating field is isolated, the pin is removed from the distal root through the use of the operating microscope and the d.l. channel has been found and treated; at the same time the old filling material is



Fig. 1. A Large osteolytic lesion at the medial root of 3.6 with partial and incomplete treatment of the root canal system and fiber pin on distal root.



Fig. 2. Closure of the four root canals present on 3.6 (Mv, Ml, Dv and Dl) with abundant leakage of Bioroot on the medial root.

removed from the m.v. and m.l. channels, which proved to be undersized in the preparation, creating an adequate preparation under continuous alternating washing of hypochlorite and 17% EDTA constantly activated with sonic tips (63-70).

The root canal closure was performed cold with gutta-percha cones, chosen according to the new canal preparation, and Bioroot RCS. The control rx performed 3 and 5 months after the closure shows that, even in a very short time, there was a complete restitutio ad integrum of the hard foil obtaining a surprising healing. (Fig. 1-4).

DISCUSSION

Bioceramics are today quite well-known materials in the literature, especially because they represent an important step forward in the search for the ideal root canal sealant. Bioroot RCS guarantees countless properties including good sealing capabilities, antimicrobial activity that stimulates periapical healing. It is a bioactive material that continues to produce hydroxyapatite for a long time after mixing, adapting to the dentinal walls and optimising its sealing capacity. It is an interesting material because it adheres tenaciously to both dentin and gutta-percha, so it can be used in cold sealing techniques. At first the cone is prepared according to the diameter and taper that is given to the canal so as



Fig. 3. *Three-month control X-Ray indicating a healing process with activation of remineralization bone.*



Fig. 4. *Five-month control X-Ray showing complete healing and complete remineralization of the bone.*

to have the correct tie-rod back to its working length, while the cement is mixed to obtain a viscous/fluid consistency (71-74).

Then the cone is wetted with cement and inserted several times until the channel is submerged by the cement and the surfaces of the master cone are completely covered by it. In this way we will be sure that our Bioroot RCS cement will seal the apical part of the canal where the greatest chance of endodontic success is played. Cold sealing, as other endodontic cements on the market can be absorbed over time, has always been considered an ineffective technique. On the other hand, with the advent of bioceramics, cement plays a crucial role because it permanently

seals the apex of the canal by itself. The gutta-percha cone - which must be brought to the apex - has the function of being only a guide for possible future reprocessing (75-80).

The case described above shows us how the Bioroot RCS, thanks to its ability to adhere firmly to both the gutta-percha and the walls of the canal and to permanently seal the third apical, can allow us to achieve extraordinary healing in a relatively short time by exploiting its antimicrobial capabilities. Further clinical studies must be carried out to evaluate the long-term effectiveness of this technique.

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A new factor involved during childbirth labor: the importance of having a good dental occlusion

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It is widely recognized that the physiology of childbirth labor largely depends on the interaction between three factors: a) the force generated by uterine contractions; b) the structure and characteristics of the birth canal and c) the fetus. Harmony between these three variables determines the initiation of maternal dynamic phenomena and the establishment of an optimal maternal-fetal balance in which both warrant for a correct delivery timing. The present study considered the above known factors and assessed if any other factor, still not recognized, could also play a role, and eventually modify the timing of delivery during the expulsive period. In particular, we focused our attention on the role played by the temporomandibular joint and dental occlusion on maternal body balance and on the stability of muscular reflected forces. The importance of assessing the temporomandibular function and the dental occlusion lies in the fact that any alteration in chewing or in temporomandibular joint (TMJ) mobility and occlusion brings to relevant modifications on the vertebral column and pelvic girdle. Our hypothesis is based on the evidence that those women who have any kind of alteration in their dental occlusion, can have an altered capability of pushing during the expulsive period, as the force applied on the pelvic floor is not expressed. Moreover, recent studies have highlighted a relationship between temporomandibular dysfunctions and sleep apnea syndrome and between sleep apnea syndromes and pregnancy. These relationships are explored in this study.

The duration of the first stage of childbirth labor is defined as the time between the beginning of the active phase and the complete cervical dilation, while duration of the second stage of labor is defined as the time between the complete cervical dilation and fetal expulsion (1). The second stage is divided into two phases: the passive second stage, during which the head of the fetus descends passively down the maternal pelvis; and the active second stage, which corresponds with the phase of expulsive efforts. The World Health Organization (WHO), current national

clinical practice guidelines in the United States and in Canada state that the diagnosis of a prolonged second stage of labor should be considered, in nulliparous women, when the second stage exceeds 2-3 hours, while in multiparous women the second stage would be prolonged when it exceeds 1-2 hours, where the upper limit is modified by the use for regional anesthesia (SOGC Clinical Practice Guidelines, 1998; ACOG Practice Bulletin No. 49, 2003; WHO, 2005).

Safety and effectiveness of management of

Key words: pregnancy, dysfunction temporomandibular joint closure, labor, expulsive period

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the second stage of labor represent a fundamental clinical challenge for laboring women and obstetric practitioners. Indeed, several systematic reviews found evidence of a strong association between a prolonged active second stage and operative vaginal delivery, an increased incidence of indication for emergency cesarean section, and a higher risk of maternal complications – such as postpartum hemorrhage, chorioamnionitis, puerperal sepsis, severe obstetric lacerations (third- or fourth-degree), anal sphincter tear (2), obstetric trauma, due to a pelvic floor damage, such as postpartum urinary incontinence (3) and postoperative pyrexia, unintentional hysterotomy extensions in case of cesarean section (4).

Prolonged labor has been reported as an important risk factor even for neonatal outcomes. Although larger studies do not confirm a direct correlation between birth depression, neonatal trauma (brachial plexus palsy, facial palsy, fractures, intracranial hemorrhage) and admission to the neonatal intensive care unit, there are reports of severe metabolic acidosis, neurological damage, meconium-stained amniotic fluid, longer neonatal stay in the hospital and perinatal death (5).

The causes of a prolonged labor are nowadays still scarcely known. Weak contractions, fetal malposition, disproportion between maternal pelvis and fetal head use of epidural anesthesia (6), maternal ethnicity, maternal body mass index (7), gestational age birth weight (8), maternal age fear of childbirth parity (9), a scarce support provided by midwives during labor and prostaglandin use are reported to influence the duration of labor. No evidence of a possible association between physical activity and duration of labor has been found (10, 18)

The present study is based on the hypothesis that women who have any kind of alteration in their dental occlusion may have a reduced capability of pushing during the expulsive period, since the force applied on the pelvic floor during labor is not properly expressed.

Malocclusion is an abnormal alignment of the teeth of the maxilla and the mandible that prevents the teeth from meeting properly. The term was coined by Edward Angle, the father of modern orthodontics, as

a derivative of occlusion, which refers to the manner in which opposing teeth meet. Malocclusions can be divided mainly into three types, depending on the sagittal relations of teeth and jaws, by Angle's classification method, based on the relative position of the maxillary first molar (19-27).

Posture is the position of the body with respect to the surrounding space. A specific posture is determined and maintained by coordination of the various muscles that move the limbs, by proprioception, and by the sense of balance, in relation with the force of gravity (28-36).

The origins and consequences of the biomechanical correlation between the cranio-mandibular complex and the cervical column have been intensely studied in last ten years, in the effort to find evidence that a dental malocclusion could cause a temporomandibular dysfunction and then a cervical spine diversion. Occlusion and mandibular movements have been investigated with open and closed eyes, in standing and sitting position, and the conclusion is that the registration proprioceptive position and the visual system can influence mandibular movements. Many research found experimental evidence of the relation between the mandibular position and head posture and with the whole body posture as well. Many studies in literature reported evidence of the dependence between mandibular movements and possible head and neck movements, led a clinical study on 130 volunteers, demonstrating the correlation between a cranio-mandibular defect, the mobility of the cervical tract and the weakness of neck and shoulders muscles. Urbanowicz documented that an increase of the vertical dimension of the mandibular posture could create a sub occipital compression and a deregulation of the postural balance of the neck and head (37-43). Ries detected an asymmetric activation of the temporalis, masseter and sternocleidomastoid muscles in patients affected by temporomandibular disorders (TMD) (44-50). A precise diagnosis is fundamental to address the actual causes. In a correct therapeutic plan, the examination should include an accurate anamnesis, especially concerning the stomatognathic system; an exam of the oral cavity; a measurement of the inferior limbs; the evaluation

of a descendant postural defect; and an overall muscular assessment. In addition to these exams, it is necessary to perform a postural analysis on a stabilometric platform, by using reflective passive markers on the body and an optoelectronic unit with infrared cameras, to analyze the postural defect, the malocclusion and the correct mandibular position, in order to guarantee the best therapeutic approach.

Various studies have already investigated the effect of a dental support device on the isometric strength of different muscles (51-55) and a number of these demonstrated an improved physical performance even in athletes. Patients with a proper dental occlusion showed greater endurance in isometric muscle performance than those who present a malocclusion. Another variable that could influence delivery and labor could be the occurrence of Obstructive Sleep Apnea (OSA) during pregnancy, as sleep disorders are linked to bruxism which is linked to occlusion, TMJ dysfunction and facial pain. A recent study defined “gestational OSA” as the respiratory sleep disorder that occurs during pregnancy, thus distinguishing it from the chronic form, already present in women before pregnancy.

A better understanding of risk factors for abnormal labor progression may impact the timely diagnosis of dystocia, active management of labor and, at the end, the method of delivery. Our aim was to investigate

whether subjects with a good temporomandibular closure could have a shorter expulsive period.

MATERIALS AND METHODS

Design of the study

Each woman underwent two different examinations: the first one at term, performed at the Pregnancy Outpatients Consultation Room of the Obstetrics and Gynecology Department of the Hospital “Vittore Buzzi” in Milan (Italy), the second examination was performed during delivery at the Hospital’s Obstetrics Ward. The Hospital Ethics Committee approved the experimental protocol and all subjects who took part in the study provided written informed consent.

A total of 246 pregnant unselected healthy singleton pregnant women, at term, attending the Pregnancy Outpatients Consultation, volunteered to enter the study at the Obstetrics and Gynecology Department, at “Vittore Buzzi” Hospital, Milan (Italy) between December 2003 and July 2004. They were all Caucasian with an average of 31.6 years of age.

Of this group, 56 were excluded from our study for the following reasons: 29 (26 nulliparous and 3 multiparous) underwent a caesarean section and 27 (20 nulliparous and 7 multiparous) needed vacuum extraction for fetal distress.

The remaining 190 women who had eutocic (physiological) deliveries were included in this study: 115 were nulliparous, 68 on their second, 6 on their third and 1 on her fourth pregnancy. Details of this population are presented in Table I.

Of the total population considered at the beginning of the study, 80% of women had jobs outside of the house (74.1% employees, 5.2% freelancers), while 20% worked at home (housewives). Fifty-five percent of the total population never practiced regular sport activity.

Regarding the obstetric history, 45.8% of women were nulliparous, 31.6% were at their second pregnancy, 16.8% at their third, 4.2% at their fourth and 1% at their fifth or sixth. Of the initial group, 60.5% of women never had a eutocic delivery before, 36% one, 3% two and 1% three while 96.8% never had a cesarean section.

Measurement

Each subject underwent two phases of examination, antepartum and during delivery, respectively. During

Table I. *Analysis on the 190 eutocic deliveries.*

Presenting part	
left occipitoanterior (LOA)	106 (56%)
right occipitoanterior (ROA)	80(42%)
right occipitoposterior (ROP)	4(2%)
Blood Loss	
<500mL	171(90%)
500-1000mL	15(8%)
>1000mL	4(2%)
N of pregnancies	
nulliparous	87(46%)
second pregnancy	60(31%)
third	32(17%)
fourth	8(4.2%)
fifth or sixth	2(1%)

the prepartum examination, healthy pregnant women attending the Term Consultation were asked about their weight, height, job, practiced sport activities, past medical history (particularly about skeletal pathologies, postural defects and temporomandibular joint disorders), as well as their past obstetric history. A dentist also assessed their stomatognathic system, their occlusal parameters, their temporomandibular joint mobility and their maximal mouth opening. Measurements of each woman's occlusal parameters and TMJ mobility were made in mm by a dentist and a chiropractor, two times each, in randomized order.

During delivery, we collected data on modality and procedure of their deliveries, timing of labor, expulsive period duration, eventual analgesia and/or induction with prostaglandin or oxytocin, newborn weight, sex, blood pH and Apgar score. Statistical analyses were established by correlation and multiple regressions as appropriate.

Statistical Analysis

The correlation between the duration of the expulsive phase (dependent variable) and other parameters (independent variables) was analysed by Statistical Package for the Social Sciences (SPSS 25.0, SPSS Inc., Chicago, USA[®]). To assess the degree of linear correlation and multiple regressions a P-value less or equal to 0.05 was accepted as significant.

RESULTS

During the first phase of data collection, we observed in our global sample of 246 women that the mean measure of the maximal mouth opening was 46.1 mm with a standard deviation of 1.5 mm (range 30-60 mm). The capability of mouth opening had a Gaussian distribution (Fig. 1), with a mean of 46 mm and a data interval included between the 5° percentile (35.5 mm) and the 95° percentile (56.6 mm).

During delivery, at the second phase of data collection, 190 women had a mean duration of the dilation phase of 210 min (range 5-780 min) and a mean duration of the expulsive phase of 43 min (range 3-185 min). The results of our analysis on the 190 eutocic deliveries are presented in Table I.

The position of the presenting part was in 56% of cases left occipitoanterior (LOA), in 42% right occipitoanterior (ROA) and in 2% right

occipitoposterior (ROP). The blood loss was less than 500 mL in 90% of cases between 500 and 1000 mL in 8% and more than 1000 mL in the remaining 2%.

A significant correlation ($R^2=26.8\%$) was found between cervix dilation time and the following parameters, taken as independent variables: number of vaginal deliveries ($p=0.008$), pre-gravidic weight ($p=0.007$), third trimester weight ($p=0.024$), analgesia ($p=0.001$), prostaglandin treatment ($p=0.018$) and oxytocin treatment ($p=0.028$). The regression analysis of these significant variables confirmed their significant correlation with the duration of labor ($R^2=28.8$), number of vaginal deliveries ($p=0.006$), pre-gravidic weight ($p=0.008$), third trimester weight ($p=0.031$), analgesia ($p=0.001$), prostaglandin treatment ($p=0.010$) and oxytocin treatment ($p=0.010$).

The expulsive period had a significant correlation ($R^2=25.5\%$) with the following variables: maternal height ($p=0.009$), number of vaginal deliveries ($p=0.000$), maximal mouth opening ($p=0.011$), oxytocin treatment ($p=0.013$), neonatal weight ($p=0.018$). The regression analysis between the duration of the expulsive period and each of the previous independent variables confirmed a significant relation ($R^2=23.2\%$), except for maternal height ($p=0.154$), as follows: number of vaginal deliveries ($p=0.001$), maximal mouth opening ($p=0.004$), oxytocin treatment ($p=0.001$), new born weight ($p=0.013$). Excluding maternal height, the regression analysis confirms the significant relation ($R^2=22.1\%$) to the number of vaginal deliveries ($p=0.001$), maximal mouth opening ($p=0.008$), oxytocin treatment ($p=0.001$) and newborn weight ($p=0.014$).

The significant negative correlation between duration of the expulsive period (dependent variable) and maximal mouth opening (independent variable), with an angular coefficient of -1.37 ($R^2=3.7\%$) is shown in Fig. 2.

To strengthen the above results, we repeated the analysis on the nulliparous group only, a sample of 115 subjects (60% of the entire initial sample). The duration of the cervix dilatation showed a significant correlation ($R^2=23\%$) with the following parameters, taken as independent variables: analgesia ($p=0.023$) and oxytocin treatment ($p=0.008$). The regression

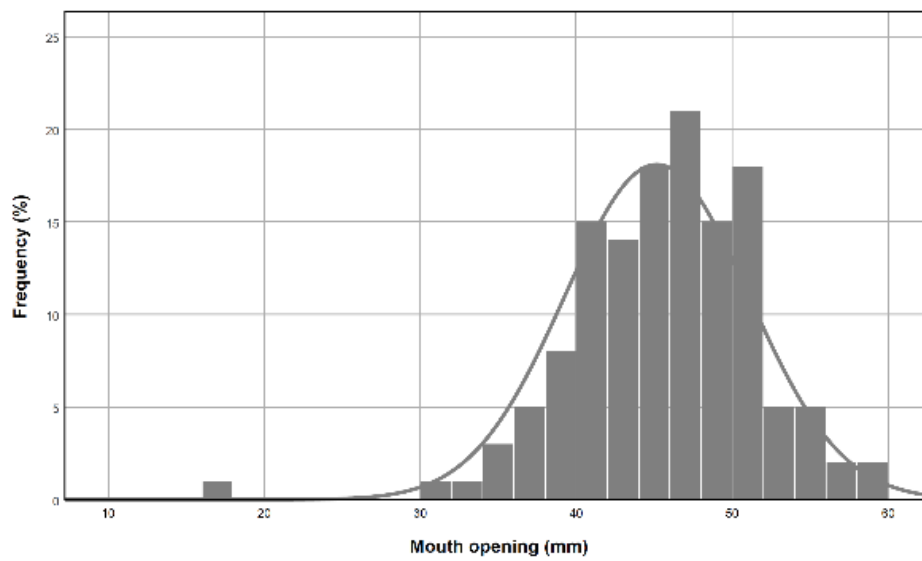


Fig. 1. Distribution of mouth opening in sample population. Underlying gray line represents normal distribution.

