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FUNDAMENTAL AND CLINICAL RESEARCH IN DENTISTRY. FROM THE ORAL CAVITY ANATOMY TO ORO-DENTAL DIAGNOSIS AND TREATMENT IN YOUNG AND GERIATRIC PATIENTS

- HABILITATION THESIS –

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CONTENTS

ABBREVIATIONS

BMI - body mass index
CBCT - cone-beam computed tomography
CC - complete concordance
CD - cluster of differentiation
CD - concordant data
CFI - caries frequency index
CK - monoclonal mouse anti-human cytokeratin
DCR - diacrylic composite resins
EAD - extreme anterior domain
ED1 - expected data 1
ED2 - expected data 2
EGFR - epidermal growth factor receptor
EMG – electromyography
GIT - gastrointestinal tract
GOHAI - geriatric oral health assessment index
HE - Hematoxylin–Eosin
HP – histopathologic
IHC – immunohistochemic
LBS - levator labii superioris muscle
LCH - Langerhans cell histiocytosis
LLLT, low-level laser therapy
MgSO47H2O - magnesium sulphate heptahydrate
MMSE - mental status score
MNA - Mini-Nutritional Assessment
NC - neural crest
OIT - oblique incremental technique
PG - pyogenic granuloma
PGCG - peripheral giant cell granuloma
RD - real data
SD - standard deviation
SMAS – Superficial muscular aponeurotic system of the face
SPSS - Statistical Package for Social Sciences
TBS - Tris-buffered saline
TGF-V - transformation factor-alpha
TGFA – genomic polymorphisms of genes encoding growth

THESIS SUMMARY

An academic career must bring together the profession of scholar mentor, researcher and medical practitioner. It lays on the top educational system and its succeeds based on dedication and self motivation, responsiveness and broad-mindedness to new ideas and concepts.

It has been proved that state of the art academic careers meets standards and highlights awareness of the need for continued training, the integration of modern methods into teaching and medical practice. Heretofore these criteria are met, they lead to an increase of the educational process quality and its outcomes, the improvement of scientific performance and the success in the medical career. The career of an academic member has and must have a significant impact upon the entire academic community. It has to harmoniously combine a series of skills and qualities, such as: solid and constantly updated scientific acquaintance, availability to communicate and teamwork, along with the desire to be part of a team.

This habilitation thesis summarizes my entire professional, academic and scientific activity after the completion of doctoral research. It is structured according to the criteria recommended and approved by CNATDCU, in three major sections. It highlights both the overall picture and the detailed overview of the main domain of interest and objectives of my academic career so far. Considering on personal experience in areas of interest, I will detail the management of the ongoing research projects, along with the studies and implementation opportunities that they open.

Encompassed by the cardinal concerns I have since the beginning of my academic career my main interest is to address new areas of research with direct implications in medical practice and, particularly, for the benefit of patients. These domains are now stretch out in a series of future projects which are describe in the corresponding section of the thesis. There are also presented detailed descriptions of the future research projects that I want to follow in the coming years.

This manuscript summarizes my research, didactic and medical work carried out since 2003, year which corresponds to the finalization and presentation of my PhD thesis entitled "The value of the early diagnosis methods in oral mucosal cancer in the senior age", coordinated by MD, PhD, Professor Maria Voroneanu.

The structure of the paper is divided into three main sections: SECTION I detailing my academic, medical and research work, SECTION II, which maps on the main results of my research activity, materialized by publication of ISI rated articles with impact factor as a point of continuation of the research started but also for future projects and SECTION III which contains the bibliographic references.

SECTION I presents the results of my professional, scientific and academic work in four chapters and summarizes the main personal contributions in the field of dentistry.

Betraying personal motivation in choosing research topics and the way ahead and implementing them reveals both the capacity to initiate and develop personal research projects, as well as my team working abilities. The ultimate goal of the efforts and work carried out by me and the teams I coordinate is the patient's benefit in everything that means comfort, social reinsertion, distinguished results and long survival.

The scientific research I have had so far has materialized in book publications, articles, and communications at congresses. All these hides behind the hard work teamwork and passion for what we do. The dissemination of the results of the scientific research would

not have been possible without the help of the medical school trainers who coordinated my medical and academic career and from whom I learned this specialty in the field of dentistry.

I am convinced that the academic level I have achieved ensures a great international visibility and, directly, the prestige of the university I represent.

The theme chosen for the doctoral study and its manners and opportunities for continuity have opened the way to research in the field of dentistry. In the doctoral study I learned and perfected my research skills so necessary for my future career. I have been familiar with the early, clinical and radiological diagnostic techniques of the most of the dental pathologies.

My research in this direction focused on highlighting the modern means of diagnosis and treatment in general dentistry and dental gerontology. The development of study models of the etiopathogenesis and evolutionary patterns of the dental pathologies, management and research of the elder patients are current and some of the main areas of interest in my career.

In the second part - SECTION II - I present my scientific and professional achievements in order to certificate my future research projects which are detailed in this section. It is divided into 3 chapters.

The first one present my expertize in the assessment of the clinical anatomy of the elder patients in both elective and emergency interventions is particularly guided to unfold the functionality of the stomatognathic system. The approach of the mimic muscles together with superficial fascia and connective tissue which form together the musculoaponeurotic sysytem of the face allows detailed training and comprehensive understanding of the stomatognathic system either. In order to be able to perform in any field, it is necessary to put some feeling into what you do and to constantly update your knowledge in the field.

This research domain has also benefited from the current advances in related fields: radiology, computing, oncology, plastic surgery, orofacial surgery and bioengineering. The results of current research practice in the field are mirrored in materials published in ISI-rated journals with impact factor.

The following two subchapters continue naturally the first one. It refers to the most related orofacial congenital malformations to dental practice.

I emphasize this section's second and third chapters. The second one contains the results of my research direction focused on dental diagnosis and tretment particularities of the elder patients. The material proves my interest in researching new methods of early diagnosis and staging of the different and particular conditions from dental gerontology. The results of my personal research on the patient management specificities from the main interest domains are also represented in the third chapter. Finally, this section puts into perspective my personal achievements in the niche areas represented by the applicability of the outcomes from the management research, didactic and medical experience which fit my utmost domain of interest - dental gerontology.

The final chapter of the thesis - entitled SECTION III - represents the scientific support of my research activity up to date and the basis for my future research plans and projects. This section contains all the bibliographic references that I have built up my current knowledge in the fields of interest and the support of their permanent updating.

REZUMATUL TEZEI

Orice carieră academică trebuie să reunească profesia de îndrumător, cercetător și practicant medical. Aflându-se la baza sistemului educațional de vârf, cariera academică se bazază pe dedicație și motivație de sine, capacitate de reacție și permeabilitate la idei și concepte noi. Cariera unui membru al corpului universitar are și trebuie să aibă un impact semnificativ asupra întregii comunități academice. Trebuie să îmbine armonios o serie de abilități și calități, cum ar fi: cunoștințe științifice solide și permanent actualizate, disponibilitatea de a comunica și munca în echipă, împreună cu dorința de a face parte dintr-o echipă.

Această teză de abilitare rezumă întreaga mea activitate profesională, academică și științifică de după finalizarea cercetării doctorale. Este structurată după criteriile recomandate și aprobate de CNATDCU, în trei secțiuni majore. Acestea evidențiază atât imaginea de ansamblu, cât și imaginea de detaliu a principalului domeniu de interes și obiectivele carierei mele academice de până acum. Având în vedere experiența personală în domeniile de interes, voi detalia managementul proiectelor de cercetare în curs, împreună cu studiile și oportunitățile de implementare pe care acestea le deschid.

În urma preocupărilor cardinale pe care le am de la începutul carierei mele de cercetare, principalul meu interes este să abordez noi domenii de studiu, cu implicații directe în practica medicală și, în special, în beneficiul pacienților. Aceste domenii sunt acum desfășurate într-o serie de proiecte viitoare, care sunt descrise în secțiunea corespunzătoare a tezei. Sunt prezentate și descrieri detaliate ale viitoarelor proiecte de cercetare pe care vreau să le urmez în următorii ani.

Acest manuscris rezumă munca mea de cercetare, didactice și medicale desfășurate din 2003, an care corespunde finalizării și prezentării tezei mele de doctorat intitulată „Valoarea metodelor de diagnostic precoce în cancerul mucoasei orale la vârsta a treia”, coordonată de Prof. Dr. Maria Voroneanu.

Structura lucrării este împărțită în trei secțiuni principale: SECȚIUNEA I în care este detaliată activitatea mea academică, medicală și de cercetare, SECȚIUNEA II, care conține rezultatele principalor activități de cercetare, materializate prin publicarea de articole cotate ISI cu factor de impact, ca punct de continuare a cercetării începute, dar și pentru proiecte viitoare și SECȚIUNEA III care conține referințele bibliografice.

SECȚIUNEA I prezintă rezultatele activității mele profesionale, științifice și academice și rezumă principalele contribuții personale în domeniul stomatologiei. Motivația mea personală se reflectă în alegerea subiectelor de cercetare iar implementarea acestora relevă atât capacitatea de a inița și dezvolta proiecte personale de cercetare, cât și abilitățile de lucru ale echipei mele. Scopul final al eforturilor și muncii desfășurate de mine și de echipele pe care le coordonez este orientat spre beneficiul pacientului în tot ceea ce înseamnă confort, reinserție socială, rezultate favorabile și supraviețuire îndelungată.

Cercetarea științifică pe care am avut-o până acum s-a materializat în publicații de carte, articole și comunicări la congrese. Toate acestea se ascund în spatele muncii în echipă și a pasiunii pentru ceea ce facem. Diseminarea rezultatelor cercetării științifice nu ar fi fost

posibilă fără ajutorul formatorilor de școală medicală care mi-au coordonat cariera medicală și academică și de la care am învățat această specialitate în domeniul stomatologiei.

Sunt convinsă că nivelul academic pe care l-am obținut asigură o mare vizibilitate internațională și influențează în mod direct prestigiul universității pe care o reprezintă.

Tema aleasă pentru studiul de doctorat, metodologia utilizată și oportunitățile de continuitate mi-au deschis calea cercetării în domeniul stomatologiei. În studiul de doctorat am învățat și mi-am perfecționat abilitățile de cercetare atât de necesare pentru viitoarea mea carieră. M-am familiarizat cu tehnicile de diagnostic precoce, clinice și radiologice ale celor mai multe patologii dentare.

Cercetările mele în această direcție s-au concentrat pe evidențierea mijloacelor moderne de diagnostic și tratament în stomatologie generală și gerontologie dentară. Dezvoltarea modelelor de studiu ale etiopatogenezei și modelelor evolutive ale patologiilor dentare, managementul și cercetarea pacienților vârstnici sunt teme actuale și unele dintre principalele domenii de interes din cariera mea.

În partea a doua - SECȚIUNEA II - prezint realizările mele științifice și profesionale pentru a-mi certifica viitoarele proiecte de cercetare care sunt detaliate în acest manuscris.

Este împărțită în 3 capitole. Primul dintre acestea prezintă experiența mea în evaluarea anatomiei clinice a pacienților vârstnici atât în intervenții elective, cât și în caz de urgență și este orientat în special apre a desfășura funcționalitatea sistemului stomatognat. Abordarea mușchilor mimicii, împreună cu fascia superficială și țesutul conjunctiv care formează împreună sistemul musculoaponeurotic superficial al feței permite formarea detaliată și înțelegerea cuprinzătoare a sistemului stomatognatic. Pentru a putea performa în orice domeniu, este necesar să pui suflet în ceea ce faci și să îți actualizezi constant cunoștințele în domeniu.

Următoarele două subcapitole continuă în mod natural pe primul. Se referă la cele mai frecvente malformații congenitale orofaciale în practica dentară.

Țin să subliniez al doilea și al treilea capitol al acestei secțiuni. Cel de-al doilea conține rezultatele direcției mele de cercetare axată pe diagnosticul afecțiunilor dentare și particularitățile de tratament ale pacienților vârstnici. Materialul dovedește interesul meu de a cerceta noi metode de diagnostic precoce și expunerea diferitelor particularități din gerontologia dentară. Rezultatele cercetărilor mele personale privind specificul managementului pacientului din domeniile de interes principal - gerontologia dentară, sunt, de asemenea, reprezentate în capitolul al treilea.

În cele din urmă, această secțiune prezintă perspectivele deschise de realizările mele personale în domeniile de nișă reprezentate de aplicabilitatea rezultatelor din cercetarea managementului pacientului vârstnic, experiența didactică și medicală care se potrivesc celui mai mare domeniu de interes al meu - gerontologia dentară.

Ultimul capitol al tezei - intitulat SECȚIUNEA III - prezintă fundamentul științific al activității mele de cercetare la zi și baza pentru planurile și proiectele mele de cercetare viitoare. Această secțiune conține toate referințele bibliografice pe care mi-am construit cunoștințele mele actuale în domeniile de interes și care vin în sprijinul actualizării lor permanente.

SECTION I - SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

Brief overview of the academic carreer

The implementation of professional standards in the university career is essential, this aspect involving a continuous increase in the quality of the educational system. The profession that I have the privilege to practice gives me the unique opportunity, but at the same time the challenge, to perform my activity in three exceptional fields: education, health, research. Each of these areas, offers multiple professional satisfactions, but requires, at the same time, a continuous learning, a desire for improvement, in order to be able to assure the high quality requirements. In addition to the activity in the field of health, which itself involves maintaining certain professional standards, but also human quality, the university environment brings with it a multitude of opportunities, but also duties.

Thus, I had the opportunity to develop myself professionally in a multicultural, multifunctional environment that stimulated me to develop propperbehavioral abilities in the spirit of a civilized, elegant, calm, but also firm dialogue. On the other hand, I had the duty to cultivate the students, besides the medical notions themselves, the passion and respect for the doctor's job, the respect for the patient and his suffering, but also the interest for the scientific research, as a source of progress and innovation.

Teaching activity

My didactic activity is extremely challenging and also stimulating. It implies having a good professional and pedagogical knowledge and skills. I have started my didactic activity in 1998 and it is currently focused on the activity within the disciplines of Oro-dental Diagnosis and Gerontostomatology of the Faculty of Dental Medicine. The permanent interaction with the students implies a permanent adaptation to their personalities, with different typologies, in order to captivate, stimulate and train them in the future medical and research activity.

I have achieved all my teaching levels by competition:

↗ Associate Professor from 2017 until now at the Department of Implantology, Removable Restorations, Technology, Oro-Dental-Diagnosis and Gerontostomatology, Faculty of Dental Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi;

↗ Lecturer from 2008, at Department of Implantology, Removable Restorations, Technology, Oro-Dental-Diagnosis and Gerontostomatology, Faculty of Dental Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi;

↗ Assistant professor between 11.2001 – 02.2008 Department of Implantology, Removable Restorations, Technology, Oro-Dental-Diagnosis and Gerontostomatology, Faculty of Dental Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi

↗ Associate instructor from 03.1998 – 10.2001, at the Faculty of Dental Medicine University of Medicine and Pharmacy "Grigore T. Popa" Iasi.

I have always been focused on professional training and development of organizational/managerial competences. I managed to obtain them by participating as a member in scientific committees of national medical manifestations.

In order to support the didactic activity, I participated as author/co-author in the publication of 15 books, monographs and other study materials dedicated to university education.

In my activity I tried to promote the active-participative methods centered on the student, to stimulate the participation and the involvement of the students during the course hours by including evidence-based concepts.

I realized the support of the practical works interactively through pro and cons discussions, which allows the students to be able to make logical correlations with other disciplines in the field of dental medicine.

Research activity

I began my scientific research projects simultaneously with my enrollment in the PhD studies. I was admitted to PhD training at the "Grigore T. Popa" University of Medicine and Pharmacy Iaşi and in 2003. I obtained the title of Doctor in Medical Sciences, (OMEC 5663 from 15.12.2003) – with the thesis entitled: " The value of early diagnostic methods in oral mucosal cancer at elder", coordinated by Prof. Maria Voroneanu, MD, PhD.

Thus, starting with 2004, I was involved in obtaining founding for research and development projects and subsequently I participated actively in their implementation as a project manager or member. Of which I present the following:

- The project "Teaching staff from the pre-university and state university education system - promoter of lifelong learning", Beneficiary Ministry of National Education, from 17. 11. 2014, according to the report with no. 25725 (project manager).
- The COST Action CA15208 project entitled "Rationing - Missed Nursing which: An international and multidimensional problem"; 2016-2020 development period (member).
- The project "Integrated system for real-time telemonitoring of patients and the elderly - TELEMON" Financing: State budget - Ministry of Education, Research and Innovation Program "PARTNERSHIPS IN PRIORITY AREAS" - PC 4, 11-067 / 18.09.2007Act additional no. 3 / 06.04.2009. Duration of the contract: 34 months (member).
- The project entitled "Computerized morphometry in the efficiency of the cyto-pathological and anatomo-pathological examinations", 2004-2005 (member).

Training during the development of the projects was of major importance for my interdisciplinary research capacity, acquiring knowledge from various related fields.

The dissemination of the research results was materialized by articles in ISI journals, by ISI indexed articles in the volumes of conferences with ISBN or ISSN, articles in BDI journals or papers in other journals, in volumes of conferences with ISBN or ISSN.

The research results are of scientific, didactic and applicative utility. From a scientific point of view, they bring a great contribution in the field of fundamental knowledge, by developing and deepening research in dental medicine. From didactic considerations, the practical research methods and procedures applied are of particular interest and usefulness. Thus, the researches bring a scientific foundation of the knowledge accumulated through the practical experience.

CHAPTER 1. NORMAL AND PATHOLOGICAL FEATURES IN THE ORAL CAVITY ANATOMY – CLINICAL AND EXPERIMENTAL STUDIES

1.1. State of the Art

A basic understanding of the development, structure and relationship of the tissues and structures which constitute the oral cavity and its associated environment is fundamental to the practice of clinical dentistry. It enables the clinician better to appreciate how subsequent pathology change may be influenced by adjacent anatomical structures or tissues and therefore helps to provide a better understanding of the rationale for potential treatment options (Ferguson, 1988; Chen et al., 2017).

Anatomical sciences, which include tooth morphology, oral histology, oral embryology, and head and neck anatomy form a core part of the formation in dental technology programs. The of anatomical sciences importance is even more relevant to dental personnel with no direct contact with patients (dental technicians) and limited discipline related contact with patients (dental prosthetists) (Bakr et al., 2016). The anatomical sciences allow dental physicians, technicians and prosthetists to a gain a better insight of how tissues function, leading to a better understanding of diagnosis, comprehensive treatment planning and referrals if needed. Patient communication and satisfaction also increases as a result of this deep understanding of oral tissues. Finally, treatment outcomes are positively affected due to the appreciation of the macro and micro structure of oral tissues (Gotjamanos, 1979; Ueno et al., 2016).

A great deal of progress has been made in the researches, with the development of different materials and fabrication techniques, with the purpose of improving the properties of the materials for this field of interest (Moharamzadeh, 2017). However, in spite of these advances, complete regeneration of dental tissues continues to be challenging. Therefore, dentists must be aware of these advances, help with the development of researches, and use the new technologies in daily clinical practice, thereby providing patients with more efficient therapy, and consequently, improvement in their quality of life.

The necessary clinical approach uses a patient-centered technique in covering to help dental professionals build a strong foundation in oral biology and basic science for dental professionals. It provides a clear understanding explanation of the anatomy of oral and facial tissues — and of normal and abnormal orofacial development — so that you can provide effective oral health care for all patients with abnormalities (Chiego, 2018).

- **Short overview of oral cavity embryological development**

In all animals, the mouth derivess from both ectoderm and endoderm layers. A direct correlation of oral ectoderm and digestive endoderm is present even in triploblastic animals, and in chordates, this region is known as the extreme anterior domain (EAD). Evidences for a single origin of the mouth is represented by a preserved set of genes that form a 'mouth gene program' such as *FOXA* and *OTX2*. The mouth of the vertebrates derives from oral ectoderm of the anterior neural crest, pharyngeal endoderm and cranial neural crest (NC). Vertebrates form a mouth by breaking through the body covering in a precise sequence including

specification of EAD ectoderm and endoderm as well as NC, formation of a ”pre-mouth array” basement membrane dissolution, stomodeum formation, and buccopharyngeal membrane perforation. Human mouth anomalies are prevalent and are affected by genetic and environmental factors, with understanding guided in part by use of animal models (Chen et al., 2017; Sadler, 2019).

The oral cavity development follows the breakdown of the buccopharyngeal membrane (oropharyngeal or oral membrane) and contributed to mainly by the pharynx lying within the pharyngeal arches. Other cells originating from the neural crest are contributing to these processes. The mouth will be separated from the nasal cavity by the development of the palate, that includes both embryonic and fetal components (Sadler, 2019).

The complex and superficially idiosyncratic embryologic patterns result from well-defined evolutionary processes, producing a subtle anatomic pattern. This heterogenity is also the source of the main developmental abnormalities such as cleft lip and cleft palate (Sadler, 2019).

The oral cavity forms between the 4th and 10th weeks of fetal development from the frontonasal prominence and pharyngeal arches. From the frontonasal prominence, medial and lateral nasal processes develop. The medial nasal processes fuse in the midline and give rise to a part of the nose and an intermaxillary segment that develops into the philtrum of the upper lip, the primary palate, and the anterior maxilla containing four incisors (Berkovitz and Moxham, 2009; Sadler, 2019).

The 1st pharyngeal arch is composed of maxillary and mandibular processes. The primitive oral cavity, called the stomodeum, initially lies in the groove bounded by the frontonasal prominence and the maxillary and mandibular processes of the 1st pharyngeal arch (Sadler, 2019).

The stomodeum is initially separated from the pharyngeal space by the buccopharyngeal membrane. This membrane will break down by apoptosis around the 5th week, making the oral cavity and pharyngeal cavity continuous.

The maxillary processes give rise to the secondary palate and the maxilla. The mandibular processes give rise to the mandible (Sadler, 2019).

The gastrointestinal tract (GIT) initially starts to grow during the process of gastrulation from the trilaminar embryogenic disc (week 3) and extends from the buccopharyngeal membrane (oral membrane) to the cloacal membrane. The GIT and associated visceras later have contributions from all the three germinative layers (Sadler, 2019). The respiratory initial development was highlighted in 26-somite embryos and it flattened and then branched at Carnegie Stage 13 (Ueno et al., 2016; Sadler, 2019).

The tongue's anterior 2/3^{rds} of the tongue derives from two lateral lingual swellings and one medial lingual swelling, also known as the tuberculum impar. All three structures arise from the mandibular process of the 1st pharyngeal arch (Larsen, 2001; Sadler, 2019).

The posterior 1/3rd of the tongue derives from two midline swellings: the copula, which is associated with the 2nd arch, and the hypopharyngeal eminence, which is associated with the 3rd and 4th pharyngeal arches. During the growth of the posterior tongue, the hypopharyngeal eminence overgrows the 2nd arch structure, and the remaining portion of the 4th arch will develop into the epiglottis (Moore, 2003; Sadler, 2019).

The lingual papillae arise on the tongue between the 8th and 11th week of development. However, the taste buds do not develop until the 11th through the 13th weeks. The teeth, which are another major structure of the mouth, begin to develop in the 6th week in the form of tooth buds from reciprocal interactions between the mesenchyme and epithelium of the oral cavity (Drake, 2005; Sadler, 2019).

In an adult, the oral cavity can be divided into two regions: the oral vestibule, which is the space between the lips and cheeks superficially, and the gingivae and teeth internally, and the oral cavity proper, which is the space medial and posterior to the maxillary and mandibular dental arches. As such, the oral cavity proper is bounded anteriorly and laterally by the alveolar bone of both arches. Its roof is formed by the palate, and posteriorly, the oral cavity is in communication with the oropharynx. At rest, this space is filled by the tongue (Larsen, 2002; Sadler, 2019).

- ***Oral mucosa main features***

Three types of mucosa line the oral cavity: lining mucosa, masticatory mucosa and specialized mucosa. As the name indicates, lining mucosa's primary function is to line the oral cavity in the areas that do not encounter frequent force or friction, such as the inner surface of the lips, the floor of the mouth, the inferior tongue, and the soft palate. Histologically, the lining mucosa of the oral cavity contains nonkeratinized stratified squamous epithelium. Nonkeratinized epithelium can be recognized by the presence of nuclei in the surface cells (Moore, 1999; Nanci, 2017).

Masticatory mucosa, on the other hand, lines the areas of the oral cavity that encounter frequent force and friction, such as the gingiva, and the hard palate. Histologically, masticatory mucosa contains slightly keratinized stratified squamous epithelium. In contrast to nonkeratinized epithelium, keratinized epithelium is composed of non-nucleated cells on the surface. Specialized mucosa is found on the dorsum of the tongue and contains a variety of papillae that provide friction and special sensory function to the tongue. Histological features of the specialized mucosa of the tongue will be covered in the later slides (Snell, 2004; Nanci, 2017).

- ***Teeth main features***

Humans have two sets of teeth. The 1st set is called the primary, or deciduous, dentition. There are ten teeth in each arch of deciduous dentition consisting of four incisors, two canines, and four molars. These teeth are eventually exfoliated and replaced by the permanent teeth, which remain in the mouth for the duration of life except when they are lost due to trauma, disease, or other factors. The permanent dentition is composed of sixteen teeth in each arch and they are four incisors, two canines, four premolars, and six molars (Rohen, 2002; Nanci, 2017).

Teeth develop from the reciprocal interaction between the loose connective tissue of the developing embryo, called mesenchyme, and the epithelium of the oral mucosa that thickens and is called the dental lamina. The dental lamina invaginates into underlying mesenchyme to give rise to a tooth bud. A tooth bud is composed of the epithelial derived bell shaped structure and the mesenchyme in contact with the bell. The bell shaped structure is called the enamel organ, and it is composed of outer and inner enamel epithelia. The enamel organ contains epithelial derived mesenchyme in the middle, called the stellate reticulum (Jirasek, 2004). The mesenchyme in contact with the concave portion of the enamel

organ is called the dental papilla, and the mesenchyme at the rim of the bell shaped enamel organ is called the dental follicle. The enamel organ plays a role in initiating the development of the tooth, developing the enamel, and establishing the shape of the crown of the tooth. Its features include inner and outer enamel epithelium, the stratum intermedium, which lies between the two, and the stellate reticulum, which synthesizes glycosaminoglycans and is lost as the tooth is formed (Agur, 2005; Nanci, 2017).

Cells of the inner enamel epithelium in contact with the dental papilla differentiate into ameloblasts. These specialized cells are only present during the development of the teeth. They produce proteins that mineralize into enamel, which is the hard outer surface of the crown of the tooth. Cells in the dental papilla, in contact with the inner enamel epithelium, differentiate into odontoblasts which produce dentin (Rohen, 2002). The rest of the dental papilla gives rise to loose connective tissue of the pulp of the tooth. The dental follicle, or sac, contains the structures that form the tooth and later differentiate into supporting structures, such as the periodontal ligament. Although not pictured, the teeth form in stages, which can be distinguished by the shape of the developing enamel organ. The three stages of tooth development are: the bud, the cap, and the bell stage (Jirasek, 2004; Nanci, 2017).

In the adult, the tooth is divided into two main structures: the crown, which is the exposed portion of the tooth above the gingiva, and the root, which lies deep to the gingiva, within a socket in the alveolar bone. The connective tissue in the middle of the tooth is called the pulp. The pulp contains odontoblasts, fibroblasts, collagen, and a rich nervous and vascular supply that enters and leaves the tooth through the apical foramen at the apex of the root. The middle layer is called dentin. It is produced and maintained by odontoblasts whose cellular processes are found within dentinal tubules. This layer forms the bulk of the tooth. The outermost layer of the crown of the tooth is enamel an acellular material comprised of rods and interred enamel that binds the rods together. Enamel on this section is not obvious due to lack of staining (Gray, 2005; Nanci, 2017).

The outermost layer of the root of the tooth is cementum, which can be cellular, acellular or afibril. The cementum is produced by cementoblasts, and it connects to periodontal ligament fibers to anchor the tooth in the alveolar bone. Between the enamel and cementum is the cemento-enamel junction. This junction point is also commonly referred to as the neck or the cervical constriction of the tooth. Additionally, note that the boney sockets for the teeth are located within the alveolar processes of the maxilla and mandible. This bone is in direct contact with the periodontal ligament. On the right is the histology of the periodontal ligament. It is derived from mesenchyme around the cementum of the tooth and is comprised of fibers in various orientations. The varying fiber orientations attach and secure the tooth to the alveolar bone. The epithelium of the gingiva around the tooth varies, such that three types can be seen. Junctional epithelium is found in the deep portion of the gingival sulcus and attaches to the tooth via a thickened basal lamina and hemidesmosomes. Distal to this is the sulcular epithelium that extends from the base of the sulcus to the free margin. At the free margin of the gingiva, the epithelium changes to that of the surrounding epithelium and blends in with the masticatory mucosa (Bosma, 1972; Nanci, 2017).

- ***Tongue main features***

The tongue is a muscular organ that serves many important roles, such as in mastication and speech. Some of the anatomical features of the tongue include the terminal

sulcus, which is a V-shaped line separating the anterior 2/3^{rds} of the tongue from the posterior 1/3rd of the tongue, the median sulcus, and the foramen cecum, which is a remnant of the embryonic thyroglossal duct, located at the apex of the terminal sulcus. The dorsum of the tongue is lined by the specialized mucosa, composed of papillae which give a velvety and raised papule like appearance to the tongue. Anatomical features of the underside of the tongue include: the sublingual fold, formed by the sublingual gland and the submandibular duct that lie deep to the mucosa, the lingual frenulum, which attaches the tongue to the floor of the oral cavity, and the sublingual caruncles, which are the sites of the submandibular glands opening into the oral cavity (Hyman, 1942; Nanci, 2017).

There are four types of lingual papillae, and each will be described. Filiform papillae are the most numerous and the smallest papillae found on the dorsum of tongue. They appear scaly or thread-like, and are conical projections of connective tissue covered by keratinized stratified squamous epithelium (Nanci, 2017). They provide friction and are sensitive to touch. However, unlike the other lingual papillae, they contain no taste buds. Fungiform papillae are scattered over the dorsum of the tongue but are most numerous near the apex. They appear mushroom shaped or as pink or red spots on the tongue. Histologically, they are taller than filiform papillae, and they contain taste buds on their apical surfaces (Bosma, 1985; Nanci, 2017).

The taste bud is an ovoid structure composed of pale staining cells within the epithelium containing the neuroepithelial cells with microvilli that project through a taste pore. Although taste buds are mostly associated with circumvalate, foliate, and fungiform papillae, they are also found on the palate, the patatoglossal arches, the posterior epiglottis, and the posterior wall of oropharynx. Foliate papillae are found on the postero-lateral margins of the tongue but are not highly developed in humans. The papillae appear as parallel ridges with leaf-like shapes, separated by a deep groove (Nanci, 2017). They also contain taste buds in the lateral surfaces. Circumvallate, or vallate, papillae are found in varying numbers in a row anterior to the terminal sulcus. They appear large with a flat top, and contain taste buds on their lateral surfaces. Each papilla is surrounded by a trench (Crelin, 1987; Nanci, 2017).