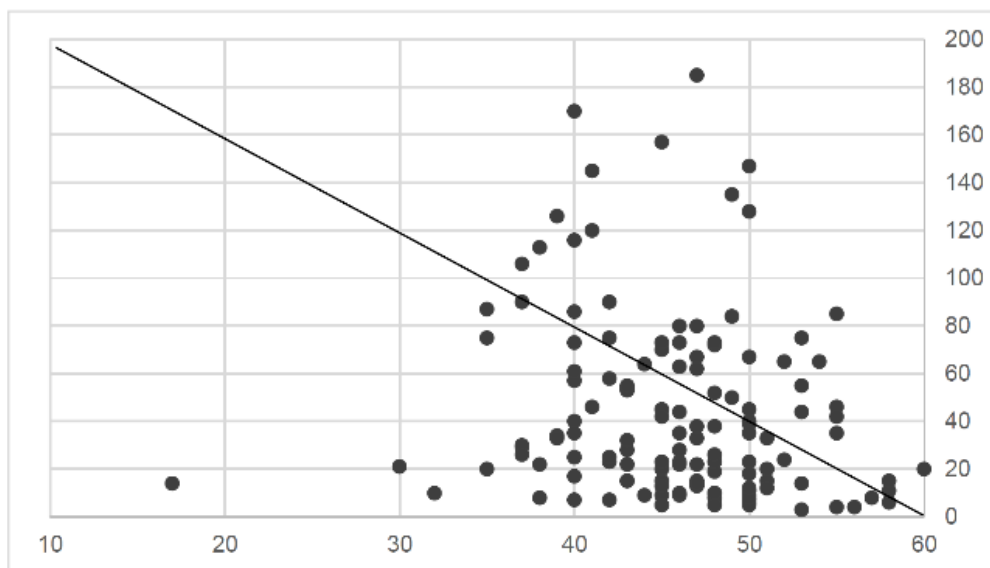


Fig.2. Scatter plot representing mouth opening on the x-axis in mm, expulsive period time in minutes on the y-axis and regression line.

analysis confirmed the level of significance ($R^2=24\%$) between cervical dilatation time and each of the previous significant independent variables: analgesia ($p=0.001$) and oxytocin treatment ($p=0.000$). As shown in Fig. 3, the duration of the expulsive period had an asymmetric distribution, with a median of 45 min, a 5° percentile of 10 minutes and a 95° percentile of 120 min.

The expulsive period is significantly correlated ($R^2=16.3\%$) to maternal height ($p=0.007$), maximal mouth opening ($p=0.003$) and oxytocin treatment ($p=0.006$), taken as independent variables. The multiple regression analysis confirmed the relation between the duration of the expulsive period (dependent variable) and maximal mouth opening (independent variable). Fig. 4 shows this significant

correlation, with a P-value of 0.004 and a $R^2=6.2\%$, with a negative angular coefficient of 1.6.

The correlation between maximal mouth opening and duration of the expulsive period showed a relative risk of 2.8-2.9. for a mouth opening of < 40 mm. It means that if a nulliparous woman has a small mouth diameter, she has a high relative risk to have a longer expulsive period during labor, with all the possible complications that can follow a long expulsive period.

DISCUSSION

The strong connection existing between the different skeletal components of the body and the continuous nervous control on muscles, by means of

the elaboration of information taken from periodontal, articular and muscular proprioceptors and of sensory stimuli, determines continuous variations in head, mandibular and hyoid bone posture, but also in vertebral column and pelvic structure. It has been demonstrated how a malocclusion could be responsible for an imbalanced muscular reaction: when the tooth has a premature contact, the homolateral elevator muscles release, allowing the contraction of the antagonist ones. If the nociceptive stimuli persist, the muscles divert the mandible in a more favorable occlusal intercuspation, modifying the overall posture. In case of pre-contact, strong descending repercussions affect the neck muscles, such as the homolateral upper trapezius muscle and contralateral sternocleidomastoid muscle. The head undergoes a

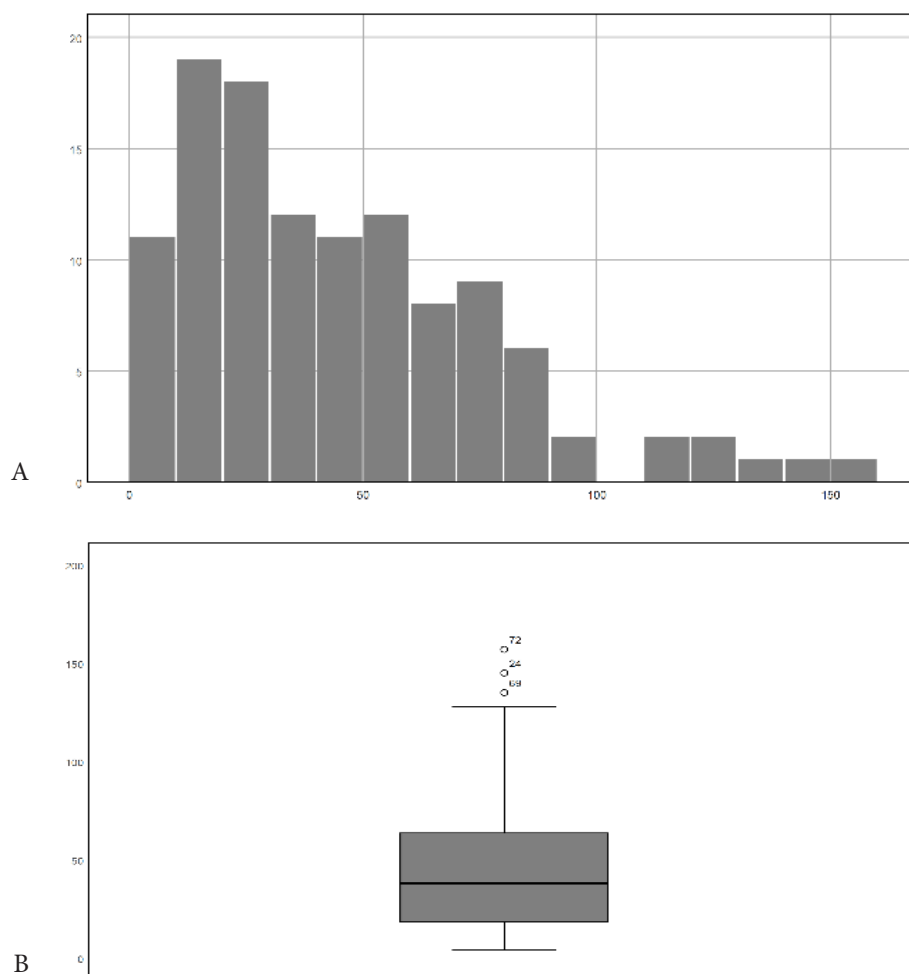


Fig. 3. a. Boxplot representing expulsive period time, box represents interquartile range, black line median and confidence interval. **b.** Patients #69 #24 and #72 resulted outliers.

flexion towards the mandibular deviation that results in shoulder asymmetry. The cervical column flattens, and this results in thoracic and lumbar compensatory curves. These modifications trigger compensatory muscle imbalances: the iliopsoas muscles cause a pelvic slope and, in worse cases, an asymmetry of the inferior limbs.

Accordingly, a good temporomandibular occlusion plays an important role in guaranteeing body balance. The harmony between all the components of the stomatognathic system, such as bones, TMJ, masticatory muscles and teeth, influences the overall posture, and the mandibular position is important in balancing the craniosacral function (56-63).

The main result of our study is that the expulsive period is strongly influenced by a general musculoskeletal balance: our findings can be of important relevance, suggesting that the dynamic of delivery is the result of maternal posture balance and can be modified by any alteration of the physiologic equilibrium determined by a series of factors, including the temporomandibular joint. It is reasonable to think that a good temporomandibular

closure, reflected by women's mouth opening, can influence the delivery timing.

In our study we also found a statistically significant correlation between dilation time and epidural anaesthesia, maternal body mass index, neonatal birth weight, parity and prostaglandin use.

Concerning expulsive period, we found a statistically significant correlation between expulsion time and the factors mentioned above and mouth opening.

While we confirm literature data concerning the effect on delivery time of epidural anaesthesia maternal body mass index, neonatal birth weight parity and prostaglandin use, we add a direct significant negative correlation

between duration of the expulsive period (dependent variable) and maximal mouth opening (independent variable): as the maximal mouth opening increases, the expulsion time (64-70).

In our study, we enrolled only Caucasian pregnant mothers who delivered at term, with an average age of 31.6 years, so that we couldn't focus our analysis on how maternal ethnicity, gestational age and

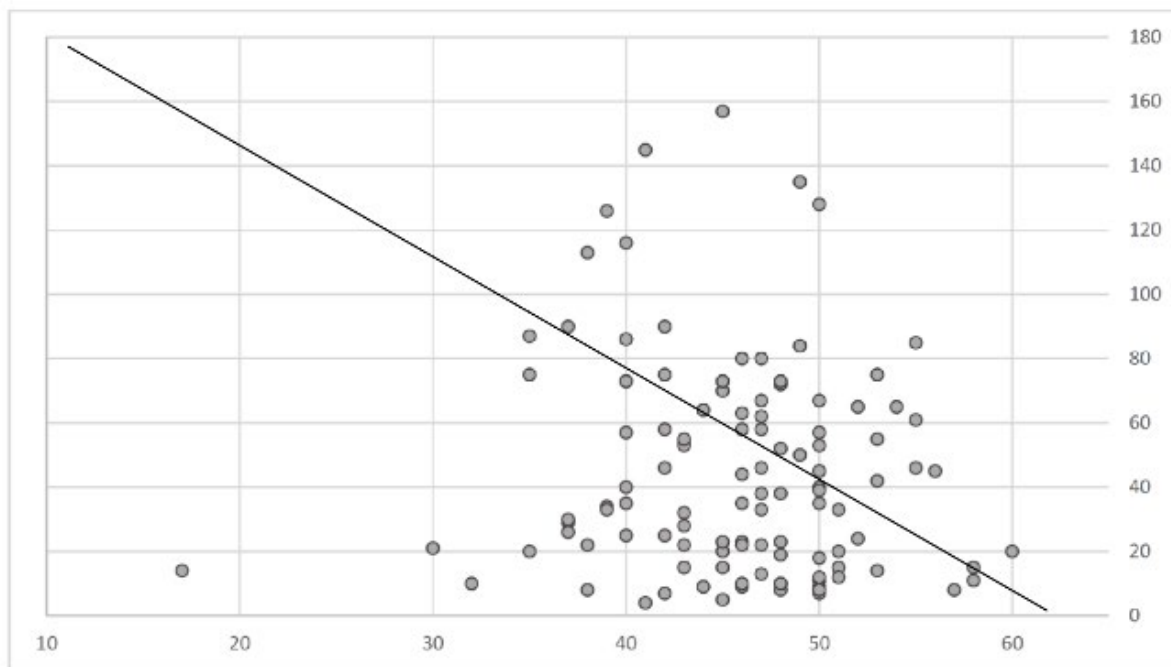


Fig. 4. Scatter plot representing mouth opening on the x-axis in mm, expulsive period time in minutes on the y-axis and regression line.

maternal age influence labor.

Moreover, we did not evaluate maternal fear of childbirth, which is a possible factor influencing delivery time, considering that maternal opening mouth diameter is an anatomical factor, independent from any maternal psychological expectation, fear or support from midwives.

Analyzing only the nulliparous subgroup, only oxytocin and epidural analgesia treatment show a significant influence on the first stage of labor, and this surely opens new perspectives for future research.

Instead, concerning the expulsive time, the second stage of labor, beside the known factors as oxytocin use, parity, and neonatal weight, we added a new factor, less commonly considered, as the mouth opening diameter. This factor was evident for all pregnant women and especially for the nulliparous. In this subgroup indeed, the only significantly influencing factors on expulsive duration were maternal height, mouth opening and oxytocin use.

Since maternal height may be related to mouth opening, we have detected that between factors significantly involved in a prolonged second stage of labor, temporomandibular malocclusion plays a fundamental role. In support to our hypothesis, a pilot study indicated that the duration of the second stage of labor was significantly shorter among patients who wore a dental support device, in comparison to the non-device group.

The maximal mouth opening reflects the correct mandibular closure and the absence of a temporomandibular joint disorder. All these findings support the hypothesis that, working on this so far ignored variable so as to improve or correct any potential malocclusion, will enable to modify the delivery timing, making the expulsive period faster and less painful.

A better understanding of risk factors for abnormal labor progression may impact the timely diagnosis of dystocia, active management of labor and, at the end, the method of delivery.

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Complications of oral piercing: a review of the literature and two case reports

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The practice of piercing has been used since ancient times, and nowadays is coming back in fashion among adolescents and young adults. Teenagers wearing oral piercings are frequently observed during routine examination, and there are, also, recurrent harmful consequences attributed to the devices. Unfortunately, piercings are usually placed by unlicensed individuals, lacking the appropriate anatomical knowledge, and therefore unable to provide sufficient information regarding the potential risks, especially when compared to the ones known by the patients. In this regard, the relevant legislation is frayed both vertically (that is within the framework of State and Regional competences), and horizontally (within the competences between the various regions). With the present study, through an accurate review of the literature and the description of two case reports, we underline the importance of informing both the patients and the dental professionals about the dental consequences undergoing the application of piercing. Both patients examined presented the common complications related to the application of piercing. The aim of this study is to underline the importance of being informed for both the dental professional and the person who undergoes piercing about its consequences for the whole oral system as well as the possible risks of infections and complications. We want to underline the key role of the dentist as a promoter of prevention of oral disorders as well as being able to deal with the possible complications which may occur.

Piercing is a widespread practice, especially among young people, consisting in the perforation of the skin or mucosal and underlying tissue of any part of the human body, to insert jewelry permanently. These jewels are objects of various workmanship and use various metals (sometimes decorated with precious stones) such as: surgical steel, gold, niobium, titanium and alloys (1).

The first evidence of piercing goes as far back as 2000 BC, when this practice was largely used as a form of art, or to identify group membership, or for ceremonial purposes, using objects made of stone or bone.² Piercing nowadays is a widespread fashionable trend among young people, the majority of whom decide to get it to express their personality,

for aesthetic reasons, for erotic reasons, for fashion and sometimes because they are influenced by friends. Piercings that are currently available for the oral cavity are 1) the barbell (straight, curved), a bar with two balls at the ends; 2) the labret, a bar with a fixed plate at one end, a small ball on the other; 3) the ring, closed or terminated with two balls. Several materials currently are used for the fabrication, mainly titanium, surgical steel, niobium, nickel alloys. Titanium and niobium are preferred due to the lightness and low percentages of allergic responses (2).

Piercings are trendy worldwide among young people, with an increasing popularity of tongue and lip piercing: Covello et al. (2020) in their sample,

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identified tongue and lip piercing in 64.4% of cases aged between 20-29 years, mostly in female (3). There is a lack of updated data on the prevalence in the literature: Hennequin-Hoenderdos et al. (2012) reported that the percentage of intra-oral and peri-oral piercings in the population is on average 5.2% (value ranging from 0.8% to 12%). The tongue is the most frequent site in the oral cavity, followed by the lip. Other areas may be the frenulum, the cheeks, the uvula (4-10).

The Pew Research Center in 2010 estimated that in the high schools of eight States of America, between the ages of 12 and 22, between 23% and 47% of young people showed body piercing in different sites than ear lobes.² The results don't differ from the estimates of a study carried out at five high schools in Latina, Italy: Between 30 to 50% of young people aged between 14-22 had at least one piercing, while all those born in the year 1991 had a piercing (11-20).

The practice of piercings is not always performed by specialists, thus, in adolescents and young adults the risk of developing complications is frequent. A sample of 108 young people wearing oral piercings revealed in 96% of cases that they had post-surgical complications even if they complied with the instructions of the piercer. Several complications of tongue piercing have been reported, which, in accordance with the classification proposed by Campbell et al. (2002), can be distinguished in local or systemic, early or late. Early complications of oral piercings are frequent, mainly include pain, edema, hemorrhage, inflammatory reaction, hematoma caused by rupture of the tongue vessels, or the typical characteristics of an inflammatory state resulting in swelling and pain during chewing, swallowing and speaking. Rare complications may be galvanic electric currents occurring between the piercing device and a dental restorations, prolonged bleeding or severe infections (21-30).

Late or chronic complications are mostly topical, including traumatic injury to the teeth such as chipping, fracturing and pulp damage, gingival recessions affecting the lower incisors and periodontal damage. The severity of localized periodontal destruction increases according to

years of wear and the barbell's stem length (30-33). Campbell reported that, among subjects who wore tongue piercing for at least two years, 50% of long-stem barbells users exhibited lingual recession. Moreover, among long-stem barbell users, the prevalence of lingual recession was significantly greater in the patients who wore the piercings for at least 2 years, compared to those who wore it less time (34-40).

Damage to the oral cavity is worsened by the piercing devices as they become reservoirs of periodontal bacteria. Ziebolz et al. (2019) observed the increased prevalence of potentially damaging periodontal bacteria and the possible relation with periodontal pockets. Moreover, the authors recorded an increased prevalence of potentially pathogenic periodontal bacteria.

MATERIALS AND METHODS

Review of the literature

From the review carried out on PubMed we found 13 studies on specimens involved with peri-oral piercing: the first was published in 2002 and the most recent is dated 2019.

This study evaluated the complications caused by oral piercings as well as by gender, mean age, and by anatomical site. We obtained interesting considerations. Data concerning gender showed that the sample was mostly made up of girls. The result obtained excluded the sample analyzed by Ziebolz et al. (2012) because they had a resulting group made up only by men. We confirmed what reported by Hennequin-Hoenderdos review according to which oral and peri-oral piercing were more prevalent in the female gender, but in a different way the resulting number in our review had shown a low disproportion (50,29% of female). This little variation, compared to the 4% in the mentioned article, can be seen as a development of the society. We also observed a high percentage of subjects aged between 20 and 29 years, rather than teenagers or older subjects who, although included in our study, they were not very represented. This data didn't differ from the recent study by Covello et al., in which they examined a population of 387 patients, of which 64.4% of individuals with piercing were between 20 and 29 years of age.

According to the literature, lingual piercings were more

popular when compared with lip piercings. Both are damaging for the periodontium and for the overall dental health (41-50). The piercing is a foreign body that interferes with physiological patterns in the oral cavity, causing the modification of lingual movements. It is often associated with the development of systemic and local complications that may occur at the time of insertion of the ornament, or later as a result of the healing process or jewelry accident. Campbell et al. classified the possible complications associated with piercing into early (acute) and late (chronic).

Early consequences of the insertion of the ornaments mainly include pain, swelling, edema, and consequently, difficulty in articulating words, chewing and swallowing. Such an insertion may perforate the numerous lingual blood vessels that irrigate the lingual musculature. In rare instances, it has been reported an increase in salivary flow, infections and generation of galvanic currents between the barbell and the metallic dental restoration (51-54).

Late complications are the most frequent. Mainly localized, they include damage to the periodontium such as gingival recessions, increased pocket depth, bone resorption, abnormal tooth wear, tooth chipping or cracking, tissue overgrowth at the pierced site, swallowing impairment, and ornament's embedment in the tongue.

In this study we decided to evaluate the effects of piercings on the soft tissues, confirming, as already ascertained, that the constant mechanical trauma causes gingival damage in the region of the lower central incisors (Table 2). The periodontal inflammation seems to accelerate the destruction of the oral tissues, which is increased by the presence of the piercing and by insufficient attention to oral hygiene. Ziebolz et al. confirmed that oral piercings promote the pathogenic bacterial population (*Treponema Denticola*, *Provetella Intermedia*). Moreover, the piercing material might also affect bacterial colonization by *S. Oralis* and *E. Corrodens*, with greater

Table 1. *Tongue and Lip Piercing Statistical Data*

AUTHORS	YEAR	SAMPLE (Male-Female)	MEAN AGE (range)	LOCATION (Tongue- Lip Piercing)
A.Campbell et al.	2002	52 (21F - 31M)	19 (15-34)	52 (TP)-6(TLP)
John K. Brooks et al.	2003	5(4F - 1M)	22,4 (19-25)	3(TP)-2(LP)
L. Chambrone et al.	2004	1F	19	1(LP)
R. De Moor, et al.	2005	50 (37F - 13M)	NR (12-40+)	47(TP)-8(LP)-5(TLP)
Kleser et al.	2005	43 (40F - 3M)	21	33(TP)-15(LP)-5(TLP)
T. Levin et al.	2005	389 (179F - 210M)	20 (18-24)	61(TP)-22(LP)
L. JW et al.	2006	91 (39M-52F)	25 (20-43)	91(LP)
D.Ziebolz et al.	2009	3(2F-1M)	26 (19-30)	3(TP)
F.Inchingolo et al.	2011	108(74M - 34F)	NR (14- 39)	45%(TP)-10%(LP)
D.Ziebolz et al.	2012	1884	NR (18-27)	46 (TP)
A.Plessas	2012	110(52M - 58F)	21 (18-35)	110(LP)-51(TP)
B. Bajkin	2014	1	16	1(TP)
J.Schmidt et al.	2019	18(14F-4M)	28 (21-38)	11(TP)-4(LP)-3(TLP)

F= Female; M=Male; TP= Tongue Piercing; LP= Lip Piercing; TLP= Tongue & Lip Piercing

susceptibility when the material is constituted of Teflon or Bioplast, predisposing to the development of gingivitis and periodontal destruction. Data were confirmed by the literature, in which numerous cases of diffuse gingivitis were reported. It must be added that often those who present piercings do not pay enough attention to oral care. A recent review by Covello et al. showed in 50% of those who wear piercing present insufficient oral hygiene. While comparing a group wearing lingual piercing to a control group that does not wear it, both with similar distribution of oral hygiene habits, Ziebolz et al. in a case control confirmed greater dental and periodontal damage in those wearing lingual piercing. Poor oral health, in addition to the presence of an ornament that favors the colonization of periodontal pathogenic bacteria, could increase the risk of developing periodontal disease.

Damage to the periodontum is the most commonly described oral complication, it is frequent and localized regardless of the gingival biotype. It is present with greater severity in the mandibular incisors area, contributing in particular to the development of gingival recessions at the lingual side in the mandibular central incisors (55-60). Our cases confirmed these complications for which the lingual side of the teeth is the most affected in those who wear

lingual piercing, unlike the damage to the vestibular soft tissues corresponding to the location of the lip piercing of the lower lip. We have also observed the most impacted periodontal tissues were found in those with tongue piercings, compared to those wearing a labial piercing. An example is the data presented by Brooks et al. who report that 64.4% of the affected sites were in patients with tongue piecing, while the percentage of affected sites in the wearers of lip piercing was 35.7%, a lower value than the first group, but still significant. This information were confirmed by the cases of Schmidt et al. (2019), according to which the periodontal parameters of the mandibular incisors are worse when compared to the rest of the dentition. Also, Leichter et al. identified gingival recessions in the area directly opposite to lip piercing in 68.13% of patients, compared to 22.2% of patients without piercings¹⁸. Therefore, as confirmed by various authors, the main damage occurs in the area on which the piercing strikes with a mechanical harmful action. Even if the percentages, the depth of gingival recessions and the period of time for the damage to occur differ in the various studies, however, it remains a common complication in the wearers of the piercing ornament.

As for the tongue piercing, Campbell et al. first

Table II. *Tongue and Lip Piercing Complications – A Literature Review.*

AUTHORS	MEAN TIME WEARING	GR	CHIPPING	OTHER COMPLICATIONS
Campbell et al.	30 ± 24 months (range 1 day - 9 years)	LR 19,2% e increased with time and long stem. 53% of cases. VR in LP	19,2% cases	NR
Brooks et al.	27,3 months (range 2 months-9 years)	LR in TP. VR in LP. 41,31	1 case	NR
Chambrone et al.	6 months	VR 41,31	tooth abrasion	Ulceration at the FGM, deposits of dental plaque, increase in dental hypersensitivity.
DeMoor al.	12, 6 months	3/5 cases VR in LP. LR in TP	chipping in the posterior teeth	Postprocedural complications: swelling, pain, hemorrhage, infection, disturbed wound healing, eating problems and interference with speech
Kleser et al.	range 2 - 5 years	LP 80% casi VR, TP LR	NR	swelling and infection
Levin et al.	13,04 months (range 1-60 months)	21 pz in sede incisivi mandibolari	NR	Swelling and bleeding
Leichter et al.	11,86 months (range 1 -86 months)	68,13% VR	NR	NR
Ziebolz et al.	7 days, 4 months, 7 years	LR in TP	NR	Chronic generalized gingivitis, swelling, demineralized enamel, pain, fibroma
Inchingolo et al.	12 +/- 4 months	25%	NR	Bleeding, perilesional edema, persistent mucosal atrophy, erythematous palatal mucosa, dentine hypersensitivity.
Ziebolz et al.	3,8 years (range 6 month-9 year)	LR in TP	93,5 % tooth damage	NR
Plessas	30,3 months (range 1-144 months)	39,70%	32,30%	Inflammation dentine hypersensitivity, increased salivary flow, taste change, generation if galvanic current, deposits accumulation. Postoperative complication: pain, chewing and speaking impairment, ornament swallowing and sever haemorrhage.
Bajkin	NR	NR	upper e lower incisor	Substantial bone loss in the mandibular incisor area
Schmidt et al.	6,7 anni (range 2 - 19 anni)	TP LR, LP VS	NR	NR

LR= Lingual Recession; VR= Vestibular Recession; TP= Tongue Piercing; LP= Lip Piercing; FGM= Free Gingival Margin; NR= Not Rated.

observed how the severity of the recession was statistically associated with the time of use and the length of the bar. The longer the length of time (years of wear) since piercing, the greater the gingival recession depth. In particular, among the group wearing the piercing from 0 to less than 2 years there were no recessions, which occurred with increasing severity in the group wearing it for 2-4 years and the one wearing it for 4 or more years.¹⁰ Also Plessas et al. organized the subjects into 3 groups based on the length of time: less than 12 months, between 12 and 36 months, greater than 36 months. They came to the same conclusion, that both the GR (gingival recession) and CAL (Clinical Attachment Level) values increased with the lengthening of the wearing period.⁸ In addition, a patient described by Chambrone et al. with lip piercing, developed a class II gingival recession on both central mandibular incisors after 6 months of wearing the ornament but showing an early impact of piercing on the gingiva.

Long piercing barbell stems affect the prevalence of lingual gingival recession in anterior teeth more than the short ones (61-65). Extremely rare are cases of buccal gingival recession associated with tongue piercings: in literature, only Campbell et al. have encountered, in a group of 52 patients examined, 2 cases whose vestibular recession appears to be due to the device.

Regarding the possible secondary outcomes to oral piercing, the damage to the dental tissues that occurs most likely is areas of abrasion, fractures and chipping that often affect the premolars and molars. This could be the result of the ensuing habit of chewing the ornament. Plessas et al. observed a statistical association between chewing, rolling, nibbling the ornament and the presence of dental defects. Dental chipping, like gingival recession, is associated with the time factor: the longer the ornament is worn, the higher the percentage of complications.

The foreign body can be associated with tissue proliferations with formations of sarcoidal-like fibroids as a foreign body reaction. These have been encountered in two studies (. Bajkin reported a case of substantial bone destruction in the mandibular incisal area following lingual piercing (66-70).

As an intervention piercing is identified as a possible vector for the transmission of blood-borne viruses HIV, HAV, HBV, HCV, Herpes Simplex Virus, and Epstein-Barr. Serious systemic complications include meningitis, Ludwig's angina, endocarditis, temporary or permanent paralysis may rarely occur.

Piercing is often performed by operators without a license and adequate clinical and anatomical knowledge. Furthermore, often there is no regulation to guide or mandate the professional education on this matter and on better patient's protection (regulations vary with different countries and states or counties). The apprenticeship hours for the piercers are in fact variable from region to region. Frequently, those who undergo the intervention are not adequately informed of the possible risks associated with the intervention. The percentage of those unaware of possible complications in the sample analyzed by Vozza et al. corresponds to 53.7%, with a similar percentage confirmed by the study of Levin et al.

In our study, both patients were not aware of the piercer license, and both had not been informed about the dental complications; in both patients, local complications, although not systemic, occurred in the periodontal tissue.

CASE REPORTS

Case one

A 23-year-old female patient was examined during a routine dental check-up. The clinical evaluation revealed a large gingival recession (Fig. 1) affecting the central mandibular incisor #41. The patient was in good general health and in a good nutritional state, and she was a nonsmoker. She reported she had a barbell piercing performed 12 months prior and had a lip piercing, corresponding to the recession region, performed 6 months prior. The dental examination presented caries-free dentition and localized gingivitis and an advanced gingival recession affecting the incisor # 41 on the vestibular side, a clear result of the mechanical damage inflicted by the lip piercing, also associated with the general inflammatory state. Two years after this visit the patient returned for a check-up visit (Fig. 2). The gingival health had improved, and the vestibular recession of the incisor # 41 is stabilize. The patient had removed the lip piercing, but not the lingual one, confirming on one hand the possible traumatic connection with the lip piercing, and on the other hand the benefit of its removal associated with regular dental follow-up.

In dental literature, the incidence of vestibular recession due to lip piercing is a frequent occurrence,

in fact Leichter et al. (2006) described the vestibular recession in the gingival area directly opposed to the labret's inner disk in 68.13% of cases. It is probable that mechanical trauma in conjunction with inflammation contribute to the development of the recession.

Case two

A 33-year-old female patient attended an oral hygiene session. She was a nonsmoker in good general health and in a good nutritional state. The patient reported that her barbell piercing had been inserted 11 years prior. The dental examination revealed generalized gingivitis and marked lingual gingival recession at the mandibular incisors (#41, #42 and #31) (Fig. 3). The patient was a dental assistant and worked as an assistant at a dental practice. She didn't remember having received information about dental complications, or receiving an informed consent, but

thanks to her profession she was aware of the possible risks and complication of piercing. Hence, she attended regularly to the follow-ups and to the dental hygiene session. The patient declined to remove the traumatic agent, regardless of the oral trauma that occurred, being aware of the complications and receiving strong recommendations to remove the piercing permanently.

DISCUSSION

It is clear that although the practice of piercing is widespread, regulations are not, being mostly regionally and unevenly applied. Whoever decides to undergo piercing should always turn to a piercer registered with the APP (Association Professional Piercer), hence someone who knows and respects the standards of hygiene, disinfection and post-

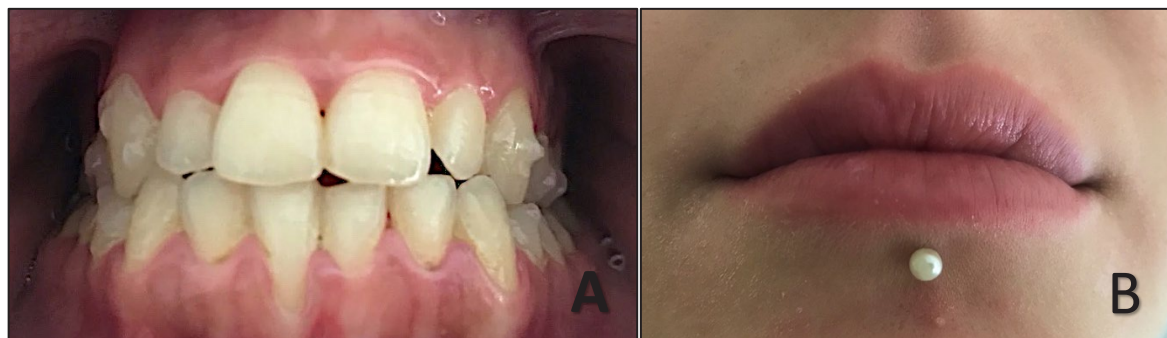


Fig. 1 Case 1. **A.** Significant dehiscence extending to the mucogingival junction along tooth #41 attributed to the lip piercing. **B.** Perioral site of piercing along the labiomental groove.



Fig. 2 Case 1 after 2 years. **C.** Stabilization of the dehiscence along the tooth #41 **D.** The piercing was removing after the recommendations.

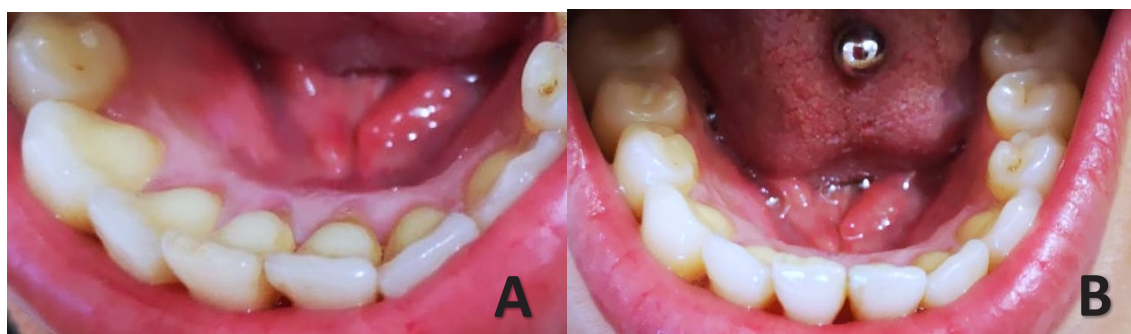


Fig. 3 Case 2. *A. Mild gingival recession along the lingual aspect of teeth #41 #42 #31. associated with tongue piercing B. Barbell shaped tongue piercing.*

operative instructions (mouthwash rinses, removal and bar brushing, use of antibiotic creams, post-piercing dental examination). Professional piercers have the obligation to attend a theoretical and practical training course on anatomy and foreseeable possible complications. They ought to be instructed about proper disinfection, starting from hand washing and the use of personal protection devices, as well as decontamination/sterilization of reusable instruments that can be performed with an autoclave (steam sterilization at 121° for 21 min), with dry heat (at 170° for 2 h), or at least with chemical disinfection (sodium hypochlorite at a concentration of 5000 ppm and glutaraldehyde at 2%). Too often the patient signs an informed consent that does not adequately indicate the complications, and as in the case of our two patients, despite the oral implications, patients are not warned of the dental consequences.

Unfortunately, even in regional official legislations, the model of informed consent focuses mainly on infections and wound healing problems but does not appear to indicate an explicit reference to any dental consequences (71-72).

In a study by Plessas et al. less than a third of the sample dedicated enough time to the sanitation of the ornament (8), by then it would be important to have information to avoid post-intervention infections. Considering the widespread ignorance of the complications and risk of transmission of HIV, hepatitis and bacterial infections and oral consequences, it is important to ensure that prospective clients go to facilities and contact

operators who comply with all hygiene standards, creating an environment as aseptic as possible, through suitable products and autoclaves that ensure the sterilization of the used instruments.

The role of the dentists, in patients who wear piercings, is to recommend their removal if they notice problems that could damage the periodontal or dental structures and, in any case, schedule periodic check-ups. In case of infection, the dental professional should prescribe antibiotic therapy and rinses with chlorhexidine while the patient should then be closely monitored for further complications. It is possible, as in one of our cases, that the patient decides to keep the ornament as a mean of personal expression. Then the education of the young person becomes of primary importance not just regarding the need for basic oral hygiene, but also regarding how to care for the ornament, the need to wash one's hands before handling the ornament, clean the insertion area and check at least twice a day that there is no adherence at both ends of the piercing. Furthermore, it is necessary for the clinician to promote oral health as much as possible in the young person with piercing, given that attentive oral care minimizes the early and late complications caused by piercing. Covello et al. found that half of the subjects in their study was misinformed about the effects of the piercing on health; the same percentage of individuals do not disinfect the ornament; 70% of cases were unaware of the impact on the gums and 60% of them was unaware of dental consequences.

The description of the two cases confirms what

we have deduced from the literature, in terms of the frequency of the female sex getting a piercing and the incidence of periodontal damage in the mandibular incisors. Our cases confirm these complications for which the lingual side is the most affected for those who wear the tongue piercing, unlike the damaging action of the vestibular soft tissues corresponding to the location of the lip piercing of the lower lip. Confirmed by the various studies, the main damage occurs in the area on which the piercing strikes due to a mechanical damaging action, as it happened in our two patients.

Based on literature review and the clinical description of our cases, we can affirm that given the spread of this trend and the complications that can occur, both systemically and locally, it is recommended to have more frequent check-ups among patients with piercings; ensure that the disinfection and sterility guidelines of piercers are respected, and that the clients requesting a piercing are also adequately informed about possible dental consequences.