Foliate and circumvallate papillae are also associated with serous glands underneath that produce watery secretions into the deep ridges around the papillae. The palate consists of a hard palate and a soft palate. The hard palate is lined with masticatory mucosa. Projections of the hard palate include: the incisive papilla, located near the incisors, the palatine raphe, which is a midline ridge of the palate, and the palatine rugae, which are a series of ridges seen running horizontally on the anterior hard palate (Nanci, 2017). Another feature that can be seen is the maxillary tuberosity, which is the distal aspect of the alveolar bone of the maxilla. The soft palate is lined with lining mucosa. The major extension of the soft palate is the uvula, but also, extending toward the tongue and pharynx, are two folds of tissue: the palatoglossal and palatopharyngeal arches (Sessle and Hannam, 1976).

Although not found directly inside the oral cavity, salivary glands are major structures anatomically and functionally associated with the oral cavity. The sublingual glands are the smallest of the 3 major salivary glands and are located under the lining mucosa of the floor of the oral cavity. The glands group together to form a U-shape of tissues on either side of the lingual frenulum. They have numerous small ducts that open into the floor of the mouth adjacent to the sublingual fold (Nanci, 2017). Histologically, the sublingual glands are

compound tubuloacinar glands containing both mucous and serous secreting cells. However, mucous secreting cells predominate (Dubner et al., 1978; Turvey 1996).

The submandibular glands lie beneath the mandible near the mylohyoid muscle. They are also a mixed gland containing both serous and mucous producing cells. However, compared to the sublingual glands, they have a more extensive ductal system. The submandibular duct, also called Wharton's duct, opens into the sublingual caruncle on the underside of the tongue (Smith, 1992; Hall et al., 2004).

The parotid glands are found on either side of the face, superficial to the ramus of the mandible and anterior and inferior to the ear. Parotid glands are compound, branched acinar glands and are composed of mostly serous secreting cells (Gould and Vrba, 1982; Nanci, 2017).

Parotid glands have an even more extensive ductal system than the submandibular glands, and contain numerous, much larger, interlobular ducts. The parotid duct travels along the buccal fat pad and opens into the oral cavity adjacent to the 2nd maxillary molar (Hopson, 1973; Bramble, 1978).

- ***Tonsils - in the oral cavity***

The palatine tonsils, commonly referred to as the tonsils, can be found within the tonsilar beds of the oral cavity which has their embryonic origins in the second pharyngeal pouches. The tonsilar bed lies between the palatoglossal and palatopharyngeal arches. The tonsils are composed of diffuse lymphoid tissues and lymphoid follicles, some with germinal centers, containing dividing and differentiating lymphoid cells. The tonsils are surrounded by an incomplete capsule that separates the tonsils from the underlying connective tissues. The oral surface of the tonsils is covered by lining epithelium, which occasionally invaginates into the tonsilar parenchyma to form crypts. Heavy infiltration of lymphocytes in the lining epithelium of the tonsils is not uncommon (Keir et al., 1985; Thorogood et al., 1988; Crompton, 1989; Smith, 1997; Lieberman et al., 2001; Miller, 2002; Nanci, 2017).

The lingual tonsils, located on the postero-lateral tongue, would look very similar to the histological images of the palatine tonsils. While they contain lymphoid follicles and diffuse lymphoid tissue, their capsule is thinner and not well defined (Parker and Lee, 2011).

- ***Embryological hallmarks correlated with craniofacial anomalies – genes and signalling pathways***

Craniofacial anomalies often involve abnormal mouth development, which may go awry frequently due to the many steps involved. These steps may occur very early during mouth formation, and involve the EAD. Later events involving cartilage or bone formation leading to development of the primary or secondary palate may also impact mouth development (Sadler, 2019). Regulation of human mouth development is complex, including genetic and environmental factors. Mouth anomalies may occur as part of a 'syndrome' if they consistently occur together with phenotypes elsewhere in the face or body, or they may specifically only affect the mouth (Saal, 2016).

Understanding EAD activity in model organisms will lend insight into human craniofacial anomalies, since defects in human EAD signaling may lead to abnormal NC development later manifesting as malformed cartilage and bone. Conversely, abnormal NC signaling to the EAD may lead to abnormal mouth morphology and delayed mouth opening. Symptoms of several syndromes such as Nager syndrome, craniofacial microsomia, and

persistent buccopharyngeal membrane may represent outcomes of abnormal EAD function, although these connections are yet unexplored (Nanci, 2017).

Human mouth defects have been associated with genes and signaling pathways identified in vertebrate models, indicating the utility of these for addressing human disorders (Van Otterloo et al., 2016). As details of mouth and other facial features may differ between animals, particularly with regard to palate formation, the model must be chosen carefully.

Some of these pathways identified in vertebrates may affect early events, including those surrounding EAD function, while others may impact much later events. For example, *TBX1* and *FGF8* are implicated in human DiGeorge syndrome (Huh and Ornitz, 2010).

The Shh pathway is associated with many craniofacial anomalies including Pallister–Hall syndrome and Grieg cephalopolysyndactly syndrome. Disrupting both SHH and β catenin WNT signaling promotes facial pathogenesis including that of palate and mouth. Genome wide association studies analyzing variation in face morphology finds associated loci harboring candidate genes important for facial development in vertebrate models (Villavicencio et al., 2000; Hill et al., 2007; Cobourne et al., 2009; Veistinen et al., 2012; Kurosaka et al., 2014).

Examples include *GLI3*, a member of the Shh pathway, and *RUNX2* a gene that interacts with Shh during bone development, members of the FGF family, endothelin pathway, and semaphorins. Genes not obviously involved in signaling such as the nucleolar protein TCOF1 in Treacher Collins Syndrome78 may impact human mouth development (Adhikari et al., 2016; Zhang et al., 2016).

Mouth development is sensitive to environmental factors including pathogens, teratogens in the form of medicines and other chemicals, especially during the first trimester (Sadler, 2019). In general it is unclear what steps in mouth formation these agents impact. Zika virus and cytomegalovirus are both associated with cleft lip and palate (Weichert et al., 2010; Moura da Silva et al., 2016).

Antiseizure medications such as valproate and phenytoin, as well as RA, are associated with mouth anomalies. Smoking and ethanol are tightly associated with facial anomalies. Other teratogens affecting the mouth have been defined in animal studies, for example, dioxins and dithiocarbamates. Maternal health challenges have also been associated with mouth anomalies, including diabetes and hyperthyroidism. Overall, the landscape of human mouth developmental anomalies is multifactorial, evolving and incomplete (Shi et al., 2008; Ornoy, 2009; Malvasi et al., 2009; van Boxtel et al., 2010; Orup et al., 2014; Murawski et al., 2015; Burns et al., 2015; Liu et al., 2015; Carmichael et al., 2015).

- ***Functional features of the oral region development ana anatomy***

The descent of the hyoid bone and larynx in early postnatal development has important implications for swallowing and respiration, as well as communication. The neonatal position of the larynx definitively separates the airway from the foodway during tidal respiration. Food accumulating in the oral cavity and oropharynx is prevented from entering the hypopharynx or the larynx by the direct contact of the epiglottis and larynx with the enfolding tissues of the fauces and soft palate. This contact is broken briefly during swallowing, when the soft palate elevates and the epiglottis folds downward, but then is rapidly restored after the swallow. The intranarial position of the larynx also prevents airflow

through the mouth, and thereby limits vocalization. The larynx is pulled inferiorly during infant cry, mimicking its adult position (Nishimura and Suzuki, 2003; Sadler, 2019).

Laryngeal descent is essential for speech, because it enables airflow through the larynx, pharynx, and oral cavity. Speech obviously carries an enormous benefit for human communication. The question then arises, given the advantage of the descended larynx for speech, why this descent does not occur prenatally. Certainly there are issues regarding the cortical development necessary for speech, and evidence that this development occurs postnatally. But the postnatal development of cerebral capacity for spoken language does not require that development of the peripheral structural mechanisms for speech occur postnatally as well. There is no need to separate the airway from the foodway in utero, because both are filled with amniotic fluid. But the neonate must adjust to huge changes in environment at the moment of birth, with a need to rapidly fill both lungs with air (Sadler, 2019). The intranarial larynx provides an upper airway that is structurally isolated from the oral cavity, with a semirigid cartilaginous framework for the upper airway. This simplifies the problem of establishing and maintaining airway patency, and reduces the likelihood of airway obstruction. Indeed, the relatively weak muscles of the neonatal pharynx and soft palate may need this structural support to maintain airway integrity for breathing, especially during sleep. The inability to maintain airway patency is a cause for obstructive sleep apnea (Crompton et al., 1997; German et al., 2008; Sadler, 2019).

The issue of swallow safety is also critical. Coordination of suckling, swallowing, and breathing is not fully developed in the neonate, creating a risk for deglutitive aspiration. The intranarial larynx prevents the bolus from entering the larynx before and after swallowing, and thus reduces the risk for airway obstruction and/or contamination by ingested milk or other material (Jeffery, 2006; Sadler, 2019).

The developmental and functional anatomy of the oral region is closely correlated with that of the superficial musculaponeurotic system of the face - SMAS. The embryology of the superficial planes of the face and neck shows that the development of facial expression muscles, superficial and deep adipose tissue, facial nerve and parotid gland is the result of divergent and confluent migratory movements (Delmar, 1994; Sadler, 2019).

The pharyngeal musculature originates in the paraxial mesoderm of the occipital somitomers and somites. The boundaries between adjacent somitomers are difficult to detect, the origin of the premuscular mesoderm of each pharyngeal arch has been clearly identified by studies of human embryos to which experimental studies have been added (Larsen, 1993).

The paraxial mesoderm of the first pharyngeal arch derives from the fourth cranial somitomer and generates the following muscles: masticators (temporal, masseter, medial and lateral pterygoid), milohioid, anterior belly of the digastric muscle, tensor of the palatine veil, tensor of the eardrum. The paraxial mesoderm of the second pharyngeal arch derives from the sixth cranial somitomer and generates the following muscles: facial expressions (orbicularis occuli, orbicularis oris, rizorius, platysma, auricular, fronto-occipital, buccinator), posterior belly of the digastric muscle, stapedius (Larsen, 1993). The muscles of the first two pharyngeal arches mix in their final location. The paraxial mesoderm of the third pharyngeal arch derives from the seventh cranial somitomer and generates a single muscle, stylopharyngeal. The paraxial mesoderm of the fourth pharyngeal arch derives from the occipital somites 2-4 and the first cervical somite and generates the muscles: pharyngeal

constrictors (upper, middle and lower), cricothyroid, palatal veil lift. The paraxial mesoderm of the sixth pharyngeal arch derives from the occipital somites 1 and 2 and generates the intrinsic muscles of the larynx (Sadler, 2019).

At the embryo 16-17 mm long (mid-6th week), the superficial layer of the mesenchyme of the second arch is divided into 4 laminae: occipital (from which occipital muscles arise, posterior auricle, transverse head), cervical (from which will form the cervical portion of the platysma muscle), the mandible from which the depressor muscles of the lower lip, mentally, rizorius, depressor of the mouth, the lower part of the orbicularis of the mouth, buccinator - sometimes, elevator of the mouth - sometimes) are formed , temporal (from which at the end of this period, the upper ear muscle will be formed) (Sadler, 2019).

At the embryo of 20-45 mm long (week 7 - mid week 8), the superficial layer of the mesenchyme of the second pharyngeal arch further differentiates two laminae: infraorbital (from which the muscles are formed: large and small zygomatic , levator of the upper lip and nose wing, upper part of the orbicularis muscle of the mouth, compressor of the nose, depressor of the nasal septum, orbicular of the eye, frontal, corugator supercilii, procerus) and occipital, for the platysma muscle (Nanci, 2017; Sadler, 2019).

The development of the muscles of the facial expressions is successive: in week 7, at the 26 cm embryo, large zygomatic muscles, mouth angle depressant, buccinator, frontal and small zygomatic develop; In week 8, the orbicularis muscles of the mouth develop, mouth elevator, orbicularis of the eye; In week 9, the anterior auricular muscles, the corrugator supercilii, the occipital, the facial portion of the platysma muscle, the upper lip lifter, develop (Sadler, 2019).

Gasser (Gasser, 2005) systematizes the stages of cervicofacial muscles in development. In the 4.2-6.5mm embryo, the second pharyngeal arch begins to become denser, but does not divide into distinct muscle masses. In the 8-20mm embryo, the prizeblasts and myoblasts extend from the superficial part of the arch into the temporal, occipital, cervical and mandibular regions. The deep pre-muscular condensations of each arch will become the stapedius muscles, the posterior belly of the digastric and the styloid. The infraorbital lamina and the occipital platysma appear in the embryo from 20 to 23 mm. The superficial muscles differ rapidly, in the embryo from 26 to 37 mm. In the 41 mm embryo most are made of myoblasts. Starting with the 80 mm embryo, all muscles contain myotubules in their definitive position. At the 80 - 140 mm embryo the myotubules begin to form young muscle fibers, and from 140 to 360 mm (term) the muscles grow in size and become definitive attachments (Sadler, 2019).

Sequence of morphogenesis of facial expression muscles: the mesenchyme of the second pharyngeal arch surrounds the end of the facial nerve (weeks 6 - 8); occurrence of premuscular facial masses (weeks 8 - 9) - occipital, cervical, mandibular and temporal lamellae (precursors of facial expression muscles); differentiation of muscles of facial expressions; new blades appear: infraorbital and occipital platysma (weeks 9-11); definitive localization of facial expression muscles; The muscles are located above the branches of the facial nerve; The superficial muscles differ later than the deep ones, and those in the cervicomandibular and occipital region earlier than those in the frontal and middle region of the face systematize the stages of cervicofacial muscles in development (Gasser, 2005).

In the 4 mm embryo, myogenesis in the head differs markedly from that of the trunk or limbs. Certain craniofacial muscles have different phenotypic properties (eg myosin isoform and possible neuromuscular control of the phenotype). They develop by moving mesodermal cells outside the paraxial mesoderm through mesenchyme (derived from neural crest or mesoderm) to the final destination. The morphogenesis of the skull muscles seems to be determined by intrinsic information in the connective tissue that connects the muscles. There is no level of specificity in paraxial myogenic cells (Nanci, 2017). This is determined by grafting somites or somitomers from one level to another in the craniocaudal sense. In this case, the myogenic cells that leave the grafted structures form normal muscles for the regions where they will migrate later, than the muscles destined for the graft somite origin level (Kablar 1999; Sadler, 2019).

Certain muscles of the head (eg, the muscles of the tongue), arise from the occipital somites in the same way as the muscles of the trunk and undergo extensive migration at the level of the head, at a level much caudal to the origin, evidenced by the innervation by the hypoglyphic nerve, which, according to some anatomists, is a modified spinal nerve. In addition, the precursor cells of the tongue muscle express *PAX-3* during migration, having the same regulatory molecules of myogenesis as the trunk muscles(Sadler, 2019).

- ***Histogenesis of facial expression muscles***

The muscles are made up of muscle fibers, connective tissue, blood vessels and nerves. Even the muscle fibers differ according to functional and biochemical criteria. At first all myoblasts are identical, the different characters being imprinted by motor innervation (Wigmore and Dunglison, 1998; Hughes and Salinas, 1999). More recently, it has been shown that in some mammals there are distinct populations of slow and fast muscle cells, even from the early stages of myogenesis, before the nerve fibers reach the developing muscles. In addition, there are early and late forms of myoblasts that require different serum factors and different nerve interactions for differentiation. Mioblasts are characterized by Myo D expression and growth factors. Thus, insulin-like factor promotes fusion and differentiation into myotubes, which express myogenin (Brand-Saberi and Christ, 1999).

The sequence of muscle fiber formation involves mononuclear myoblasts that fuse into multinucleated myotubes, from which primary myotubes are born, the initial basis of embryonic muscles, before the motor axons enter the forming muscle. In the 41 mm embryo most muscles are made of myoblasts. The motor nerves enter the muscle in formation. Muscle masses form step by step with the muscular branches of the facial nerve that develop deep against them. Secondary myotubes are formed around primary myotubes by elongating them. Starting with the 80 mm embryo, all muscles contain myotubules in their definitive position (Gasser, 2005).

Initially, the associated primary and secondary muscle fibers are surrounded by a common basal lamina, are electrically coupled, and synthesize a variety of contractile proteins (multiple sets of myosin isoform subunits). At the 80 - 140 mm embryo the myotubules begin to form young muscle fibers, and from 140 to 360 mm (term) the muscles grow in size and become definitive attachments. Backup mononuclear cells (satellite cells) can proliferate and merge, to increase or mechanically stress muscle fibers. Fast and slow nerve fibers select the corresponding muscle fibers based on information contained on their surface. Initially, a motor fiber can end up on both fast and slow muscle fibers, so that it

remains only for compatible muscle fibers. The phenotype of the muscle fibers depends on the nature of the specific proteins in their contractile apparatus, but this is not irreversibly fixed, after the birth of the muscle fibers having a high plasticity (Carlson, 2004).

As the muscles form, the myoblasts mix with the mesenchymal tissue of the future connective tissue. The capillaries penetrate into the training muscle to provide nutrition, and the motor nerves will penetrate shortly after the myoblasts begin to form myotubules. At a high level of organization, development involves the formation of identifiable muscles. The external shape of the muscles is determined more by the connective tissue, than by the muscle fiber itself. The component cells of the connective tissue of the muscles seem to have imprinted the morphogenetic plane. SMAS is a superficial, strictly surgical anatomical structure derived from the primitive platysma muscle and has no bone insertion. It is composed exclusively of platysma and rizorius muscles, it is not parotid aponeurosis (Carlson, 2004).

- ***Facial nerve morphogenesis (VII)***

Initially, the 12 pairs of cranial nerves are segmented and sequentially organized. This serial pattern is not obvious in adults due to nerve migration to the target organs. Adult nerve pathways follow embryonic migration routes to the target organs.

The initiation of ontogenetic development of the sensory ganglia of the cranial nerves begins at the end of week 4 and the beginning of week 5. The epipharyngeal plaques are four thickening of the ectoderm, located dorsally to the four external pharyngeal grooves. The ontogeny of the sensory ganglia of the cranial nerves is closely related to the embryological development of the pharyngeal apparatus, a transient region responsible for the development of the inferior part of the face and neck (Carlson, 2004).

The trigeminal, facial, glossopharyngeal, lymph nodes and parts of the vestibulocochlear nerve and vagal ganglion derive from the neural crest. Neuroblasts derived from epibranchial ectodermal placodes contribute to the formation of V, VII, IX, X.

Epibranchial neuroectodermal plocods contribute to the taste components of these nerves. The vestibulocochlear ganglions appear from the otic vesicles, neural crest cells and from an epibranchial ectodermal plocode. The embryology of the facial nerve is complex, which is why we will present, chronologically, aspects of its morphogenesis, orienting us on the established studies (Sataloff 1990, 1991; Sataloff and Selber, 2003).

In weeks 3-4, facio-acoustic primordium appears during week 3 and is attached to the rostral metencephaly to the optic vesicle. It becomes more superficial and rostral as it advances ventrally, its extremities being adjacent to the deep surface of the epibranchial plate on the dorsal and caudal side of the first pharyngeal groove. It has no branches, and the geniculate lymph node is not yet present.

In weeks 5-6 the mesenchymal condensations that will generate the cephalic muscles are found associated with their nerves. The geniculate lymph node is visible, as is the large stony nerve. The tympanic nerve enters the mandibular arch and terminates near a branch of the trigeminal nerve that will become the lingual nerve. The posterior auricular nerve appears near the tympanic cord nerve. From the middle of week 5 (10 mm embryo), the facial nerve gives small branches to the premuscular masses of the posterior belly of the digastric. The nerve ends in the mesenchyme. In the 8 mm embryo, all cranial nerves except olfactory and optic nerves can be recognized. Sensitive fiber nerves, including the facial nerve, have

prominent lymph nodes near the site of connection with the brain. In the 8-14 mm long embryo, the posterior auricular nerve in the face is located near the tympanic cord (Sataloff and Selber, 2003). The separation of facial and acoustic nerves is complete. The intermediate nerve develops discreetly (Bascom and Schaitkin, 2003).

At the 14 mm long embryo, the geniculate lymph node is well developed and the epibranchial plaques have disappeared. In the embryo 16-17 mm long (mid-week 6), the superficial layer of the mesenchyme of the second arch is divided into 4 laminae: occipital, cervical, mandibular and temporal, and in the embryo 10-18 mm long, from the layer deep of the mesenchymal blade the posterior digastric complex is differentiated (stapedius muscle, posterior belly and tendon of the digastric muscle, the styloid muscle) (Sadler, 2019). At the end of week 6, in the 18 mm long embryo, the intermediate nerve is smaller than the motor root of the facial, and the ribs of the tympanum and lingual are located near the submandibular gland (Carlson, 2004).

In weeks 7-9, several branches of the peripheral portion of the facial nerve begin to become visible: the most caudal establish communications with the second and third cervical ganglion and form a plexus located in the second pharyngeal arch, some branches have ventral pathway and terminate deep to the myoblastic lamina of the platysma, other branches are directed to the corner of the mouth, the rest of the branches are directed to the first pharyngeal arch. All branches have intimate relationships with the deep face of the myoblastic lamellae that will generate the muscles of facial expressions. At this time, the parotid gland appears as a lateral evagination of the oral cavity, undivided, located rostral to the branches of the facial nerve. Only a small branch approaches the superficial buccal region of the parotid gland (Rohen, 2006).

At the 26 mm long embryo (end of week 7), a considerable number of separate peripheral branches are found, all peripheral divisions being identifiable. Only the front branches do not yet reach the corresponding region. The connections with the branches of the trigeminal nerve (infraorbital, buccal, auriculotemporal and mental) are also established. At the embryo 20-45 mm long (week 7 - mid week 8) the mandibular-cervical marginal combined branch is evident. At this time, the superficial layer of the mesenchyme of the second pharyngeal arch further differentiates two laminae: infraorbital and occipital, for the platysma muscle (Rohen, 2006).

A branch of the temporal division reaches the frontal region, but at this moment, there is no branch to the merged eyelids. In the 37 mm long embryo, the orbicularis muscles of the mouth, the levator of the mouth and the orbicularis of the eye develop, and in the embryo of 50-60 mm long (week 9), the occipital muscles, the mandibular portion of the platysma muscle are formed. , levator of the upper lip, muscles towards which the peripheral branches of the facial nerve gradually advance (Carlson, 2004).

In weeks 10-15, at the embryo of 58-80 mm in length, the peripheral branches of the facial nerve extend much, some reaching the midline. Also, there are numerous communications with the trigeminal nerve in the perioral and infraorbital region. In week 11, at the 80 mm long embryo, the branches for the lateral part of the eyelids are visible, the nasal muscles develop, and the relations with the parotid are similar to those established in week 7. In week 12, the relations of the nerve to the parotid gland are complicated by the connections that are established between its superficial and deep portions. In weeks 16-20 all definitive

communications of the facial nerve are established at the embryo of 146 mm length (week 16) (Carlson, 2004). During this period, the mesenchyme around the facial nerve differentiates into connective tissue and will form adjacent structures.

This research direction has been materialized by publishing the following articles:

1. Hînganu D, **Scutariu MM**, Hînganu MV. The existence of labial SMAS — Anatomical, imaging and histological study. Ann Anat 2018; 218: 271-275.
2. Romanec C, Pacurar M, Decusara M, **Scutariu MM**, Hinganu D, Hinganu MV, Ciupilan, C. Labio-palatine cleft, morphological substrate. Rev Chim (Bucharest) 2018; 69(4):1002-1005.
3. Ciupilan C, Hinganu MV, Hinganu D, Ciurcanu OE, **Scutariu MM.** Study of the occurance of labio-palatine cleft embryogenic disorders of the stomatognated system by using lots of wistar rats females. Rev Chim (Bucharest) 2017; 68(11): 2694-2699.

1.2. Morphofunctional concepts of SMAS

1.2.1. Introduction

Superficial fascia of the different regions of the body is universally accepted that it exists and proceeds from one region to another, except the face. In parotid, masseteric and jugal regions its existence is evident, while in the middle regions of the face - nasal and oral - it is long disputed.

Not long ago (Tessier, 1989) it was proposed the existence of a morphofunctional cervicofacial system formed by platysma muscles, mimic muscles and superficial cervical and facial fasciae. At the facial and anterior cervical regions, the superficial fascia does not have a role of separation between superficial muscles and skin, but a role of cohesion and connection between them.

The soft tissue architecture of the face can be described as being arranged in a series of concentric layers: skin, subcutaneous adipose tissue, superficial fascia, mimics muscles, deep fascia (parotid masseteric), facial nerve plane, parotid duct, and oral fat pad (Hînganu et al., 2017).

The term SMAS introduced in 1974 defines a musculoskeletal sheath that continues the frontal muscle to the platysma and acts as a "contraction amplifier of the facial muscles". SMAS action is to transmit muscular contraction to the skin: each muscle contraction follows a preferential direction in the network. An infinite number of actions are possible because, on the one hand, SMAS conveys the contraction of the muscles along the parallel network to the skin plane, and on the other hand it transmits the resulting effect in a direction perpendicular to the skin through fibrous expansions from SMAS towards dermis.

At the beginning the notion of SMAS was proposed to designate an anatomical-surgical structure that is individualized from the platysma muscles. The main description was made in the parotid and cheeks regions, which stimulated many surgeons to perform dissection on each planes of the face regions (Mitz and Peyronie 1976). As a result, the initial description has been

completed over the years, without full consensus of all the authors. The most complete descriptions are made in the cheek region (Bosse and Papillon 1987). It is admitted that the fascial plane that cover the parotid gland it is made by parotid and masseteric part of SMAS and skin (Gardetto et al., 2003).

Some researchers (Gola 2005) consider this extension to all face regions to be abusive without sufficient data.

Other researchers (Delmar 1994) considers SMAS as a strictly surgical superficial anatomical structure derived from primitive platysma and risorius muscle. Another group of researchers consider as beeing formed by a distinct fibromuscular layer consisting of platysma muscle, parotid fascia and fibromuscular layer of the cheek (Gossain et al., 1993).

In our previous studies we have investigated the disposition of this musculofascial structure in the lateral regions of the face, dissecting and exploring the fixation means for the soft facial structures to the proximal periosteum. We have identified the ligamentary adhesions, ligaments and septa that fix SMAS to the bone, these being the main mechanism of antigravitational support for the soft facial tissues. From this point of view, the existence and particularities of the superficial musculoaponeurotic system plays a crucial role against the phenomenon of „aging face".

We have also identified and researched the morphology and function of SMAS in the Moebius Syndrome. Congenital atresia of the facial nerve leads to important functional deficiencies of the perioral muscles which, over time, affect adjacent regions by elongation of fixation means and appearance of superficial soft tissue prolapse. Surgical reconstruction technique which use the temporal muscle tendon transposition is based on the existence of an oral superficial musculoaponeurotic system.

There is a great variability in the histological aspect of SMAS in different facial regions, describing individual and regional peculiarities at the same person (Rubin, 1974). Due to the large microanatomic variability, SMAS can be histologically identified in the continuation of some reference structures such as platysma. It is characterized by extreme variability in terms of thickness, from one individual to another, but also in the same individual, right / left. A particular attention should be given to patients with a history of malignant tumors or metabolic disorders such as diabetes mellitus. These patients require particular attention due to tissue fragility caused by paraneoplasic syndrome.

In support of this theory there are several aspects, such as:

- It explains the functional mechanisms of the facial expression muscles;
- Development of the superficial face and neck muscles and fasciae shows that the development of the facial expression muscles, superficial and profound adipose tissue, facial nerve and parotid gland is the result of some divergent movements and migratory confluences (Standring, 2016), which eventually led to anatomical shaping of a superficial and unique layer at face-level;
- It explains the anatomo-functional mechanisms of the „aging face" phenomenon;
- Nowadays cosmetic surgery would not be possible without SMAS. Old cosmetic surgery techniques, which did not take SMAS into account (or maybe it was not known at that time), could cause downright mutilating scars to patients.

The aim of our study was to evaluate the oral structures in anatomo-functional terms, from a lesser-known perspective: the existence of a unique superficial layer that is closely related to both the skin and subjacent muscular layer.

1.2.2. Materials and Methods

Our study was carried out on a material consisting of twenty-four dissected hemifacieses formalized and dissected at the Institute of Anatomy „Ion Iancu" within University of Medicine and Pharmacy „Grigore T. Popa" Iasi. The macroscopic study on the dissection specimenswas performed using the SOM 62 Kaps operator microscope owned by „Ion Iancu" Institute of Anatomy, University of Medicine and Pharmacy „Grigore T. Popa" Iasi. There were identified the following overlapping layers on the dissection specimens: dermo-epidermic, subcutaneous adipose, superficial fascia, superficial muscular layer, deep fascia, profound musculo-glandular elements and periosteum layer, partially sampled in order to stabilize the fragments. The conclusive aspects taken over by an image acquisition system were subsequently examined and processed to spot regional topographic differences.

As regards the qualitative micro-anatomical study of SMAS within the topographic pattern of the face, we sampled soft tissues from oral region, starting from the skin to the bone layout, in the form of small blocks. The sectioning was made perpendicular to the epidermis surface in order to be able to examine the correct sequence of the planes. Sampled fragments were processed using paraffin and H&E stained technique as well as special techniques for connective and muscle tissue (Verhoeff). For stereology technique we used PRODIT 5.2 programme in order to make quantitative measurement of connective and musculartissue.

Our casuistry also included a group of fifteen patients imagistically explored for aesthetic surgery purposes or for congenital malformations surgical treatment (Moebius syndrome, congenital facial nerve paralysis) in the Maxillofacial Surgery Clinic within "St. Spiridon" Emergency Clinical Hospital, Iasi – a University based hospital.

MRI is the imaging exploration method that provides the most conclusive images on the cervicofacial soft structures. It is able to demonstrate the stratygraphic anatomy data in detail, obtaining images similar to CT but with a better differentiation of the soft tissues.

1.2.3. Results

Oral region has as its main feature the existence of an infraSMAS space filled with adipose tissue. This space is a way of spreading for an infection into neighbouring regions. The infraSMAS connective tissue from this level contains fibres organized as conjunctive tracts which separate the fat lobules (**Figure 1).**

Its structure includes also muscle fibres from the buccinator muscle. Muscle adhesion to the skin through SMAS leads to the conclusion that SMAS actively intervenes in the masticatory act, pulling the skin in direction of the movements of masticatory muscles (**Figure 2).**

Starting from the zygomatic arch and progressing to the mandible, SMAS and infraSMAS tissue are giving the cheek appearance by the amount and shape of the adipose tissue, which they delimit it. Here, it also offers skin firmness at the level of the deep planes.

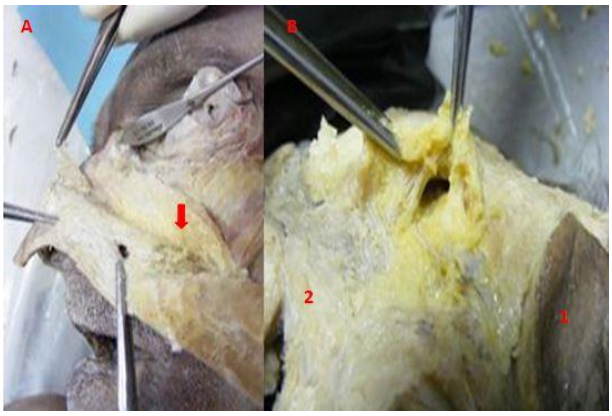


Figure 1 - *Connective-adipose tissue from the cheek, prebuccinator and perioral region. Red arrow from picture A points the examined region from picture B. 1 represents the infraorbital region and 2 the labial commissure. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 10/1 scale in the right image.*

Oral muscles adhere intimately to the deep surface of the skin, crossing the superficial fascia. At this level, into the thickness of the musculoaponeurotic system there is adipose tissue trabeculated by conjunctive septa and labial vascular nervous structures. The insertion is firm, almost impossible to dissect. Because of this, superficial fascia is extremely difficult to be shown by classic anatomical dissection (macroscopic). Thus, it is preferred to choose the operator microscope for examination of this region.