It would be appropriate that the regional legislators in the discipline of piercing activity, foresee all possible complications expressly indicated in the model of informed consent, especially in relation to the possible dental consequences (such as those examined in this paper) and to the need for periodic check-ups by the dentist.

However, the desire to wear the piercing as a way of expressing individuality is sometimes stronger than worries about their side-effects, but we as professionals must respect this need.

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Proposal of use of the autotransplantation of the third molar as space maintainer in growing patients: a review of literature and a clinical case

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The review of literature shows that 48,22% of the autotransplanted teeth were orthodontically treated. A 13-year-old male patient presented a destructive decay in the element 3.6, which was underoccluded. To maintain the arch space and the quantity of bone necessary for a potential implant, it was decided to perform an autotransplantation of the impacted element 3.8. Tooth autotransplantation is a valid and durable alternative to the prosthetic and the implant solutions with high success rate.

The transplantation of teeth is a surgical procedure performed for the first time by Ambroise Pare in 1554 and the autotransplantation is a valid and durable solution in the case of loss or agenesis of permanent elements. The first publication of the autotransplantation of teeth was the study of Horace Miller, published in 1956 (1), which describes fourteen case reports citing previous experimentations.

According to Mendes et al. (2), the indications for the autotransplantation of the teeth are: impacted ectopic teeth, premature tooth loss, traumatic tooth loss, tumours, iatrogenic grounds, congenitally missing tooth in one arch with clinical signs of tooth crowding in the opposing arch, replacement of developmentally absent teeth, teeth with bad prognosis and developmental anomalies of teeth and related syndromes (3-10).

The autotransplantation is very versatile between the different dental elements: it is not used only for third molars, but it's widespread also for the canines, the premolars and the other molars (11-16).

Based on the review of the literature, the studies which included both orthodontically treated and untreated autotransplanted teeth, the authors found an average percentage of teeth orthodontically treated of 48.22%, so it can be confirmed that in about half autotransplantations the orthodontic therapy is needed.

The work of Lagerström and Kristerson did not find any significant difference in final root length between orthodontically treated and untreated autotransplanted premolars, whereas the study of Bauss et al. found a significant difference between the final root length of the derotated immature third molars and the control group, conversely it was not found between extruded immature third molars and the control group (17-25).

Furthermore, the 4-year observation by Tanaka et al. of 28 autotransplanted premolars in orthodontic patients reported a success ratio of 100%, whereas the study of Mendoza-Mendoza et al. about 12 autotransplanted premolars in orthodontic patients reported a success ratio of 100% in 5 years and of 83.3% in 10 years.

Key words: third molar autotransplantation, space maintenance, incomplete root formation

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INTEREST RELEVANT TO THIS ARTICLE.**

CASE REPORT

A 13-year-old male patient comes to the Dental Department for an orthodontic evaluation; functional analysis, and control lingual frenum. He presented an Angle's second class, a reduced space for the eruption of the superior canines, a deepbite and a destructive decay in the element 3.6, which was underoccluded (Fig. 1); the patient revealed a physiological lingual frenulum and no needing of myofunctional therapy.

The authors tried to look after the tooth in relation to its reduced eruption, but then it was decided to extract the element, because it was compromised. To maintain the arch space and the quantity of bone necessary for a potential implant, it was decided to perform an autotransplantation of the impacted element 3.8.

At the same time, a fixed orthodontic treatment was performed using metal brackets in both dental arches to create the space to the element 1.3 which had not enough space to erupt, to create the space in



Fig. 1. Pretreatment orthopantomogram radiograph

the area of the 3.6 to insert the 3.8 autotransplanted and to correct the Angle's second class (Fig. 2).

After the successful procedure of autotransplantation and the orthodontic correction of the malocclusion the patient can wait the end of the growth to choose about the maintenance or not of the autotransplanted molar, the potential extrusion of it to solve the underocclusion and the possibility of substitute the tooth with an implant (Fig. 3).

DISCUSSION

The autotransplantation of teeth with incomplete root formation have a very high survival rate and success rate: according to Rohof et al. , the average survival rate is of 97.4 % after 1 year, 97.8% after 5 years and 96.3% and the success rate is of 96.6%.

Whereas, considering also the autotransplantations of teeth with complete root formation, according to Yang et al. , the survival rate decrease to 88% at 1 year, 78% at 5 years and 74% at 10 years; the same retrospective study revealed that, according to the long-rank test univariate analysis, the application of an orthodontic force after autotransplantation significantly increases the survival rate and that early orthodontic force prior to 4 weeks is significantly effective for the prevention of tooth ankyloses, while Kokai et al. affirmed that the early application of orthodontic force may increase the success rate of the autotransplanted teeth (26-34).

Considering only the autotransplantations of children and adolescents, there is a favourable PDL healing in the 87.6% of the cases and there is no

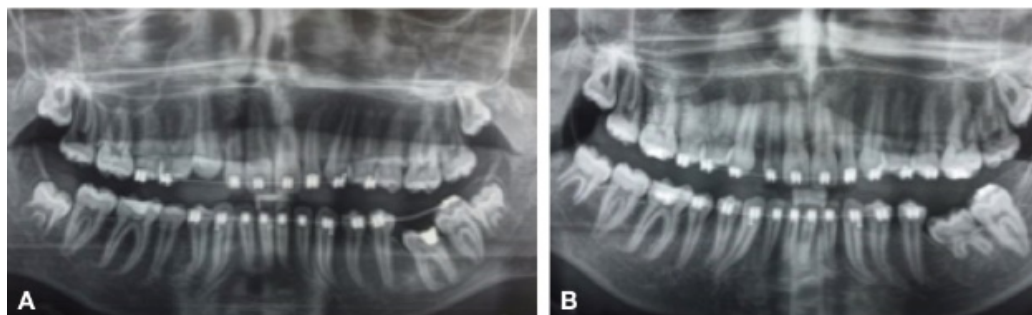


Fig. 2. A. Orthodontic treatment panoramic radiograph. B. Panoramic radiograph after 3.6 extraction and 3.8 autotransplantation.



Fig. 3. *Occlusal inferior photograph after 3.6 extraction and 3.8 autotransplantation.*

resorption in the 73% of the cases; moreover, the survival rate is of 94.4%.

In the literature there are lots of studies that investigate the use of the autotransplantation as definitive substitute of a lost element, as an alternative to dental implants or dental prosthesis, but none debates the use of the autotransplantation as space maintainer, as an alternative to metal space maintainers. (35-46)

This article yearns to expand the field of use of the autotransplantation to the space maintenance, because it permits to maintain efficiently also the bone and it is very long-lasting; this use of the autotransplantation is especially indicated in the growing patients, who must wait the end of the growth to undergo to an implant-prosthetic rehabilitation.

These patients can take advantage of autotransplantation whether as a definitive or as a temporary solution and this versatility offers to the dentist the possibility to wait and evaluate at the end of the growth the success of the autotransplantation and if the dental implant is needed or not a good way to seek constantly the patients to intercept the end of the growth can be the use of the teleorthodontics (47-55).

The tooth autotransplantation is a very useful technique to maintain the space and the bone in the growing patients who loose permanent elements and very often it needs to be performed in combination with the orthodontic treatment, but it demonstrated to be also a valid and durable alternative to the prosthetic

and the implant solutions, because it grants very good survival rate and success rate (56-63).

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Does a short lingual frenulum affect body posture? Assessment of posture in the sagittal plane before and after laser frenulotomy: a pilot study.

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The factors that characterize posture are neurophysiological, biomechanical, psychoemotional. Neurophysiological factors concern the modulation of tone, muscle tone is the result of a series of neuropsychological processes within the tonic-postural system. The tonic-postural system can become unbalanced for various reasons, including a tight lingual frenum. The aim of this pilot study was to evaluate the benefits of frenulectomy by laser on body posture and on the scapular (shoulders) anteroposterior movement. Twenty-four healthy subjects were selected, between the ages of 10 and 26 years (mean age 15.22) who presented a short lingual frenum and a low posture of the tongue and jaw. They were examined using the Marchesan Protocol for Lingual Frenum and the Spinometry® Formetric and underwent laser frenectomy by diode laser (Siro Laser Blu. 660 nm) without any post-surgery complications. The release of the frenulum immediately brought benefits to patients, reorganizing the physiological modulation, and the movement of the tongue within the normal parameters of temporomandibular kinematics which were within physiological parameters. Frenectomy improved the anterior-posterior flexion of the scapulas (shoulders) in the sagittal plane but a larger sample is required to have statistically significant results.

Although posturology has increasingly focused attention on the stomatognathic receptor in the modulation of the tonic-postural system, due to the anatomical relationships that connect the tongue / jaw / hyoid bone, in the implementation of physiological functions — primary and not — the correlation swallowing-chewing-occlusion-posture requires a multidisciplinary approach.

Muscle, functional and postural alterations due to short lingual frenum

Neurophysiological factors concern the modulation of tone, muscle tone is the result of a series of neuropsychological processes within the tonic-postural system. This system has specific inputs consisting of information from specific posture receptors: the foot, the eye, the stomatognathic

Key words: posture, short lingual frenulum, laser, frenectomy, tongue

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system, the skin, the musculoskeletal system. Biomechanical factors concern the relationships between bodily attitudes and the force of gravity. Psychoemotional factors can affect the postural system. (25-30)

In the case of a short lingual frenulum, which reduces the mobility of the tongue and its position, almost resting on the buccal floor, numerous and complex modifications of the physiological mechanisms are determined. The alterations in the physiological function of swallowing are widely documented in the literature. In particular, the difficulty in lactation /

feeding in newborns with short lingual frenum often indicated surgical treatment. A rather small number of scientific studies have instead addressed the issue of the short lingual frenum in the young adult. The study aimed to investigate, by means of clinical and instrumental evaluation, whether in young adults there is a correlation between a short lingual frenum and the tonic-postural system before and after a laser frenectomy. In particular, the measurements focused on the anterior-posterior flexion of the scapula (shoulder) in the sagittal plane (VP-DM) as anecdotal reports from patients mention

Table I. *Lingual Frenulum Protocol by Marchesan 2012.*

Measurements using a caliper. Larger or equal 50,1% (0) – Less or equal 50% (1) FINAL RESULT =		
Take measurements from superior right or left incisive to the inferior right or left incisive.	Value in millimeters	
Consider the same tooth for all the measurements.		
Open mouth wide		
Open mouth wide with the tongue tip touching the incise papilla		
Difference between the two measurements, in percentage	%	

Alterations during tongue elevation (best result = 0 e worst result = 2) FINAL RESULT =		
Open mouth wide; raise the tongue without touching the palate	NO	YES
1. Tip of the tongue's shape: oblong or square	(0)	(1)
2. Tip of the tongue's shape: like a heart	(0)	(1)

Frenulum fixation. Add A and B (best result = 0 e worst result = 3) Final result =		
A – Mouth floor:		
Visible only from the sublingual caruncles	(0)	
Visible from inferior alveolar crest	(1)	
Fixation in another point:		
B – Sublingual:		
In the middle of the tongue	(0)	
Between the middle and the apex of the tongue	(1)	
At the apex	(2)	

Clinical frenulum classification (best result = 0 e worst result = 2) Final result =		
Normal (0)	Borderline (1)	Altered (2)

If the frenulum was considered altered it would be because:			
The frenulum seems normal but it is attached between the middle and the apex of the tongue	The frenulum is short	The frenulum is short and it is fixed between the middle and the apex of the tongue	
Ankyloglossia (frenulum attached to apex of the tongue)	Another reason	Unsure	

Measurements using a caliper. Larger or equal 50,1% (0) – Less or equal 50% (1) FINAL RESULT =		
Take measurements from superior right or left incisive to the inferior right or left incisive.	Value in millimeters	
Consider the same tooth for all the measurements.		
Open mouth wide	46,00	
Open mouth wide with the tongue tip touching the incise papilla	37,55	
Difference between the two measurements, in percentage	81,63 %	

that right after the frenectomy they feel that they can stand up straighter and the “shoulders open”. The parameter spinometry of the antero-posterior plane (VP-DM) indicates retroflexion and it was chosen because it refers to (31-33):

VP = prominent vertebrae;

DM = midpoint of the straight line join;

DR and DL = right and left dimples (Michaelis' dimples).

MATERIALS AND METHODS

Twenty-four patients were selected, between the ages of 10 and 26 years (mean age 15.22) who present with ankyloglossia as defined as: a short lingual frenulum, or a frenulum with an anterior insertion closer to the dental arch, or a short one with an anterior insertion. All participants provided the informed consent to the study.

The study was conducted according to the principles outlined in the Declaration of Helsinki and in accordance with the principles outlined by the scientific commission of the biomedical sciences department of the University of Sassari, Italy. The study was approved by the scientific commission of the Department of Biomedical Sciences, University of Sassari, Italy.

The subjects were evaluated using a portion of the “Lingual Frenulum Protocol” by Marchesan 2014 (Table I) which was considered sufficient to diagnose the condition. Measurement of the lingual frenum (Fig. 1) was taken with a digital caliper, as per protocol instructions, and expressed in mean millimeters +DS. It was performed by asking the patient to open his/her mouth wide and the distance between the upper and the lower incisors was registered. Then the patient was asked to elevate the tip of the tongue and touch the retro-incisor papilla (often clinically referred to as the “spot”) and a second measurement was taken between the upper and the lower incisors. Then a difference between the two measurements was calculated, in percentage. A difference of less or equal 50% was assigned a score of 1 while a percentage of 50.1% and above was assigned a score of 0. The limit score for that section was equal to/greater than 3 on a maximum score of 8 (worse score, suggesting a significant ankyloglossia). Best score for this section of the protocol would be 0, suggesting an anatomically normal lingual frenum.

For body postural evaluation, all subjects underwent instrumental evaluation by using the Spinometry® Formetric 4D (Fig. 2, 3). This is an analysis system that performs a detailed and extensive (without the use



Fig. 1. Steps to evaluate the tongue elevation in the presence of a short lingual frenum, before and after surgical laser frenulum release.

Table II. *The myofunctional therapy protocol's home assignment list.*

Exercise #1	Raise your tongue up onto the “spot” (retro-incisive papilla), repeat X times
Exercise #2	Lift the tip of the tongue just behind the spot, repeat X times
Exercise #3	Place the tip of the tongue on the spot, hold it there and close your mouth, repeat X times
Exercise #4	Hold the tip of your tongue on the spot and open your mouth, repeat X times
Exercise #5	Stick your tongue out, repeat X times
Exercise #6	Make circular movements with the tongue first to the right then to the left following the inner surface of the teeth, repeat X times
Exercise #7	Make circular movements with the tongue first to the right then to the left on the outer surface of the teeth, repeat X times
Exercise #8	Move the tip of your tongue as close as you can to your nose, repeat X times

Table III. *Tongue elevation improvement (open mouth, tongue on the retro-incisive papilla) and scapular anteroposterior movement (shoulder opening) expressed in mean millimeters ($\pm$ standard deviation) as measured with the Spinometry.*

	Before surgery	After surgery	Significance
Average lingual frenulum protocol score	4.7 $\pm$ 1.1	0.5 $\pm$ 0.7	P<0.01 (Significant)
Max mouth opening	18.7 $\pm$ 22.2	17.7 $\pm$ 19.0	N.S.
Tongue to palate with mouth open	21.0 $\pm$ 22.5	18.9 $\pm$ 19.1	N.S.

previous findings that laser frenectomy avoids bleeding, use of sutures and post-surgical pain (6); furthermore, the release of the frenulum and the immediate post-operative support of myofunctional rehabilitation of the tongue mobility showed improvement of the range of motion and movement pattern of the tongue, possibly related to the neuroplastic influence of repeating new movements with the tongue now freer to move in all planes of space.

Regarding posture, unfortunately the small sample showed no statistical significance although in all of the patients the spinometry revealed “shoulder opening” (Table III-IV), a change to the anterior-posterior flexion in the sagittal plane of the reference anatomical landmarks previously described. The pre- and post-operative comparison of measurements taken by spinometry, during tongue elevation to the retro-incisive papilla, via paired samples showed a mean base of 18.67 $\pm$ 22.8 vs 17.67 $\pm$ 19.02 and a

value during tongue elevation of 21.08 ± 22.52 before surgery versus 18.91 ± 19.17 after surgical release. Both differences showed no statistical significance as mentioned above for the sample size which is small.

DISCUSSION

We know that an alteration of the lingual frenulum

can affected the normal function and development of the orofacial complex. The presence of a short lingual frenum in children and adults has been linked to problems in swallowing, sleep breathing as well as posture, and often it requires a surgical release. Our study confirmed the utility of laser frenectomy and even though this is a pilot study and has some limitations due to the limited sample, it allowed us

Table IV. *Overview of individual results.*

Patient	SEX	Frenulum protocol test results pre - frenectomy (worse 8, best 0)	Frenulum protocol test results post - frenectomy (worse 8, best 0)	VP-DM Shoulder Retroflexion pre-frenectomy measure in mm	VP-DM Shoulder Retroflexion post-frenectomy measure in mm
L.L.	M	5	0	-14	-11
C.M.	F	6	1	35	55
D.M.	F	6	1	51	55
D.A.	M	7	1	-27	-22
P.A.	M	5	0	-3	13
O.M.	M	4	0	22	26
F.W.	M	3	0	26	34
C.F.	M	5	0	-8	5
C.M.	F	4	0	-11	5
D.L.	F	6	2	34	54
F.L.	M	4	0	29	37
G.A.	M	4	0	25	34
D.E.	F	4	0	38	46
F.L.	M	4	0	-12	0
I.A.	M	5	1	7	18
I.F.	M	3	0	-11	8
M.V.	M	3	0	-3	9
M.D.	M	4	0	27	30
P.S.	M	7	2	32	38
S.L.	M	5	1	37	47
T.M.	F	6	2	31	33
T.L.	F	5	1	-12	0
T.E.	M	4	0	-11	8
S.L.	F	4	0	2	4

to develop a protocol for future studies that could clarify the effects of frenectomy on overall posture.

The relationship between tongue mobility and body posture is evident when considering the tonic-postural system, where a dysfunction on the sagittal plane manifests itself as a protraction of the scapular plane, more rarely a retraction (backward position), with respect to the pelvic plane, in proportion to the degree of restriction of the lingual frenulum (34-40). The forward projection of the line of gravity with respect to the pubic symphysis produces a cascade of postural compensations with tensions that are particularly evident at the cervical or lumbar level. At the cervical level, we may encounter superior crossed neuromuscular imbalance in the presence of hypertonic muscles (scalene, superior trapezius and levator scapulae; pectoralis major and minor muscle; suboccipital and paravertebral neck muscles) which is countered by a weakness of their respective antagonists. Moreover, the postural pattern is expressed with a flexion of the atlas-occipital joint, cervical hyperlordosis and anteriorized protracted scapular plane. At the lumbar level, the inferior cruciate neuromuscular imbalance is present when the hypertonicity of some muscles (iliopsoas, paravertebral, loin quadrant and tensor fascia lata) is accompanied by weakness of the antagonist muscles. In postural terms, hyperlordosis is a highlighted lumbar position with an anterior rotation of the pelvis. All these changes can be documented by the Spinometry® Formetric diagnostic system (41-50).

A lingual frenectomy may have a positive impact on swallowing as well. During the first phase of swallowing, the oral preparatory phase, if the tongue is restricted in its movements by a short lingual frenum or by a true ankyloglossia, compensatory movements develop to process the food bolus in the oral cavity and eventually propel it towards the pharynx and the esophagus. If the apex of the tongue cannot reach the retro-incisive papilla due to the short lingual frenulum, even in adulthood, the pattern of forward tongue position anteposition towards the incisors is maintained. Considering the risk of postoperative scars that can limit tongue mobility and require subsequent surgeries, we suggest that a rehabilitation treatment is implemented both pre- and

post-surgery. The histological structure of the lingual frenulum is characterized by type I collagen fibers, also present in ligaments and tendons, which have a high tensile strength but do not stretch, therefore myofunctional therapy is indirectly very useful on the lingual frenulum as it repatterns the musculature of the tongue. A previous research confirmed that the frenectomy associated with myofunctional therapy improves orofacial and nasal functions by way of muscle tone and increase of tongue mobility.

Our limited sample size pilot study allowed us to verify that the release of the frenulum acts on the posture, specifically on the antero-posterior plane of the shoulders, as measured with spinometry. Future studies should include measurements of movements of other anatomical landmarks such as hips, neck, head etc. (51-55).

This study suggests that it is useful to evaluate and treat a short frenulum not only in children but also in young adults, especially because it affects posture and therefore it may trigger many other studies on scoliosis and other skeletal deformities as well.

Today spinometry represents a very interesting diagnostic method, noninvasive and helpful to study the entire vertebral column. We propose future studies on restricted frenula, before and after laser release, with larger samples analyzing other parameters of the spinal column; future studies on the effect of myofunctional therapy and the restoration of swallowing and breathing on various postural body landmarks; or studies on the spinometric aspects on patients suffering from various orofacial dysfunctions and obstructive sleep apnea (OSA). It would also be interesting to evaluate in growing patients whether an orthodontic pathology or orthodontic treatments may affect posture. We often talk about the correlation occlusion-posture but given that there are only a few studies on this subject we propose spinometry as an instrumental diagnostic to investigate this aspect (56-63).

Even with a small sample size, the question “does a short lingual frenulum affect body posture?” the answer is yes, at least anecdotally. The assessment of the tonic-postural system, in particular the scapular retroflexion (shoulder opening) before and after frenectomy by laser showed a small but measurable

difference in favor of surgically released lingual frenulum. This aspect is very interesting for future studies. This study also confirmed the efficiency of a laser frenectomy on tongue mobility and pattern of movements, which eventually improves all other orofacial functions that depend on it.

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The relationship between TMJ symptoms and orthodontic treatments: a survey on 236 orthodontic patients

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The study analyzes how and if temporomandibular joint symptoms are influenced by different types of orthodontic therapy. Two-hundred-and-thirty-six adult orthodontic patients treated by different clinicians, were asked to complete a survey in which factors as the age, the gender and the type of device were considered. The questions were about the typical symptoms of temporomandibular disorders, in particular headache, bruxism, clenching, pain while opening the mouth and joint's noise. It was highlighted if these symptoms changed during the therapy and if they increased or decreased. The answers to our questionnaire revealed that the only statistically significant difference was related to bruxism, because we found a higher rate in patients treated with aligners than patients treated with metal braces, so we can suggest the fixed technique in the orthodontic patient who suffers of bruxism, even if further studies are required.

Malocclusion is an improper position of the teeth associated, in some cases, with an abnormal relationship between the jaws that don't respect some parameters. In particular there can be problems which were divided according to Angle classes; these are defined considering the relationship between the lower and upper first molars and the upper canine with the lower teeth; furthermore, there are other kinds of malocclusions which consist in transversal discrepancy, sagittal problems, cross bites and overbites. In the recent literature the types of malocclusions which are more related to symptoms, commonly known as temporal mandibular disorders, TMDs, are the ones with an evident overbite and a unilateral crossbite, but it is still controversial.

The malocclusions are quite common nowadays as human lifestyle changed, if we consider the past; now people are used to having a soft diet which is considered one of the reasons of malocclusion.

Another important factor that causes malocclusion to spread between people is the presence of habits during adolescence, which influence the development of the jaws and the teeth. It is undeniable that in the last years the TMDs are more common between people, comparing to the past, which brought the scientific community to look for the reason and the treatments that can help in solving the issue.

For all these reasons, there are many types of orthodontic treatments, as the need for them increased; the orthodontists really believe that each case has its own type of treatment and device. The clinicians refuse the idea that each device can be used in any case, but the choice should be made according to the problems and symptoms that the patient has.

According to the recent studies the malocclusion and TMD are more prevalent in females than males and related also to the adolescents, even if there are few studies that consider them. (1-12)

Key words: TMJ symptoms; braces; aligners; orthodontics; survey

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Liu et al. in 2013 showed how TMD concerns more than 5% of the population and that there is a peak between the ages of 20 to 40, although it can affect all ages, while Lipton et al. highlighted that about 6% to 12% of the people experienced clinical symptoms of TMD (13-16).

There are few theories which support that some hormones and systemic aspects can influence this kind of issue. We also know that there is also a connection between TMD and rheumatological disease, like juvenile arthritis, as rheumatoid arthritis (RA), seronegative spondylopathies, such as ankylosing spondylitis, psoriatic arthritis, gout, and infectious arthritis.

The main symptoms thought to be related to the malocclusion were bruxism, headache, articular noise, orofacial muscle pain and ear pain. TMD can be divided in articular or muscular issues and in a few cases, they can both be present.

These disorders are synonymous with intracapsular and extracapsular conditions, respectively. most non-articular disorders present as myofascial pain focused on the muscles of mastication. In fact, more than 50% of TMD is myofascial pain. Other non-articular disorders include chronic conditions, such as fibromyalgia, muscle strain, and myopathies. Myofascial pain and dysfunction are theorized to be caused also from clenching, bruxism, or other parafunctional habits. The result is masticatory musculature strain, spasm, pain and functional limitation.

For this reason, in the last 30 years there was the idea to treat these symptoms improving the dental occlusion, giving a stable one, an orthopedic stability. It was a common belief in the 90s that the dental occlusion was the main reason of the temporal mandibular disorders, TMD, that can develop in different ways. Since 1950 it was thought that a dental pre contact could exacerbate or cause bruxism and clenching. Till now there is the idea that bruxism and clenching can increase the other symptoms which we said before, in particular the muscle soreness and pain. For that reason at the beginning, it was given great importance the muscle balance which was thought to be reached when there was a stable and proper occlusion.

Nowadays the role of the dental occlusion is not

more centric in the treatment of the TMD, because many clinicians noticed that the benefits of the change of the mandible position were not so much lasting in the time. In some way the change of the position of the mandible, in many patients gives an initial benefit to their symptoms, but these benefits were not always continuous. Anyway these aspects are controversial, because there are clinicians still today who think that occlusion has still an important role in this problem even if it is not the only one. The most common and equilibrated idea is that the TMD is a multifactorial disease; in the last years they found an important relationship between the OSAS (Obstructive Sleep Apnea Syndrome), TMD and bruxism and it seems that the OSAS can increase this unwanted movement of the teeth during the night; it was also remarkable in few studies a reduction of the bruxism when OSAS was solved. Moreover, it was also discussed if the devices used to treat the OSAS, like MADs, can influence TMJ and TMD. It is good, as said before, while deciding to start an orthodontic treatment in a patient with TMDs, to consider many aspects, also systemic problems which seem, apparently, far from the alignment of the teeth. As well emotional stress can contribute to increase bruxism and clenching.

This study/survey was done to analyze the connection between the orthodontic treatment and the disorders related to the temporal mandibular joint (TMJ) and how the technique and the devices used can influence the most common symptoms (17-22).

We tried to see how the TMJ's symptoms in patients, who needed an orthodontic treatment because of their malocclusion, can change in according to their age and sex, the type of orthodontic device used.

MATERIALS AND METHODS

We enrolled 236 consecutive patients (217 females and 19 males) admitted to a private dental clinic suffering from teeth misalignment. All patients underwent a complete dental evaluation comprehensive of x-rays and dental impressions. After evaluation braces were prepared personalized based on previously taken impressions and cephalometric measures to correct the misalignment.

After 3 months from treatment, questionnaires were administered to all patients from 05/3/2020 to 05/30/2020 by an online form service (Google Form service, Google LLC, 1600 Amphitheatre Parkway, Mountain View, California, U.S.) and the sample was randomly selected. Demographic data are plotted in table I.

The form, presented in bilingual Italian English, was composed of nine multiple choice question (23-33):

- Are you female or male? - Sei femmina o maschio? (male; female)
- How old are you? - Quanti anni hai?
- What type of braces do you wear? - Quale tipo di apparecchio porti? (Ceramic, lingual, traditional transparent aligners)
- When did your orthodontic treatment begin? - Quando è iniziato il tuo trattamento ortodontico?
- Do or did you suffer from headache? - Hai sofferto o soffri di mal di testa?
- Do or did you suffer from articular noise? - Hai sofferto o soffri di rumore articolare?
- Do or did you suffer from pain during the opening and/or the closure of the mouth? - Hai sofferto o soffri di dolore durante l'apertura e/o la chiusura della bocca?
- Do or did you suffer from bruxism? - Hai sofferto o

soffri di bruxismo?

- Do or did you suffer from teeth clenching? - Hai sofferto o soffri di serramento dei denti?

For the above item the answers were:

1. Before and during with the same intensity - Prima e dopo con la stessa intensità
2. Before and during, but less before - Prima e durante, ma meno prima
3. Before and during, but more before - Prima e durante, ma più prima
4. Before the orthodontic treatment - Prima del trattamento ortodontico
5. During the orthodontic treatment - Durante il trattamento ortodontico
6. Never - Mai

All data were taken in anonymized plot and identified by time signature. Patients wearing ceramic and lingual braces were excluded from the analysis as too scarce to be representative, 208 patients were included in statistical analysis. Distribution homogeneity age group for the two brace types and distribution of answers were evaluated by Kruskal-Wallis test. Homogeneity for sex was assessed using chi square test. All data were analyzed using Statistical Package for the Social Sciences (SPSS 25.0, SPSS Inc., Chicago, USA")

RESULTS

All 236 patients responded to the online questionnaire, and we included 208 patients that used transparent or traditional braces. The only significant difference between the two groups was Bruxism.

DISCUSSION

We evaluated different symptoms usually related to malocclusion to see if there are changes according to the type of the device used for the treatment.

We considered many factors, the age, the gender, the habits like bruxism and clenching which are two of the most debated aspects that are related to headaches and muscles soreness; moreover, we considered the articular noise, which, according to Ribeiro et al. (6), involves a part as well of the patients who are TMD's symptoms free and 86% of the TMD's patients (23-30).

Table I. Demographic data

Demographic data	
<i>Age Group</i>	
18-29	106 (44.9%)
30-49	110 (46.6%)
50 and older	20 (8.5%)
<i>Sex F/M</i>	217/19
<i>Bracket type</i>	
Ceramic Braces	27 (11.4%)
Lingual Braces	1 (0,4%)
Metal braces	75 (31,8%)
Aligners	133 (56.4%)

The TMJ noise affects, according Naieje et al. (7), between the 18% and the 35% of the general population. In this study there was no evidence of difference of articular noise in the patients treated with different devices, in particular braces and aligners. We know that orthodontics is not very effective on articular noise. In the same article, we can see that the splint therapy is more indicated for this kind of problem (34-42).

We also analyzed the pain while opening the mouth, as it is common in patients with malocclusion and eventual TMD, temporal mandibular disorders, to have a limited opening and pain during that time.

We considered the study of A.M. Heredia-RIizo et al., in which it was proved that in the orthodontic patients the maximal opening of the mouth was major than the non-orthodontic patients, even if the pain is affected by many factors and the same pain influences the maximal opening.

In our survey, we also considered the general headache, which, we know is not a very objective symptom, as it is influenced, and it is caused by many aspects. In literature we can find different opinions, but there is the general idea, according to a lot of studies, that an improved and balanced occlusion can determine an improvement of pain and headache (43-50).

Table II. Results

Results			
	<i>Bracket type</i>		
	Metal braces	Aligners	Significance
Females/ Males	69/6	122/11	N.S
<i>Age Group</i>			
18-29 Y.O	49	47	N.S.
30-49 Y.O.	25	69	
50 and older	1	17	
<i>Do or did you suffer from headache?</i>			N.S
Before and during with the same intensity	14	28	
Before and during, but less before	5	11	
Before and during, but more before	7	15	
Before the orthodontic treatment	11	9	
During the orthodontic treatment	10	19	
Never	28	51	
<i>Do or did you suffer from articular noise?</i>			
Before and during with the same intensity	8	14	N.S
Before and during, but less before	1	5	
Before and during, but more before	3	6	
Before the orthodontic treatment	6	9	
During the orthodontic treatment	2	8	
Never	54	91	
<i>Do or did you suffer from pain during the opening and/or the closure of the mouth?</i>			N.S
Before and during with the same intensity	5	7	
Before and during, but less before	4	4	
Before and during, but more before	3	6	
Before the orthodontic treatment	4	11	
During the orthodontic treatment	6	15	
Never	53	90	
<i>Do or did you suffer from bruxism?</i>			P<0.05
Before and during with the same intensity	5	9	
Before and during, but less before	2	6	
Before and during, but more before	4	9	
Before the orthodontic treatment	2	14	
During the orthodontic treatment	2	13	
Never	60	81	
<i>Do or did you suffer from teeth clenching?</i>			N.S
Before and during with the same intensity	13	14	
Before and during, but less before	9	6	
Before and during, but more before	7	17	
Before the orthodontic treatment	3	10	
During the orthodontic treatment	20	35	
Never	22	50	

In this study we found a significant difference in the patients affected by bruxism, when treated with different devices. The bruxism, according to Eduardo E. Castrillon et al. (9), is an oral behavior that may lead to repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible with 2 distinct circadian manifestations: sleep bruxism or awake bruxism (51-57). They share common risk factors and lead to similar consequences for the masticatory system but may have different etiology and pathophysiology. This oral behavior has been associated with tooth wear, masticatory muscle tenderness, headaches, and painful temporomandibular disorders. Available scientific evidence does not support the view that bruxism is a direct cause of pain, which should be taken into account when treating/managing patients.

In this survey we noticed that the fixed braces induced a reduction of this habit, which, at same time, is connected to TMJ pain (8-12), more than the aligners. That confirmed a general opinion which developed in the past years that at least during the orthodontic treatment, the patients suffer bruxism less than before or less than the ones untreated. The newest thing that this study revealed was that the fixed braces seem to determine this improvement more than the aligners.

Until today there were many studies about bruxism, but they still did not find a clear etiology and a definitive cure for this problem. Interesting it was the study conducted by R. Needham et al. (9), that through a grindcare, an oral device that gives impulses, they were able to reduce the bruxism in a great part of the patients.

However, it is clear that bruxism is a multifactorial problem which has many features, of course a balanced and stable occlusion would help. Our study is confirming the conclusion which was reached by T. that aligners do not help or influence this habit (58-61).

According to our study, we can suggest fixed oral braces, not to treat the bruxism itself, but we can advise fixed braces in patients who need orthodontic treatment. In the meantime, they suffer from bruxism, because there is less invasive procedure to treat bruxism in patients, with no

need of orthodontic treatment, and that in a way or another helped patients who just suffer of bruxism and not have malocclusion. Therefore, in the light of the data obtained from this study we can say that in the presence of bruxism we should not treat the symptom but investigate the diagnosis.

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Comparative analysis of the peri-implant tissue response of standard versus short implants in smoker, non-smoker and former smoker patients: a one-year retrospective study

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The aim of this clinical study is to evaluate, through some clinical and radiographic tests, the different biological responses of hard and soft tissues adjacent to the standard or to reduced-length implants in smokers, non-smokers and former smokers. This study was a comparative analysis of three groups of patients. Patients were eligible if they needed an implant in the posterior areas of the maxilla and mandible, both with a sufficient amount of bone, using implants that are standard or with small heights, and using small implants so that they could avoid any regenerative bone therapy aimed at increasing vertical size. The clinical and X-ray assessment was performed after 4/6 months (T₁) and after one year (T₂). The main results were recordings of clinical aspects such as presence of inflammation, color and gum appearance assessments, and X-ray assessment differentiating maxilla and mandible implants. Averages were conducted to estimate differences between study groups. One-hundred patients were included, with a total of 200 implants: 100 standard sized implants and 100 short implants. In the individual study groups, there is no difference between the types of implants, and we have favorable and unfavorable conditions. The reported data and the analyzed studies are insufficient to define the influence of smoking in the assessment of the long-term predictability of implants.