We found the continuation of the SMAS with the superficial fascicles of orbicularis oris muscle, suggesting that this layer represents SMAS into superior and inferior lips, separated by the overlying fascial layer (**Figure 3**).

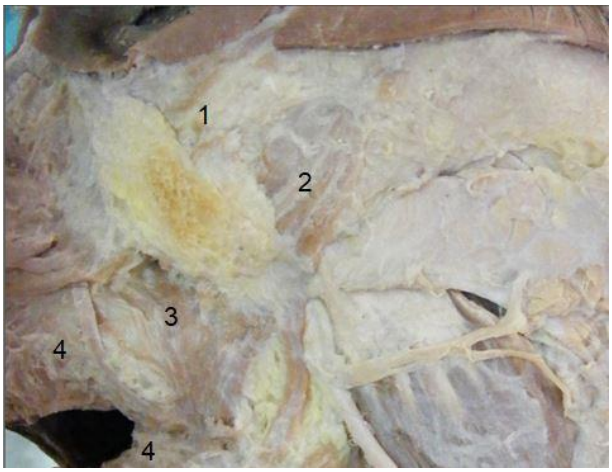


Figure 2 - Muscles of facial expressions at the mouth angle: 1 - zygomaticus minor, 2 - zygomaticus major, 3 - levator anguli oris, 4 orbicularis oris. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 3/1 scale.

We have to emphasize once again the importance of zygomatic ligament, which also contributes to raising the angle of the mouth, applying traction to the superficial fascia of the region. Traumatic injury or its involvement in another pathology may lead to the appearance

of some deformities when attempting a facial expression. Its structure is unitary, robust, with connective fibers in its axis (oriented in the superior-medial direction, in the sense of the traction forces vectors acting on it), macroscopically visible. It has two portions: medial (periorbital) and lateral (tragally) (**Figure 4**).

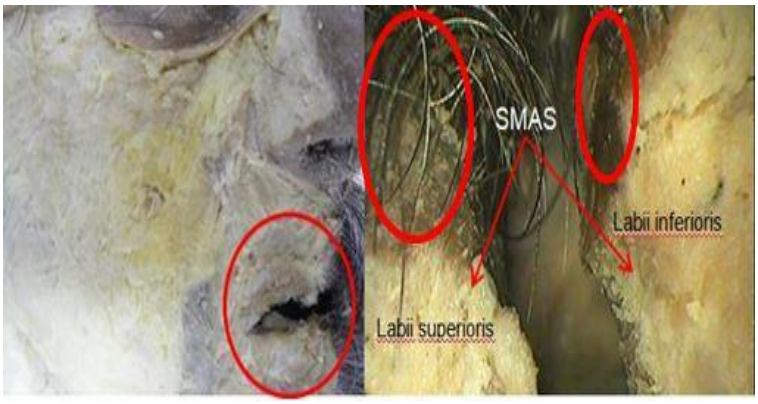


Figure 3 - SMAS at the level of the lips. Red circle in the left picture points the interest area, magnified into the right one. The two red circles from the right picture mark the areas of both lips where SMAS ends. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 20/1 scale.

Dissecting downward to inferior lip, we have easily revealed a musculo-conjunctival infradermic layer on anterior mandibular surface, which offers attachment for mental muscles.

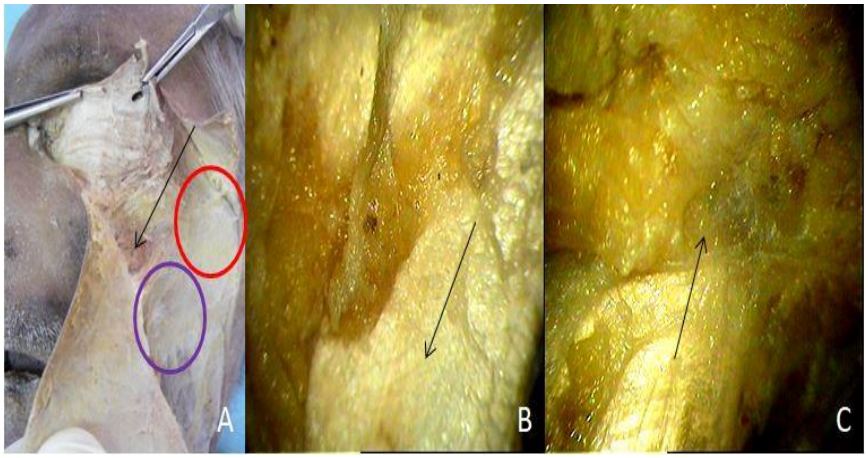


Figure 4 - Image A shows within the two circles both parts of zygomatic ligament. First part, marked by the red circle represents the tragal part, magnified in picture B. Purple circle represents the medial part which adheres on the skin of the cheek, magnified in picture C. The arrow points the direction of ligamentary fibers, from zygomatic arch to the cheek, in all 3 pictures. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 20/1 scale in B and C images.

Usual staining, specific to connective and muscular tissue, reveals that collagen fibers at this level are thinner, rarefied and placed in disorder, along with more elastic fibers and with muscular fibers longitudinally disposed in the SMAS structure at the upper lip level (**Figure 5**).

Interconnecting muscular fascicles of orbicularis oris muscle, we will find collagen fibers with the same longitudinal disposal, from which thin paths descend to the skin (**Figure 6).** The conjunctive fibers of SMAS, medial of the nasolabial groove, have longitudinal disposal.

At the angle of the mouth, the numerous collagen fibers lose their fibrous structure making dense clamping strips, which also provide resistance to muscle contraction (**Figure 7**). They are intertwined with fascicles of muscular fibers, while the elastic fibers are almost absent.

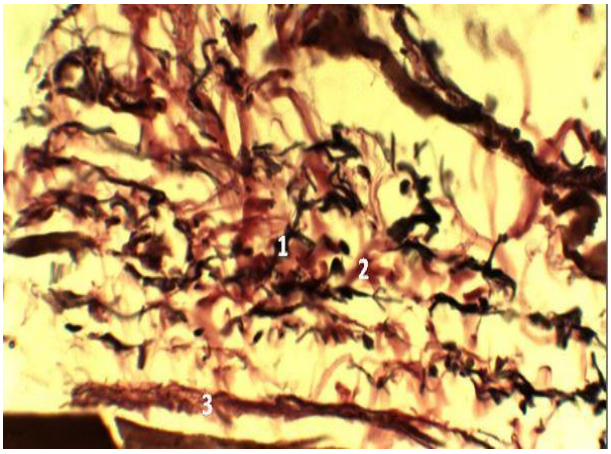


Figure 5 - Thinner collagen fibers (1) and more elastic fibers (2) along with longitudinal muscle fibers (3) in the SMAS structure from the upper lip, middle third level.Col. Verhoeff, ob. 40x.

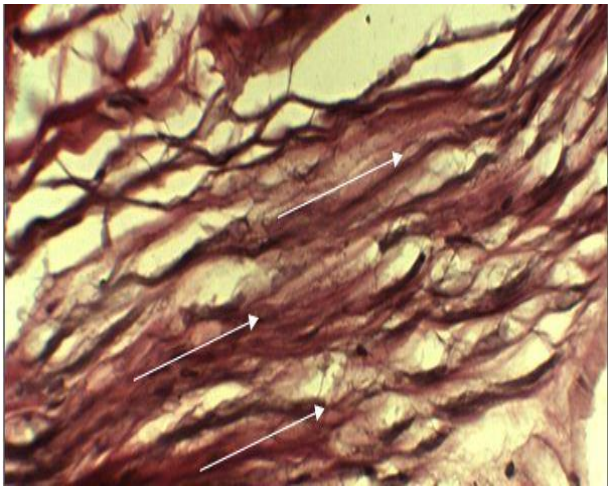


Figure 6 - SMAS medially to nasolabial sulcus with longitudinal disposal of collagen fibers, marked by white arrows. Col. Verhoeff, ob. 40x.

On the lamella stained with Verhoeff`s method we quantified the volume percentages of connective tissue, muscular fibers and interstitium compared with the other components, including adipose tissue using.

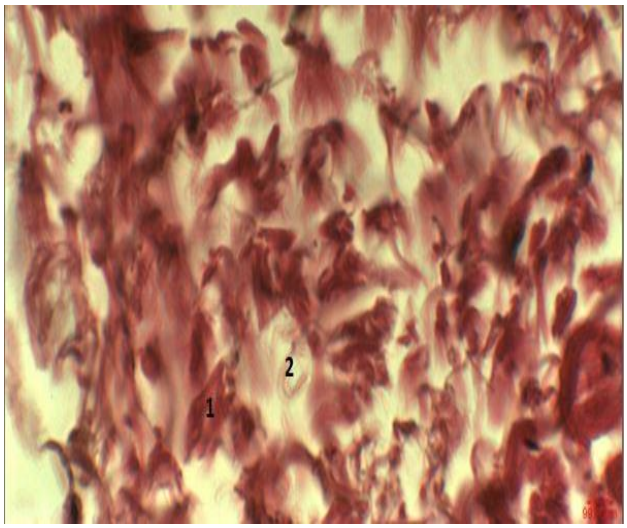


Figure 7 - The SMAS structure at the mouth angle, with many interwoven collagen fibers (1) and with elastic fibers almost absent (2). Col. Verhoeff, ob. 60x.

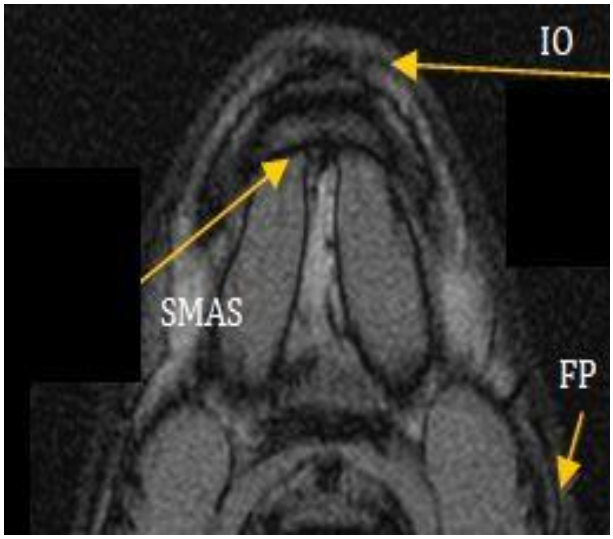


Figure 8 - MRI horizontal section through inferior mandibular margin; SMAS and profound fascia (PF) between parotidian and mental region; TransSMAS insertion of orbicularis oris, inferior fascicle (IO).

Quantitative measurements show the following volume percentages quantified by stereological examination of the lamellas:

- connective tissue – 45.56%;
- muscular fibers – 38.15%;
- interstitium – 16.30%.

SMAS cannot be identified in the free margin of lips, both macroscopically and microscopically. Verhoeff staining shows the lack of elastic fibers and the fact that the interstitial tissue prevails.

The MRI images show that above the superior margin of the mandible, the superficial fascia (SMAS) also behaves differently:

a. medially, it gives insertion to orbicularis oris muscle (inferior fascicle) and then to depressor anguli oris muscle (**Figure 8**);
b. laterally, it becomes mobile, ascending first above jugal fat pad, buccinator and then masseter muscle (**Figure 9**).

At superior lip, superficial layers become fixed once again. This happens due to insertion of orbicularis oris muscle (superior fascicle) and levator labii superioris on the profound surface of the skin, transfascially.

Even if superficial fascia gets thinner and thinner to modiolus, its thickness is still enough to appear as a clear tissue strip on MRI. The same thing is revealed on dissected specimens.

Ascending to the nasal septum base, the two fasciae (superficial and profound) are united into a dense conjunctive structure. The more we are going to the nasolabial groove in its medial part, the more clearly superficial fascia becomes.

It provides protection for the superior branch of angular artery and for superior labial fascicles of buccal branch from facial nerve. Injures of these branches of the facial nerve or of its trunk cause static deformities of this region, alimentation and phonetic disorders, depending on the scale of the injury.

These disorders are reflected in the skin insertion of the muscles and the continuity of SMAS towards the other regions that involves their step by step transmission (**Figure 10**).

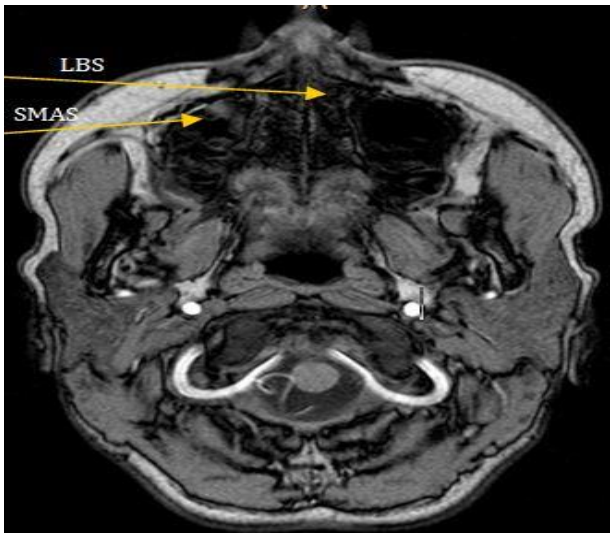


Figure 9 - Transversal MRI which illustrates TransSMAS insertion of levator labii superioris muscle (LBS).

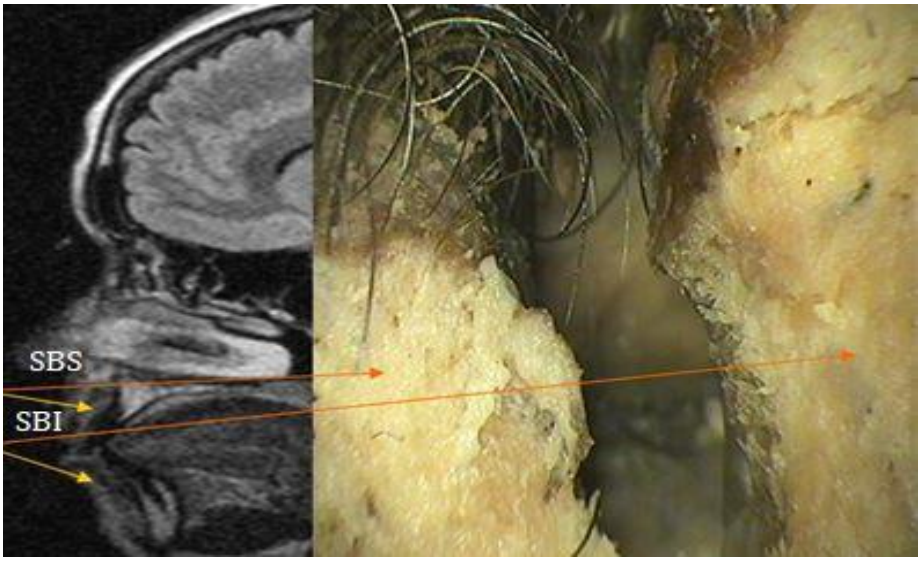


Figure 10 - SMAS at superior lip (SBS) and inferior lip (SBI).

1.2.4. Discussions

The perioral region provides a framework through which the observer views the dentition. Often, costly and time-consuming dental work remains hidden behind a less-than-optimal soft tissue window**.** In the aged patient, a more complex situation exists. Predictable changes that occur in the perioral region with aging include loss of lip volume and architecture, lip lengthening and inversion, and rhytid formation secondary to accumulated actinic damage and repeated muscle movements. These changes are progressive and are generally considered unaesthetic. For the patient who has undergone dental rehabilitation, in addition to facial rejuvenative procedures such as rhytidectomy (facelift) or browlift, inattention to this area is often the ‘‘give-away’’ of the patient’s true age (Perenack, 2005).

There are many instances in which the dentoalveolar complex influences the drape of the facial tissues. For example, because the lips frame the dentoalveolar complex, it is important to consider them when performing any esthetic procedure. The practitioner must evaluate lip support, in both relaxed and animated positions, because it may be affected by the placement of anterior restorations. This must be considered if lip augmentation is planned, to achieve an ideal esthetic result. It is important to understand the underlying processes that cause facial aging before attempting to correct any age-related changes in the face. Facial aging occurs at every level of the soft tissue envelope. Changes occur in the skin, subcutaneous fat, superficial muscular aponeurotic system (SMAS), deep fat, and muscles of facial expression (Kenneth, 2015).

Thus, SMAS limits are:

- Backward, the cartilaginous part of the external auditory conduct and the mastoid region, on which is firmly attached (fixed point)
- Anteriorly (mobile point), it is extended superiorly by greater zygomatic muscle and inferior to the comissure is gradually reduced, the cutaneous muscles of this level not beeing included in SMAS. In fact, the color of these muscles is violet, not pink-salmon like platysma and rizorius muscles. SMAS sets only a few adhesions with perioral cutaneous muscles. The lack of SMAS in the lower part explains the reduced

efficacy of the SMAS flaps in the nasolabial groove. SMAS sets adhesions with the lateral edge of the orbicular eye muscle (pars orbitalis).

- Inferiorly – clavicular region (mobile point)
- Superiorly - the zygomatic arc on the periost of which joins SMAS (fixed point). From there, it continues with the temporal or superficial temporoparietal fascia and covers the temporal region (temporal aponeurosis and homonymous muscle).

SMAS, as well as the superficial fascia, separates the skin's superficial adipose tissue from the deep cellular tissue. For this reason, when it is isolated by dissection, it appears more or less fat.

The soft tissue planes are supported in a normal anatomical position by a series of restraining ligaments coming in from the depths, fixing the facial structures to the superjacent.

SMAS relations

We underline the SMAS connection with the skin of the region but, in particular, its relations with the superficial muscles of the face (the muscles of the facial expressions) with the deep fascia and the neurovascular elements underneath. From a functional point of view, the attachments means of SMAS to viscerocranium are of utmost importance. These gives the firmness of the face skin and plays the role of fixed point in the movements of the mimics muscles. They prevent the appearance of wrinkles and the phenomenon of aging face.

Superficial relations of SMAS

SMAS forms with skin a functional, adipose and neurovascular unit, physiologically inseparable, cutaneous-mucoid-aponevrotic cervicofacial unit. The superficial surface of SMAS is united by the deep face of the dermis through fine, perpendicular or oblique fibrous tracts that isolate adipose lodges that form adipose paniculi. This superficial subcutaneous fat tissue has particular functions (elasticity, retractility). With aging, these tracts relax, the paniculum diminishes, and the skin, which in turn loses its elasticity, becomes too large and hangs (Pan, 2017).

The function of SMAS is to convey the activity of the mimics muscles to the skin and thereby to adjust the expression of emotional states. In the latero-facial region, the subcutaneous fat, more or less thick, is disposed as follows:

- superior - the zygomatic arch or adipose tissue mentioned above and the jugulopalpebral fold under which the palpebral skin adheres to the orbicular muscle,
- inferior – the superior part of the neck under the basilar edge of the mandible where the platysma muscle adheres to the skin,
- anterior - nasolugal fold which is determined by the jugal subcutaneous adipose tissue, the adherence of the labial region and by the tonus and contraction of the levator and dilation labial muscles,
- posterior - the ear pavilion and the parotid region where the adipose tissue gradually decreases from anterior to posterior.

As for the zygomatic bone, there is a particular adipose deposit that participates in the projection of the cheekbones and covers the zygomatic muscles and the inferolateral part of the orbicular eye muscle. This adipose deposit adheres to the skin and is united with SMAS.

Deep relations of SMAS

The cutaneous muscular aponeurotic cervicofacial unit is separated from underlying planes, through deep subcutaneous tissue that has the function of a sliding plane. If deep subcutaneous cellular tissue is reduced, SMAS adheres to the underlying planes and the skin loses some of its mobility.

The neurovascular elements in its immediate neighborhood are numerous and complex. From this point of view, we have to emphasize the complexity of the parotid region where the facial nerve is found (it is divided into the terminal branches within the parotid gland), as well as branches of the external carotid artery. Also in this region the external jugular vein is formed. SMAS covers the parotid fascia, coming in close proximity to the above-mentioned elements.

The course of the facial nerve branches is also in the vicinity of the SMAS, as well as branches of the trigeminal nerve. The facial nerve branches are considered the most variable anatomical elements, and the use of landmarks allows them to be located with precision (Sataloff and Selber 2002, Terzis et al., 2004).

The vascular supply of the skin through the perforating arteries coming from the underlying muscles allowed the description of the musculocutaneous territories, later known as angiosomes (Taylor and Palmer 1987; Stuzin et al., 1989; Thesleff 1998).

With age, there is a reduction in vascularization by fragility of terminal capillaries, by diminishing blood flow and elasticity of the vascular wall. This reduction in vascularity is accelerated by arteriosclerosis and tobacco. Sensitive skin innervation is ensured by the auricular branch of the superficial cervical plexus through branches of the trigeminal nerve: the auriculotemporal, the buccal and the mental.

SMAS relationships with nasolabial groove are still controversial. Literature studies (Mitz et al., 1976) described anterior continuation of SMAS into the superior lip, overlying the muscular layer. Barton et al., (1992) described a thin fascial layer covering the zygomatic muscles and extending into the superior lip, but they do not identify the subcutaneous extension of SMAS. Pensler et al., (1985) distinguished a SMAS layer which is medial to the nasolabial groove, whereas Yousif et al., (1994) consider that there is an adipose supraSMAS layer on the cheek and superior lip.

On the dissected specimens we have identified, starting from superficial to profound, the following layers:

- *cutaneous, with a denser profound part, acting for insertion of the facial expression muscles;*
- *subcutaneous adipose layer;*
- *superficial facial muscular layer, arranged as follows:*
    - ***oral region**: zygomatic, rhizorius, orbicularis oris, levator labii superioris, and at the angle of the mouth, modiolus, levator anguli oris and the buccinator;*

- ***mental region****: depressor labii inferioris, depressor anguli oris, platysma, mentalis.*

This particular SMAS architecture into oral region is the most important mechanism of support against the "aging face" phenomenon, preventing the occurrence of perioral creases, and downturned of oral commissures (Jeffrey et al., 2015).

Anatomy studies conducted with focus on superficial muscular and fascial structures support the Duchenne`s statement in 1862: "*the law governing the expression of the human face can be discovered by studying the action of the muscles*" (Duchenne de Boulogne GB, 1990).

Surgical techniques of facial rejuvenation and dynamic resuscitation of the oral sphincter are based on the existence of this functional musculoaponeurotic facial system (Pidgeon et al., 2017)

In this context, SMAS, the musculofascial sheath that goes downward the frontal muscle to platysma, plays an essential role, acting as a "**facial muscular contraction amplifier**". In fact, it is a distributor of muscle contraction towards the skin: each muscle contraction follows a preferential direction in the network.

An infinite number of actions are possible because, on the one hand, SMAS conveys the contraction of the muscles along the parallel network towards the skin area, and on the other hand it transmits the resulting effect into a perpendicular direction towards the skin, through fibrous expansions from SMAS towards skin. Lifting of the upper lip for facial rejuvenation is based on these features of the regional muscles (Bai-lin Pan, 2017).

SMAS is present in the upper lip and represents the superficial portion of the orbicularis oris muscle but it is not revealed at the free margin of the lips. The main feature of this region is the fixity of the superficial structures to the profound ones. This feature is the basis for the principles of oral facial lifting (Le Louarn et al., 2006; Fogli, 2017).

1.2.5. Conclusions

This is caused by strong and flared insertions of the muscles from this level to the profound face of the skin. We can state that it is the real visceral segment of the face. Some oral muscles (orbicularis oris fascicles) play the role of sphincter of the orifice they surround while the others are functional extensions of them. The more we move toward the midsagittal plane, the thinner the skin becomes. The same disposition is revealed in the subcutaneous adipose tissue, with the mention that the supraSMAS adipose layer is almost non-existent. The superficial and deep fasciae lose their elasticity, being composed of dense connective tissue.

Facial SMAS is a unitary structure that fixes the dermis to the facial bones providing a multiligamentary fibrous support system. We showed that oral SMAS exists and facilitates the skin insertion of the oral muscular apparatus. Through SMAS, the oral muscles can exercise their controlled contraction and sphincter function. Zygomatic ligament is the key structure in the dissection process for successful mobilization of the middle part of the face. The facial nerve branches are located between the muscular layer and SMAS. At the upper lip, SMAS has a mixed structure with quite small quantitative differences between the connective and muscular tissue. SMAS is also realizing connective tunnels for the branches of

the angular and buccal artery. These anatomical findings could be useful for the understanding of the SMAS concept and when performing various types of facial surgery.

1.3. Embriological errors: the labio-palatine clefts – a clinical morphological study

1.3.1. Introduction

It is well known that both genetics and the environment play an important role in the etiology of labial (cheiloschizis) and palatinal (cheilognatopalatoschizis) clefts. Embryogenesis of the cleft can take place via several mechanisms: the lack of contact between the maxillary buds which may occur as a result of the volume change of the buds and, also, the lack of contact can occur (Bayerlein et al., 2006; Sadler, 2019) by distortion of some cranial-facial partitions. This can happen even if the buds have normal dimensions. The lack of epithelial fusion, even under the contact of buds is a poorly accepted mechanism. Perforations of the epithelial fusion spots by the occurrence of epithelial cysts and pearls along the fusion area, which may increase and cause failures of buds' union (Bing et al., 2011). It has been found that palatine clefts are more common in females (Carstens, 2004; Sadler, 2019). This might be due to the fact that the ascendancy of the palatine processes would later occur in female gender during the organogenesis. Overall, male is more offen affected by labio-palatine clefts than the female gender. It is generally accepted that there is a genetic component in the cause of clefts, about 40% of cases presenting a family history of labio-palatine cleft. This genetic predisposition is increased by environmental factors (Cash, 2012).

The failure of mesenchymal migration is considered to be the most important mechanism in the etiopathogenesis of cleft. Mesenchymal migration may be defective due to initially low amounts, or under the influence of various environmental factors.

From an etiological consideration, most of the cases of clefts can have a complex morphogenesis, which consist of the interactions between propensing genetic factors and environmental factors (Daw and Patel, 2004).

Identifying environmental factors that increase or decrease propensity to oro-facial clefts continues to be of major concern. Disclosing the mechanism underlying maternal smoking and oral clefts could reveal the possible pathways leading to the defect (Enemark et al., 2001).

Etiopathogenesis of the clefts has been intensively studied but is still poorly elucidated. Nowadays it is considered to be the result of intercourse between different genetic factors and the environment (exogenous). Teratogenic action depends on the so-called embryological schedules. After the end of the critical period specific to organ development, the teratogenic agents no longer have any malformative action (Evans, 2004). The same teratogenic agent may cause various malformations, depending on when they act (Gkantidis et al., 2012).

Between the chemical agents an important role is played by drugs. The intent of drug action on the embryo increased after the impact caused by the use of thalidomide.

Aminopterin and other folic acid antagonists (such as Dilanthin) also have a teratogenic action. A series of antibiotics (Hadacidin) or antimitotic (cytostatic) are capable of producing malformations. Although, no malformations have been observed in humans. Trasler recommends avoiding aspirin that caused clefts in mice.

The actualy knowledge about the influence of several factors and radiation on cell division is due to the discovery of uranium radioactivity by H. Becqerel. The effect of radiation depends on: the penetration capacity; energy absorbed by irradiated tissue; relative ionization density; cell radiosensitivity that is directly proportional to proliferation capacity and inversely proportional to the degree of differentiation and varies with species. With regard to sources of contamination of the human body, they can be grouped into two categories: natural radiation and artificial radiation.

Basic nutritional deficiencies (nutritional factors): proteins, lipids, carbohydrates do not seem to play a role in the etiopathogenesis of malformations in humans. The same can be said about mineral deficiencies. Probably some congenital dysplasia seen in some nutritional deficiencies are due to vitamin deficiencies (Hout et al., 2011).

Infectious factors may have teratogenic effects, causing under certain conditions structural changes, chromosomal and nuclear mutations, disruption of the cell division. The most well-known are the malformative consequences after viral infections of the pregnant mother. The cytomegalovirus inclusions were also teratogenic, herpes virus, urliane virus, influenza virus.

The uterine environment can influence the development of the embryo: anatomical and physiological variations in the uterus, such as altered blood supply (hypoxia) or changes in uterine pressure or uterine fluid quantities may more frequently cause malformations at the level of the palate; metabolic alterations have significant teratogenic potential. A series of hormones and metabolic degradation products can cross the placenta. Diabetes is a high-risk disease for the embryo. Thyroxine deficiency is another factor that is experimentally proven to be a producer of facial cleft.

The family study of children with facial malformations can bring forward a series of features that indicate a genetic predisposition (Izuka et al., 2005). Thus it is suggested that the parents of the children with a cleft have a lower development of the middle floor of the face, but unfortunately it is not possible to have a relevant control of the adult population. It also appears that the parents of the children with cleft can have a recurved upper lip, a larger transverse diameter of the face and a tendency of hypertelorism (Losee and Kirschnner, 2008). This suggests a deficiency of the skeletal component that derives mainly from embryonic mezenchim, in families with a history of positive facial malformations.

From the results of the research om nirth defects transmission, we noticed the large incidence of multiple cases in the same generation in families without a teratological history, which advocates the possibility of mutations under the influence of environmental factors in germ cells of the past generation (Josip et al., 2006).

In cases where malformations of the face, tramsmitted to 2-3 generations, it could be observed a great variability of the morphogenetic mechanisms. Probably the intervention of the numerous endo- and exogenous factors settle the irregular type of the transmissibility of these defects, the persistence or their extension. It can be surmise that the transmissibility of such congenital defects has a dynamic character (Kapp-Simon, 2004).

The same impression is left behind by the comparative study of clinical-anatomical types. Two major tendencies can be observed: to maintain the type of malformation and polymorphism, with a high risk of aggravation (Karsten et al., 2006). This latter trend suggests the alteration of the hereditary substrate, which can be expressed variably under the influence of environmental factors.

The finding that sometimes the labio-maxilo-palatine clefts are accompanied by malformations of other segments of the body, may prove the damage of genetic "nodules" with somatic consequences at different levels. Thus, the clefts can be included in some chromosomal anomalies that cause general syndromes such as Treacher-Collins, Pierre-Robin and Klippel-Fiel.

Treacher-Collins Syndrome, or mandibulo-facial disostosis has an autosomal dominant substrate. It is characterized by hypoplasia of the zigomatic bone and the mandible, which has a retrognative position, along with palpebral fisures, malformations of the ear structure and auditory deficiencies; 30% of cases have labio-palatine clefts (Laletin and Iastremski, 2012).

Also, alongside the cleft, a number of other congenital defects associated with certain systemic complications may occur to a greater extent than in the case of labial clefts (Leonard et al., 2004). These include cardiac, central nervous system, kidney and skeletal defects, which are associated with 20-25% of palatine clefts and often complicate treatment management.

Pierre-Robin's syndrome is characterized by a retrognate mandible, hypoplasia, glossoptosis and palatine clefts, leading to a respiratory distress, a hallmark of this syndrome (Lore and Medina, 2005).

Klippel-Fiel syndrome is manifested by an irregularity of cervical vertebrae, along with clefts. It should be noted that these syndromes are encountered with a fairly low frequency. Labio-palatine cleavage syndromes are associated with genomic polymorphisms of genes (TGFA) encoding growth and transformation factor-alpha (TGF-V), a ligand-containing epidermal growth factor receptor (EGFR) comprised of the majority of the epithelium (Lupan, 2004).

Labio-palatine clefts are embryogenesis disorders of the stomatognathic system that occur due to the action of genetic or non-genetic factors in weeks 5-6 of intrauterine life. The clefts fall into the group of congenital malformations of the face. They appear from birth as a slit in the upper lip, hard palate and/or soft palate (Marazita and Mooney, 2004).

If the maxillo-facial development is almost normal in unoperated labio-palatine clefts, there is a developmental deficit in all three directions of the space, due to postoperative scars and bone hypoplasia, with consequences on dento-maxillary functions (Marsh, 2004). The monstrous face from the moment of birth, as well as the feeding difficulties of the infant, the defect of speech and hearing of the child with labia-palatine cleft causes psychic trauma over the families from which it originates.

Treatment of labio-palatine clefts is complex and interdisciplinary and lasts for a long time, from birth to late, after puberty.

Early treatment steps are: preoperative orthopedic treatment, especially between 1 and 4 weeks of age, this phase being used in a small number of patients where the alveolar

segments are very large and may complicate an appropriate surgical treatment (McAllister et al., 2007).

The lip plasty is also known as cheiloplasty. It is practiced within 10-12 weeks of life. Surgery is performed when the immune system is more developed. A rule that has successfully passed the time trial and applies for the first surgical step is that the child must have over 10 weeks old, over 10mg% hemoglobin and about 5kg (Mulliken, 2004).

Restoring the continuity of the orbicularis oris muscle by cheiloplasty allows the alveolar segments to fit the anterior region into a functional matrix; eliminates abnormal lateral traction by the orbicular, especially the premaxilary fragment. These two effects allow the alveolar segments to occupy a position as close to normal as possible by repositioning the protruding fragments.

Plasty of the palate is the most controversial stage in terms of timing, staging and surgical technique; it can be practiced around the age of 18-24 months.

The controversies arise from the fact that it was clinically established that a adequate phonation requires a morphological and functional integrity of the secondary palate (the osseous and soft palate) as early as the child begins to speak. For this reason, surgeons prefer to make the interventions around the age of 12 -18 months (Nollet et al., 2007).

Another controversy regards the area of surgical intervention: palatine plastic surgery is initially limited to the restoration of the soft palate (the palatine veil and the lueta), and then to make the osseous palate plasty (the two-stage restoration), or to practice palatin raffia in one time. The techniques most commonly used are the von Langenbeck technique (bipedicular palatine muco-periostic flaps) and the V-Y-push-back technique.

Early orthodontic treatment refers to the alignment of teeth and the correction of crossed occlusions or inverse angles in deciduous dentition or mixed teeth. The aim of orthodontic treatment is to correct any frontal inverse angles, reverse posterior lateral occlusion. As a rule, the disturbances that occur during this period, especially the posterior crossed occlusion, are an indicator of the severity of late dental-alveolar involvement (Palarie, 2011).

Logopedic/phoniatric treatment is more intense in the 6-11 years time period, but can begin earlier than this age. The most disturbing disorder that occurs and causes phonation disorders is the air discharge on the nose during speech (rinolalia aperta). Their appearance occurs when the palatinal vein can not come into contact with the posterior wall of the pharynx, or when it presents mobility disorders or the presence of oro-nasal fistulas. Misalignment of the incisors and tight labial and alveolar scarrs may cause phonation disorders. The primary objective of speech therapy is to correct articulation errors.

The plumage of the alveolar defect is indicated in the clefts that affect the primary palate, where the continuity of the alveolar arcade in the lateral incisors and canines areas is interrupted (Ranko et al., 2008); bone resorption at this level can occur with root denudation and tooth loss (lateral incisor, canine).

The necessity of realizing the dento-alveolar arcade continuity, of its symmetry and of preventing the periodontal damage of the teeth limiting the defect is evident. It uses autogenous or homogenous bone materials, or even materials that induce neoosteogenesis.

The aim of the study was to analyse the main characteristivs of a group of patients with labio-palatine clefts (cheilognatopalatoschizis), Veau Class II-IV. Surgical technique

does not only create a continue layer of mucoperiostic flaps over the defect, but also initially places the bone graft, covering later with the mucous membrane. This intervention technique is not practiced early, with the primary or secondary palate plasty, since the removal of the muco-periostic flaps may affect the maxillary growth.

1.3.2. Materials and methods

The study was performed on a group of patients with labio-palatine clefts, admitted to the Oro-Maxilo-Facial Surgery Clinic, during the period 2012-2016, in order to establish the proper surgical treatment or to perform plastic surgery at the level of postoperative sequelae.

The study group consisted of 22 patients with labio-palatine clefts, of which 16 boys (72.72%) and 6 girls (27.27%), with a weight that varies between 1900g and 3400g, who presented in the clinic for the purpose of establishing specialized treatment.

The anamnesis of the mothers showed they suffered at least one viral infection during pregnancy. All of them denied having taken a medication with a possible teratogenic effect or smoking. We followed a number of parameters, such as: the status of newborns, gestational viral infections, medication administered during pregnancy.

1.3.3. Results

In the studied group, the frequency distribution of the anatomical-clinical forms of the labio-palatine clefts, the highest percentage being recorded by the total bilateral clefts - 0%; unilateral anterior cleft 0%; anterior bilateral cleft 0%; total unilateral cleft - 27.27%; total bilateral cleft - 54.54%; incomplete posterior cleft-9.09%; complete posterior cleft-9.09% (**Figure 11**).

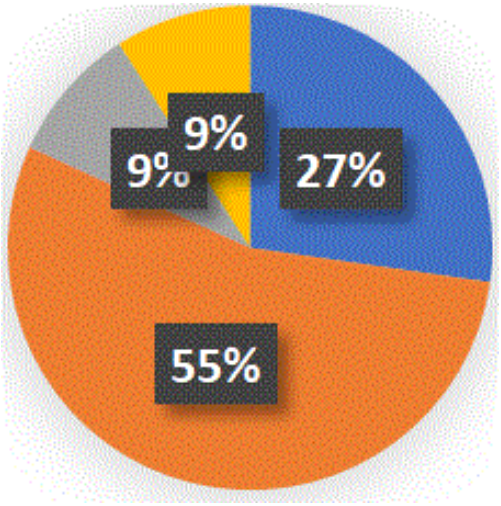


Figure 11 - Types of labio-palatine cleft, within the study group: 27.27%=total unilateral cleft; 54.54%=total bilateral cleft; 9.09%=incomplete posterior cleft; 9.09%=complete posterior cleft

In terms of urban or rural origin, their percentage is urban - 82%; rural area -18% (**Figure 12**).

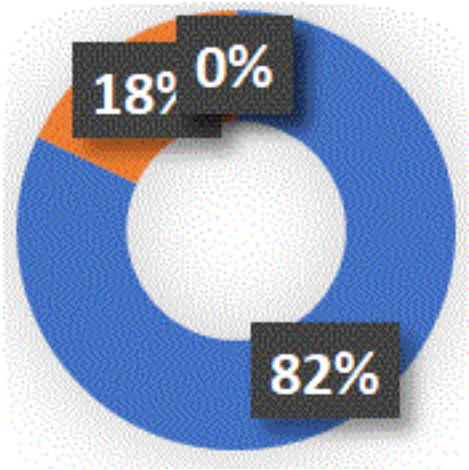


Figure 12 - Regional features within the study group: 82%=urban; 18%=rural

A correlation can be made between the presence of anterior and total clefts in rural children and anterior and total clefts in urban children. When the causal factor acts earlier, decay occurs later, since mesodermization starts from the posterior to the anterior. The more the disturbing factor acts longer, the greater the cleavage.

The cleft cases with normal birth weight have a frequency of 72.72%, 18.18% for dystrophic cases, and 9.09% for overweight patients (**Figure 13**).

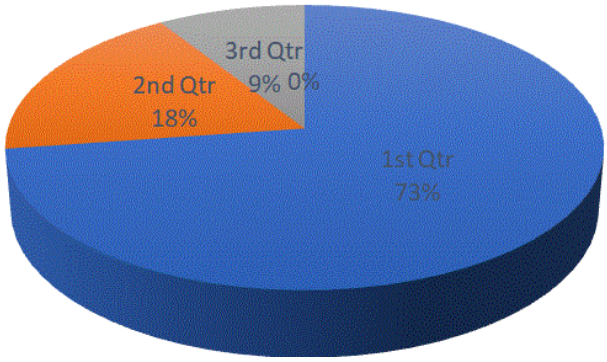


Figure 13 - The correspondance between birth weight and labio-palatine frequency, within the study group: 72.72%=normal; 18.18%=dystrophic; 9.09=overweight

It is found that in approximately 73% of cases birth weight is unaffected, the affection being secondary to an embryopathy that occurs in the 4-12 weeks of intrauterine life.

1.3.4. Discussions

Treatment of labio-palatin clefts is a complex treatment involving a team of specialists. Each of them intervenes at some point in order to ensure that the next therapeutic steps are carried out (Salyer et al., 2004; Rene 2005).

Regarding the surgical management of the applied treatment, the following surgical interventions were carried out: the plasty of oro-nasal oriffice in two planes-18.18%, chyloplasty-27.27%, veloplasty-0.09%, plasty with palatal flap-18.18%; plasty with vestibular flap-27.27%.

Orthodontic active treatment begins around the age of 7 years (Sandove et al., 2004). *The stage of alveolar grafting (8 to 10 years) is followed; to the age of 12-14 years, complex and individualized orthodontic procedures are performed for the final dentition. For some patients, orthodontic treatment may continue up to 18-20 years.*

Approximately 65% of cases still have postoperative sequelae of the labio-maxilo-palatine clefts. The causes are multiple, the most important being the intrinsic changes caused by splitting, as well as the fact that the surgery is naturally followed by the organization of a fibrous scar tissue. Therefore, it is necessary for the primary intervention to achieve a restoration of the heads of the lip muscles continuity. In the case of large bilateral clefts, to allow a smooth passage of the muscle fibers into the prolabium (Semb et al., 2012).

Also, muscle suture should reorient muscle fibers in a physiological manner to allow for a good morphofunctionality. Vestibuloplasty is most often required after bilateral clefts restoration procedures when the vestibular denture is tight or scarred (Shetye, 2004). A dense, normally conformed vestibular ditch determinates the normal functionality of the orbicularis oris muscle. Thus, as secondary changes in the lip were observed either the shortening and the prominence of the lateral lip area. In these cases the lip was too short or too long on the affected side, or where the restored lip is too strained (Vieira, 2012).

The causes of lip and palate clefts among most infants are unknown. Cleft lip and cleft palate are thought to be caused by a combination of genetic and enviromental factors during pregnancy. Understanding the morphogenetic factors that are more common among babies with a birth defect will help us learn more about the causes. Large studies such as the National Birth Defects Prevention Study and the Birth Defects Study to Evaluate Pregnancy Exposure are insisting to understand the causes of and risks for birth defects, including orofacial clefts:

- smoking — women who smoke during pregnancy are more likely to have a baby with an orofacial cleft than women who do not smoke.
- diabetes — women with diabetes diagnosed before pregnancy have an increased risk of having a child with a cleft lip with or without cleft palate, compared to women who did not have diabetes.
- use of certain medicines — women who used certain medicines to treat epilepsy, such as topiramate or valproic acid, during the first trimester (the first 3 months) of pregnancy have an increased risk of having a baby with cleft lip with or without cleft palate, compared to women who didn't take these medicines (ACPCA, 2009; Mai et al., 2019).

Children with cleft lip with or without cleft palate face a variety of challenges, depending on the type and severity of the cleft:

- difficulty in feeding,
- ear infections and hearing loss,
- dental problems,
- speech difficulties,

- challenges of coping with a medical condition. Children with clefts may face social, emotional and behavioral problems due to differences in appearance and the stress of intensive medical care. Transplantation allows to replace included teeth on the arcade and to bring osteogenic potential in the cleft; in order to compensate or at least to stabilize the rapid osteolysis of the previously grafted bone (Sinha et al., 2017).

A new restoration technique for the perioral muscles in lip and palate cleft follows the rotation-advancement method without skin measurement. A curvilinear skin incision is made from subnasale to the Cupid's bow peak. Muscle dissection is continued to the contralateral nostril floor beneath the columellar base to facilitate downward rotation in the medial lip. Wide muscle dissection will be performed in the lateral lip segment from the nasal mucosa passing the alar base. The lateral lip muscle is advanced and sutured to the medial lip muscle in a Z-plasty fashion. A small skin backcut is made above the Cupid's bow peak. Primary nasal correction is now performed. The new technique of perioral muscle reconstruction facilitated to obtain lip lengthening and symmetry in the repair of complete unilateral cleft lip (Jung et al., 2020).

Previous genome-wide association study of nonsyndromic cleft lip with or without cleft palate (NSCL/P) identified a susceptible variant (*RS4791774*). New studies highlight that the potential functional SNP (*RS4791331*) is identified by bioinformatic analysis. Decreased enhancer activity and reduced NTN1 expression following transfection of the T allele were observed.

Carriers of the CT/TT genotypes showed significantly lower expression of NTN1 than CC carriers. The *NTNA* (-/-) zebrafish showed relatively wider intermaxillary clefts. These results indicate that *RS4791331* (C > T) disrupted motif binding and led to abnormal expression of NTN1, which may be involved in the development of NSCL/P. Also, they found that the C > T base change of *RS4791331* resulted in abnormally low expression of NTN1, probably through disrupted motif binding, which may be involved in the development of NSCL/P. These findings will help elucidate the etiology and genetic factors of NSCL/P (Li et al., 2020).

1.3.5. Conclusions

In cases of the bilateral cleft lip, there was a crash of the nasal dome, the nostrils were horizontal and the columela was shortened. Congenital malformations are a major and complex public health problem to be addressed systematically and interdisciplinary, according to modern principles.

The goal of these researches is, of course, to achieve the best possible individual results, ensuring that the recovery is as close to normal as possible. The morphological aspects in the case of labio-palatine clefts are mainly characterized by the same elements. The individual variants change between the variations of a classic developmental pattern. Viral infections during pregnancy is one of the most common cause for labio-palatine cleft.

Our study investigated the demographical characteristics, health status, and associated communication disorders in patients with orofacial clefts as a retrospective research.

None of the demographic variables was associated with lip and palate cleft type. Our results suggested that families who had children with lip and palate cleft displayed poor socioeconomic status and low educational level which may impede the delivery of health education by health practitioners. Increased risk of comorbid communication disorders and malformations in OFC patients must be emphasized and disseminated to health professionals involved in the management of this particular type of patients.

There is no evidence that these factors contribute to either sex or cleft status differences. Further investigations are needed, and they should include a larger, more diverse sample, a matched control group and a focus on the mothers. As direct clinical assessment, our study lays the groundwork for a better understanding of the etiology of lip and palate cleft birth defects that present challenges for long-term dental management.

1.4. Embriological errors: the labio-palatine clefts – an experimental study

1.4.1.Introduction

Facial malformations and, especially labio-palatine clefts have always prompted a great interest both in hereditary transmissio (Moller et al., 1990), morphogenetic mechanisms, teratogenic factors, and especially on facial aesthetics and treatment issues (Evans, 2004). They represent a great public health problem due to the significant increase of incidence, but above all through early diagnosis and treatment issues raised (Soares et al., 2012).

Surgical treatment is the main therapeutic approach, but due to the multidisciplinary approach it can be compulsory achieved with a significant increase in cosmetic and orthodontic aspects (Bucur, 2009). In practice, there are three situations in which preoperative orthodontic treatment is extremely necessary: complete unilateral collapsed labial-palatine clefts with collapse of the small maxillary segment; unilateral labial-maxillary clefts with obliquity of the small segment; complete bilateral clefts with collapses of lateral segments and moderate protrusions of the medial segment (Shetye, 2004).

Labio-palatine clefts can coexist in craniofacial malformations such as Pierre-Robin syndrome, Down syndrome (trisomy 21), Patau syndrome (trisomy 13-15); Optis syndrome; ”cri du chat” syndrome, Edwards syndrome (trisomy 17-18) (Sava et al., 2013).

The mandibular and maxillary buds are derived from the first pharyngeal arch, that, during the embryonic development will form (Marsh, 2004): bone structures: upper jaw, mandible, hard palate, internal ear bones (hammer and anvil), a part of the sphenoid and temporal bone; muscles structures, including: masticatory muscles (temporal, masseter, pterigoids), soft palate tensor muscles and tympanic tensor muscle (Sandove and Culp, 2004). 50% from the case of familial labial and palatine cleft have autosomal dominant transmission (Marazita and Mooney, 2004).

The aim of the study was to develop an experimental model on Wistar rats in order to analyze the occurrence of cranial malformations, following the administration of teratogenic substances. The teratogenic potency of vitamin deficiency over the whole

period of gestation and of food restriction during the critical period of palatogenesis were investigated as well as the potential benefit of vitamin B supplementation/treatment. It was demonstrated for comparison with work on a teratogenetically induced cleft palate model.

1.4.2. Material and methods

In order to study the occurrence of labio-palatine cleft, we used eight groups of Wistar females rat, weighing between 200 and 250 g. Each sample consists of 10 females rats.

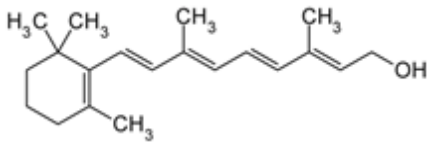
The animals were kept in standard laboratory conditions and received the same diet, to ensure their caloric support, vitamins and minerals. Each rat was numbered in each lot from 1 to 10, respectively from 1 to 12.

Female rats were accommodated in the same container with normal adult males who did not receive any substance, one night after which the males were removed.

We mention that the rate of spontaneous occurrence of labio-palatine clefts for Wistar rats is 4.5%.

Lot A of rats females was the control group and did not receive any substance. Lot B of female rats received retinoic acid derived from vitamin A, known as teratogen, which determine labio-palatine cleft inrodent.

Each animal received 50mg of retinoic acid / kg body weight per day, intraperitoneal, single dose per day until pregnant females gave birth.



Retinoic acid-belongs to the family of chemical compounds called retinoids

The lot C of females rets received retinoic acid, the same dose and the same mode of administration, plus complex of B vitamins (B1, B2 and B6), 50 mg B complex / kg body weight / day, intraperitoneal administration, single daily dose, complex B being administered one hour before the administration of retinoic acid.

The lot D of females rats received retinoic acid, the same dose and the same route of administration plus magnesium acemamate, 0.5mEq / kg body weight / day, intraperitoneal dose, single dose per day, magnesium acemamate being administered one hour before administration of retinoic acid.

The rat group E received retinoic acid, the same dose and the same administration as group B plus nickel chloride, 0.5mEq/kg body weight/day, intraperitoneal administration, single dose per day, nickel chloride being administered with a hour before retinoic acid administration.

The lot F lot received retinoic acid. Each animal received 50mg of retinoic acid/kg body weight/ day, intraperitoneal administration, single dose per day, only week of gestation

Rat G lot received retinoic acid. Each animal received 50 mg retinoic acid/kg body weight/day, intraperitoneal administration, single daily dose, only in the second week of gestation.

Rat group H received retinoic acid/kg body weight/day, intraperitoneal administration, single dose per day, only the last week of gestation.

To each lot was noted the number of females who developed gestation, the number of newborns to each pregnant female and the malformations presented at the head of each of them.

Also, it has been observed up to a month the number of newborns that survived in each female. One month after the birth all animals (both females with newborns and non-pregnant females) were anesthetized with Pentotal and sacrificed for carotid sections.

There was a final examination of the animal's head. From each sacrificed animal, were extracted lungs, kidneys, gonads, spleen and liver, in order to perform histopathological examination of these organs. The harvested organs were fixed in 15% formol contained in paraffin, then longitudinal serial sections were made at distances of 50 microns, sections of 4 microns thickness.

The sections were stained with the following methods: hematoxylin-eosin; Van Gieson's tricromatic staining; PAS staining (with Schiff's acid); Gordon Sweat staining for reticulin; Scharlach red staining for fat; Szekely's tricromatic staining. The colored lamellae were then examined with the optical microscope.

1.4.3. Results

Lot A (witness) who did not receive any substance showed the following results: 7 out of 10 rats remained pregnant.

Each of these pregnant rats gave birth to live newborns, of which only two (one from the 4 and 7 rats) died after birth, but because of their small birth weight. It should be noted that *no newborn presented any malformation in the skull* (**Table 1**).

Histopathological examination of the organs taken from both the mother and the baby presented a normal pattern of the organs architecture.

In the case of the lung in the control group - the normal structure of the alveoli and the bronchiole is noted.

Liver-control group with normal structure of the liver lobe.

Kidney-control lot with normal structure of the urinary tubes. Spleen-control group, normal structure of lymphoid follicles.

Test-control group-normal structure of seminiferous tubes. Ovary – control lot-normal ovary structure.

Lot B-It is noted from the table that several rats (four) have not remained pregnant, presumably retinoic acid also affects fertility in rats (**Table 2**).

What is important to note is that, three of these rats gave birth to *a newborn with a malformation at the head*. In the case of the rat 1 of this lot, the newborn with malformation presented only a slight labial cleft, which did not affect its feeding capacity (it survived one month after birth and showed a normal development). The newborn with labia cleft was male.

Table 1 - Group A - Female rat distribution by weight, gestation and newborns in terms of number, head malformations and at1 month survival.

Female rat In group A Criteria/pregestational weight	Gestation	Number of newborns	Number of Newborns With head Malformations	Number of Newborns Survived For 1 month
1/250g	*YES*	*5*	*0*	*5*
2/210g	*NO*	-	-	-
3/225g	*YES*	*4*	*0*	*4*
4/200g	*YES*	*3*	*0*	*2*
5/240g	*NO*	-	-	-
6/230g	*YES*	*3*	*0*	*3*
7/250g	*YES*	*6*	*0*	*5*
8/220g	*NO*	-	-	-
9/235g	*YES*	*2*	*0*	*2*
10/215g	*YES*	*3*	*0*	*3*

Table 2 - Group B - Female rat distribution by weight, gestation and newborns in terms of number, head malformations and 1 month survival.

Female rat In group B Criteria/pregestational weight	Gestation	Number of newborns	Number of Newborns With head Malformations	Number of Newborns Survived For 1 month
1/240g	*YES*	*4*	*1*	*4*
2/220g	*YES*	*3*	*0*	*3*
3/230g	*NO*	-	-	-
4/215g	*YES*	*3*	*1*	*2*
5/250g	*YES*	*4*	*0*	*4*
6/210g	*NO*	-	-	-
7/245g	*YES*	*5*	*1*	*4*
8/200g	*NO*	-	-	-
9/245g	*YES*	*3*	*0*	*3*
10/235g	*YES*	-	-	-

Rats females 4 and 7 also gave birth, but their newborns had a more serious malformation, palatine cleft, which affected their feeding potential, and the two pups died about two weeks after birth. Both newborns with palatine clefts were female.

Histopathological changes are also seen in the liver of animals in group B that received retinoic acid. The first manifestation that can be seen in all the sacrificed rats in the B group is the expansion of all capillary vessels from the periphery of the liver lobe and

sometimes also of the centrolobular veins.

The kidney of the rats from lot B also presents histopathological changes. First, it can be seen a slight retraction of the glomeruli in the Bowman capsule. In the urinary tract, there is a slight dilation of the capillaries and a slight decrease in the lumen of the urinary tubes.

In **group C** we monitored the newborns and possible malformations in the skull, obtaining *one newborn with skull malformation* (**Table 3**).

From the analysis of this table it is observed that unlike lot B, which received only retinoic acid, group C that received retinoic acid and vitamin B showed a decrease in the number of newborns with malformations in the skull.

Only one newborn showed cranial malformation, but this was a minor malformation (small lip cleft in one male newborn), which allowed the chicken to survive at 1 month.

From the experiment on a small number of rats females, the table allows us to conclude that vitamin B has a beneficial effect in labio-palatal clefts induced by retinoic acid: the number of newborns with these malformations is much smaller and the malformation is minor; compared to lot B, more female rats were pregnant.

From the analysis of the organs collected from the group C of female rats, it can be seen that the harmful effects of retinoic acid have been diminished. Thus, in the lung, although there is the same vasodilatation in the pulmonary capillaries, no thickening of the capillary walls or their destruction is observed.

The liver of the rats in group C also exhibit the same changes induced by retinoic acid like in the B lot but more diminished. The kidney keeps exactly the same changes as in B lot; there is a slight retraction of the glomeruli at the Bowman capsule. The spleen retains its normal architecture. The collected rat testicles show the same vasodilation as in group B but without affecting the normal testicular architecture.

Summarizing the changes found in organs collected from animals in group C, we can conclude that vitamin B positively modulates the harmful action of retinoic acid, both on the cranial malformation and on the studied organs.

Lot E - and at this lot we monitored newborns and possible malformations in the skull; there was an *increased number of newborns with malformations in the skull*, as well as an increase in the number of newborns that did not survive at one month. Female 3 gave birth to a baby with a palatine cleft (female) that survived one month after birth.

Rat female 5 gave birth to two malformed newborns, of which one with a minor lip cleft, which survived at birth and one with a palatine cleft that died three weeks after birth. Both newborns were male.

Rat female 7 gave birth to a single malformed newborn, with micrognathia (female), which allowed one month's survival. Female 10 gave birth to a single newborn, with palatine cleft (female) that died 18 days after birth.

In the **lot G**, *no skull malformations* were found, although pathological changes in the studied organs at one month after birth were similar to those of F lot. One newborn did not survive at one month but from cause of low birth weight.

Table 3 - Group C - Female rat distribution by weight, gestation and newborns in terms of number, head malformations and 1 month survival.

Female rat In group C Criteria/pregestational weight	Gestation	Number of newborns	Number of Newborns With head Malformations	Number of Newborns Survived For 1 month
1/210g	*YES*	*2*	*0*	*2*
2/250g	*YES*	*3*	*1*	*3*
3/200g	*NO*	-	-	-
4/220g	*NO*	-	-	-
5/240g	*YES*	*4*	*0*	*4*
6/230g	*YES*	*1*	*0*	*1*
7/235g	*YES*	*3*	*0*	*3*
8/205g	*NO*	-	-	-
9/225g	*YES*	*2*	*0*	*2*
10/245g	*YES*	*2*	*0*	*2*

It is noticed that in the **lot H** lot no rat babies were born with malformations in the skull more than all the newborns survived one month after birth. The histopathological changes found one month after birth were similar to those found in in lot G.

The lot D of females rats has the results presented in **table 4**.

Table 4 - Group D - Female rat distribution by weight, gestation and newborns in terms of number, head malformations and 1 month survival.

Female rat In group D Criteria/pregestational weight	Gestation	Number of newborns	Number of Newborns With head Malformations	Number of Newborns Survived For 1 month
1/230g	*NO*	-	-	-
2/220g	*YES*	*2*	*0*	*1*
3/200g	*YES*	*1*	*0*	*1*
4/210g	*NO*	-	-	-
5/250g	*YES*	*5*	*1*	*5*
6/215g	*YES*	*2*	*0*	*2*
7/230g	*YES*	*4*	*0*	*4*
8/205g	*YES*	*2*	*0*	*3*
9/220g	*NO*	-	-	-
10/240g	*YES*	*3*	*1*	*3*

Newborns that showed malformations in the skull showed only minor malformations that were found to be lip cleft in one case, and micrognation in other case, both malformations endangering the life of the newborns, both surviving one month after birth.

After harvesting more organs from mothers and babies, we found an improvement in anatomo-pathological changes.

1.4.4. Discussions

Hundreds of studies described more than seventy types of anomalies affecting almost every organ system related to excess intake of retinoids: eye defects, cleft lips, cleft palates, exencephaly and brachygnathia. Researchers inferred from this data that developing embryos are particularly sensitive to the developmental effects of retinoids during early development (Cohlan SQ, 1953; Jick H. 1988; Ross et al., 2000; Soprano and Soprano, 1995).

What is interesting to note in group B of the rats is that all the rats females presented more or less significant changes in the studied organs. Thus, in the lungs there is a dilatation of the capillary vessels, as well as the thickening of the alveolar wall (Bjork et al., 2010) with the accumulation in the alveoli of an eosinophilic material and inflammatory cells (especially lymphocytes). In some animals there are accumulations of lymphoid elements around the bronchiole and around the dilated vessels, suggesting a pro-inflammatory potential of retinoic acid. These elements are also highlighted by special stains.

Retinol is derived from isoprene and has a hydroxide as functional group.The first complete synthesis of this compound was found by David Adriaan van Dorp and Josef Ferdinant Arens in 1947. George Wald won the 1967 Nobel Prize for Physiology and Medicine for his work with retinal pigments (also called visual pigments), which led to the understanding of the role of vitamin A in vision. Many of the non-visual functions of vitamin A are mediated by retinoic acid, which regulates the appearance of genes by activating intracellular retinoic acid receptors (Daw and Patel, 2004).

Of all the studied organs, the spleen showed the fewest changes under the influence of retinoic acid. As for the gonads, they also presented histopathological changes, especially female gonads in mothers. It should be noted that the organs of the newborns did not show the same histopathological changes as the mothers.

To conclude the effects of administration of retinoic acid in the first week of pregnancy, we find that it increases the incidence of malformations in the skull but also affects the organs that we studied: it produces vasodilation, sometimes marked, and in some organs (lung, liver, kidney, ovaries) causes obvious histopathological changes: thickening of alveolar walls, sometimes with their destruction, alteration of hepatocytes in area I (from the periphery of the classical hepatic lobe), glomerular retraction and urinary tract alterations, and alteration of ovarian follicles.

Vitamin B is known to have prophylactic action on the appearance of labio-palatine clefts. It can be speculated that retinoic acid is less harmful in the presence of vitamin B due to the fact that it modulates the harmful action of retinoic acid on the studied organs (Berkowitz 2013; Salyer et al., 2004). They shown that orthaghathic surgery, when indicated in conjunction with perisurgical orthodontic treatment, yields a stable, pleasing, functional, and esthetic result which eliminates the cleft dysmorphogenesis. This can result in a normal, attractive face for the patient. The main goal for all patients when observed at conversational distance, is normal appearance, speech, and occlusion of the teeth without the stigmata of perceived deformity (Berkowitz 2013; Salyer et al., 2004).

Summarizing the changes observed in the harvested organs from the animals in group

D, we can conclude that magnesium acemamate (magnesium sulphate is a solid, crystalline substance, white, odorless, hygroscopic, bitter; there are several hydrated forms of which the most important is magnesium sulphate heptahydrate MgSO47H2O called epsomite or bitter salt) positively modulates the harmful action of retinoic acid on both the malformations of the skull and on the studied organs.

It should be noted that magnesium acemamate, although having a somewhat protective action against the harmful effects of retinoic acid, its action on some of the studied organs is not as strong as that of vitamin B complex but comparable to it.

It is found that nickel potentiates congenital defects induced by retinoic acid, with an increase in the number of newborns, which presents either lip cleft or palatine clefts.