In medicine, smoking is considered a risk factor for many diseases. In the dental field, smoking increases the risk factors of cancerous and precancerous lesions, but mainly has a strong impact on dental support tissues and peri-implant tissues. In the latest classification of periodontal diseases issued by the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, held in Chicago in November 2017 and jointly organized by the American Academy of Periodontology (AAP) and the European Federation of Periodontology, it was not only stated that smoke is a risk factor, but also that the number of cigarettes helps to establish the degree of disease (1-10). Smoking also affects the disease to implantate: a systematic review, based on 107 articles,

shows how the insertion of implants in smoking patients significantly increases the failure rate due to a higher risk of post-operator infections, such as marginal bone loss. The study states that other factors related to smoking activity should also be considered.

In a study conducted from 2010 to 2017 (31-42) on 500 patients, divided into smokers and non-smokers, it was attempted to establish the influence of smoking on the survival of the implant. The evaluation was carried out over a period of 3, 6, and 12 months using X-rays. This study shows that crestal bone loss around dental implants is significantly greater in smokers (Group I) than in non-smokers (Group II) regardless of load duration ($P < 0.001$). Marginal bone loss varied significantly according

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to the position in both groups. Bone reworking and bone tissue formation depends on blood and clot formation (11-19): smoking changes blood flow in the neck head region and oral cavity. Smoking habit is also associated with delayed bone healing, reduced bone height, increased bone loss rate, poor quality bone formation, and increased incidence of perimplantate (20-32).

Our study, taking note of these considerations, is responsible for assessing and comparing whether this event also occurs on short implants, comparing standard implants and short implants in the same location and in the same patient. It also tries to compare the assessment using the same method for nonsmoking patients and former smokers. Dental implants are considered the treatment of choice to replace one or more elements in the edentulous of patients (33-35). There are many clinical situations where insufficient bone volume could be a limiting factor for implant placement and for the success of osteointegration. Several surgical techniques have been introduced to achieve adequate bone volume and to allow the placement of implants with conventional height, including bone grafts, breast lifts; surgical procedures such as the placement of tilted implants or zygomatic, transposition and laterization of the lower alveolar nerve. Although these procedures have been successful in modifying the patients' anatomy and allowing the insertion of standard height implants, recent systematic reviews (36-38) and randomized clinical trials have emphasized that these surgeries are complex and invasive, and they need a long period of treatment and replacement materials to be used. They also increase the risk of infections, complications, patient morbidity, and costs. For all these reasons, patients are often reluctant to undergo these procedures (39-45).

The use of short implants simplifies rehabilitation in the back areas, minimizing the risks related to the invasion of the lower breast and alveolar nerve; the nonuse of bone augmentation techniques or invasive surgical procedures makes the patient more inclined to undergo the surgery. The aim of our work was to evaluate the effectiveness of the use of short implants in implant-prosthetic rehabilitation by assessing the X-ray and clinical condition with a one-year follow-up.

MATERIALS AND METHODS

Study design

This study is a three-arm comparative analysis with an allocation ratio 1:1:1. It was conducted between October 2017 and February 2019. Patients involved underwent treatment at a private practice in l'Aquila. All patients received detailed information about each treatment and provided informed consent on treatment and compliance with follow-up procedures. The interventions were in accordance with the ethical standards of the revision of the Helsinki Declaration for Biomedical Research involving human subjects ("WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects," 2013).

Study population

Patients enrolled in this protocol are adult patients who needed an implant to rehabilitate the posterior areas of the mandible and maxilla. The following selection criteria have been applied:

1. age >18 years,
 2. good oral condition and systemic health
 3. single edentulia in the posterior region (premolars/molars) of the mandible and maxilla associated with a vertical defect of the posterior ridge,
 4. a period of partial edentulism of less than 3 months before the implant placed
 5. sufficient bone volume to place a short (6mm long) implant,
 6. antagonistic arch to obtain occlusive contact.
- Subjects who had undergone CT scans in the last 5 years and heavy smokers (more than 10 cigarettes per day) were not included in the study.

Surgical procedure

Local anesthesia was obtained by infiltrating articaine (4%) containing 1:100,000 adrenaline. A midcrestal incision was made at the side of implant placement. The mesial and distal aspects of the crestal incision were connected to 2 releasing incisions. Full thickness flaps were reflected exposing the alveolar ridge, and preparation of implant sites was carried out with spiral drills of increasing diameter, under constant irrigation.

Following implant placement, all patients were prescribed oral antibiotic, 2g of amoxicillin and

clavulanic acid each day for 6 days (Augumentin®; GlaxoSmithKline Beecham, Brentford, UK), as it does not experience allergic to penicillin. Post-operative pain was controlled by administering anti-inflammatory every 12 hours for 2 days, and detailed instructions about oral hygiene were given, with mouth rinses containing 0.12% chlorhexidine administered for 7 days. The stitches were removed after 8-10 days.

The two-stage positioning technique allowed to achieve tissue healing and osteointegration in 6 months for the maxilla and 4 months for the mandible. A temporary resin crown was placed to monitor the stability of the implant and to achieve soft tissue conditioning around the implant before the final artifact, made of metal-ceramic.

Statistical analysis

Descriptive statistics were calculated as an average and/or standard proportion to report differences and clinical characteristics of the included samples. Among the groups, average differences and standard deviations were calculated for all the results. The influence of smoking, analyzed as the presence of inflammation and evaluation of color and gum appearance has a physiological value to 0 at T1 and T2 while for pathological values can vary between 1-3, mild degree, moderate and severe. The absence of bleeding assumes as a physiological value 0, value 1 in presence of bleeding and 2 in presence of bleeding and suppuration. The X-ray assessment and clinical probing were evaluated considering 1 mm as physiological value of peri-implant tissues at t1 and t2.

RESULTS

A total of 100 patients, 39 women and 61 men aged 19 to 83 (average 51.89 years) were included and received treatment from October 2017 to November 2019. Among them, there were 34 smokers, 29 former smokers and 37 non-smokers.

100 short implants were placed, 62 in the maxilla and 38 in the mandible and 100 conventionally length implants, with a total of 200 single crowns. The most frequently used diameter for short systems was 4.25 mm with 42 implants, followed by the diameter 5.00 mm with 58 implants; for standard-length systems the diameter used was 4.25 for 46 implants, 3.80 for 40 implants and finally 3.30 with 14 implants. The results at t0 and t1 are shown in Tables I, II and III. No implants were lost during the follow-up period. The results of smoking patients are shown in Table I. The results of the statistical analyses show that the parameters taken into account in the two categories of implants placed in the mandible at t1 are overlapping, while at t2 we observe a better condition in standard implants. In maxilla at t1 we show overlapping results, while at t2 we show better hard tissue behaviour in small implants, whereas the behavior of soft tissues adjacent to standard sized implants is worse. The results of nonsmoking patients are shown in Table II. From the analysis of the results that emerged in the mandible, we show that there are no substantial differences between the

Table I. Smokers

<i>SITE</i> <i>IMPLANT TIPE</i> <i>TIME</i>	JAW (11)				MAXILLA (23)			
	STANDARD	SHORT	STANDARD	SHORT	STANDARD	SHORT	STANDARD	SHORT
<i>RBL</i>	1.093	1.36	1	1	1.30	1.34	1	1.08
<i>PPD</i>	1	1.36	1	1.72	1	1.56	1	1.43
<i>ASPECT</i>	0.63	0.18	0.45	0.54	0.60	0.56	0.60	1.04
<i>BLEEDING</i>	0.27	0	0.18	0.27	0.17	0.21	0.17	0.43

two types of implants studied. While in the analysis of the maxilla, the results show a better behavior of standard sized implants. The results of ex-smoking patients are shown in Table III. The results show that in the mandible both categories of implants taken into account, we observe a worsening of the parameters in the different temporal assessments. On the other hand, in maxilla the attitude remains unchanged for standard implants, while in small implants they improve over time. From the comparison of the three tables, we can see that Table II (non-smoking patients) and Table III (former smokers) are overlapping, while in Table I (smokers) compared to Table II (non-smoking), we see how the parameters of bleeding with the same appearance are better, but this is because we know that smoking causes vasoconstriction (46-50).

Finally, we can say that in the individual study groups there is no difference between the types of implants, and we have favorable and unfavorable conditions. This makes us understand how, regardless of the type of implant, the habit of the subject taken in the study has a higher impact.

DISCUSSION

The definition of short plant, in the literature and by various authors, has been described as an implant with a length of less than 11 mm (das Neves et al. 2006) ,10 mm (Morand & Irinakis 2007) (26), or 8 mm (Renouard & Nisand 2006). In the past, these implants have been associated with low survival and an unpredictably long-term result. The success of short implants could depend on several factors, biological or prosthetic, including bone density, seating, implant placement and smoking habits.

This work investigated the influence that cigarette smoking can have on soft and hard tissue stability adjacent to standard sized and small implants, using three groups of patients. The influence of smoking in the state of general health, but especially at the level of dental and implant health, has been emphasized in several reviews and clinical investigations. In the description of the new periodontal classification, cigarette smoking is recognized as the predominant risk factor for periodontal disease and peri-implantitis. Tonetti et al. in 2018 defined how the

Table II. *Non-smokers*

<i>SITE</i> <i>IMPLANT TIPE</i>	JAW (16)				MAXILLA (21)			
	STANDARD		SHORT		STANDARD		SHORT	
<i>TIME</i>	T1	T2	T1	T2	T1	T2	T1	T2
<i>RBL</i>	1	1.12	1	1.18	1	1.04	1	1.23
<i>PPD</i>	1	1.31	1	1.25	1	1.09	1	1.38
<i>ASPECT</i>	0.75	0.68	0.43	0.5	0.85	0.61	0.66	0.52
<i>BLEEDING</i>	0.37	0.18	0.06	0.18	0.28	0.14	0.38	0.19

Table III. *Former smokers.*

<i>SITE</i> <i>IMPLANT TIPE</i>	JAW (11)				MAXILLA (18)			
	STANDARD		SHORT		STANDARD		SHORT	
<i>TIME</i>	T1	T2	T1	T2	T1	T2	T1	T2
<i>RBL</i>	1.09	1.27	1	1.09	1	1.05	1	1
<i>PPD</i>	1	1.63	1	1.36	1	1.55	1	1.11
<i>ASPECT</i>	0.54	1	0.54	0.54	0.22	0.83	0.66	0.44
<i>BLEEDING</i>	0.27	0.63	0.18	0.09	0	0.33	0.27	0.05

number of cigarettes consumed by each individual patient can be an indicative factor of the degree of periodontal disease. Smoking also affects the periodontal disease, in fact, in a systematic review based on 107 articles shows how the insertion of implants in smoking patients significantly increases the failure rate, favoring risk of post-operator infections, as well as marginal bone loss.

The results reported in this study seem to confirm this evidence, i.e., the risk of inflammation of peri-implant tissues in smoking patients, unlike nonsmoking subjects who reported a lower risk of inflammation in that study (51-54). A study of 500 patients, divided into smokers and non-smokers, tried to establish the influence of smoking on the survival of the implant. This study shows that the loss of crestal bone around dental implants is significantly greater in smokers than in non-smokers. This study confirms the results highlighted by the comparison reported in our study, in fact it can be highlighted the difference in probing depth in the results reported in the tables for smokers and non-smokers. Our clinical study also included the behavior of small implants due to the great use in clinical practice. From the reported results of these implants, we can define that there is no significant difference in the behavior of peri-implant tissues adjacent to standard sized implants, in that study the prosthetic load is not lost so we are not able to give indications regarding the predictability of short implants under prosthetic load. Smaller implants have been widely used in different clinical situations over the past decade but are still being studied today. In a further study cited by Berglundh et al. (4) describes how bone reworking and bone tissue formation depends on blood and clot formation, showing how smoking changes blood flow in the neck head region and oral cavity. This study can be considered as a response to the differences between smokers and non-smokers, where the difference shown in the table. In terms of probing depth and clinical aspect of peri-implant tissues, this is explained by the fact that in smoking patients, irrespective of the number of cigarettes consumed daily. The smoke-induced vasoconstriction reduces the excellent and physiological bone neo apposition around the implants and above all by varying the

vascularization of soft tissues varies the structural integrity of the gum tissue predisposing all those factors that induce inflammatory processes that can induce a mucositis up to a peri-implantitis, in turn reducing the predilection. In this case also, we would like to point out that there are no significant differences in the analyzed parameters of short implants, because the biological factors necessary for implant osteointegration remain the same.

Our goal was to be able to evaluate and to know, analyzing the data present in the literature, the effect that smoking can have on the stability of peri-implant tissues, comparing biological responses in nonsmoking, smokers and former smokers with standard and small implants. The reported data and the analyzed studies are insufficient to define the influence of smoking in the assessment of the long-term predictability of implants, but we trust in the expertise of the scientific world.

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Fibre post cementation: instrumental analysis

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The loss of dental substance due to the presence of decay, to wide conservative repairs, to the preparation of prosthetics or to enamel-dentine fractures, often causes an important structural weakening in the element that is endodontically treated (1-15). It is therefore necessary to use endocanal posts in the coronal reconstruction. Mineral fibre posts and epoxy resins that are available at present, fixed with resinous cements, thanks to their excellent integration, exceed the concept of reconstruction, meant as assembly of heterogeneous materials, arranging homogeneous materials according to a similar flexibility module (16-18). This allows to get a “post dentine cement monobloc”, which is ideal for the morpho-functional tooth resetting (19-35). The aim of our experimental study was to analyse and compare the surface characteristics of same posts that are available at present by observing them in their relationship with cement and dental materials (36-44).

MATERIALS AND METHODS

To create instrumental tests, some of the nowadays marketed posts have been used: carbon fibre posts CARBONITE (H. Nordium sa.), COMPOSIPOST (Cabon); in glass fibre ENDO LIGHT POST (RTD), GLASSIX (H. Nordium sa) and posts in mineral fibre FOTOTECH, TECH 2000, TECH 21 (Isasan). In the first test, post surfaces have been observed through scanning electron microscope at a controlled pressure (Phillips 30 XL CP) without metalizing. The created images were later elaborated through Software Image Pro Plus 3.0 Media Cybernetics which allows their elaboration and their quantitative and qualitative analysis. The use of the Pro Plus Image allowed us to highlight through a graph the surface roughness of the single posts. This graph comes out from the different thickness existing between the surface post fibres and the epoxy matrix in which the fibres are plunged. In the second experimental study, in

14 teeth, extracted by orthodontic or paradontal treatment that were treated by Ni-Ti (Quantec) instruments through crown-down technique and filled through the gutta-percha vertical condensing technique have been placed the posts with Panavia F FLOW CHART SHEET (KURARAY CO LTD) cement according to the instructions that were foreseen by their maker through self-polymerization even in translucent post, in order to realize the same conditions in each of them. The obtained samples have been immersed in 5% fucsina colouring matter for 48 hours, then longitudinally cut and observed through stereomicroscope Wild M8 (Leitz) in order to value their level of marginal infiltration.

In the last instrumental test, 14 teeth that were prepared according to the same criteria have been cut on two orthogonal planes to tooth major axis, near tooth inferior third and at enamel-cement junction level and the sections have been observed by metallization, on SEM with the aim of analysing the cement-dentine interfaces.

Key words: endocanal post, prosthetics, post-cement-tooth

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RESULTS

The graph analysis provided by the elaboration of images by Software Image Pro Plus, highlights that orientation and spacing of fibres are more

homogenous and even among the samples of carbon fibre posts than among the mineral and glass ones.

The SEM analysis has revealed a discontinuity in any sample, in order of micron, in post cement fastening interface.

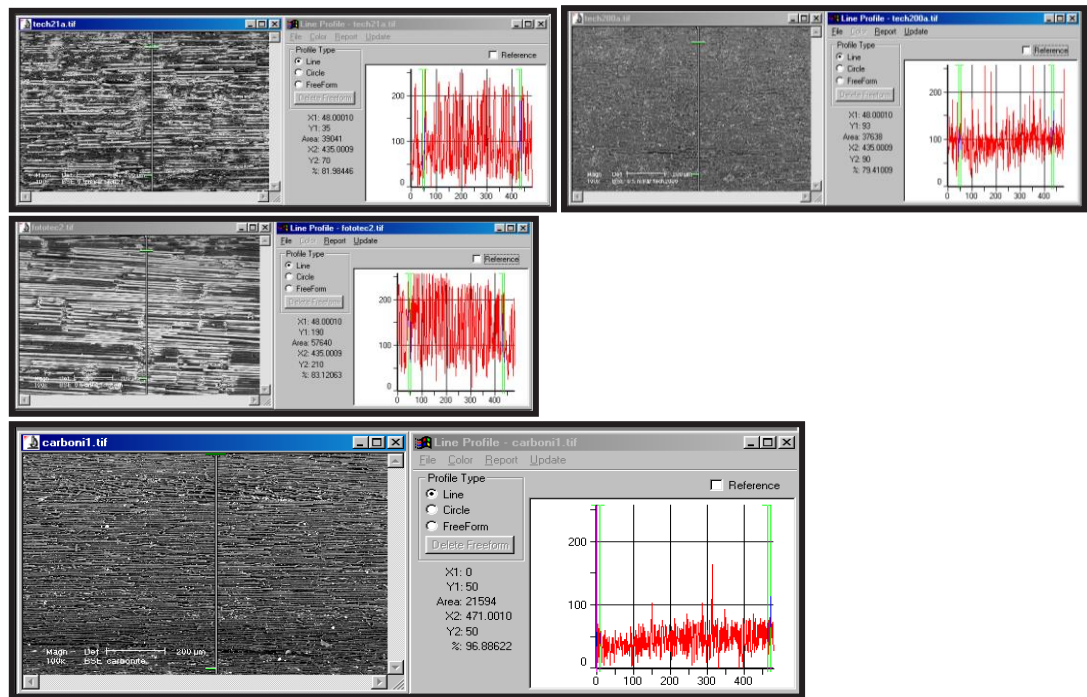


Fig. 1. Graph analysis provided by the elaboration of images by Software Image Pro Plus.

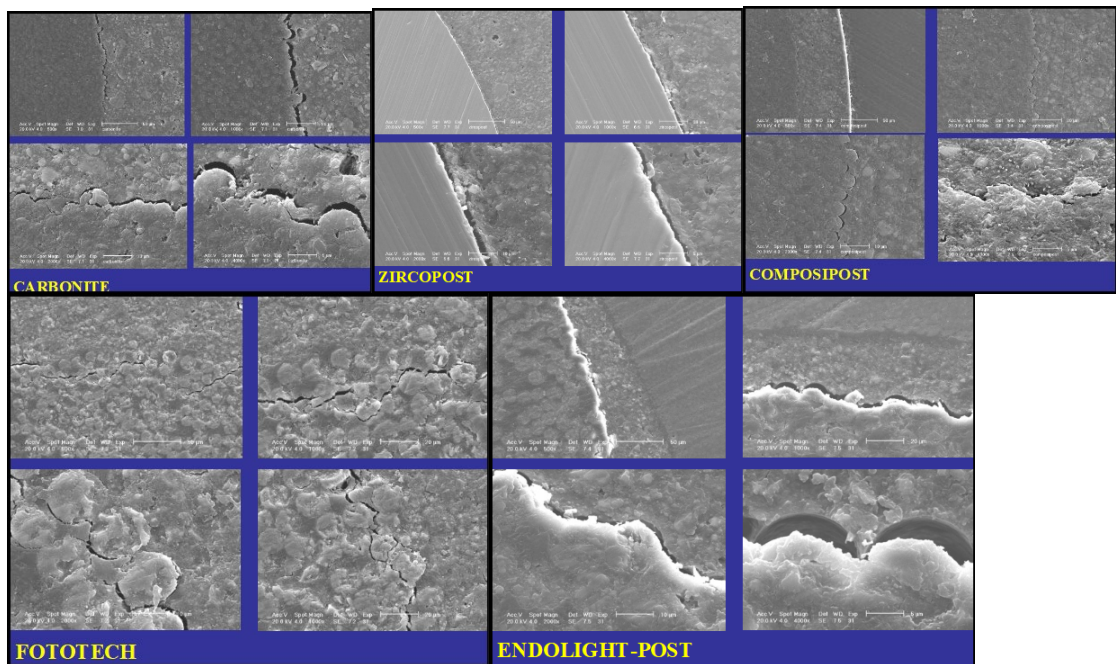


Fig. 2. The SEM analysis.

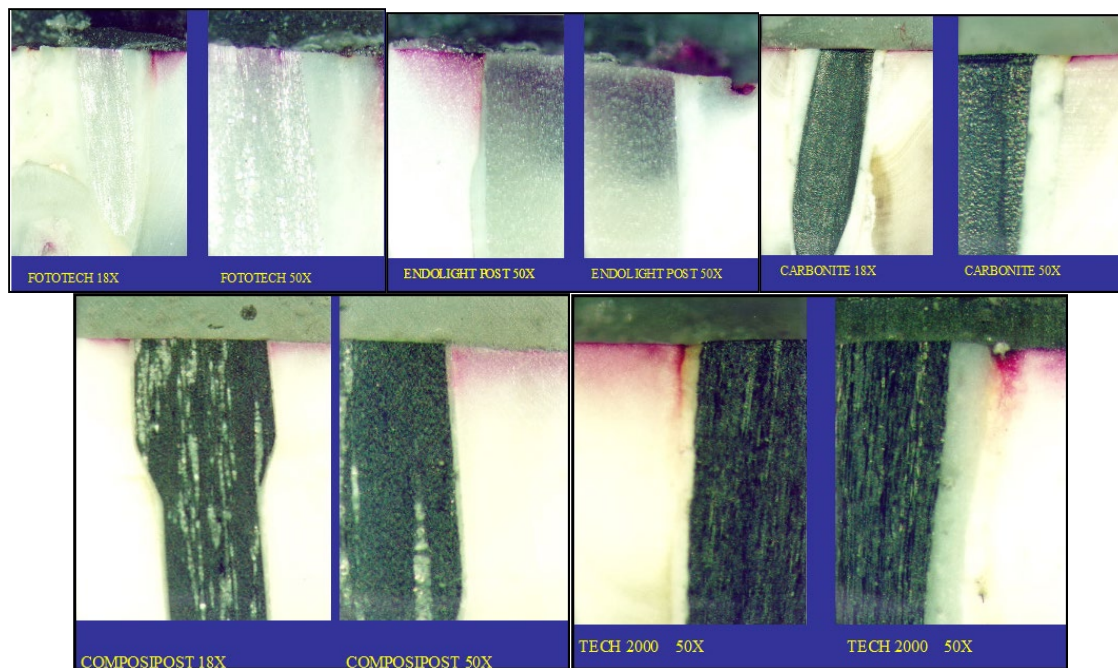


Fig. 3. *The stereomicroscope analysis..*

This discontinuity concerns contact zones between post fibres and resinous cement, while there is a contact between cement and post matrix: in fact, cement gets a semicircle shape that appears like that of post surfaces.

The stereomicroscope analysis of longitudinal cuts of teeth samples immersed in fucsina dye revealed no infiltration between post and cement fastening in any tested sample. The dye traces in some cuts are due to the permeability of the dentine-tubuli next to canal light.

CONCLUSION

Leaving out aesthetic characteristics, carbon post fibres are those that gave instrumental tests the best surface features for uniformity, orientation and thickness of fibres (45-50). The analysis of the cement layer around the post, points out that it is important not to exceed 15-100 micron optimal cement thickness, beyond which the polymerisation contraction leads to microfractures. (51-54). The

tested interface quality has a fundamental role in the rehabilitation system in lashing time, through post-cement-tooth and it highlights the possibility of the achievement of a post-cement-tooth as a monobloc. Furthermore, the realization of a good adjustment of the post to the canal is also fundamental, even if this is quite hard because of the discrepancies of the pulpal zone of its anatomy.

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Qualitative and quantitative analysis of the CTX in relation to the period of intake of bisphosphonates: A systematic review

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The aim of this systematic review was to determinate the true value of C-terminal cross-linking telopeptide test (CTX) in patient who takes Bisphosphonate. A comprehensive search of studies published up to March 2020 and listed in the PubMed/MEDLINE and Cochrane Library databases, was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search identified 99 publications; 6 studies were finally deemed eligible for inclusion according to the study criteria. These studies included a total 104 patients and was selected 101. The CTX value in the various study groups is less than 150 pg/ml. There is a difference between the age of the patient and the period of taking the drug. This systematic review indicates that the CTX test has diffent predictive value in determining the risk of osteonecrosis in patients taking bisphosphonate compared to previus standard.

Osteonecrosis of the jaws is one of the most common complications in patients taking bisphosphonates, these drugs are taken to avoid metastasis or multiple myeloma. They are also taken in patients with osteoporosis (1-2). The use of bisphosphonates (BPs) as drugs for the treatment of bone pathologies originates from a discovery in the early 1960s which was due to Neuman and Fleisch (1961), who while studying the calcification mechanism induced by collagen, realized that organic fluids such as plasma or urine contained calcification inhibitors. Specifically, they showed how inorganic pyrophosphate, present in serum and urine, could prevent calcification by binding the forming hydroxyapatite crystals (3). The first descriptions of bisphosphonate-related osteonecrosis of the jaw (BRONJ) were reported in 2003 by Marx (4). A wide-range study on osteonecrosis indicates an incidence of BRONJ between 0 to 27.5%, relevant for individuals subjected to intravenous amino-

bisphosphonates with an average incidence of 7% (5). The American Association of Oral and Maxillofacial Surgeons (AAOMS) define BRONJ as an exposed or probeable bone in the maxillofacial region without resolution for longer than 8 weeks in patients treated with an antiresorptive or an antiangiogenic agent who have not received radiation therapy to the jaws (6). Osteonecrosis of the jaw bones is a disabling necrotizing pathology with a progressive nature and with a low tendency to heal, which affects only the maxilla in 68% of cases, the jaw in 28% and both bones in 4% of cases. The mandible is more commonly affected than the maxilla (2:1 ratio), and 60% of cases are preceded by a dental surgical procedure (7).

A lot of types of bacteria (8) have been isolated, generally belonging to the resident flora of the oral cavity and germs commonly isolated in periodontal diseases and dental abscesses (9). Among these the most common one is Actinomyces. Spontaneous

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or provoked bleeding is possible. It has a tendency to extend to adjoining regions (10), such as (a) the skin (with secreting skin fistulas), (b) the mandibular canal, (c) the maxillary sinuses, (d) the pterygo-palatine fossa, (e) the oral floor and (f) the submandibular regions.

The selectivity for the jaw has been studied but we still do not have a specific cause but we have added many theories at this point still equally useful: (i) physiologically higher bone turnover of the jaws compared to the remaining skeleton (11) (ii) terminal vascularization of the jaw (12) (iii) presence of a thin mucoperiosteal coating to protect the underlying bone tissue, easily subject to traumatism (11) (iv) peculiar biofilm, microflora of the oral cavity (13) (v).

There is general consensus that dento-alveolar surgery and simple tooth extractions are the most significant risk factors associated with osteonecrosis in cancer patients taking anti-resorption drugs (14). Implant placement in cancer patients (15) is considered a potential trigger for osteonecrosis, although the true risk has not yet been assessed. Dental and periodontal infection significantly increases the risk of osteonecrosis in cancer patients undergoing anti-resorptive therapy.

Indeed, periodontal disease was diagnosed in 84% of cases (16) in a large sample of patients with osteonecrosis. However, periodontal disease is commonly observed in the general population aged > 40 years, which may represent a confounding factor in the epidemiological association assessment.

The first clinical manifestations of osteonecrosis are characterized by unexposed alveolar bone necrosis which can mimic clinical and radiological manifestations (17) of periodontitis (tooth mobility, bone loss, loss of attachment and presence of pus), which can lead to incorrect diagnoses and overestimation of the association between osteonecrosis and periodontitis.

There are many factors influencing the onset of BRONJ: i) ADMINISTERED DRUGS, in hematological and oncological patients, zoledronic acid appears to carry a statistically higher risk of ONJ than pamidronate. Insufficient data do not allow a definitive comparison with ibandronate (17-18) although it appears to be low risk, Clodronate is

associated with a lower risk of ONJ than zoledronic acid ii) TYPE OF ADMINISTRATION (IV or OS): there is a higher risk with regard to intravenous intake especially of amino-bisphosphonates (14) this factor can be closely related to their prevalent use in patients with cancer, iii) DURATION OF INTRAVENOUS N-BP TREATMENT: on average, patients with ONJ were treated for longer periods than those without ONJ. The duration of intravenous treatment with N-BP is generally related to the total dose of drug administered. In a recent literature review, the mean / minimum time for the onset of ONJ was 1.8 years and 10 months (19) iv) COMBINED THERAPY WITH OTHER DRUGS: the combined use of the latest generation antiangiogenic agents and N-BP has recently been associated with an increased risk of developing ON (14). v) INDIVIDUAL GENETIC FACTORS: the largest study currently conducted (n = 94 ONJ cases) suggests that class II MHC polymorphisms is a genetic risk factor related to the development of ONJ (20) vi) BONE MINERALIZATION DISORDERS: a single study demonstrated the possible contributing effect of secondary hyperparathyroidism after administration of BP to the development of ONJ (21). Recently, a strong association between osteomalacia and ONJ was identified and the potential triggering effect of vitamin D deficiency on secondary hyperparathyroidism and bone mineralization defects has already been demonstrated in animal models and is currently being studied (22) Marx, in 2007, suggested the use of the "C-terminal cross-linking telopeptide" (CTX) as an indicator of BRONJ risk. The C-terminal telopeptide of collagen type 1 (CTX) is a marker of bone resorption. It is a peptide fragment that is formed starting from the C-terminal end of the proteins forming the bone matrix. Marx analyzes the CTX value of 30 patients with BRONJ. The authors reported that a value less than 100 pg / ml represented a high risk of BRONJ; from 100 to 150 pg / ml a moderate risk; higher than 150 pg / ml minimum or no risk. There are many studies that refute Marx's theory of CTX.

The aim of this prospective study was to determinate the efficacy of CTX test to prevent the development of BRONJ.

MATERIALS AND METHODS

This systematic review was structured according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines²³. The following inclusion criteria were applied:

a) randomized controlled trial or prospective study or retrospective study; b) performance of the CTX test for each patient include in the study; c) study with more than five patients; d) patients taking oral and/or intravenous bisphosphonates; e) study with number list of patients; f) that include the period of taking the drug; and g) that include the sex and the age of the patients.

The following exclusion criteria were applied: i) studies with patients who had received radiotherapy to the head and neck region; ii) single case reports; iii) no presence of age or sex; iv) study with CTX average; v) drug not specified; and vi) period of taking a drug not specified.

The PICOS Table I (patients, intervention, comparator, outcomes, study design) question recommended in the PRISMA statement was defined as follows: 1) population: patients taking bisphosphonates; 2) intervention: assess the level of CTX based on the hiring period; 3) comparison: standar values of CTX; 4) outcome: analyze in relation to type of intake if the CTX is affected by the period of taking a drug; and 5) study design: randomized controlled trial or prospective study or retrospective study.

The author performed the selection of articles. Searches were performed in the PubMed/MEDLINE and Cochrane Library databases for articles published up to January 2020. The key words used in this study were: “*ctx & osteonecrosis*”. The studies were first classified according to the inclusion and exclusion criteria.

After performing searches in the selected databases, a careful analysis was performed to identify any cases of disagreement between the authors. Studies were selected based on their titles and abstracts and assessed against the

inclusion and exclusion criteria. After the first selection stage, the selected articles were analyzed based on their full content. The research strategy was according to PRISMA (Fig. 1.).

RESULTS

The keyword research provides 80 results on PubMed / MEDLINE and 19 on The Cochrane Library, for a total of 99 articles. Of these 99 articles 8 was duplicates, so total selected articles were 91. Studies were selected for analysis based on their title and abstract, and in accordance with the inclusion

Table I. PICOS=patients, intervention, comparator, outcomes, study design.

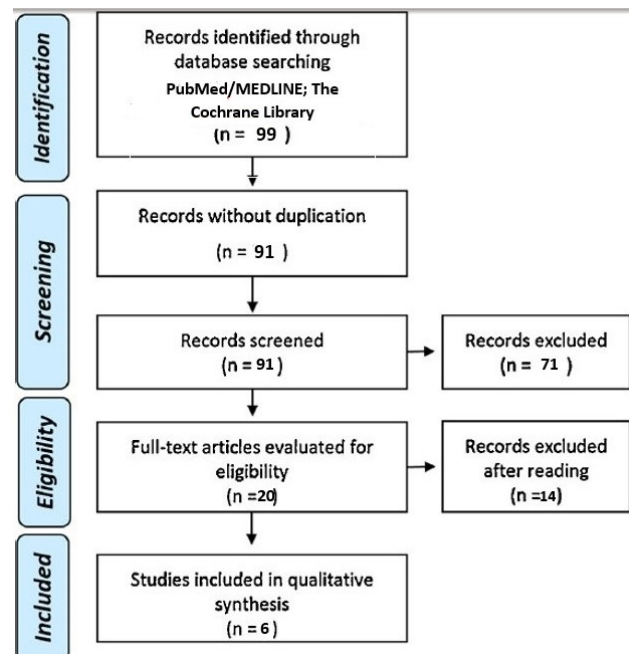


Fig. 1. PRISMA flowchart.

P	PATIENTS TAKING BPs
I	Assess the level of CTX based on the hiring period
C	comparison with standard values (Marx RE 2007)
O	analyze the ctx value and the change in value
S	randomized controlled trial or prospective study or retrospective study

and exclusion criteria. This process produced 20 studies for full text examination. Following the full-text review, 14 studies were excluded as they failed to meet the inclusion criteria. Thus, 6 studies were analyzed that form the basis of this review (Table II). From these 6 studies 104 patients were listed, of these 3 were excluded because the CTX value = 0 pg/ml or > 1000 pg/ml. The average CTX values was 126 pg / ml on the total of 101 patients, the average intake period is 50.3 months. The most common drug is ALENDRONATE taken by 88 patients, RISENDRONATE is taken by 8 patients, the intake of intravenous drugs is present in 7 patients, respectively 6 ZOLEDRONIC ACID and 1 PAMIDRONATE. The included studies run from 2007 to 2016, there are no recent studies that have included a list of patients with unique CTX values. 5 studies are prospective and only one is retrospective.

The author most involved in these studies is Yong-Dae Kwon, Director of Catholic Institute for

Healthcare Management/Professor (2009, 2011 and 2016), the other studies are conducted by O'Connell conducted in Dublin at a University-based oral and maxillafacial surgery (24) in 2012, Kunchur professor of Plastic and Reconstructive Surgery in Australia, in 2009, Marx, Professor of Surgery and Chief in Miami, in 2007.

DISCUSSION

There are many variables associated with CTX assessment, in the study of CTX and PIMP assessment (31) we know how the variables that influence blood sampling are many and although not having a lot of power alone in association they can modify the evaluation.

First of all, it would be the case to standardize the time and the modalities of the blood sampling. Among the most important factors we certainly have

Table II. *List of studies analysed in this review.*

AUTHOR	NUMBER PATIENS/ SEX (M/F)	AGE	TYPE OF DRUGS	INTAKE PERIOD	CTX VALUE
O'Connell et al ²⁵	22/1	59 (± 9.68)	19alendronate 2zoledronic acid IV 2risendronate	30,39 (± 16.40)	180(± 94.0)
Kwon et al ²⁶	0/9	76,6 (± 9.36)	6 alendronate 2risendronate 1 zoledronic acid IV	38 (± 33)	84 (± 36.7)
Kunchur et al ²⁷	8/4	72,9 (± 10.20)	6 alendronate 2risendronate 4 zoledronic acid IV	51,25 (± 39.7)	210(± 127)
Kwon et al ²⁸	16/2	74,3 (± 6.8)	17 alendronate 1 risendronate	47.3 (± 25.2)	111.7(± 74,2)
Marx et al ²⁹	17/0	64.8	16 alendronate 1 risendronate	79.1 (± 26.0)	72.8(± 25.2)
Kwon et al ³⁰	22/1	73,65(± 7.10)	23 alendronate	57.9(±31.3)	93.1(± 49.4)

the circadian rhythm, in fact we know that the CTX has high levels after midnight and low in the early afternoon (32), the power supply causes a decrease of about 20% of the circulating ctx, therefore it underestimates the value (33).