*After the anatomopathological examination of the organs harvested from the group E rats, it reveals a worsening of the effects induced by retinoic acid. Thus in the lungs there is a more pronounced thickening of the alveolar walls and the intense dilatation of the pulmo*nary capillary vessels. Around some of them is an accumulation of inflammatory elements.

Extremely interesting histopathological changes are seen in the liver of animals in group E that received retinoic acid and nickel chloride, data corresponding to those in the literature (Malek 2001).

The first manifestation that can be seen in all slaughtered rats in group E is the expansion of all capillary vessels at the periphery of the classical hepatic lobe and of the centralobular veins, more obvious effects compared to group B.

And in the kidney, changes induced by retinoic acid are accentuated by nickel. First of all, glomerular retraction is accentuated, sometimes accompanied by micro-hemorrhagia. As for urinary tubes, vasodilatation is accentuated, and narrowing of lumen of urinary tubes is almost complete. Some tubes have ruptured tubular epithelium and their lumen is occupied by eosinophilic material and red blood cells.

These elements could lead to the idea that this combination of retinoic acid and nickel chloride, because of its elimination through the kidneys would have a toxic effect on the tubular epithelium or, from the combination of retinoic acid with nickel chloride would result a metabolite with urinary excretion with the same toxic effect on the tubular epithelium.

Also, in the spleen collected from animals of group E, there is a slight change of the normal splenic architecture, in the sense of the presence of a slight vasodilatation and a moderate dispersion of the lymphatic follicles. At the level of the testicles harvested from the males newborns, there is a accentuated vasodilatation at the level of the seminiferous tubes, but with the preservation of the testicular architecture and the spermatogenesis. The adult rat ovaries in group E macroscopically presents the same changes as in group B, but with a more pronounced reddish tint. Microscopically, there is an increased capillary vasodilatation as well as a much larger number of cystic degenerated ovarian follicles.

Summarizing the pathological changes from the studied organs we can conclude that nickel in its form of administration (nickel chloride) potentiates the negative effects of retinoic acid, emphasizing in particular the vasodilation and proinflammatory effects at all the studied organs and inducing severe negative changes related or not to this vasodilatation. Nickel is otherwise known by its negative effects on various segments of the body.

In group F, it is found that the results are similar to those obtained in group B, founding three newborns with malformations in the skull, of which two (both males) with

minor lip clefts, who survived one month after birth and only one newborn (female) with major palatine cleft, who did not survive a month.

Regarding the pathological changes encountered at the level of the various organs, the same changes as in the B group were found but slightly attenuated.

The explanation for the differences between these three groups F, G, H, is given by the time of administration of retinoic acid during gestation. In group F, where malformations are noted, retinoic acid was administered in the first week of gestation, which demonstrates that the critical period for intervention of the teratogenic agents, for skull malformations, in rats, is the first week of gestation. If the teratogenic agent intervenes after the first week of gestation, when the cephalic extremity organogenesis has ended in rats, it produces far less obvious manifestations.

Researches on animals have shown that there is a dose-response relationship for teratogens. However, it is known that the doses used to produce anomalies in animals are much higher than those to which humans are exposed. For this reason, animal data are not applicable to pregnant women. For a drug to be considered teratogenic, it must be observed a dose-response relationship; for example, if exposure during pregnancy is greater, the phenotypic effects will be more severe.

Most of the lip and palatine clefts are the result of multiple, genetic and non-genetic factors, each producing a minor developmental defect, called multifactorial heritage (Firth et al., 2005); it can be represented by a model where the "responsibility" for a disease is a variable determined by the combination of genetic and environmental factors. Labio-palatine clefts in humans are transmitted through multifactorial inheritance. The prevention of these abnormalities (Walker et al., 2009) can be done by reducing exposure to exogenous factors to zero. The postnatal assessment of malformations is done through a detailed clinical examination; children (Saperstein et al., 2012) with anomalies should be registered in a national or local registry, in order to be treated appropriately and in time, avoiding facial defects in adults (Kapp-Simon 2004).

The sagittal growth of the jaw appears to be limited in patients with unoperated palatine cleft (and which becomes more severe with aging) but also to those who are operated (Shetye 2012). The basis of this jaw growth deficit is not a single factor, as evidence being the studies performed on unoperated patients and those operated for palatine cleft.

Dental anomalies in patients with cleft occur more frequently than in those who did not suffer from this disorder: changes in number of inclusions, dental agenesis, supernumerary teeth; shape changes, especially in upper lateral incisors.

The size of the teeth was smaller both in the mid-distal and vestibulo-oral ways in all types of clefts compared to the control group. The smallest teeth were found in the palatal cleft, although in earlier studies they were the same size as the control group. The smallest tooth was recorded as the upper lateral incisor on the cleft side, aspect which was also found in other studies. These results show a clear difference between the two types of clefts, which are genetically and embryological distinct. The treatment of these facial defects depends on the close collaboration between the pediatric surgeon, the oro-maxillo-facial surgeon and the family (Pegelow et al., 2008).

1.1.1. Conclusions

Retinoic acid, a teratogenic agent for rats, when given in the first week of gestation (when it takes place the the organogenesis of cephalic extremity, at the studied animals), determine various malformations in the skull. The most common malformations induced by retinoic acid are labio-palatine clefts. In our studies, lip clefts are more common in male newborns and palatine clefts are more frequent in female newborns. Retinoic acid induces histopathological changes in several organs. Our study additionally proves that the labio-palatine clefts in humans are transmitted through multifactorial inheritance. The prevention of these anomalies can be done by reducingto zero the exposure to exogenous factors, especially during the first 10 weeks of pregnancy.

The therapeutic outcome depends on the complexity of the malformation, on the moment of surgery and on the selection of the most modern and appropriate techniques and equipment for the patient. The finding that sometimes the labio-maxilo-palatine clefts are accompanied by both antecedents and collaterals, with malformations of other segments of the body, may prove to be affected by genetic nodules with somatic consequences at different levels. Thus, the clefts can be included in some chromosomal anomalies that cause general syndromes such as Treacher-Collins, Pierre- Robin and Klippel-Fiel.

CHAPTER 2. CHALLENGING IN ORO-DENTAL DIAGNOSIS AND TREATMENT

2.1 State of the Art

Dental and oral health is an essential part of your overall health and well-being. Poor oral hygiene can lead to dental cavities and gum disease, and has also been linked to heart disease, cancer, and diabetes. Maintaining healthy teeth and gums is a lifelong commitment. The earlier you learn proper oral hygiene habits — such as brushing, flossing, and limiting your sugar intake — the easier it'll be to avoid costly dental procedures and long-term health issues (Kemparaj et al., 2018).

The oral health is a general term which refers directly to oral pathological conditions. These influence routine medical maneuvers in examining the oral cavity and the method of treatment.

Dental cavities and gum disease are very common. According to the World Health OrganizationTrusted Source: between 60 and 90 percent of school children have at least one dental cavity; nearly 100 percent of adults have at least one dental cavity; between 15 and 20 percent of adults ages 35 to 44 have severe gum disease; (Kornman, 2005) about 30 percent of people around the world ages 65 to 74 don't have any natural teeth left; in most countries, out of every 100,000 people, there are between 1 and 10 cases of oral cancer; the burden of oral disease is much higher in poor or disadvantaged population groups (Imhoff et al., 2020).

There are many steps you can take to keep your teeth healthy. For example, dental and oral disease can be greatly reduced by: brushing your teeth with fluoride toothpaste at least twice a day, flossing your teeth at least once a day, decreasing your intake of sugar eating a diet high in fruits and vegetables, avoiding tobacco products, drinking fluoridated water and seeking professional dental care (Featherstone, 2003; Thomson et al., 2019).

Symptomes

Symptoms of dental and oral problems are counting: ulcers, sores, or tender areas in the mouth that won't heal after a week or two, bleeding or swollen gums after brushing or flossing, chronic bad breath, sudden sensitivity to hot and cold temperatures or beverages, pain or toothache, loose teeth, receding gums, pain with chewing or biting, swelling of the face and cheek, clicking of the jaw, cracked or broken teeth and frequent dry mouth (Armitage and Douglass, 2003). If any of these symptoms are accompanied by a high fever and facial or neck swelling, you should seek emergency medical treatment. Learn more about the warning signs of oral health issues (Wang et al., 2019).

Causes

Bacteria near gumline thrive in a sticky matrix called plaque which accumulates, hardens, and migrates down the length of your tooth if it isn't removed regularly by brushing and flossing. This can inflame your gums and cause the condition known as gingivitis. Increased inflammation causes your gums to begin to pull away from your teeth. This process creates pockets in which pus may eventually collect. This more advanced stage of gum disease is called periodontitis (Papapanou, 1999; Bernabe et al., 2020).

There are many factors that contribute to gingivitis and periodontitis, including: smoking, poor brushing habits, frequent snacking on sugary foods and drinks, diabetes, the use of medications that reduce the amount of saliva in the mouth, family history, or genetics certain infections, such as HIV or AIDS, hormonal changes in women, acid reflux, or heartburn, frequent vomiting, due to the acid (NIHCDP, 2001).

Diagnosing

Most dental and oral problems can be diagnosed during a dental exam. During an exam, your dentist will closely inspect your: teeth, mouth, throat, tongue, cheeks, jaw and neck. Your dentist might tap or scrape at your teeth with various tools or instruments to assist with a diagnosis (Thomson et al., 2019). A technician at the dentist's office must take dental X-rays of your mouth, making sure to get an image of each of your teeth. Be sure to tell your dentist if you're pregnant. Women who are pregnant shouldn't have X-rays (Arabi et al., 2018).

A tool called a probe can be used to measure your gum pockets. This small ruler can tell your dentist whether or not you have gum disease or receding gums. In a healthy mouth, the depth of the pockets between the teeth are usually between 1 and 3 millimeters (mm). Any measurement higher than that may mean you have gum disease. If the dentist finds any abnormal lumps, lesions, or growths in your mouth, they may perform a gum biopsy. If oral cancer is suspected, your dentist may also order imaging tests to see if the cancer has spread.

Tests may include: X-ray, MRI scan, CT scan, endoscopy (Reddy et al., 2012; Wang et al., 2019).

Types of dental and oral diseases

Cavities are also called caries or tooth decay. These are areas of the tooth that have been permanently damaged and may even have holes in them. Cavities are fairly common. They occur when bacteria, food, and acid coat your teeth and form a plaque. The acid on your teeth starts to eat away at the enamel and then the underlying dentin, or connective tissue. Over time, this can lead to permanent damage (Roisin et al., 2019).

These can cause the inflammation of the gums, which results of plaque building up on your teeth due to poor brushing and flossing habits. Untreated gingivitis can lead to periodontitis, a more serious infection. As periodontitis progresses, the infection can spread to your jaw and bones. It can also cause an inflammatory response throughout the body (Orentlicher et al., 2019).

A tooth can crack or break from an injury to the mouth, chewing hard foods, or grinding the teeth at night. A cracked tooth can be very painful. You should visit your dentist right away if you've cracked or broken a tooth (Chandra et al., 2016).

If your teeth are hypersensitive, you might feel pain or discomfort after having cold or hot foods or beverages. Tooth sensitivity is also referred to as "dentin hypersensitivity." It sometimes occurs temporarily after having a root canal or a filling. It can also be the result of: gum disease, receding gums, a cracked tooth, worn-down fillings or crowns, Some people naturally have sensitive teeth because they have thinner enamel. Most of the time, naturally sensitive teeth can be treated with a change in your daily oral hygiene regimen. There are specific brands of toothpaste and mouthwash for people with sensitive teeth (Kemparaj et al., 2018).

Clinical aspects of these dental problems, such as gingival overgrowth, features of local anesthetise, dysfunctional syndrome or dental pain are all about oral health management. Oral health has risen in importance in recent years, as researchers have discovered a connection between declining oral health and underlying systemic conditions (Orentlicher et al., 2019). It turns out that a healthy mouth can help you maintain a healthy body. According to the Mayo Clinic, oral bacteria and inflammation may be associated with: heart disease, endocarditis, or inflammation of the lining of the heart, premature birth, low birth weight (Shivpuje, 2016).

Treatment

Cleanings

A professional cleaning can get rid of any plaque you may have missed while brushing and flossing. It'll also remove tartar. These cleanings are usually performed by a dental hygienist. After all the tartar is removed from your teeth, the hygienist will use a high-powered toothbrush to brush your teeth. This is followed by flossing and rinsing to wash out any debris. A deep cleaning is also known as scaling and root planning. It removes tartar from above and below the gumline that can't be reached during a routine cleaning (Orentlicher et al., 2019).

Fluoride treatments

Following a dental cleaning, your dentist may apply a fluoride treatment to help fight off cavities. Fluoride is a naturally occurring mineral. It can help strengthen the enamel of your tooth and make them more resilient to bacteria and acid.

Antibiotics

If you show signs of a gum infection or you have a tooth abscess that has spread to other teeth or your jaw, your dentist may prescribe antibiotics to help get rid of the infection. The antibiotic may be in the form of a mouth rinse, gel, oral tablet, or capsule. Topical antibiotic gel may also be applied to the teeth or gums during surgical procedures (Du et al., 2020).

Fillings, crowns, and sealants

A filling is used to repair a cavity, crack, or hole in the tooth. The dentist will first use a drill to remove the damaged area of the tooth and then fill the hole with some material, such as amalgam or composite. A crown is used if a large portion of your tooth needs to be removed or has broken off due to an injury. There are two types of crowns: an implant crown that fits over an implant, and a regular crown that fits over a natural tooth. Both types of crowns fill in the gap where your natural tooth appeared. Dental sealants are thin, protective coatings that are placed on the back teeth, or molars, to help prevent cavities (Imhoff et al., 2020). Your dentist may recommend a sealant for your children as soon as they get their first molars, at around age six, and again when they get their second set of molars around age 12. Sealants are easy to apply and completely painless (Ahmed et al., 2014).

Root canal

You might need a root canal if tooth decay reaches all the way inside the tooth to the nerve. During a root canal, the nerve is removed and replaced with a filling made of a biocompatible material, usually a combination of a rubber-like material called gutta-percha and adhesive cement (Orentlicher et al., 2019).

Probiotics

Probiotics are mostly known for their role in digestive health, but new research has shown that the healthy bacteria may be beneficial for your teeth and gums. Probiotics have been shown to prevent plaque and treat bad breath. They also help to prevent oral cancers and decrease inflammation from gum disease. While large clinical trials are still needed to prove their effectiveness, results to date have been promising. You can take a probiotic supplement or eat foods high in beneficial bacteria, such as yogurt, kefir, and kimchi. Other popular probiotic foods include sauerkraut, tempeh, and miso (Canullo et al., 2017).

Changing daily habits

Keeping your mouth healthy is a daily commitment. A dental hygienist can teach you how to properly take care of your teeth and gums on a daily basis. In addition to brushing and

flossing, your daily routine can include mouthwash, oral rinses, and possibly other tools, such as a Waterpik water flosser (Imhoff et al., 2020).

Surgery for dental and oral problems

Oral surgeries are usually performed to treat more serious cases of periodontal disease. Certain dental surgeries can also be done to replace or fix missing or broken teeth caused by an accident. During a flap surgery, a surgeon makes a small cut in the gum to lift up a section of the tissue. They then remove tartar and bacteria from underneath the gums. The flap is then stitched back into place around your teeth (Canullo et al., 2016).

Bone grafting

Bone grafting is needed when gum disease causes damage to the bone surrounding the root of your tooth. The dentist replaces the damaged bone with a graft, which can be made from your own bone, a synthetic bone, or a donated bone (Orentlicher et al., 2019).

Soft tissue grafts

A soft tissue graft is used to treat receding gums. A dentist will remove a small piece of tissue from your mouth or use a donor tissue and attach it to the areas of your gums that are missing (Collins et al., 2014).

Tooth extraction

If your dentist can't save your tooth with a root canal or other surgery, the tooth will likely need to be extracted. You may also need a tooth extraction if your wisdom teeth, or third molars, are impacted. Sometimes, a person's jaw isn't large enough to accommodate the third set of molars. One or more of the wisdom teeth will become trapped or impacted when it tries to emerge. A dentist will typically recommend that wisdom teeth be extracted if they cause pain, inflammation, or other problems (Imhoff et al., 2020).

Dental implants

Dental implants are used to replace missing teeth that are lost due to a disease or an accident. An implant is surgically placed into the jawbone. After the implant is placed, your bones will grow around it. This is called osseointegration. Once this process is complete, your dentist will customize a new artificial tooth for you that matches your other teeth. This artificial tooth is known as a crown. The new crown is then attached to the implant. If you're replacing more than one tooth, your dentist may customize a bridge to fit into your mouth. A dental bridge is made of two abutment crowns on either side of the gap, which then hold the artificial teeth in between in place (Collins et al., 2015).

Risks and complications of untreated periodontal disease include: tooth abscesses, other infections, migration of your teeth, pregnancy complications, exposure of the roots of your teeth, oral cancer, tooth loss, increased risk of diabetes, heart disease, cancer, and respiratory diseases. If left untreated, an infection from a tooth abscess can spread to other parts of your head or neck. It can even lead to sepsis, a life-threatening blood infection (Roisin et al., 2019).

Oral health has an effect on more than just the teeth. Poor oral and dental health can contribute to issues with individual self-esteem, speech, or nutrition. They can also affect people comfort and overall quality of life. Many dental and oral problems develop without any symptoms (Kayaoglu et al., 2016).

This research direction has been materialized by publishing the following articles:

1. Nemțoi A, **Scutariu MM**, Nemțoi A, Eva L, Dumitrescu GF, Plămădeală P, Ferariu D, Haba D, Costea CF. Clinical, imaging and histopathological correlations of gingival overgrowth: a retrospective analysis in northeastern Romanian population. Rom J Morphol Embryol 2019; 60(3):811–822.
2. Macovei G, Beldiman AM, **Scutariu MM**, Armencia AO, Surlari Z, Tofan N, Moldovan A, Pancu G. Interdisciplinary therapeutic approaches based on the relatioship between dental pain perception and niti archwires types, evaluated in the first months of orthodontic treatment. Rev Chim (Bucharest) 2017; 68(12): 2860-2864.
3. **Scutariu MM**, Danila V, Ciupilan C, Ciurcanu OE. Semiology of the pain syndrome - identifying the ideal methods of locoregional anesthesia based on their rationale and features. Rev Chim (Bucharest) 2017; 68(10): 2373-2377.
4. Surlari Z, Ioanid N, Niţescu D, Cotea C, **Scutariu MM**, Virvescu D, Aungurencei O, Țănculescu- Doloca O, Ifteni G. Affectation by the dysfunctional syndrome of an adult group of population - statistical study. Romanian Journal of Oral Rehabilitation, 2018; 10(1): 149-154.
5. Romanec C, Rosu S, Macovei G, **Scutariu MM**, Dragomir B, Olteanu ND. Morphofunctional features in angle second class malocclusion on dental gypsum models. Materiale Plastice 2018; 55(4): 686-690.

2.2. Gingival overgrowth – a multifaced lesion

2.2.1. Introduction

Many types of lesions share the same site, which is the gingiva (Shamim et al., 2008). It is commonly affected by non-neoplastic and neoplastic lesions; the latter is usually characterized by a progressive growth, which can be either benign or malignant.

A great number of gingival localized overgrowths are however considered to be reactive lesions rather than non- neoplastic ones (Gandolfo et al., 2009) and can develop as a result of many underlying both gingival and periodontal diseases. There have been recorded cases of idiopathic hyperplasia (gingival elephantiasis) and of secondary hyperplasia, as well caused by a bacterial infection and associated with other local iatrogenic factors like unadjusted prosthesis or malocclusions. There are also those cases generated in the course of systemic diseases, such as leukemia (Chowdhri et al., 2018, diabetes, amyloidosis, and immunodeficiency disease or as a side effect of specific medications, such as phenytoin, cyclosporine or calcium channel blockers (Mironiuc-Cureu et al., 2014). Also, attention should be given to the new materials used to improve dental implants osseointegration, like strontium ranelate, because maxillary bone regeneration could cause an inflammatory

response of the maxillary bone and as such an overgrowth of the superjacent gingiva.

Even though almost all gingival overgrowths are reactive, they demonstrate at the same time tumor-like hyperplasia, which renders very difficult the differential diagnosis with a neoplastic lesion. According to the size of the affected area, gingival overgrowths can be localized, regional or generalized. In the first case, the lesion is painless and has the aspect of a pedunculated or sessile mass of dissimilar colors, ranging from light pink to red. The outside appearance varies from non-ulcerated to ulcerated mass. Lesion dimension extends from a few millimeters to several centimeters (Gandolfo et al., 2009).

The clinical characteristics of the above-mentioned reactive gingival lesions seem to reflect their various developmental phases. In the early stages they look red, raw, have ulcerated surfaces and bleed spontaneously or on slight touch whereas in the late stages they may be pedunculated, sessile or leaf-shaped growths and look mature, firm, avascular and fibrous (Arduino et al., 2011; Bobic et al., 2016).

For those gingival overgrowths that cannot be diagnosed on the basis of clinical and radiographic screenings alone, oral tissue biopsy may be required. Biopsy and histological examination represent the golden rule in oral pathology diagnosis and are used to confirm the clinical prognosis (Ababneh 2006).

When further information is required for the indicated therapy, oftentimes the ultimate procedure, which can provide tissue for microscopic analysis, is biopsy (Gandolfo et al., 2009).

As stated above, biopsy is oftentimes the only way to find the cause of a gingival hyperplasia. Furthermore, microscopic analysis is necessary in order to discern neoplasms from non-neoplastic lesions, which range from granulation tissues to avascular masses of collagen (Stablein and Silverglade 1985).

In the medical literature, many studies were aimed at analyzing the occurrence of oral reactive gingival lesions with regard to age, gender and location, but only a few have focused on the histopathological (HP) features of them (Macleod and Soames 1986). Similarly, there are only few Romanian studies on this subject that have considered large number of patients and analyzed their clinical and HP features (Pisoschi et al., 2014; Draghici et al., 2016), but there is really no study regarding this issue targeting the northeastern Romanian population.

Therefore, the aim of the present study was to analyze and to correlate the clinical, epidemiological, imaging and HP features of gingival overgrowth in northeastern Romanian population. Gingival enlargement (overgrowth), also mistakenly used synonymously with gingival hypertrophy and gingival hyperplasia, describes a condition that occurs when the size of the gingiva increases. Gingival hyperplasia typically refers to the increase in the number of cells, whereas gingival hypertrophy deals with the increase in cell size. These microscopic distinctions are both indicative of a disease process.

2.2.2. Materials and methods

We started by selecting 98 patients who needed a medical checkup for the diagnosis and management of a gingival overgrowth, either localized or regional, from a total of 300 that had been referred to the Office of Oral and Maxillofacial Surgery, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania, during a 14-month period (January 1, 2018 to

February 28, 2019). The following exclusion criteria were used: edentulous subjects, generalized gingival enlargement, patients using anticonvulsant drugs, calcium-channel blockers, and immunosuppressants.

For each case, demographic data, such as age and gender, smoking habits and alcohol consumption patterns, also clinical features of the lesions, their location, imaging aspects, and HP diagnosis were recorded.

An oral and maxillofacial surgeon observed and described the clinical characteristics of all the localized gingival overgrowths. After the clinical examination, all the patients with localized gingival enlargements were referred to a panoramic radiograph and a cone-beam computed tomography (CBCT), in order to detect possible lesions in the bone. The radiologist described the images performed by a Panoramic X-ray Machine (Planmeca ProMax® 3D, Planmeca Oy, Helsinki, Finland) and CBCT images that were obtained using an X-ray device (Planmeca ProMax® 3D, Planmeca Oy, Helsinki, Finland) and a spiral technique with 0.2 mm thickness [200 μm voxel size, 200 mm field of view (FOV), 90 kV, 10 mAs, 1 mm pass].

Next, the surgeon performed the excision of the gingival overgrowth and sent the surgical specimens to the Laboratory of Pathology for HP examination.

The tissue samples were labeled, fixed in 4% neutral buffered formalin, dehydrated in a mixture of acetone and xylene, and then embedded in paraffin. Using a microtome, 3 μm thick serial sections were cut and stained with Hematoxylin–Eosin (HE) staining. Representative sections were stained with Szekely staining, in order to identify the fibrous connective tissue presence. Also, other representative sections were used for an immunohistochemical (IHC) two-step staining technique using the En Vision TM+ detection system (Dako, Carpinteria, USA).

Briefly, histological slides were dried overnight, at 37°C, then deparaffinized in three washes of xylene and rehydrated in three graded ethanol washes (70%, 80%, 100%). The slides were treated with Dako target retrieval solution sodium citrate, pH 6, 1:10 dilution (Dako, Carpinteria, USA) before antigen retrieval was done by heating them at 95°C, in a steamer, for 30 minutes. Then, the slides were cooled to room temperature (RT) for 30 minutes, and treated with diluted 3% hydrogen peroxide to block endogenous peroxidase activity.

Four primary antibodies [monoclonal mouse anti-human cytokeratin (CK) 19, 1:100 dilution, Dako, Denmark; monoclonal mouse anti-vimentin, 1:100 dilution, Dako, Denmark; monoclonal mouse anti-human cluster of differentiation (CD) 34, class II, 1:50 dilution, Dako, Denmark; mono- clonal mouse anti-human CD1a, 1:50 dilution, Dako, Denmark] were applied on the corresponding slides, at RT, for 30 minutes.

After washing them with Tris-buffered saline (TBS), the slides were incubated for 30 minutes, at RT, with Dako EnvisionTM+Dual Link System (Dako, Carpinteria, USA), followed by a 5-minute incubation with 3,3'-Diamino- benzidine tetrahydrochloride (Dako Liquid DAB+ Substrate Chromogen System, 20 μL:1 mL substrate, Dako, Carpinteria, USA) for color reaction, and then counterstained with Mayer's Hematoxylin (three minutes) to visualize the nuclei. Slides were then immersed in distilled water, dehydrated in graded alcohols (70%, 90%, and 100%), cleared in xylene and mounted in Entellan. The slides were

viewed on a Leica DMC 2900 (Germany) light microscope and assessed for the presence of IHC staining.

CK19 immunostaining and vimentin immunostaining, showing an epithelial origin and a mesenchymal origin of cells, respectively, were considered positive when, in each case, definite brown expression was observed in the cytoplasm of the cells. CD34 immunostaining, expressed on capillary endothelial cells and embryonic fibroblasts, and CD1a immunostaining, useful in differentiating Langerhans cells, were considered positive when a definite brown expression was observed in the membrane of the cells.

Negative controls were obtained through omission of the primary antibody.

All demographic, clinical, imaging, and pathological data collected from each patient were analyzed using descriptive statistics; continuous variables were expressed as mean ± standard deviation (SD). Values were considered significant at $p<0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (SPSS® for Windows, version 11, SPSS Inc., Chicago, IL, USA).

The correlation between clinical, imaging and pathological diagnosis was classified as follows: (1) expected data 1 (ED1) – provisional clinical diagnosis; (2) expected data 2 (ED2) – provisional imaging diagnosis; (3) real data (RD) – final histopathology diagnosis; (4) concordant data (CD) – correspondence between the expected data and real data. The correlation was calculated as follows: CC (complete concordance) = CD×100/ED, this expressing the percentage in which the clinical or imaging and the histological diagnosis overlapped. This method was adapted from the one previously reported by Patel (Patel et al., 2011).

Our observational study involves using a descriptive statistic method (in the count noun sense) which, generally is a summary statistic that quantitatively describes or summarizes features from a collection of information. Descriptive statistics (in the mass noun sense) is the process of using and analysing those statistics. It is distinguished from inferential statistics (or inductive statistics) by its aim to summarize a sample, rather than use the data to learn about the population that the sample of data is thought to represent.

2.2.3. Results

Our study group involved 98 patients from the northeastern part of Romania. There were 56 female and 42 male patients (F:M 1.33), with a mean age at presentation of 49.8 years for women (SD ±12.3) and 52.3 years for men (SD ±11.9).

When considering sampling sites, the majority of surgical samples were obtained from the maxillary gingiva (51 patients, 52.04%), followed by the mandibular gingiva (47 patients, 47.95%).

The most frequently observed and biopsied gingival lesions were the exophytic masses, caused by local inflammation.

Within the male group, 55% of the patients were tobacco smokers, 19% were chronic alcohol drinkers and 26% did not use any of these two products. Within the female group, 63% were tobacco smokers, only 7% of them were occasionally alcohol drinkers and 30% were non-smokers and non-alcohol drinkers. 34% of all patients had regular routine dental visits, but 76% had not had a dental checkup for a long period of time.

All patients presented an enlargement of the gingival tissue, which has been located in different regions of the gums, namely marginal, papillary and diffuse. The extension of the gingival overgrowths ranged from limited (gingiva adjacent to a single or two teeth), to regional (gingiva around three or more teeth) or even generalized. In some cases, isolated tumor-like enlargements, sessile or pedunculated, were discovered. Clinical features of the cases presented include lesions which begin as a slight swelling of the papilla or marginal gingiva (**Figure 14A; Figure 15, A and B**) and which may progressively increase in size and extension until it becomes generalized. Clinically, the enlargements may appear bluish or deep red in color. The gingival overgrowths are often friable and soft; they have a smooth shiny surface and usually bleed easily.

Other types of enlargements, such as firm, pink, non- inflamed mass were clinically presented, which seemed to grow from below the free gingival margin/interdental papilla (**Figure 16A**). Most often, the lesions were painless. Pain was associated due to secondary trauma via brushing, flossing or chewing. A particular type of gingival over- growth we had to deal with were lesions with significant growth potential, purplish-red in color, and a tendency to bleed and to penetrate interdentally (**Figure 17, A and B**).

A single case presented a gingival overgrowth on the right part of the mandible between the teeth 4.3–4.6, covering the vestibular and oral surface of the two right premolars, with a soft tissue purple swelling (**Figure 18A**). On the panoramic and CBCT scan, an osteolytic lesion was found, which had developed on the right side of the body of the mandible and had obliterated the outer cortical (**Figure 18, B–E**). Another case presented a gingival tissue swelling in the region of the right and left mandibular molars and which was mimicking a gingival overgrowth. The molars had been extracted two months before (**Figure 19, A and B**). On panoramic and CBCT scan, osteolytic lesions were found (**Figure 19, C–G**).

Out of 98 cases, 43.87% of them were diagnosed as pyogenic granuloma (PG); fibrous hyperplasia accounted for 22.44% of all biopsied gingival lesions; peripheral giant cell granuloma (PGCG) was identified in 18.36% of cases, while epithelial hyperplasia represented only 13.26%. Only two of them (2.04%) presented localized gingival overgrowth as a consequence of a deep intraosseous lesion. One of them was diagnosed with central giant cell granuloma and one with Langerhans cell histiocytosis (LCH).

Correlating the HP aspect of the gingival lesion with the patients' gender, we found out that fibrous hyperplasia, PG and PGCG occurred more frequently in female patients, whereas male patients were more often diagnosed with epithelial hyperplasia (**Figure 20**).

Regarding the correlation between the HP diagnosis and gingival overgrowth location, we found out that the mandible was the most common site for the development of fibrous hyperplasia (59%) and PGCG (55%), whereas maxilla was the preferred location for epithelial hyperplasia (76%) (**Figure 21**). Gingival overgrowth in cases with a deep intraosseous lesion developed on both maxillary bones in an equal manner (**Figure 15**).