Age and sex are high impact variables, we know that ctx is high in childhood, decreased in early school age and then increased during puberty (34-35), the period in which it is lowest in men is in the fifth decade, fourth decade in women and then fluctuations in menopause (36), for men instead there is the possibility after 70 years of an increase in the value of CTX or at most of a constant trend (37).

The woman's state of menopause is also of great importance, in the post-menopausal phase the CTX increases (38). A study conducted in Spain (39) describe that CTX is closely related to hormones, in fact in this study conducted on women aged between 16 and 25, divided into two groups, one that does not take contraceptives oral and a group that takes it, it is clear that those who take the drug have a lower level of CTX.

Also, the Osteoporosis is correlated with hormones, it is estimated that osteoporosis affects around 5,000.000 people in Italy, 80% are postmenopausal women. (Italian ministry of Health). A study on 17251 patients conducted by the "International Osteoporosis Foundation and European Calcified Tissue Society Working Group" (40) found that after 3 months of taking bisphosphonates the CTX drops significantly, the alendronate on average causes a decrease in the CTX of 73%, l'ibadronato of 81% and Risedronate of 68%. Marx describes the variation of the ctx after a 6-month drug holiday, 17 patients are taken as the object of study, the average of their CTX is 71.2 pg / ml, after 6 months it is 231 pg / ml.

In this systematic review we try to indicate the

parameters for standardize the CTX value. In a first investigation Table 4, the patients (84) were divided into groups by age and by the period of taking the drug, the age groups are ≤ 59 years, 60-69 years, 70-79 years and 80-89 years, the period of taking the drug is <36 months and ≥ 36 months. The investigation shows that only two groups have a CTX > 150 pg / ml and for both groups the common discriminant is an intake of less than 3 years. For the other groups the value is less than 150. Of these patients we do not know how many have osteonecrosis and how many, however the value of 150 pg / ml is not considered a correct parameter for this evaluation as a discriminant. The sample is small, but the data shown indicate that only two groups have value of CTX > 150 pg/ml, so we could hardly ever perform low-risk surgery. In the first two groups (< 59 years, 60-69 years) that have a similar population, we see that subjects who have been taking the drug for less than 36 months have higher CTX values than those who have been taking the drug for more than 36 months.

A second investigation Table 5 (101 patients) instead related the period of taking the drug, regardless of the age of the subject or sex, the results are divided into <20 months, 20-39 months, 40-59 months, 60 -79 months, 80-99 months, > 100 months. Only the first group shows a ctx > 150 pg/ml, all the others have a lower value. The results obtained in this study show us that only one group has a CTX value with minimal risk, all the others are instead at medium risk; moreover, we are confirmed that at low risk there are those who have been taking the drug for relatively little time, of course the standard deviation has also been included because the values have a fair oscillation. The previously cited research showed that it was still only 3 months from the start of tak-

Table III. CTX value in relation to age and intake period.

AGE(years)	<59 (13F/ 1M)		60-69 (20F/ 6M)		70-79 (22F/ 6M)		80-89 (13F/ 6M)	
INTAKE	<3 anni	>3 anni	<3 (14)	>3 (12)	<3 (4)	>3 (24)	<3 (4)	>3 (15)
PERIOD	(8)	(6)						
(years)								
CTX	176	131	137	122	97	120	203	138
(pg/ml)								

ing the drug to have a high decrease in the CTX value. However, it can be noted that the decrease is not constant but settles in all the other groups, remaining in the range of 101 pg / ml to 134 pg / ml with an average of 114 pg / ml.

The last study table 6 have a low-quality relevance, that because we have a small sample size, we also assessed how the parameter is influenced by the type of drug. The drugs as mentioned are ALENDRONATE (88 patients), RISENDRONATE (7), ZOLEDRONIC ACID (6).

It's difficult to critically analyze these data, the samples are very disproportionate to each other, not only as regards the number of subjects but also as regards the intake period. In the case of zoledronic acid the intake period is 27 months on average, for alendronate 53 and Risedronate 37, they are also different drugs that have different power of action and

mode of intake (41). Despite this we see that by setting an ideal parameter of CTX before administration of the drug, common to all subjects and related to the period of intake and the potency of the drug, the data obtained are the expected ones, as zoledronic acid is the more powerful but it is taken for less time, compared to Risedronate which has an average power between the three drugs taken into consideration but taken for longer and alendronate which is the least powerful, 10 times less than Risedronate and 100 times less than zoledronic acid (42-43).

CTX is one of the few parameters that has not been absolutely excluded to assess the risk of osteonecrosis associated with bisphosphonate drugs, in the evolution of the studies certain bone markers have been introduced and subsequently eliminated.

In the literature, however, there are no articles confirming the usefulness of CTX, in the systematic review of Del Prà made in 2017 it is stated that CTX does not have a predictive value, the review is based on a total of 1447 patients and after the evaluation of the CTX, which considered the parameters imposed by Marx as reference parameters, saw the development of the pathology of osteonecrosis in only 24 patients, corresponding to 1.7%.

Another systematic review conducted by Awad in 2019, gives us an idea about the test itself stating that the sensitivity of the CTX test, considering a value greater than 150 pg/ml as a positive value, is 34.26%, while the sensitivity of the test is 77.08%. At the conclusion of this study it is therefore stated that the use of CTX before dental procedures is unjustified and that the value greater than 150 pg / ml is not predictive for determining the development

Table IV. *CTX value in relation to intake period.*

INTAKE PERIOD in months (patients number)	CTX	STD DEV
<20 (15)	177	132,7064
20-39 (34)	119	99,99702
40-59 (15)	103	133,0314
60-79 (16)	134	123,019
80-99 (9)	101	149,3557
>100 (12)	116	134,9194

Table V. *CTX value in relation to type of drug.*

DRUG	CTX (pg/dl)	DEV STD
ZOLEDRONIC ACID	145	114,5059
ALENDRONATE	124	84,11
RISENDRONATE	89	87,77813

of osteonecrosis. The study says that you must start looking for new markers.

The results obtained show us that the value of 150 pg / ml is in fact disproportionate, since it is difficult to find patients with higher values and above all it is impossible to think that for lower values, we would always encounter osteonecrosis (43-51).

The study carried out must necessarily be in-depth and must necessarily concern a larger sample but invites to formulate values in relation to more stringent parameters, considering not only the drug but also the age of the subject and the period of intake in the first place.

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Early childhood caries in Tirana, Albania

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Early Childhood Caries (ECC) is a destructive form of caries that affects the temporary teeth and may be present in children of very young age as early as teeth erupt. The distinctive characteristic of caries in this age is that it affects initially a limited number of teeth which if not treated in time spread rapidly across all deciduous teeth. The purpose of this study is to determine the prevalence and severity of early childhood caries in children 3-5 years old in the public kindergartens in Tirana.

This is a transversal epidemiological study type (cross - sectional). The superiority of this type of study consists in the acquisition of point information (at a certain point in time) about the size (magnitude) or the prevalence of a particular characteristic or specific health event in the population of interest (1-3). It is this feature of transversal studies in this paper which was used to assess the prevalence of early childhood caries in children aged 3-5 years in the city of Tirana. The entire study duration was about 3 years. It began in September 2011 and was completed in late 2014.

MATERIALS AND METHODS

The intraoral examination process and registration of children in selected kindergartens lasted for a period of 3 months. The sample size was estimated by WIN program - PEPI. According to sample size calculations done a priori and based on conservative assumptions, the minimum sample size for this survey was 734. Conservative assumptions tend to maximize sample size. In this regard, a size of 734 children was estimated as the maximum possible to achieve the purpose and objectives

of this study. However, it was decided to include a number of 1000 children to increase the power of the study and was considered the refusal rate, the absence of children in the kindergartens on the day of the examination and the possibility of exclusion of children who did not submit their questionnaire completed by parents.

In total the study included 904 children, who were present in the respective kindergartens on the day of examination, children who did not refuse the examination and had submitted the questionnaire completed by parents. So, the participation rate in the study was 90.4% and the final sample of this study (n=904) was of a much larger size than the minimum required (n=734) to achieve the purpose and objectives of this study. The collection of the necessary information for this study was conducted with the approval of the Regional Education Directorate in Tirana and the relevant departments of kindergartens selected for this study. The collection of these data was conducted through structured questionnaires completed by parents and children's intraoral examination in each kindergarten.

Statistical analysis

Statistical analysis was conducted in SPSS

Key words: Pediatric Dentistry, Early Childhood Caries, Survey, Deft Index, Care Index

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(Statistical Package for the Social Sciences, version 15.0). Size of central tendency was calculated for numerical variables (arithmetic average, median and the mode) and the dispersion size (variance, standard deviation and interquartile distance). Absolute values and their respective percentages were calculated for categorical variables. Comparison of the average values between the two groups was made possible through the test “t” test of Student and Mann - Whitney (a non-parametric version of the test “t” Student). Comparison of proportions (percentages) for categorical variables was made possible through Hi square test and Fisher’s exact test. The general linear model was used to calculate the average values of the indexes. Average values and respective confidence interval 95% were calculated for all indicators. In all cases, it was considered statistically significant a value of $p \leq 0.05$. The results were presented in absolute values and in percentage and illustrated by the tables.

RESULTS

The coefficient of compatibility beyond chance

(Kappa Index) between the two experts involved in the study is presented in Table I. As may be seen from the table, this coefficient ranges from 0.699 to 0.802 of the deft index. These results represent a moderate to high compatibility, which means that the examination is reliable.

The prevalence of early childhood caries

Table II presents early childhood caries (ECC) in the children involved in the study, the absolute number, average value, standard deviation, minimum and maximum value. By analyzing the collected data, we were able to report the overall prevalence of early childhood caries in children included in this study. The prevalence of Early Childhood Caries (ECC) in 3- to 5-year-old children in Tirana public kindergartens was $823/90 = 91\%$.

Table III presents in detail in absolute numbers and relevant percentages, the distribution of early childhood caries according to the ECC classification defined in 2008 by the AAPD (18). The table shows that most of the children involved in this study (62.1%) suffer from a severe form of the ECC.

Table I. KAPPA Index (compatibility beyond chance) Variable

Variable	KAPPA Index
deft index	0.802
ft index	0.701
et index	0.766
dt index	0.699

Table II. Early childhood caries in the study subjects.

Number	904
Average	2.7
Standard deviation	0.749
Minimum	1
Maximum	4

Deft index

By analyzing the data collected we were able to report average values 6.2, 0.07 and 0.2 respectively for specific indexes dt, et and ft, and the average value 6.45 of the deft index. Table IV. presents the average values for dt, t, ft and deft indexes, and the standard deviation for each index, while Table V shows the distribution date, t, ft and deft indexes in children involved in the study.

Care Index

Table VI presents the Care Index (CI) value in absolute numbers and average value, together with deviation or standard deviation and the minimum and maximum values. These data show that the average value of Care Index is 3.1%, meaning only 3.1% of the affected deciduous teeth are treated.

DISCUSSION

The results of our study reported that the

prevalence of early childhood caries in children 3- to 5-years-old in public kindergartens in Tirana was 91%, a value that is very high considering that Tirana is the capital city of Albania. Various authors report that the prevalence of caries in preschool has declined in most of the developed countries (4, 5), but in developing countries and even some developed countries there is a growing trend (6, 7). Conclusions based on the results from these studies show that early childhood caries are considered an epidemic in developing countries (8-12). Increasing urbanization and the rapid changes related to eating habits are probably the factors that contribute most to the deterioration of dental health in developing countries (13-15). In a recent survey of the literature review conducted by the authors in 2013, important data was extracted, data obtained from various studies regarding the prevalence of early childhood caries and deft index (16). The maximum age of the children involved in these studies, selected for review is 5 years which coincides with the maximum

Table III. Distribution of ECC in the study subjects based on the AADP classification.

Variable	Number	Percentage (%)
ECC Form:		
Without ECC	81	9.0
Simple ECC	184	20.4
Severe ECC	561	62.1
Maxillary ECC	78	8.6

Table IV. Average value of dt, et, ft and deft index.

	dt index	et index	ft index	deft index
Number	904	904	904	904
Average	6.20	0.07	0.20	6.45
Standard deviation	4.236	0.424	0.781	4.258

age of children part of our study. A closer look at the literature review showed that the prevalence of ECC has a very big margin in various places ranging from 17 to 94%. (17-20).

Meanwhile, a study done in Kastriot municipality

in Kosovo showed that the prevalence of ECC in children 3- to 5-years-old was 25% and the dmft index was 12.5 (21) These comparative data, including the data from our study, are presented in detail in Table VII.

Table V. *Distribution of dt, et, ft and deft index in children included in the study.*

Value	Index*			
	dt	et	ft	deft
0	87 (9.5)	867 (95.8)	826 (91.4)	80 (8.8)
1	26 (2.9)	23 (2.5)	32 (3.5)	24 (2.7)
2	102 (11.3)	11 (1.2)	19 (2.1)	92 (10.2)
3	36 (4.0)	2 (0.2)	11 (1.2)	37 (4.1)
4	116 (12.8)	-	8 (0.9)	105 (11.6)
5	47 (5.2)	-	6 (0.7)	49 (5.4)
6	95 (10.5)	-	1 (0.1)	99 (11.0)
7	46 (5.1)	-	-	38 (4.2)
8	113 (12.5)	-	1 (0.1)	125 (13.6)
9	37 (4.1)	1 (0.1)	-	38 (4.2)
10	73 (8.1)	-	-	78 (8.6)
11	17 (1.9)	-	-	15 (1.7)
12	52 (5.8)	-	-	62 (6.9)
13	8 (0.9)	-	-	10 (1.1)
14	24 (2.7)	-	-	28 (3.1)
15	1 (0.1)	-	-	1 (0.1)
16	8 (0.9)	-	-	7 (0.8)
17	1 (0.1)	-	-	-
18	3 (0.3)	-	-	3 (0.3)
19	-	-	-	-
20	12 (1.3)	-	-	13 (1.4)
Total	904 (100)	904 (100)	904 (100)	904 (100)

Table VI. *Value of CI (Care Index).*

Number	904
Average	3.1
Standard deviation	3.52

Table VII. *Prevalence of e ECC and dmft/deft index in several studies.*

Study (authors, year)	Place (city, country)	No. of children	Age (years)	% (deft>0)	dmft/deft (avg. value)
Mantonanaki et al. (2013)	Attica (Greece)	605	5	17	3.23
Ferro et al. (2006)	Veneto (Italy)	290	5	27	1.34
Mora et al. (2000)	Granada (Spain)	173	2 – 3	37	3.52
Pitts et al. (2007)	Scotland (UK)	11161	5	46	2.16
Wigen et al. (2011)	Oslo (Norway)	1348	5	11	1.4
Ferreira et al. (2007)	Canoas (Brazil)	1487	0 – 5	40	1.53
Simratvir et al. (2009)	Ludhiana (India)	609	3 – 5	59	4.76
Sufia et al. (1974)	Lahore (Pakistan)	700	3 – 5	75	1.85
Autio-Gold et al. (2005)	Florida (USA)	221	5	48	2.5
Wanjau et al. (2006)	Philadelphia (USA)	269	5	53	2.18
Li et al. (2012)	Shanghai (China)	1850	5	64	2.96
Cleaton-Jones et al. (2008)	Johannesburg (South Africa)	7185	2 – 5	59	3.4
Cariño et al. (2003)	Northern Philippines	448	5	94	9.8
Begzati- Rexhepi et al.	Kastriot (Kosovo)	108	3 – 5	25	12.5
Petro. E. (2015)	Tirana (Albania)	904	3-5	91	6.45

As observed by the comparative data offered by the literature, the prevalence of ECC in our study (91%) is slightly lower than the prevalence of ECC in the Philippines (94%), but this is a much higher figure than most authors present in their studies, including neighboring countries like Kosovo, Italy and Greece (22-25). In Kastriot, Kosovo the study was conducted in one of the municipalities with the lowest income level and the 25% figure of the prevalence of ECC in this municipality is much smaller than that reported in our study (26-30). In addition to the alarming prevalence figure, most of the children involved our study (62.1%) suffer severe form of the ECC indicating greater severity of early childhood caries.

The results of our study show that the average value of the deft index is 6.45, while average values for individual dt, et and ft indexes are respectively 6.2, 0.07 and 0.2. Comparative data from the literature, show that the deft index in our study is a much higher figure than in most studies carried out by various authors, apart from Philippines which has a higher deft index i value (9.8) and Kosovo which has a dmft index much higher (12.5) nearly double the value of our index (31-40).

The results of our study show that the average value of CI is 3.1%. This index provides data indicating that only 3.1% of the decayed deciduous teeth were treated by a dentist and the majority is untreated. This shows a very low level of dental care for children involved in the study. This figure is lower than the results of a study conducted in South Africa where the level of care was only 20% and most children 4-5 years old had ECC untreated by the public service or the private one (41-47).

Based on data obtained from the results and cross-checking them with data from the literature on similar studies by various authors, we can draw the following conclusions, i.e., this is the first study in our literature in the field of Pediatric Dentistry, which provides data on the prevalence of ECC and caries experience in primary teeth in children of preschool age (3- to 5-years-old) in the public kindergartens of the city of Tirana and which identifies factors associated with early childhood caries (48-53). The prevalence of early childhood caries in children 3-

to 5-years-old in the public kindergartens of Tirana is very high (91%) and most children (62.1%) suffer Severe ECC that indicates the gravity of this condition (54-59). The average value of the deft index of 6.45 (SD±25.4) shows a high level of caries experience and the lower average value of the Care Index 3.1%, indicates a very low level of dental care in children with ECC (60-65).

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