The patients who presented a gingival overgrowth due to soft gingival tissues enlargement did not show any change of the radiological investigations (**Figures 16B, 17C, 18C and 19C**). In the two cases with deep intraosseous lesions, the imaging investigations revealed an osteolytic lesion of the maxillary bones (**Figure 18, B–E; Figure 19, C–G**).

The HP and IHC analysis of the surgical samples revealed some interesting facts.

The gingival epithelial hyperplasia cases showed an ulcerated gingival surface epithelium that displayed an excessive acanthosis; this resulted in branched epithelial ridges descending deep into the lamina propria, which contained numerous dilated capillaries and inflammatory infiltrate around. IHC staining with anti-CK19 antibody revealed the epithelial ridges that branched and adhered to each other (**Figure 14, C and D; Figure 15, D–F**).

The gingival fibrous hyperplasia cases displayed an epithelial hyperplasia with parakeratosis and acanthosis, and also long epithelial ridges that looked like "gloves fingers" and descended into the lamina propria (**Figure 16D**).

At the same time, lamina propria has been replaced in almost all of its thickness by a heavy proliferation of fibrous tissue.

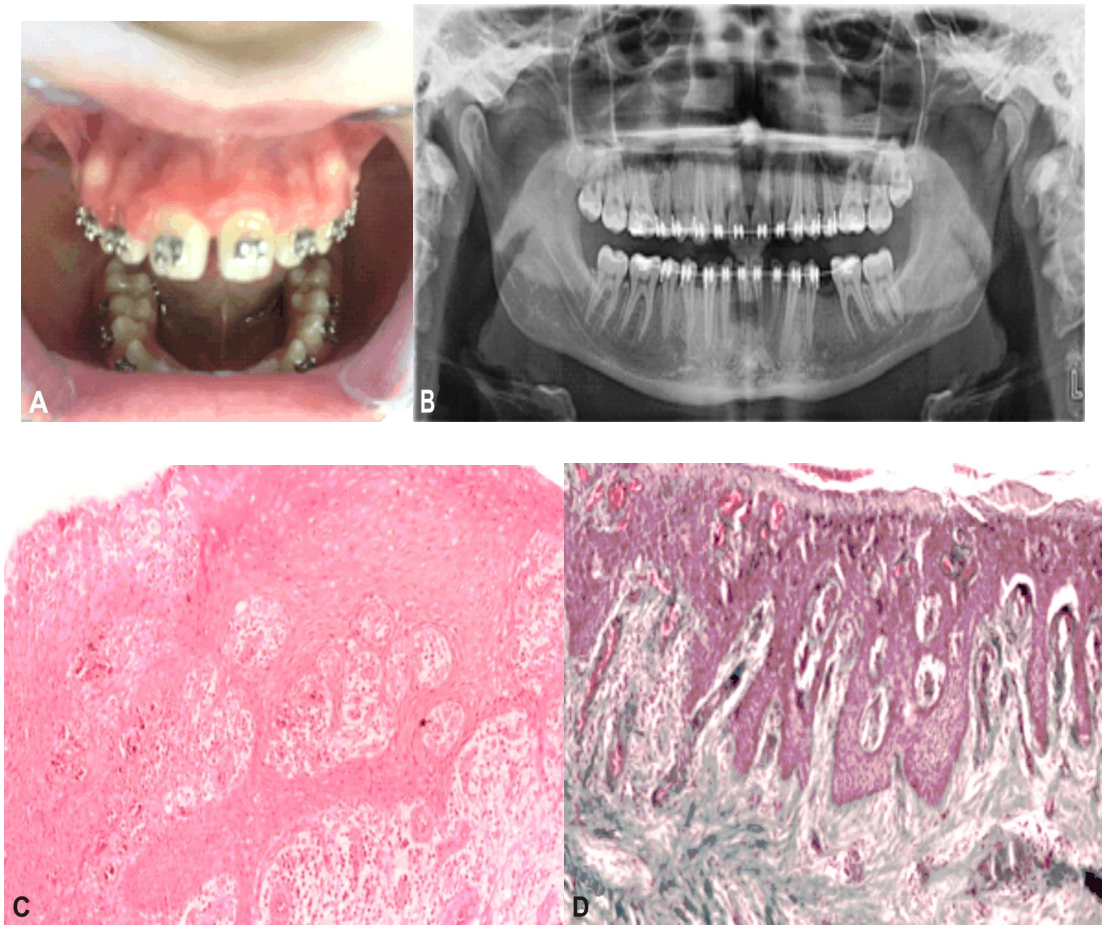


Figure 14 – Female patient, 26-year-old, with epithelial hyperplasia in the region of the right maxillary bone: (A) Clinical aspect of gingival tissue enlargement in the region of right maxillary incisors and canine due to the inflammatory reaction in the presence of fixed orthodontic treatment; (B) Imaging aspect without any bone modification in the region; (C) Photomicrograph showed an excessive acanthosis of gingival stratified squamous epithelium, with many branched epithelial ridges descending deep into the lamina propria (HE staining, ×200); (D) Lamina propria was also enlarged due to proliferation of the fibrous connective tissue and of extensive inflammatory cell infiltration (Szekely staining, ×200).

The fibrous tissue consisted of thick bundles of collagen fibers hazardly arranged and mixed with numerous dilated new capillaries, presenting inflammatory infiltrate around them (**Figure 16E**). I

HC staining with anti-vimentin antibody revealed reactive proliferation of fibroblasts, the presence of numerous capillaries and lymphocytic inflammation (**Figure 16, F and G**).

The PGCG cases displayed a non-encapsulated mass consisting of numerous multinucleated giant cells and covered by a hyperplastic gingival epithelium (**Figure 17D**). The lesion was covered by the gingival squamous cell epithelium, which was ulcerated in its superficial part and presented acanthosis, with thick epithelial ridges descending into the lamina propria. Also, lamina propria was enlarged due to heavy chronic inflammation (**Figure 17E)**.

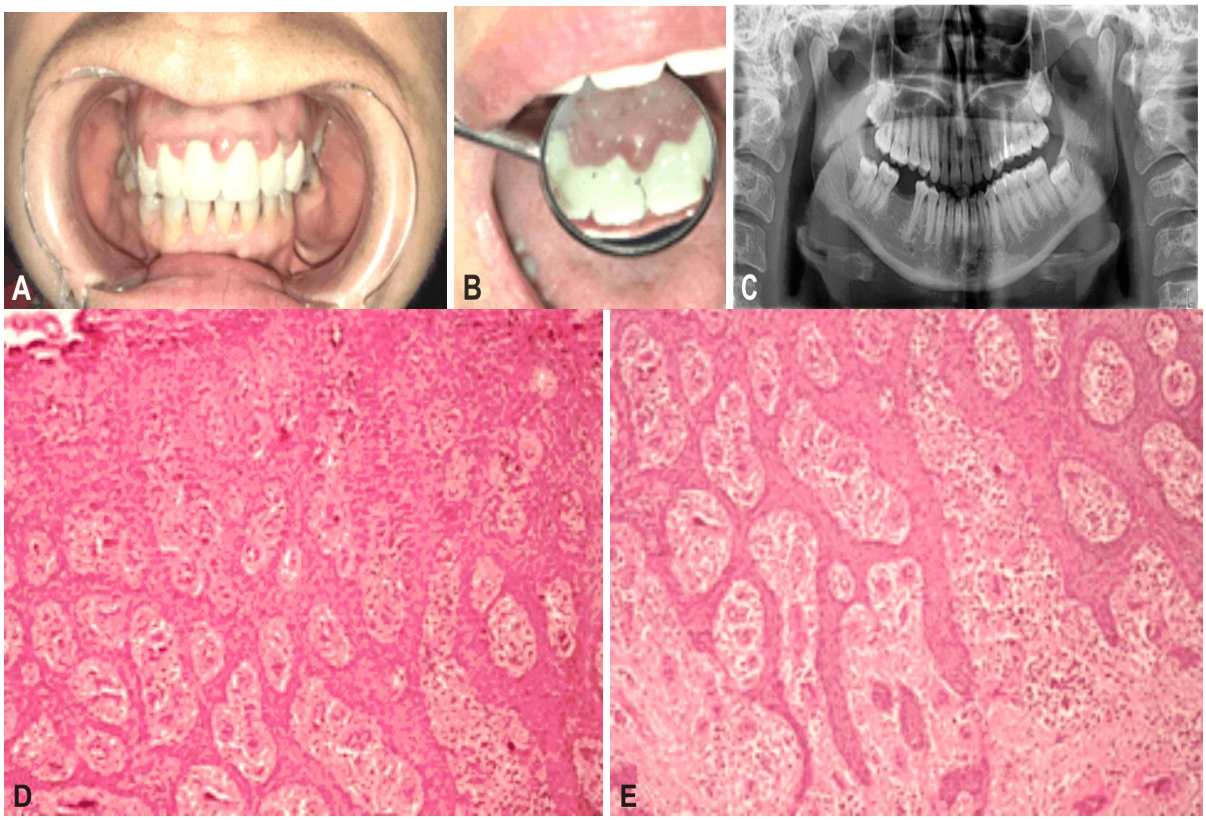


Figure 15 – Male patient, 49-year-old, with gingival epithelial hyperplasia in the maxillary region: (A and B) Clinical aspect of gingival tissue enlargement in the region of papilla between maxillary incisors; (C) Imaging aspect without any bone modification in the region; (D and E) Photomicrographs showed ulcerated gingival surface epithelium with excessive acanthosis leading to branched epithelial ridges and extensive inflammatory cell infiltration in the lamina propria (HE staining, ×100) – superficial part of the epithelial hyperplasia (D); deep part of the epithelial hyperplasia (E).

The non-encapsulated mass consisted of a fibrillar connective tissue containing abundant plump mesenchymal cells, ovoidal or spindle-shaped, and many multinucleated giant cells scattered throughout the lesion, along with extravasated red blood cells (**Figure 17F**).

Gingiva covering the central giant cell granuloma also displayed epithelial hyperplasia due to acanthosis (**Figure 18F**) and an enlarged lamina propria too, as thick collagen bundles

were arranged in a haphazard fashion and admixed with a few dilated new capillaries (more numerous at the papillae level) and few chronic inflammatory cells (**Figure 18G**).

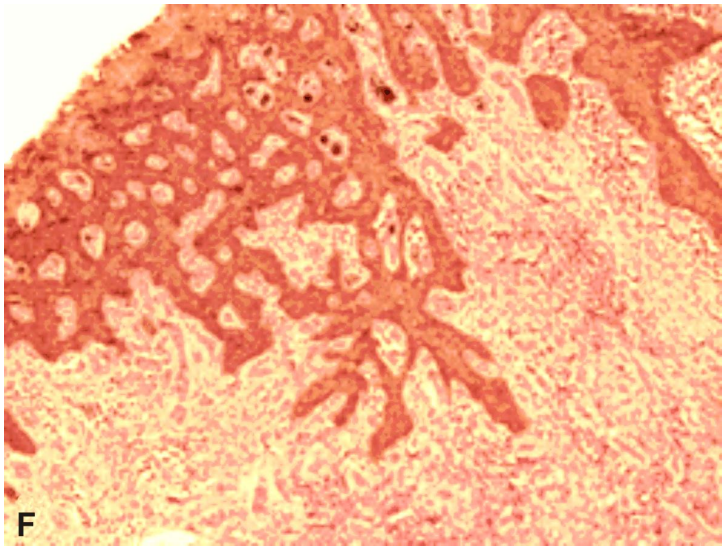


Figure 15 (continued) – Male patient, 49-year-old, with gingival epithelial hyperplasia in the maxillary region: (F) Strong immunopositivity for CK19 of the gingival epithelium revealed the heavy branched epithelial ridges that descended deep into the lamina propria (Anti-CK19 antibody immunostaining, ×50). CK19: Cytokeratin 19.

IHC staining with anti-CD34 antibody revealed numerous newly formed capillaries with moderate inflammation, made up of mononucleated and polynucleated cells in the lamina propria (**Figure 18H**). T

he deep part of the surgical sample showed a lesion made up of a proliferation of osteoclast-like multinucleated giant cells, with five to 20 nuclei.

The stroma also contained plump spindle-shaped mononuclear cells, numerous vascular spaces with erythrocytes inside, and some newly formed bone at the edge of the lesion (**Figure 18, I and J**).

In the LCH case, gingiva covering the deep lesion showed almost the same morphological changes as those encountered in the LCH case (epithelial hyperplasia with acanthosis and enlarged lamina propria due to bundles of collagen fibers arranged haphazardly).

On the other hand, LCH was identified as a proliferation made up of Langerhans cell, presenting irregular nuclei, incisions and fine chromatin, as well as frequent eosinophils. The hallmark of the lesion was CD1a diffuse positivity in Langerhans cells (**Figure 19, H–J**).

We found a very good match between clinical aspects and pathological diagnosis in the epithelial hyperplasia cases (92.3%), PGCG cases (83.33%), and PG cases (81.39%), but only a moderate one in the fibrous hyperplasia cases (54.54%).

We did not find any match (0%) between clinical aspects and pathological diagnosis in the LCH or central giant cell granuloma cases.

Regarding the correlation between the imaging aspects and pathological diagnosis, we found a strong match (100%) in lesions with bone destruction, such as LCH and central giant cell granuloma and a poor one in epithelial hyperplasia (15.38%) and PGCG (16.66%).

We did not consider any correlations for the fibrous hyperplasia and PG, because these two pathologies did not present any changes in the bone structure in the imaging tests.

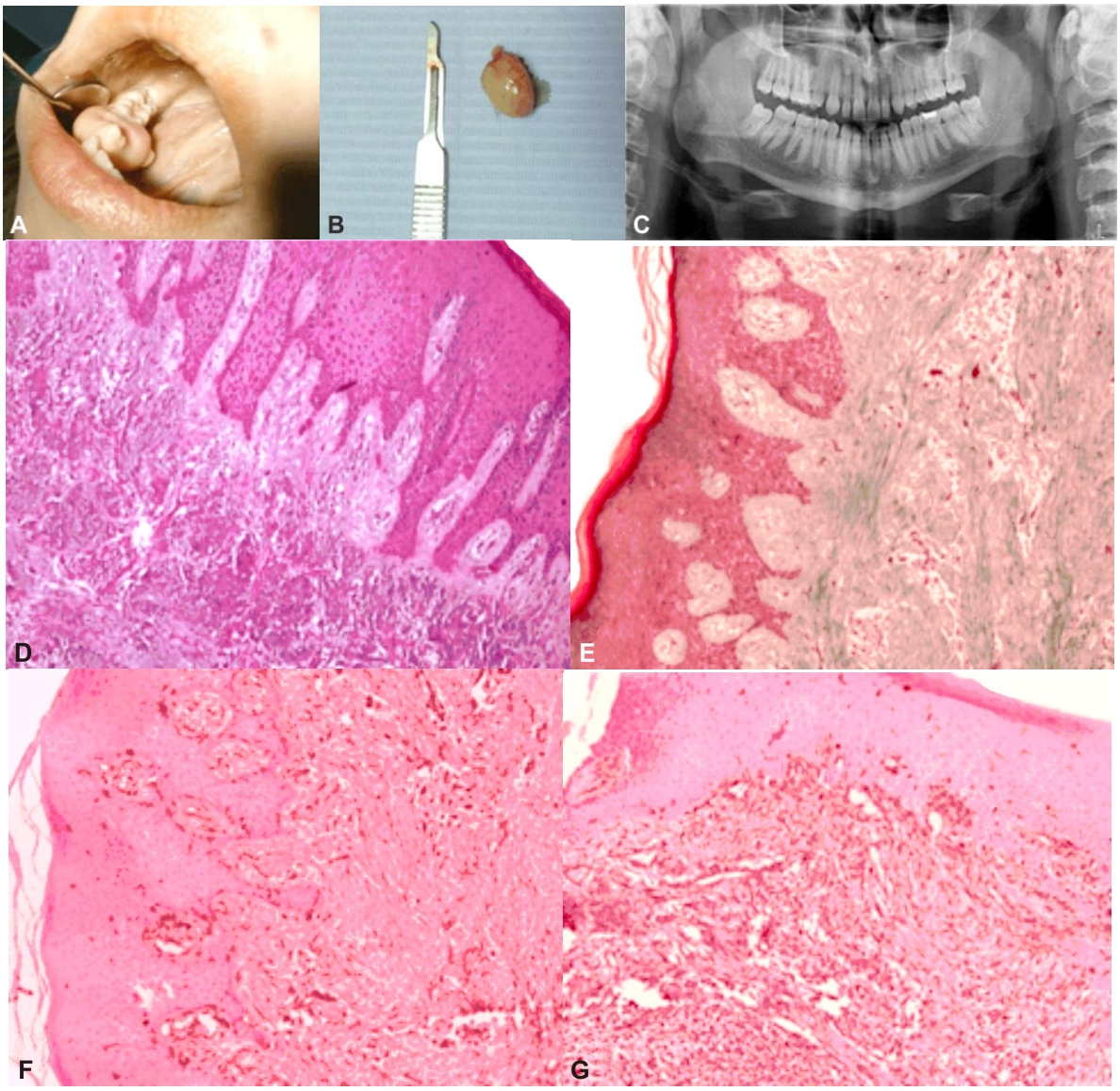


Figure 16 – Female patient, 24-year-old, with gingival fibrous hyperplasia in the region of right mandible: (A) Clinical aspect of gingival enlargement in the region of right mandibular premolars; (B) Macroscopic aspect of lesion – a raised sessile mass with a smooth surface, having its external surface of the same color as the surrounding gingiva; (C) Imaging aspect without any bone modification in the region; (D) Gingival epithelial hyperplasia with parakeratosis and acanthosis, with long epithelial ridges having "gloves finger" features and elongated papillae – the hyperplastic epithelium covered a heavy proliferation of fibrous tissue that replaced the lamina propria (HE staining, ×200); (E) The exceedingly dense fibrous connective tissue presented thick collagen bundles arranged in a haphazardly fashion, numerous dilated new capillaries and inflammatory infiltrate around (Szekely staining, ×200); (F and G) Two different areas of gingival hyperplasia revealing strong expression of vimentin immunostaining with different degrees of fibrous hyperplasia (Anti-vimentin antibody immunostaining, ×200) – moderate proliferation of fibroblasts (F); heavy proliferation of fibroblasts and lymphocytic inflammation (G).

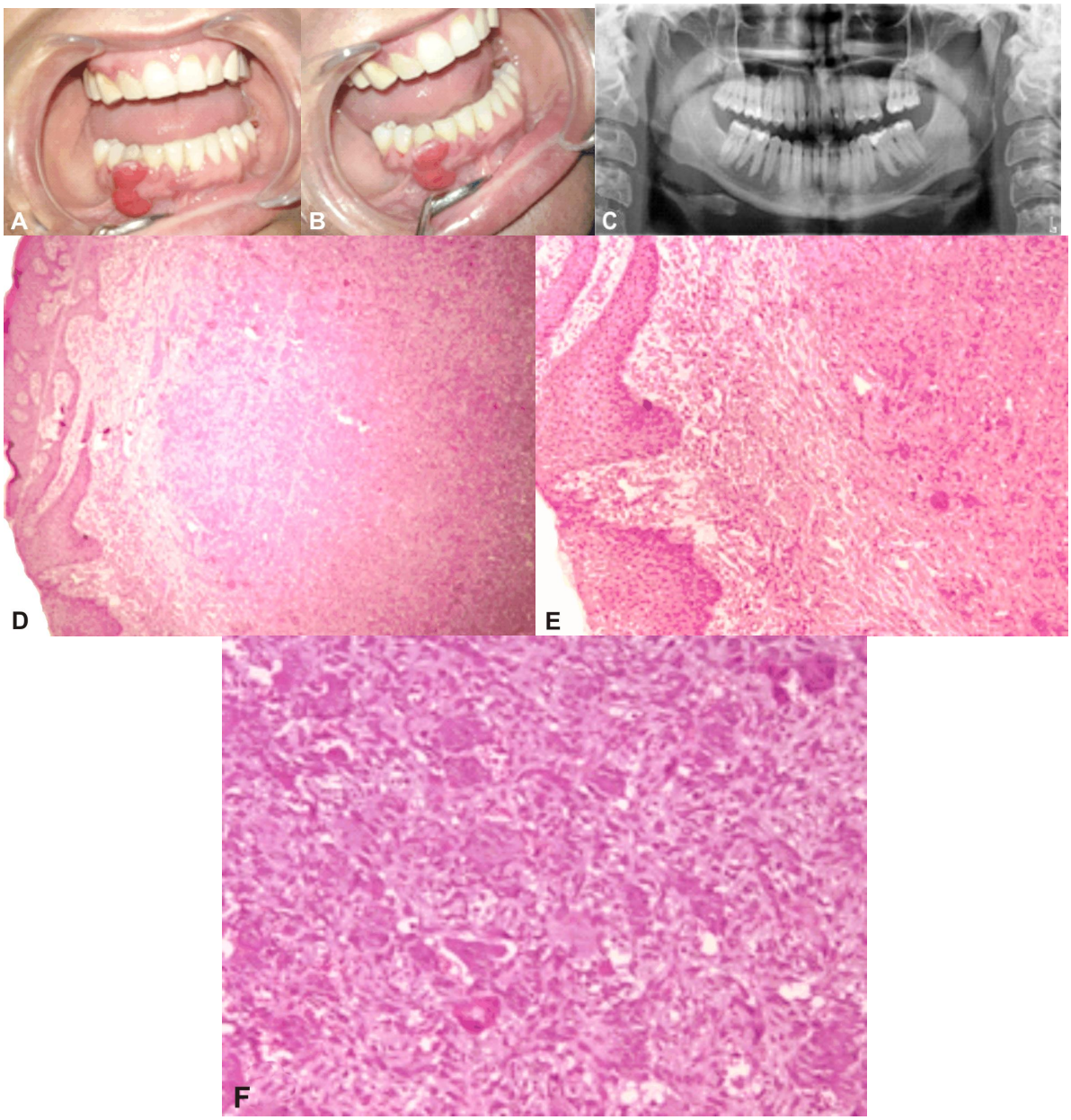


Figure 17 – Male patient, 51-year-old, with peripheral giant cell granuloma in the region of right mandible: (A and B) Clinical aspect of gingival tissue enlargement in the region of right mandibular incisors and canine; (C) Imaging aspect without any bone modification in the region; (D) Gingival overgrowth appeared due to a non-encapsulated mass covered by a hyperplastic gingival epithelium and an enlarged lamina propria due to chronic inflammation; (E) The gingival squamous cell epithelium is ulcerated in some areas and showed acanthosis with long irregular epidermal ridges – the subjacent lamina propria was enlarged due to heavy chronic inflammation and dilated capillaries; (F) The pseudo-tumoral mass consisted of a fibrillar connective tissue containing abundant plump spindle-shaped mesenchymal cells, numerous multinucleated giant cells scattered throughout the lesion, and extravasated red blood cell – multinucleated giant cells presented an abundant cytoplasm and five to 20 nuclei. HE staining: (D) ×50; (E) ×100; (F) ×400.

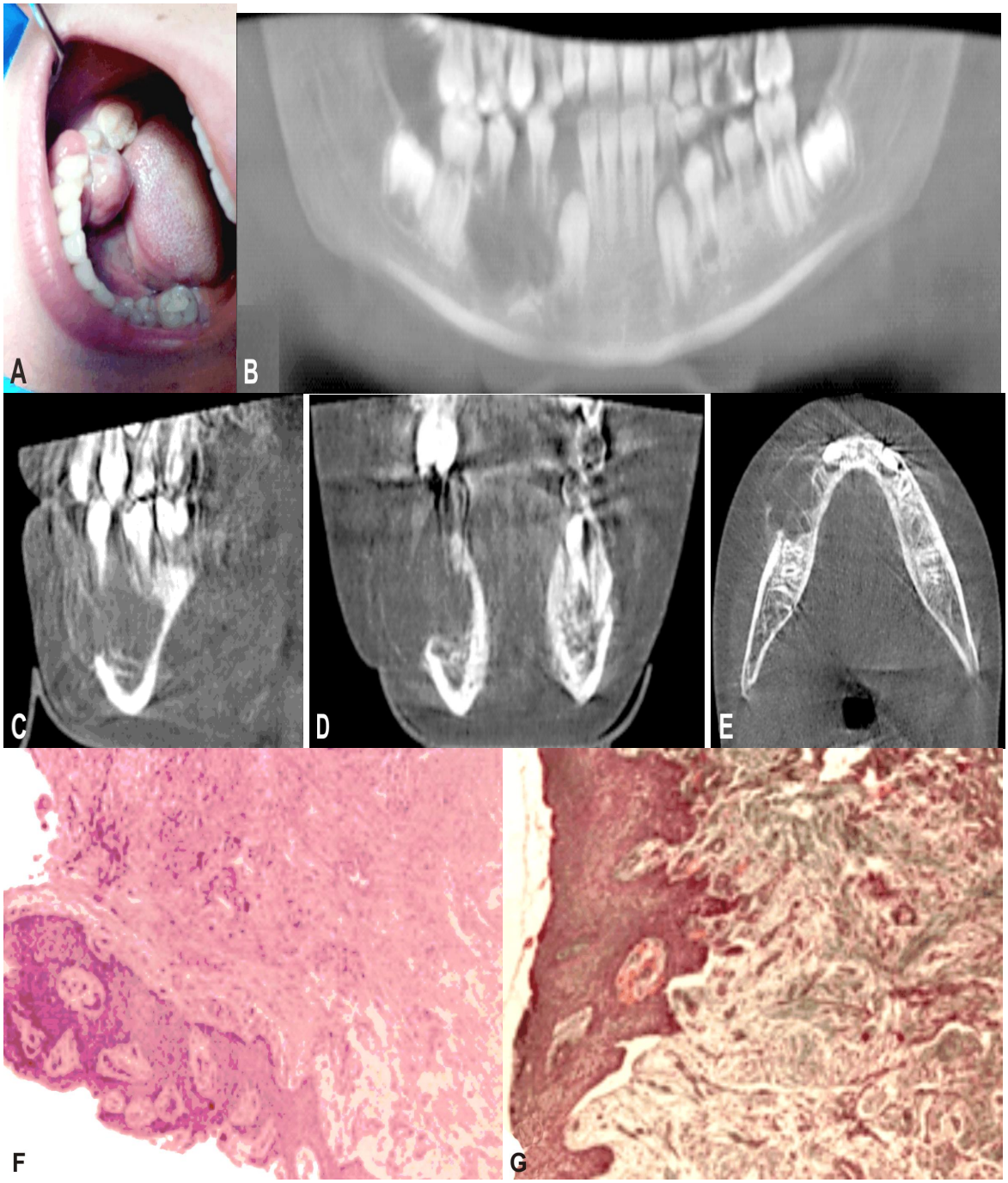


Figure 18 – Male patient, 10-year-old, with central giant cell granuloma in the mandible region: (A) Clinical aspect of enlargement gingival tissue in the region of right mandibular premolars; (B–E) Imaging aspects of an osteolytic lesion developed adjacent to dental apexes 4.3–4.6, measuring 23.21/22.63/31.55 mm (vestibulolingual/craniocaudal/transversal), which destroys the outer cortical of the mandible with obvious extension in the soft parts. (F) Gingival enlargement in the close proximity of the lesion showed epithelial hyperplasia and lamina propria with moderate inflammation made up of mononucleated and polynucleated cells located around the capillaries; (G) Epithelial hyperplasia and fibrous connective tissue proliferation in the lamina propria, with collagen bundles haphazardly arranged together with numerous newly-formed vessels;

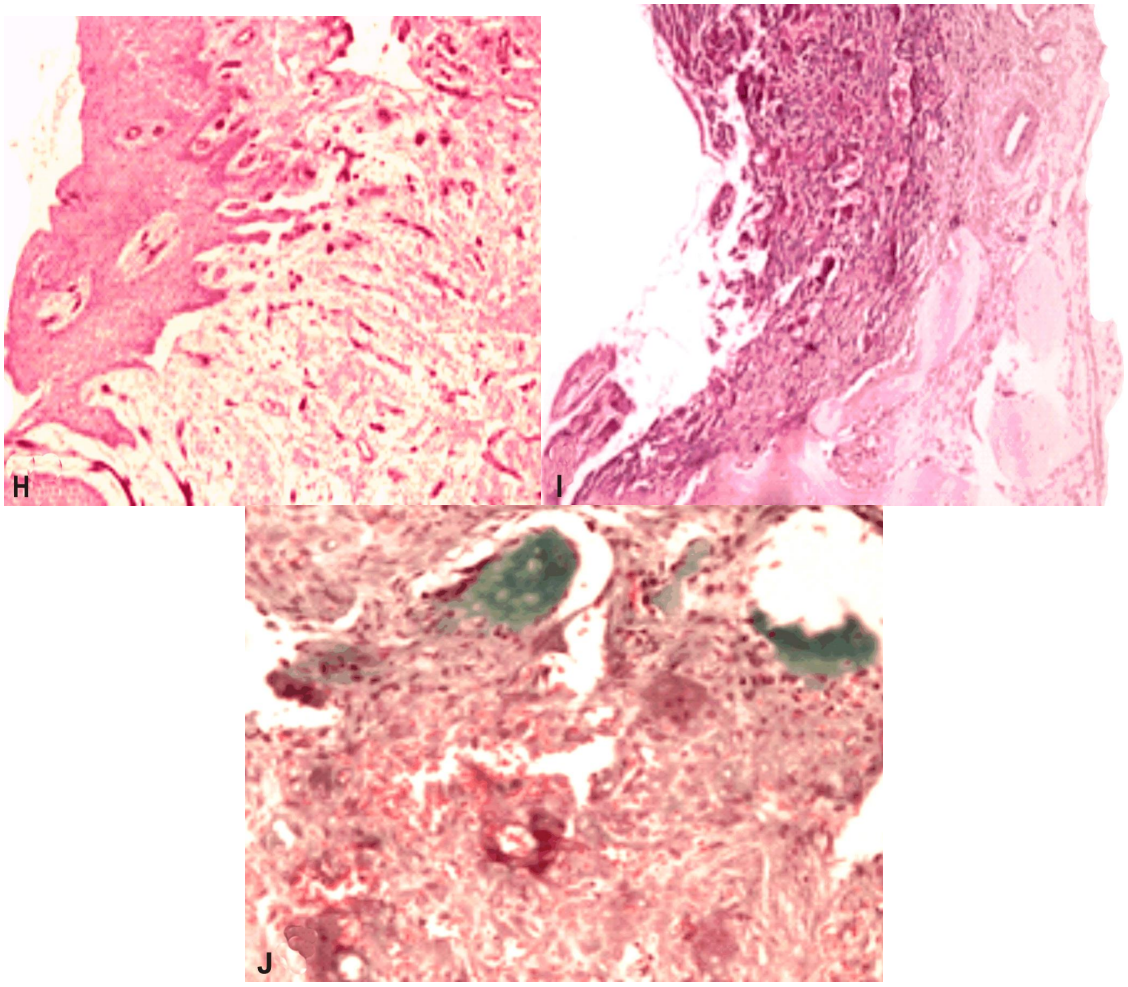


Figure 18 (continued) – Male patient, 10-year-old, with central giant cell granuloma in the mandible region: (H) Epithelial hyperplasia and numerous newly-formed vessels in the lamina propria; (I) The deep intraosseous lesion was made up of a proliferation of osteoclast-like MGCs dispersed amongst a fibrous stroma; (J) The stroma contained plump spindle-shaped mononucleated cells with smooth oval nuclei, numerous vascular spaces with erythrocytes inside, and some new bone formation at the lesion edge. MGGs contained between five and 20 nuclei. Anti-CD34 antibody immunostaining: (H) ×100. HE staining: (F and I) ×100. Szekely staining: (G) ×100; (J) ×400. MGCs: Multinucleated giant cells.

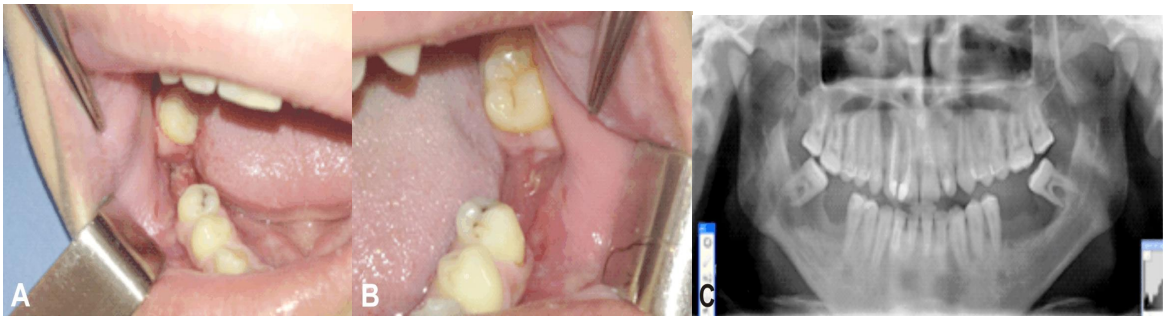


Figure 19 – Male patient, 19-year-old, with Langerhans cell histiocytosis in the region of mandible: (A and B) Clinical aspect of inflammation of gingival tissue in the region of right and left mandibular molars;

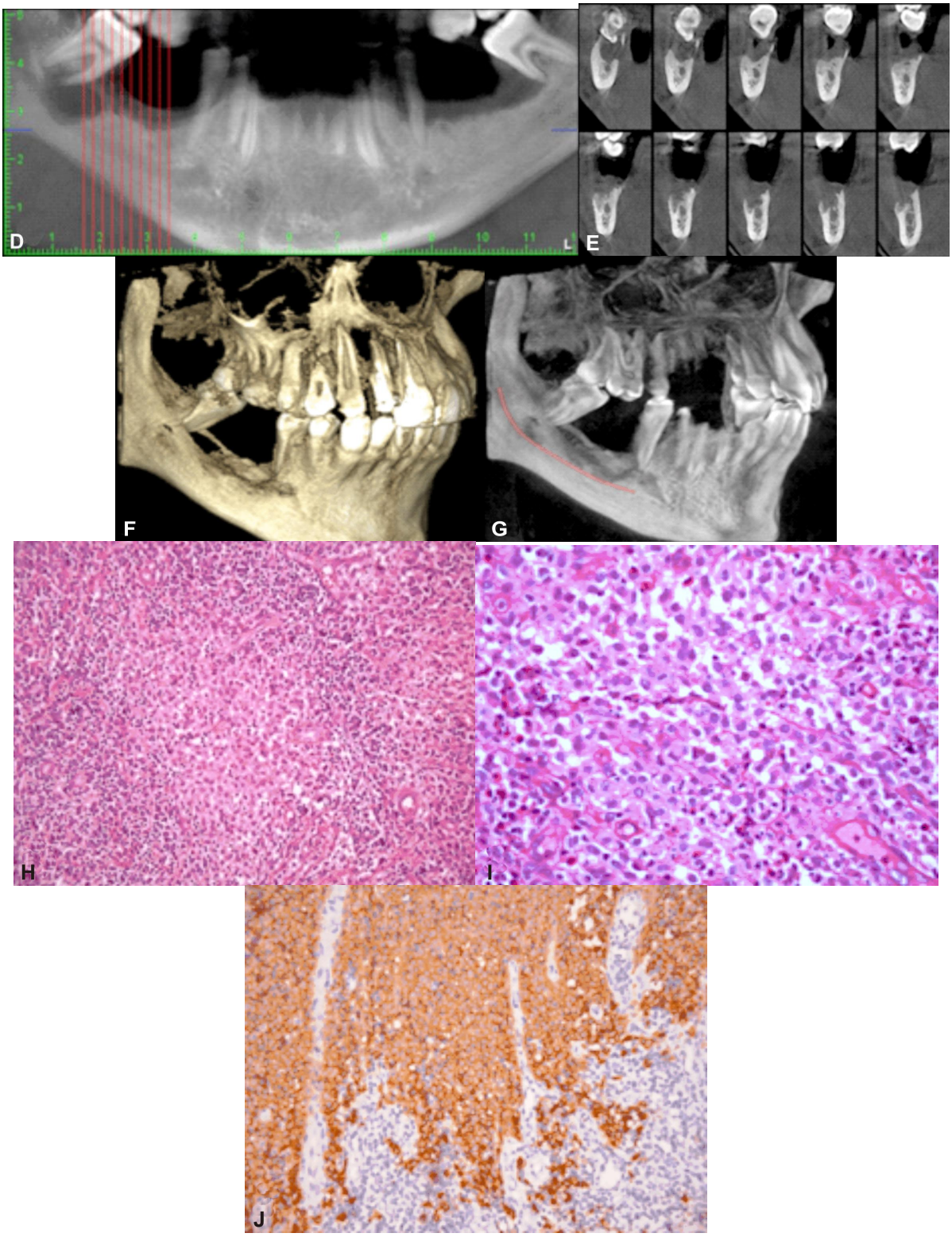


Figure 19 (continued) – Male patient, 19-year-old, Clinical aspect of inflammation of gingival tissue in the region of right and left mandibular molars; (C–G) Imaging aspect of an osteolytic lesion, which involves the roots of the second molar and the second premolar, in close relationship with the inferior alveolar nerve on both sides of the mandible; (H) Histiocytosis-type proliferation that associates frequent eosinophils; (I) Langerhans cells with irregular nuclei, incisions and fine chromatin – frequent eosinophils dispersed; (J) CD1a diffuse positive in Langerhans cells. Anti-CD1a antibody immunostaining: (J) ×200. HE staining: (H) ×200; (I) ×400. CD1a: Cluster of differentiation 1a.

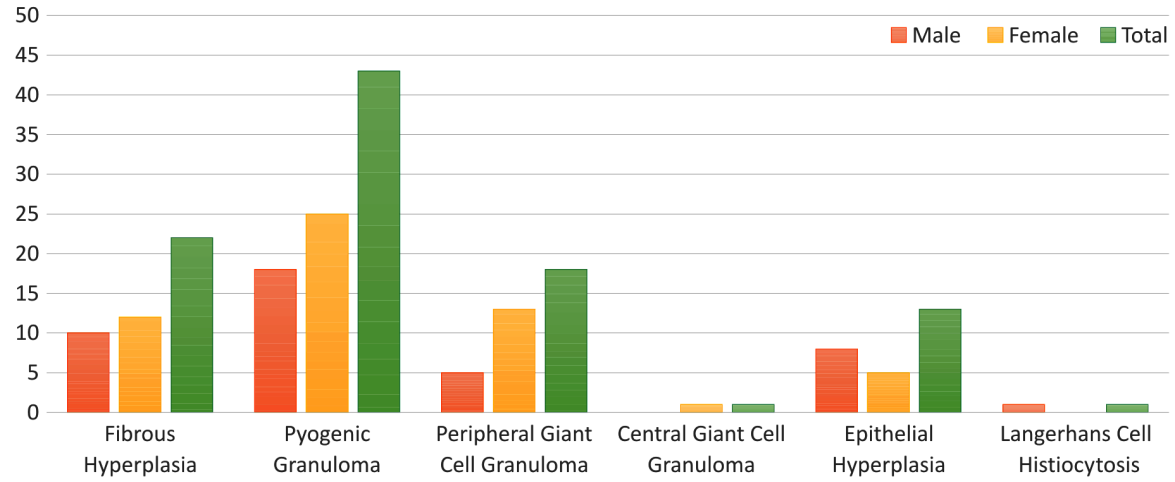


Figure 20 – Distribution of patients with gingival overgrowth by gender.

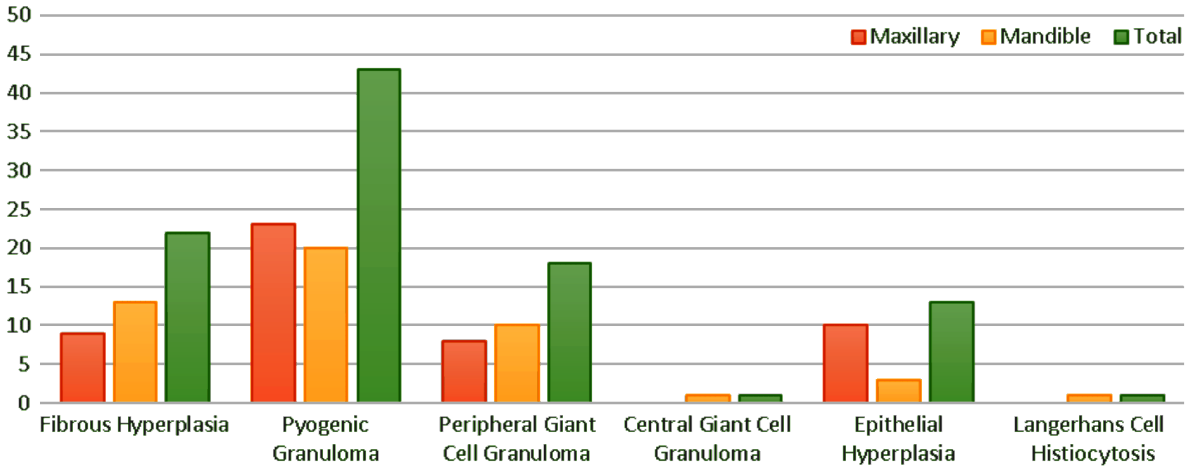


Figure 21 – Distribution of patients with gingival overgrowth by localization.

2.2.4. Discussions

To the best of our knowledge, this study represents the first report on the frequency, location, clinical, radiological, and HP features of gingival overgrowth within the northeastern Romanian population. The data we produced in our survey share many similarities with those previously reported in other regions of Romania or other countries (Carbone et al., 2012; Gambino et al., 2017), but we also recorded some essential differences that can be attributed to geographic or ethnic factors; yet further studies of larger sample size are needed.

Literature reported that reactive hyperplastic lesions of the gingiva have a high female predominance irrespective of continent or region and the patients' age can vary from the 1st to the 7th decade of life (Sangle et al., 2018; Dutra et al., 2019). *We also found a slight predominance of female patients, even though our patients were adults in their 5th–6th decades of life.*

From a histological point of view, the researchers reported the following entities

among their cases of gingival overgrowths: (1) PG (pregnancy tumor included); (2) peripheral ossifying fibroma (POF) (also referred to as ossifying fibroid epulis; (3) focal fibrous hyperplasia (FFH), or fibrous epulis; and (4) PGCG.

In literature, the most common lesion was reported to be fibrous hyperplasia (diagnosed in more than 50% of patients) (Vidyanath et al., 2015), or PG a in some other studies (Kashyap et al., 2012), where it made more than 40% of all cases. The HP findings varied from one country to another. *However, we did not find any POF. In our survey, the fibrous hyperplasia was the most common form of reactive gingival hyperplasia. Similar to all other studies, PGCG was the form with the lowest occurrence.*

Our HP analysis also revealed that an overgrowth of the gingiva consisted of the reactive hyperplasia of both gingival elements: the epithelium and its lamina propria, but in various degrees of development, according to the cause underlying it. However, when an epithelial hyperplasia predominated, the gingival epithelium expressed many epithelial ridges that branched and adhered to each other and went deeply in the lamina propria. At the same time, the lamina propria presented a moderate fibrous hyperplasia. On the other hand, when the fibrous gingival hyperplasia predominated, we also found an epithelial hyperplasia of the surface epithelium, showing parakeratosis and acanthosis. We also found epithelial and fibrous hyperplasia of the gingiva in all cases, even in those diagnosed with deep intraosseous tumor. This can be explained by the fact that gingival tissue is likely to be a very plastic one that promptly reacts to any irritating stimulus by hyperplasia of both elements that constitute its histological structure; the mechanism is correlated with the immune system as numerous inflammatory cells were identified in the lamina propria in all cases.

One of the aims of our study was to establish a correlation between clinical, imaging and pathological findings in gingival overgrowth as some other authors reported a high concordance rate (82.5%) between the clinical and HP diagnosis (Dutra et al., 2019). *In our study, we found a stronger correlation rate (more than 90%) in the epithelial hyperplasia cases and more than 80% in the PG and fibrous hyperplasia cases. Also, PG was the most frequent among gingival overgrowth, and fibrous reactive hyperplasia was the most common pathological diagnosis.*

Furthermore, we did not find any correlation between the radiological and pathological tests in the cases with reactive gingival hyperplasia developing in the absence of a deep subjacent lesion; still, we identified a strong correlation between radiological and subsequent pathological diagnosis in the cases with deep intraosseous lesions. It is worth mentioning that in the latter situation there were no correlations whatsoever between clinical and pathological diagnosis.

We only identified one case of LCH, which is an extremely rare dendritic cell disorder with variable clinical evolution and currently described as an inflammatory myeloid neoplasia (Badalian-Very et al., 2010). LCH occurs less commonly in adults compared to children, with an incidence of 1–2 adult individuals per million and an age ranging between 29 and 38 years old (Berres et al., 2015). In the present study, we discovered incidentally one case, who was a 19-year-old young adult. Manifestation of the disease in the oral cavity can be the first and sometimes the only sign of LCH, often resulting in periodontal involvement (Milian et al., 2001; Stockschlaeder and Sucker 2006; Cantu et al., 2012; Chiong et al., 2013). Periodontal manifestations were similar to those observables in severe periodontal diseases,

more exactly the presence of deep periodontal pockets, furcation involvement, recession, gingival bleeding and mobility (Vassallo et al., 2002).

In our study, the patient was admitted for proliferative inflammatory condition of the gingiva, which developed after tooth extractions. This clinical aspect, coupled with the rarity of this disease, resulted in an initial incorrect diagnosis. In fact, dentists and periodontists might lack familiarity with the exceptionally rare oral manifestations of LCH. Impaired soft tissue healing after extraction can be suggestive of LCH; however, multidisciplinary approaches are warranted. In everyday clinical practice, dentists and periodontists should be aware that rare systemic diseases, such as LCH, might lead to manifestations in the oral cavity as the first clinical sign (Merglová et al., 2014). Patients would benefit from a correct multidisciplinary approach for the identi- fication and clinical management of this rare entity.

The accurate diagnosis and treatment of gingival overgrowths should be a crucial aspect of general dental practice. Valuable diagnostic information is often provided by the appearance of the lesion itself. That is why many experienced clinicians use visual inspection and palpation (Ababneh. 2006) in order to get an accurate provisional diagnosis.

Drug-induced gingival overgrowth is a tissue-specific oral disease that involves hyperplasia and hypertrophy of the gingiva. It is an adverse drug reaction related largely to three types of medicines: antiepileptic drugs, immunosuppressants and calcium channel blockers (Han et al., 2020). Gingival overgrowth is a major problem in maintaining oral hygiene, increasing the patient's vulnerability to oral infection, inflammation and periodontal disease. Currently, the treatment for this condition includes good oral hygiene, periodontal therapy, gum resection and dose control of harmful drugs. However, when it is not possible to remove or replace the drug, postoperative recurrence and treatment of gingivitis are common (Livada and Shiloa, 2014). Although gingival overgrowth is not directly life-threatening, the quality of life of affected individuals is impaired. To improve treatment options in the future, the molecular mechanisms of drug-induced gingival overgrowth need to be characterized (Khocht and Schneider, 1997).

Recent studies show that gingival hyperplasia could occur after the administration of cyclosporine A. Up to 90% of the patients submitted to immunosuppressant drugs have been reported to suffer from this side effect. The role of fibroblasts in gingival hyperplasia has been widely discussed by literature, showing contrasting results. In order to demonstrate the effect of cyclosporine A on the extracellular matrix component of fibroblasts, we investigated the gene expression profile of human fibroblasts after cyclosporine A administration (Ponnaiyan and Jegadeesan, 2015). The administration of cyclosporine A was followed by down-regulation of other genes: *COL7A1*, the transmembrane receptors *ITGB2* and *ITGB4*, and the basement membrane constituents *LAMA2* and *LAMB1*. Data collected demonstrate that cyclosporine inhibits the secretion of matrix proteases, contributing to the accumulation of extracellular matrix components in the gingival connective tissue, causing gingival overgrowth. Patients affected by gingival overgrowth caused by cyclosporine A need to be further investigated in order to determine the role of this drug on fibroblasts (Lauritano et al., 2020).

Limited study evidences indicate apositive effect of systemically administered azithromycin on the reduc-tion of cyclosporin A mediated gingival overgrowth.

Azithromycin may be con-sidered as an alternative first approach to a surgical reduction of gingival-overgrowth (Fuchs et al., 2019).

NF-KAPPA B plays a crucial role in collagen overproduction in dihydropyridine-induced gingival overgrowth fibroblasts. Recent studies investigate the role of the *KAPPA B* (*I KAPPA B*) kinase *(IKK)-NF-KAPPA B* pathway and downstream collagen type I (Col I) synthesis in DIGO cells and demonstrate the therapeutic strategy of interference of this pathway with proteasome inhibitors. The results demonstrated that both drugs additively mediated *NF-KAPPA B* activity by activating IKK alpha/beta phosphorylation. They also triggered nuclear translocation of *NF-KAPPA B*, Rela, and p50 (*p < .05) and increased Col I production in both healthy and DIGO cells. The addition of proteasome inhibitors, including bortezomib and *MG132*, promoted the accumulation of phosphorylated p-I kappa B alpha, prevented the subsequent cytosol-to-nuclear translocation of p50 and Rela (*p < .05), and abbreviated the biosynthesis of Col I in DIGO cells. These findings suggest that IKK-I kappa B alpha activation is mediated by proinflammatory cytokines and CCBs in DIGO cells and triggers downstream NF-kappa B-Col I synthesis. Proteasome inhibitors may strategically interfere with the *IKK-I KAPPA B ALPHA-NF-KAPPA B-COL I* pathway and inhibit the etiopathogenesis of DIGO (Lu et al., 2020).

Reactive gingival hyperplasia includes a group of lesions that are commonly the result of either injury or chronic irritation (Shah et al., 2012; Scrieciu et al., 2015). From a histological point of view, chronic trauma leads to granulation tissue formation, which comprises new capillaries running in an edematous matrix; this matrix is made up of both fibroblasts and mononuclear and neutrophilic inflammatory cells. Later on, this new tissue begins to display fibroblasts differentiation and vessels maturation and to manifest as an overgrowth, which is called reactive hyperplasia. Under the circum- stances, even though at first sight they can appear as tumor-like lesions, the gingival overgrowths are not neoplastic; rather they suggest the existence of a chronic process in which an intensified restoration occurs (Scrieciu et al., 2015). The favored treatment of choice in gingival overgrowth is surgical excision, when removal of local irritants occurs in order to prevent recurrence. If the lesions fail to resolve, they should be surgically excised. A follow-up checkup is needed as it normally shows a tendency to recur (Shah et al., 2012; Manjunatha et al., 2014).

2.2.5. Conclusions

We found out in our study that gingival overgrowth can be triggered by a lot of different diseases, which stresses the importance of pathological examination and differential diagnosis for practitioners. This gives grounds for more prospective studies that can better explain the real incidence of various related gingival diseases.

The data gathered by the present study are similar to those reported in the specialized literature, but there are also some differences, especially regarding the HP features. Furthermore, it was difficult to compare studies carried out in different countries around the world, because of differences in people's attitude towards oral health, on the one hand, and the accessibility of the various population groups to biopsy services, on the other. We believe that similar studies should be conducted in other oral facilities in Romania in order to further investigate not only the epidemiology of gingival overgrowth, but also the numerous different

HP facets of this lesion because the fact should not be overlooked that a gingival overgrowth can sometimes hide a deep intraosseous tumor. Moreover, the data presented in this study can be used as a (good practices) guide for additional multicenter studies in our country

2.3. Advantages and limits in locoregional anesthesia

2.3.1. Introduction

Pain is altogether a functional phenomenon, arising due to an excitation of certain intensity acting on the surface nerve terminations or on the profound ones. It is the result of the stimulation of specialised nociceptor receptors and/or their related senzory fibres. This symptom is a global phenomenon requiring scientifical approach on two essential and distinctive aspects: the perception of and the reaction to pain (Imhoff et al., 2020). The perception is a neurological phenomenon, while the reaction to pain is a group of somatic, vegetative and mental phenomena. Each of these aspects has its own programme and pathway. The threshold of pain perception is the lowest intensity of a stimulus which is recognised as pain. The threshold of the reaction to pain is represented by the intensity of the painful sensation triggering somatic phenomena (Aggarwal et al., 2009).

Pain represents an important alarm system which triggers defense reactions towards the removal of the harmful agent. Also, it works like a landmark for the diagnosis. The decoding and acknowledgement of a painful message, which has reached the superior nervous centers by different sensory pathways, bears the generic term of sensation of pain (Adler 2007).

Pain is a multidimensional experience, caused by characteristic properties of the neuroprint of nervous impulses type, generated and distributed by a neurocerebral network: the body's own neuromatrix. This Neuromatrix is genetically determined and modified by sensory experiences, representing the primary mechanisms inducing the neuronal character of the pain (Aggarwal et al., 2009).

The neurochemistry of pain refers to the endogenous chemical substances intervening in the triggering process of the pain signal and the transmission of specific information to the brain and also in the descending control of the brain over the intensity of this signal (Anderson 2004). The brain exercises a descending control over the intensity of the pain signal by means of neurons containing opioid peptides, catecholamine, serotonin (Bernabe et al., 2020).

Serotonin's role in modulating the pain is a slight electric stimulation of the gigantocellular reticular nucleus, facilitating the transmission of painful information through the spine, by means of a serotonergic mechanism in which 5HTI receptors are involved. The global effect of serotonin (an indole derived amine) on pain is an algebraic sum between the exaggeration tendencies and the diminishing tendencies of the pain sensation, ensuring one the whole the effect of an analgesic (Babl et al., 2007).

The patient is first and foremost afraid of pain, which causes an experience each person encounters more or less frequently, in a higher or lower intensity (Imhoff et al., 2020).

It is thus normal to have the patient be focused with the method, technique and quality

of the anesthesia and the surgical performance, the possibility of deficiences in the induction or the extend of the anesthesia, which might cause pain during the intervention or right afterwards. The perception of pain in patients is strongly correlated with their mental state. A solitary therapy is not 100% self-sufficient in dental practice. Only the combination of several factors has a cummulated positive effect: mental preparation, pharmaco-therapeutical sedation, local anesthesia (Brad et al., 2001; Bucur et al., 2009).

Although the modern anesthesia procedures have considerably changed the dental practices and the ambulatory care for oral and maxillofacial surgery, the emotion and the anxiety, the concerns persist still with patients that will have to be subjected to dental treatments and care. The relief of the pain represents a highly important objective for the dentist or the oral surgeon (Caracas and Martins 2009).

Local anesthesia came into current practice after 1905, when Einhorn synthesized procaine (novocaine). Step by step, anesthesia methods and techniques were perfected, new anesthetic substances were synthesized, which allowed all the surgical branches, and of course maxillofacial surgery, as well as other stomatological specialties, to develop intensly. After that, general anesthesia was replaced by local anesthesia, considered to be a method of anesthesia with the lowest risks, provided the indications and warnings are closely followed (Dau et al., 2017).

Local anesthesia is the method in which the use of chemical, physical substances or electricity creates a temporary desensitization on a specific anatomical region, while retaining intact the consciousness. Due to the fact that this type of anesthesia abolishes only nociception, preserving the thermic, tactile and pressure perception. American authors called it local analgesia (Babl et al., 2007).

The most used methods for pain control in dental practice consist of the blocking the ways of pain impulses. This blocking is made by depositing an adequate chemical agent extraneurally, close to the nerve or the nerves to be anesthesied. The chemical agent, represented by the anesthetic solution, is absorbed by the lipoid tissue of the nerve, thus preventing the depolarization of the nerve membrane (Bucur et al., 2009).

To achieve a maximal effect, the local anesthesic must be in contact with at least 8-10 mm of nerve, in order to block 2-3 Ranvier nodes. If, however, a smaller part of the nerve is the focus, it is believed that the anesthesia will be incomplete. The thickness of the nerve's fibre is another important factor in local anesthesia; the thicker nerve fibers are more difficult to block, requiring a higher concentration and quantity of anesthetic solution (Imhoff et al., 2020). This is the result of a larger surface, in relation with the volume and the size of the internodal distance. If an inappropriate concentration of local anesthetic makes contact with the nerve fibre or if less than a minimum number of fibres is affected, the anesthesia may be incomplete. These manifests itself by insensitivity to a single stimulus, but a possible painful response to a repeated series of stimuli. If a nerve or nerve fibres are unable to transmit impluses, because of a local anesthetic agent, it means that the regional analgesia is functioning well over the area innervated with these nerves (Adler 2007).

Anesthesia ensures the relief of pain, the undettered functioning of the vital systems and apparatus, facilitating the surgical performance. The usage of analgesic-anesthesic substances preceded, by far, the anatomical and physiological research and discoveries on pain. Today, modern anesthesia allows the adjustment of methods and techniques for each

specific case individually, so that it ensures a perfect operating security, with minimal risks. A good local or local anesthetic should be harmeless to the tissues, should not have a local or general toxicity, should determine a qualitative and enduring anesthesia, and should not cause injuries at the level of nerve terminations. It should also be hydrosoluble and not cause allergic (Dau et al., 2017).

Local anesthesia is successfully used for patients with a balanced mental state, calm and cooperative. For anxious, aggitated and non-cooperative patients one recommends as an ideal anesthesia method, for extended oral surgical interventions, that can be both time-consuming and traumatising, the local anesthesia in combination with a safe and efficient pre-anesthesia (Imhoff et al., 2020). During the intervention, an intravenous infusion is carried out to administer the anxiolytic medication or those meant to prevent and treat potential side effects.

Pre-anesthesia comprises all the measures taken to better prepare the organism for anesthesia; knowledge of safe pre-anesthesia methods, of pain and anxiety control, establishing a good relation with the patient are the most important managing methods for the patient harbouring negative emotions in the dentist's practice. The purpose of premedication must be unconditionally determined by the anxiolytic effect and the analgesic one for the improvement of pain toleration (Douketis et al., 2015).

The objectives of premedication – inducing calmness, a certain degree of amnesia, causing induction, the preservation of the anesthesia; reducing salivary secretion. To remove the reflex hyperactivity, vegetative hyperactivity, using a vagal as well as a sympathetic-adrenaline pathway. Both manifest themselves by rhythm disturbances. To increase the threshold of pain sensitivity; to reduce the painful effects of certain substances used in premedication (atropine, antispasmodic substances or barbiturates). During operation the basal metabolic rate must be decreased (Hila and Kwon, 2009).

Pre-anesthesia is carried out by combining four fundamental drugs: (alone or in various association): Analgesics (opiates); Vagolytics (atropine, scopolamine – cerebral depressor); Sedatives (barbiturates); Tranquilizers (derivatives of phenothiazine).

Mental and drug-related preparation (pre-anesthesia - premedication) do not have as a primary objective the control of intraoperative pain (covered by the anesthesia), but mainly the control of the mental reaction to pain (Douketis et al., 2016).

There can be no premedication if there is no infrastructure in place with a surgical treatment room and a waking room, in which the patient can be supervised by qualified staff and has at its disposal oxygen therapy equipment and instruments, keeping the breathing airways free, with monitors for the vital functions and cardio- respiratory analeptic functions (Hila and Kwon, 2009). We bear witness thus to the limits of dental medicine practice, especially for the stomatological specialists.

The search for, the research and the assessment of optimal methods of local anesthesia to successfully address distressed patients as an alternative to the combined anesthetic method implies thorough knowledge of the physiologic, physiopathologic and, last but not least, the psychological aspects of pain. Knowledge of pharmacology and pharmacodymanics of sedative, analegesic and anesthetic medication is a major prerequisite in the creation of alternative pre- anesthesia schemes (Dau et al., 2017).

For a surgical intervention the anesthetic is more dangerous for the patient than the

scalpel itself.

Analgesia is a peripheric anesthesia; regional analgesia refers to the loss of pain sensation for an anatomical area, without affecting the patient's consciousness (Wiener, 2009; Bernabe et al., 2020).

Basic anesthesia is a very strong pre-anesthesia, a partial anesthesia. The hypnotic effect of the medication is surpassed, without reaching the stage of anesthesia without reflexes. The characteristic of analgesia is the action on the fiber. Regional anesthesia represents more than the loss of the pain sensation for a specific anatomical area, without affecting the consciousness; regional anesthesia interrupts all other sensations, including temperature, pressure, contact, motor function (Dau et al., 2017).

The terms of regional analgesia and regional anesthesia are presented as an expression of local anesthesia and are often used indiscriminately. As long as the pain is the main sensation of the tooth, the analgesia may offer a satisfactory state for the patient. If one wishes to interrupt the painful sensibility, the motor function, the thermic and pressure sensation, then regional anesthesia offers the best results (Hila and Kwon 2009).

Analgesia and regional anesthesia do not exclude one another. They interfere with one another, completing each other, with the ultimate purpose of relieving pain for the patient's comfort and security (Imhoff et al., 2020).

A few criteria are to be considered in selecting the method of local anesthesia, on which the success or failure of the method often depends. These criteria are: the anesthesia area, the necessary depth, the duration of the anesthesia, the presence of infection, the patient's age, the general state of the patient, hemostasis, if it is absolutely necessary (Bernabe et al., 2020).

The action of local anesthesics is represented by the blocking of Na channels. From a chemical viewpoint, they are categorised in 2 large groups: aminoesters: procaine, tetracaine, aminoamides: lidocaine, prilocaine, mepivacaine, bupivacaine, ropivacaine, etidocaine.

The choice of the local anesthetic is made depending on the size of the nerve to be blocked and the type of block we want to achieve (Imhoff et al., 2020).

Pain is one of the most common reason for patients scheduling or avoiding appointments in the dental office. It is an unpleasant sensation that cannot be endured for a long time by the patient and which, in correlation with fear and anxiety, may exacerbate their symptoms. Symptomatology commonly associated with fixed orthodontic treatment, this may have a negative impact on the patient's quality of life and may result in discontinuation of treatment or absence from periodic appointments (Imhoff et al., 2020).

Orthodontic treatment is often an integral part of interdisciplinarity, creating through it the premises of a complex oral rehabilitation. One of its goals is to achieve the aesthetic aspect of the smile as well as a functional occlusion. Thus, besides the conventional stainless steel archwires, the Nickel-Titanium alloy (NiTi) archwires are used. Nitinol is the name for Nickel Titanium alloy which has two interesting properties: memory of forms and superelasticity. Due to its molecular structure, at low temperatures the alloy can be deformed and manipulated, known as martensitic state, but it returns to its form at high temperatures-known as austenitic state, as seen in **figure 22** and **figure 23.** This property of returning to the original form is known as memory shape (Gherghescu et al., 2016). Nitinol memory shape is the ability to mechanically deform, subjected to a certain temperature, called transition

temperature, and then to recover its original shape, non-deformed by heating over transformation temperature.

Along with its memory shape, Nitinol also has extraordinary elasticity, making it extremely useful for archwires. Superelasticity goes hand in hand with the memory shape and, even if some criticism has been brought to its use in the medical field due to its nickel content, Nitinol proves to be very useful in applications involving movement and flexibility as is the orthodontic treatment. Among the properties of Nitinol that make it appreciated and preferred for medical use are: biocompatibility, torsional strength, physiological compatibility, shape memory, fatigue resistance, and imaging compatibility.

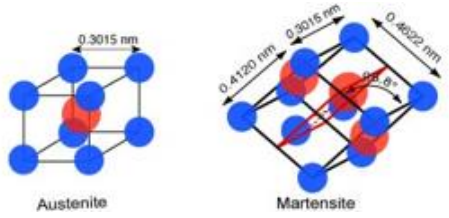


Figure 22 - Nitinol's molecular structure

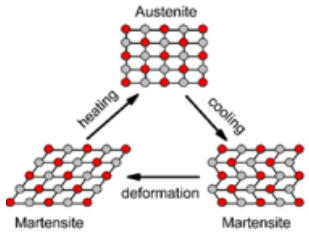


Figure 23 - Nitinol superelastic molecular transformation due to temperature

Pain is an undesirable side effect of orthodontic tooth movement, which causes many patients to give up orthodontic treatment or avoid it altogether. Several studies have evaluated the effects of different types of drugs on the control of orthodontic pain. Researchers compared the effects of paracetamol, ibuprofen, aspirin, and placebo initially administered 1 hour before the insertion of elastic separators and every 6 hours after the insertion of elastic separators. They reported that all drugs had effects superior to placebo and that the best results were observed in the group that used aspirin, followed by the group that used ibuprofen. Paracetamol generated less pain reduction compared to the other drugs tested (Sudhakar et al., 2014). Other researchers (Patel et el., 2011) observed that ibuprofen had a greater effect on orthodontic pain compared to naproxen sodium, in contrast to the study by Polat who observed that naproxen sodium was more effective than ibuprofen (Polat et al., 2005). With respect to drug administration, positive results were obtained in terms of pain control with the use of ibuprofen, paracetamol, naproxen sodium, aspirin, etoricoxib, meloxicam, piroxicam, and tenoxicam. Nevertheless, there is no consensus on the most effective dose and the protocol to be used (Topolski et al., 2018).

The reduction in pain perception after insertion of elastic separators by the effect of LLLT was reported by researchers. Almallah tested the effect of LLLT after separator

insertion by comparing a protocol with a single application, which was administered immediately after separator insertion, and another with double application, which was administered immediately after the separator insertion and once again after 24 hours. The authors reported that LLLT was effective in reducing pain, with no significant differences between the single and double application protocols (Almallah et al., 2016). With respect to LLLT, although most studies report favorable results in terms of pain reduction, there is a need to establish an ideal clinical protocol (Topolski et al., 2018).

The selection of a local anesthetic for intraoral injection must include considerations of efficacy, safety, and individual patient and operative needs. Drug selection has to consider both components of the anesthetic solution. The local anesthetic agent must have a high intrinsic activity and a low systemic toxicity. The complications due to the local anesthetic solution itself demands a knowledge of the pharmacology of the drugs used and an awareness that it is important to obtain an accurate medical history (Singh, 2012).

The aim of our study was to evaluate the perception of pain occurring after the use of 0.14 NiTi and 0.16 NiTi. We have also tried to demonstrate the value of local anesthesia methods. In order to apply these techniques, the knowledge of anatomical features of the oral-maxillofacial territory becomes a vital prerequisite. We highlighted that, although the local anesthetics used today do not meet all the criteria, they do comprise the majority. The management of dental pain in a medical setting must follows specific guidelines for elderly patients in order to achieve either definitive treatment or to provide relief before referral for dental treatment.

2.3.2. Materials and methods

2.3.2.1. Evaluation of the dental pain perception correlated with NiTi archwires types

The study group consisted of a total of 39 patients with fixed orthodontic appliances who have given their consent freely to answer the questions. One group consisted of 11 patients with esthetic braces with NiTi coated esthetic archwires (8 female, 3 male), another group of 14 patients with metallic braces and NiTi archwires (8 female and 6 male), and last group of 14 patients with metallic braces and thermal NiTi archwires (9 female and 5 male).

Using the 0-10 , patients were asked to assess and quantify the appearance or persistence of the sensation of: tension, pressure, dental sensitivity, dental pain on a scale from 0 to 10, where 0 is no pain and 10 is the maximum intensity of pain / sensation experienced in: the first day after the archwire insertion (Z1), at the end of the first week (S1) and at the end of the fourth week (S4) from the archwire insertion.

2.3.2.2. Evaluation of local anesthesia in pain syndrome

We carried out a study on a representative human sample comprised of 10,123 patients, treated in the Oral and Maxillofacial Surgery Clinic (Ambulatory Care), from the County Clinic Emergency Hospital, ”St. Spiridon” Iasi, between 01.01.2015- 31.12.2016 for dentoalveolar surgical interventions. The selection of cases was random, in relation with the addressability of the patients for the duration of the research.

The investigation algorithm of the general sample involved the analysis of a multitude of data concerning the patient and the recommended intervention. Thus, before establishing the indication of anesthesia, a general compulsory clinical examination was carried out, in order to assess whether the patient's state does not contraindicate any of the substances used; three compulsory stages are followed: the psychological study stage, the anamnesis stage and the patient's organic state.

In our study we included the following surgical interventions, generically referred to as: surgical intervention by appointment: dental extraction, apical resection, canine or molar odontectomy, regularization of alveolar ridge, gingivectomy.

We deemed as necessary the removal of 3131 patients from the sample, presenting: a special conditions background, children under 18 years old, elderly persons over 60 years old.

The reason for excluding from research these categories of patients was: the patients with special conditions background require personalised pre-anesthesia schemes correlated with the nature of their general preexisting condition, and even more so, approved by the attending specialist physician: cardiologist, internist, diabetologist; children under 18 years old have a high threshold of anxiety; a high percentage of elderly patients over 60 years old have a combination of general problems, thus requiring a special approach.

We selected an optimal number of patients – 6992 (69.07%), aged between 20-60 years old, in order to apply a coherent comparative statistical method with a practical and scientific conclusion.

The local anesthesia methods used were: local anesthesia – 57%; local anesthesia + topical anesthesia – 19%; local anesthesia + pre-anesthesia medication – 24 %.

2.3.3. Results

2.3.3.1. Results of the study regarding the evaluation of the dental pain perception corelated with NiTi archwires types

Percentage distribution by gender did not show significant differences depending on the type of archwires used (p = 0.371) (**Figure 24**).

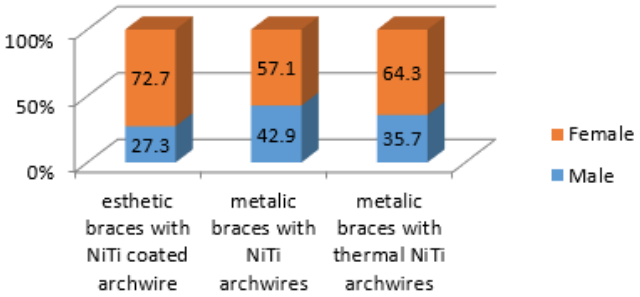


Figure 24 - Structure of study groups by gender

After the use of 0.14 NiTi archwires we noticed that the average level of sensation of tension at Z1 moment did not differ significantly depending on the type of archwire used ($p>0.05$), while the in S1 ($p <0.05$) and S4 ($p <0.001$) moments, for the patients with thermal

NiTi archwires the average level was significantly lower than the others used (**Figure 25**). For the sensation of tension there were decreases in the mean score in moments S1 and S4 as compared to day 1, but the differences were significant only in patients with thermal NiTi archwires.

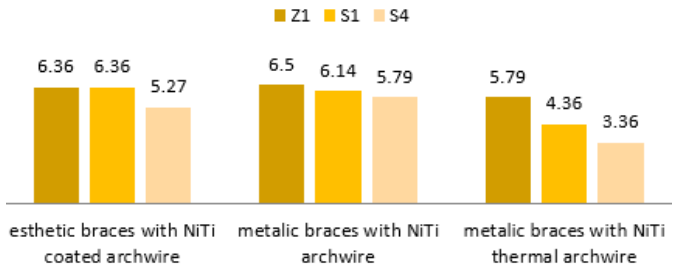


Figure 25 - Tension perception assessment for 0.14 NiTi coated, NiTi and NiTi thermal archwires

We notice that the average level of pressure sensation at Z1 moment did not differ significantly depending on the type of archwire used (p> 0.05), while in weeks S1 (p <0.05) and S4 (p <0.001) for patients with the thermal ones the average level was significantly lower than the other types. For the sensation of pressure there were decreases in the average level during the study period, but the differences were significant only for the patients with thermal NiTi archwires (**Figure 26)**.

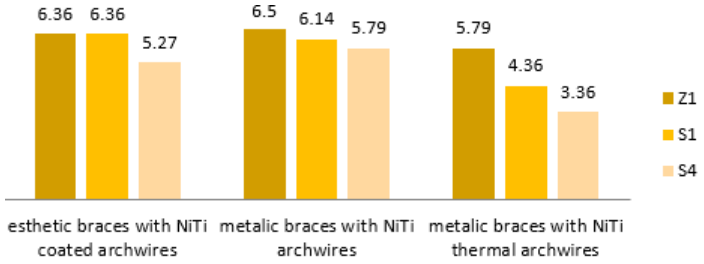


Figure 26 - Pressure sensation assessment for archwires 0.14 NiTi coated, NiTi si NiTi thermal

The average levels of dental sensitivity (**Figure 27**) at moments Z1 and S4 did not differ significantly depending on the type of applied archwires (p> 0.05), while at week S1 the average level in patients with thermal NiTi archwires was significantly lower than the other types (p <0.05). Dental sensitivity decreased from an average score of 6.27 (Z1) to 4.09 (S4) for patients with NiTi coated archwires (p <0.05), from 6.71 (Z1) to 4.07 S4) for patients with NiTi archwires (p <0.001) and from 5.79 (Z1) to 2.93 (S4) for patients with thermal NiTi archwires (p <0.001).

The average levels of dental pain (**Figure 28**) at moments Z1, S1 and did not differ significantly depending on the type of applied archwires (p>005). Dental pain has decreased from an average score of 6.27 (Z1) to 4.09 (S4) for patients with NiTi coated archwires (p

<0.05), from 6.71 (Z1) to 4.07 S4) for patients with NiTi archwires (p <0.001) and from 5.79 (Z1) to 2.93 (S4) for patients with the thermal NiTi archwires (p <0.001).

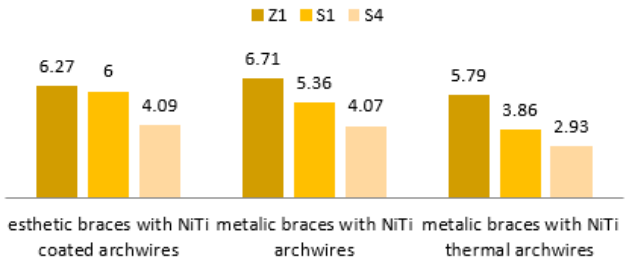


Figure 27 - Dental sensitivity assessment for 0.14 coated NiTi , NiTi si NiTi thermal archwires

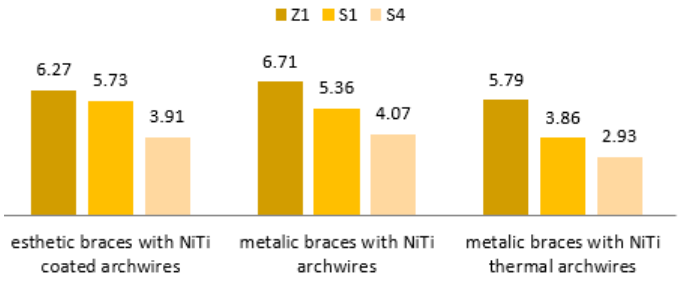


Figure 28 - Dental pain assessment for 0.14 coated NiTi , NiTi and NiTi thermal archwires

In the case of 0.14 NiTi and NiTi thermal archwires we can say that the sensation of tension, pressure, dental sensitivity and dental pain has a peak at Z1, then a slight decrease at S1, then at S4 the decrease is more importance. The NiTi thermal archwires have determined the lowest recordings due to the most likely lower and constant forces they exert on the teeth (**Table 5**).

Table 5 - Average perception of tension, pressure, dental sensitivity, dental pain depending on the type of niti 0.14 archwire

Perception	Braces with 0.14 NiTi archwires								
	NiTi coated archwires			NiTi archwires			NiTi thermal archwires		
	Z1	S1	S4	Z1	S1	S4	Z1	S1	S4
Average Tension± SD	6.31± 1.91	6.31± 1.91	5.27± 1.62	6.50± 1.40	6.14± 1.66	5.79± 1.67	5.79± 1.48	4.36± 1.87	3.36± 1.65
Average pressure±SD	6.36± 1.91	6.31± 1.91	5.27± 1.62	6.50± 1.40	6.14± 1.66	5.79± 1.67	5.79± 1.48	4.36± 1.87	3.36± 1.65
Average dental sensitivity ±SD	6.27± 1.95	6.00± 2.05	4.09± 1.92	6.77± 1.77	5.36± 2.06	4.07± 1.64	5.79± 1.67	3.86± 1.96	2.93± 1.59
Average dental pain ±SD	6.27± 1.95	5.73± 2.05	3.91± 2.02	6.77± 1.77	5.36± 2.06	4.07± 1.64	5.79± 1.67	3.86± 1.96	2.93± 1.59

Regarding patients responses following the application of 0.16 NiTi archwires, the following aspects were noticed. The average levels of tension sensation (**Figure 29**) at moments Z1, S1 did not differ significantly depending on the type of archwires applied (p> 0.05), while at week S4 the average level in patients with thermal NiTi archwires was significantly lower than the other types (p<0.05). For the tension sensation there were decreases in the average score from 6.45 (Z1) to 4.91 (S4) for patients with NiTi coated archwires (p <0.05); from 6.57 (Z1) to 5.21 (S4) for patients with NiTi archwires (p <0.05) and from 5.79 (Z1) to 3.36 (S4) for patients with thermal NiTi archwires p<0.001).

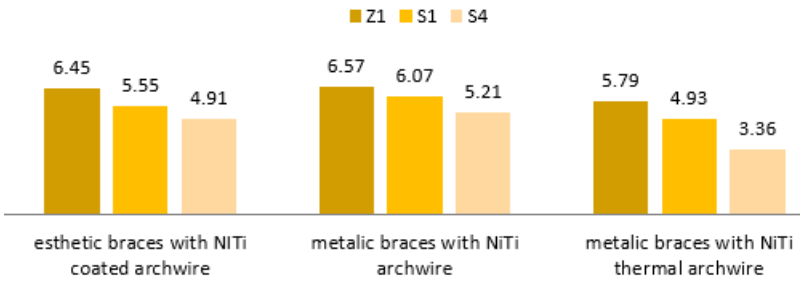


Figure 29 - Tension perception assessment for 0.16 NiTi coated, NiTi and NiTi thermal archwires

The average level of pressure sensation for the moments Z1, S1 did not differ significantly according to the type of archwires used (p> 0.05), while the average level in S4 week for the patients with thermal NiTi archwire was significantly lower than the other types (p <0.05).

For pressure sensation there was a decrease in average score from 6.45 (Z1) to 4.91 (S4) for patients with NiTi esthetic archwires (p <0.05); from 6.36 (Z1) to 4.43 (S4) for patients with NiTi archwires (p <0.001) and from 5.79 (Z1) to 3.21 (S4) for the patients with thermal archwires (p<0.001).

The average level of dental sensitivity (**Figure 30**) at moments Z1, S1 and S4 did not differ significantly depending on the type of archwires used (p> 0.05). Dental sensitivity decreased from an average score of 5.45 (Z1) to 3.18 (S4) for patients with esthetic archwires (p <0.05), from 5.57 (Z1) to 3.21 (S4) for patients with NiTi archwires (p <0.001) and from 5.36 (Z1) to 2.79 (S4) for patients with thermal NiTi archwires (p <0.001).

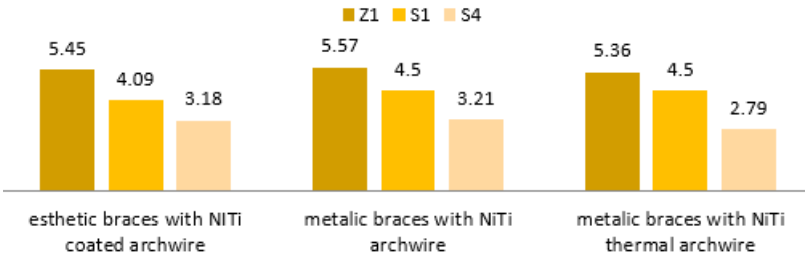


Figure 30 - Dental sensitivity assessment for 0.16 coated NiTi , NiTi and NiTi thermal archwires

The average levels of dental pain at Z1, S1 did not differ significantly depending on

the type of archwire applied (p> 0.05), while at week S4 the mean level in patients with thermal NiTi archwires was significant less than the other types (p <0.05). Dental pain has decreased from an average score of 5.45 (Z1) to 3.18 (S4) for patients with esthetic archwires (p <0.05); (p <0.001) and from 4.79 (Z1) to 2.07 (S4) for patients with thermal NiTi archwires (p < 0.001).

As can be seen in **table 6**, in the case of the thermal 0.16 NiTi archwires and NiTi archwires, we can say that the sensation of tension, pressure, dental sensitivity and dental pain has a peak at Z1, then a slight decrease at S1, then at S4 the decrease is more important. Both 0.14 and 0.16 NiTi Thermal archwires have produced the smallest record due to better biocompatibility in terms of spring force expression.

Table 6 - Average perception of tension, pressure, dental sensitivity, dental pain depending on the type of niti 0.16 archwire

Perception	Braces with 0.16 NiTi archwires								
	Esthetic archwires			NiTi archwires			NiTi thermal archwires		
	Z1	S1	S4	Z1	S1	S4	Z1	S1	S4
Average Tension± SD	6.45± 1.44	5.55± 1.44	4.91± 1.38	6.57± 1.28	6.07± 1.21	5.21± 1.05	5.79± 1.48	4.93± 1.44	3.36± 1.65
Average pressure±SD	6.45± 1.44	5.55± 1.44	4.91± 1.38	6.36± 1.15	5.36± 1.22	4.43± 1.02	5.79± 1.48	4.64± 1.50	3.21± 1.67
Average dental *sensitivity* ±SD	5.45± 1.57	4.09± 1.70	3.18± 1.33	5.57± 1.34	4.50± 1.02	3.21± 0.70	5.79± 1.87	4.50± 1.91	2.79± 1.58
Average dental pain ±SD	5.45± 1.57	4.09± 1.70	3.18± 1.33	5.57± 1.34	4.29± 0.91	3.14± 0.66	5.79± 1.85	4.00± 1.66	2.07± 1.64

2.3.3.2. Results of the study on local anesthesia in pain syndrome

During stomatological therapy or dentoalveolar surgical therapy, for the anxiety-ridden patients, with DASS-2, DASS-3, DASS-4, the negative emotion's main source lies with the anticipation of pain (28%), followed by fear of the specialist physician (22.3%), fear of anesthesia (20.6%), fear of surgical intervention (17.3%) and the sight of blood (11.6%). A patient, even a mentally balanced one, confident in his/her emotional reactions, may become anxiety-ridden when unexpectedly introduced in the dentist's office, surrounded by instruments, seringes, blood-soaked dressings, imprinting materials, high rotating equipment, enhancing and internalising his/her concerns.

The surgical intervention ranking at the top was: dental extraction -3241 cases (46.35%); apical resection -1720 cases (24.59%), odontectomy-621 cases (8.88%); periapical curretage -583 cases (8.33%); removal of small tumors -507 cases (7.25%); regularization of alveolar ridge -320 cases (4.57%).

Regardless of the accuracy of the stomatological technique and the skill of the medical practitioner, a faulty anesthesia indication – disregarding the specific conditions of each patient may result in complications carrying hidden risks.

The value of the proposed anesthesia methods was demonstrated by means of a cross-comparative analysis, whose assessement was based on the following indexes: the tolerance

score of intraoperative pain; onset of an unpredictable medical event (allergy, a cardiovascular one); onset of postoperative pain – the patients' statements.

Out of a total of 6992 patients examined and treated, we were interested in their distribution on the medical emergency variable. We observed the following percentage scores: 37% triggered an acute hypotonia failure (vasovagal syncopes, ortostasis syncopes, pregnancy syncopes, carotid sinus syndrome); 36% triggered cardio-circulator y emergencies (rhythm disturbances, HTA crises, angorpectoris crises); 12% triggered allergic accidents; 8% triggered neurologic emergencies; 7% triggered other types of emergencies.

The analysis of data on variable indication of anesthesia *vs* onset of the medical emergency, revealed the following preliminar y data: local anesthesia vs. emergencies – 94%; local anesthesia + topical anesthesia vs. emergencies – 5%; local anesthesia + pre- anesthesia medication vs. emergencies – 1%.

Three major factors may alter the indication of anesthesia with the immediate consequence of a medical emergency: dental anxiety score - holds the highest percentage; the appearance of a general state of health; incomplete anamnesis.

Local anesthesia is far from being a perfect anesthetic technique. If one forgoes the patient's general state of health and his mental state at the moment of surgery, the LRA cannot eliminate the risk of an unpredicted medical accident.

The alternative in indication of anesthesia is the combined method of local anesthesia and pre-anesthesia medication.

The indication of anesthesia + premedication (with a computerized average of 70%) is the method that proved to be most efficient in managing postoperative pain.

After correlating all the analysed indexes, we can conclude that the combined method of local anesthesia + pre-anesthesia medication is the closest to the ideal anesthesia technique in dental practice or in the oral surgery ambullatory care, managing to reduce to a minimum the risks associated with the performance of anesthesia.

2.3.4. Discussions

Contemporary dentistry offers a wide range of biomaterials and techniques and modern technologies, which lead to substantial improvements in the treatment plans, both from the point of view of biocompatibility as well as from the biomechanical one, adding new possibilities of rebuilding morphology and affected stomatognat system functions.

Aesthetic treatment is very important in the diagnosis and treatment plan. The aesthetic requirement must be understood and dosed in the context of a complete treatment solution with the importance of other factors: mechanical, functional, biological and psychic, all the subordinate concept of a complex oral rehabilitation.

For each and every case / situation, the physician must choose the best clinical and technological solution, while ensuring optimum results (Bergius et al., 2000).

In the literature, there are a series of studies on pain assessment during orthodontic treatment. Although all studies agree that pain occurs during orthodontic treatment, there are large variations between reported prevalence rates, intensity and duration of this type of pain (Jones 1984; Krishnan 2007; Scott et al., 2008; Krukemeyer et al., 2009; Tecco et al., 2009).

It has been observed that pain and discomfort last longer than 4 weeks after the onset of fixed orthodontic treatment, which has led us to follow the perception of pain from the point of view of the patient when introducing two archwire sizes into the initial stages of treatment.

Factors influencing the pain sensitivity may be physiological or psychological. The physiological factors are represented by: age, sex, circadian rhythm, blood pressure variation, menstrual cycle, intensity of applied force, individual pain threshold. Psychological factors refer to anxiety, as its core or environment-induced, past or previous experiences (Scott et al., 2008).

As pain is generally a subjective symptom, it is also extremely difficult to quantify (Xiaoting et al., 2010; Rakhshan 2015). In the scale used by us the rate 0 indicates absence of pain, 1-3 is considered mild pain, 4-6 moderate, severe (Mccaffrey and Pasero 1999).

The fear of pain is most often triggered by influence exercised even before entering the dentist's practice; the impact of the waiting room in a dentist's practice; the overall outlook of the waiting room, the agitation of the medical staff, noises, the specific odour.

Anxiety, fear of a dental treatment that may cause pain are frequently indicated factors in DASS. The reasons for this fear are plenty, starting with previous traumatising medical-surgical experiences of any type, therapeutic stomatological experiences and, last but not least, the mental-affective load particular for each patient.

The importance of identifying the source factors for the negative emotion is of high practical interest, since this state of anxiety is characterised by an extremely powerful vasovagal response, sometimes with dramatic consequences for the patient.

The general state of health, reflected in the waist and the weight, allowes for an objective study, unlike general notions such as obesity and weight loss, since both are deemed as organic defects.

Complementary examinations are guided based on the data obtained from the anamnesis, the careful examination of the oral cavity, so as to control and remove potential mobile dental prostheses, decemented prostheses, easily detachable tooth fillings, macroglossia, possible causes of serious accidents, such as mechanical obstruction of airways.

If the medical background and the general clinical examination highlighted organic and functional defects or deficiencies, paraclinical laboratory examination will always be requested.

We suggested the improvement of local anesthesia methods through: substitution: technique – intraosseouss anesthesia or the anesthetic agent; combined: topical anesthesia or pre-anesthesia medication.

The value of the proposed anesthesia methods was demonstrated by a cross-comparative analysis, whose assessement was based on the following indexes: tolerance score of intraoperative pain; onset of an unpredictable medical event (allergy, a cardiovascular one); onset of postoperative pain –the patients' statements.

In order to assess the manner in which the proposed anesthetic option manages to modulate the patient's reception of the maneuvers considered to be painful a system was put in place to assess the degree of pain during surgery.

In addition to alveolar pain caused by dental movements, and mucosal lesions on the internal face of the cheeks, on the edges of the tongue can cause pain. Most studies conclude that the maximum moment of pain may occur within the first 24 h of application of the appliance (Ngan, et al., 1989; Jones et al., 1992; Erdinc and Dincer 2004) but may persist even after removal of the appliance (Miyawaki et al., 1999), there are some authors who did not consider significant the pain of low intensity. Changing eating habits in favor of softer foods is recommended to alleviate pain in the idea that many patients are accusing pain of hard or fibrous foods.

As a mechanism of production, the orthodontic pain is caused primarily by the compression made by the orthodontic forces on the periodontal ligament. The response occurs immediately after archwire placing and is characterized by ischemia and compression of the periodontal ligament. Age and periodontal status have influence in orthodontic response and also in pain. After the application of mechanical forces, the cells of the periodontal ligament produce some quantities of mediators that they are diffused into the gingival crevicular fluid and afterwards into the saliva. Dental movement is effected through processes of bone resorption and apposition that occur subsequent to the existence of an inflammatory process localised at this level. Delayed responses, especially hyperalgesia, begin some hours later. During this response, released prostaglandins may increase the sensitivity of pain receptors to harmful agents such as bradykinin, acetylcholine, substance P and histamine. This phase continues with neurogenic inflammation, osteoblastic and osteoclastic activity, periodontal vasodilatation and pain. It is now widely accepted that lighter forces are less traumatic and painful and are considered to be ideal for orthodontic treatment (Scott et al., 2008; Krukemeyer et al., 2009).

Nowadays the orthodontic therapy is spreading both among young patients and aged patients due to increased esthetic and functional needs. Effective communication between the clinician and patient, warning the patient about the occurrence and existence of pain during orthodontic treatment and attention to the psychological well-being of the patient can improve pain tolerance and reduce pain perception (Scheurer et al., 1996).

For pain relief, non-steroidal anti-inflammatory and analgesic drugs may be used, but should not be exaggerated with administration because they would disrupt the dental movement due to prostaglandin antagonism.

Eating habits have an influence on the onset of dental pain during orthodontic treatment, so the fibrous or harsh foods can aggravate the pain, while a softer, more protective diet will significantly reduce this unpleasant aspect. This finding was consistent with the results of other authors and suggests that patients should change their diet to provide comfort. It is generally recommended that harder foods be cut into pieces for easier chewing, however, patients tend to underestimate or ignore advice on dietary changes (Scheurer et al., 1996).

Owing to the multiple advantages presented, local anesthesia is considered a common method in routine stomatological care.

The fundamental principles and the clinical data support the anesthesia techniques, indicating the fact that it can successfully replace the sensation of pain arising during the surgical intervention, depending on the various clinical situations, so as to ensure the optimal comfort of the patient during surgery.

In the past years new intraosseouss anesthesia techniques were developed and launched on the market, and progress was made on the injection method of anesthetics, with the introduction on the market of electronic computer systems for the administration of anesthetics. Some of these are: the Confort Control Syringe sysetm from Dentsply, The Wand system, Sleeper One system from Dental HITEC. Some of these systems are adjusted for the administration of intraosseouss anesthesia as well.

The locally applied anesthetic is absorbed and carried into the blood flux. This is important, since the incorrect use of anesthetics may induce toxicity, by a single application or by association with an injection-conducted anesthesia.

The quantity of anesthesia is important when discussing topical reactions. The concentration of the anesthetic in topical medicine is higher than those in the injectable versions. Topical administration reaches high levels in comparison with slow intravenous injection. Although the plasmatic level is of 1/3 of the one obtained by intravenous administration, the speed with which it enters the blood flux is faster. What is certain is the fact that toxic reactions caused, even in the case of topical application, death.

The use of a topical anesthetic before the anesthetic puncture is something common in stomatology. Lidocaine and benzocaine are the most used topical anesthetics.

Lidocaine, aslo known commercially as xylocaine, is a local amide anesthetic, derivative of cocaine but without the euphoric or addiction effect of cocaine.

Benzocaine is a local anesthetic used for pain relief at mucosa level.

Control of postoperative pain, edema, and trismus has been the subject of continuous research in the field of oral and maxillofacial surgery, since pain can significantly reduce the quality of life of the patient (Neychev et al., 2017). Researchers found that the main local anesthetics are lidocaine (2%), mepivacaine (2%), prilocaine (3%), and articaine (4%). Since these anesthetics have different onsets of action and potency, this finding is clinically relevant for evaluating the efficacy and duration of analgesia (Filho et al., 2020).

Lidocaine is widely used as a local anesthetic after minor or major surgeries. It produces an analgesic effect that is greater than that of other locally administered analgesic drugs (Song et al., 2017). Mepivacaine shows a high affinity for the protein components of nerves and is, therefore, less liable to diffuse from the injection site and be absorbed into the systemic circulation. According to Gazal, prilocaine shows better clinical performance in terms of providing rapid dental anesthesia, allowing earlier surgical removal of teeth, than lidocaine but the differences were not significant, generating similar clinical responses among the patients evaluated in their study (Gazal et al., 2019; Filho et al., 2020).

The principle on which intraosseouss anesthesia systems are based is common and consists of the insertion of the anesthetic solution in the periradicular cancellous bone of the treated tooth, after a previous perforation of the cortical bone; the following advantages of the intraosseouss anesthesia are confirmed: immediate anesthesia of a number of teeth (2-6) from the maxilla and the mandible via a single anesthetic puncture, without an additional palatal or tongue injection; eliminates the numbness sensation in soft parts (tongue, cheeks, lips); the use of vasoconstrictors without the risk of necrosis.

The technical limitation lies exclusively in the inability to penetrate the cortical bone, due to its density or rigidity. In these rare cases (aprox. 4% of cases), another local anesthetic technique is recommended.

Discomfort and pain caused in the initial stage of fixed orthodontic treatment may be moderate to severe and may last for a long time. Tooth brushing can also cause discomfort and eating soft foods can minimize pain. Effective communication between the clinician and patient, warning the patient about the occurrence and existence of pain during orthodontic treatment and attention to a good quality of life of the patient can improve pain tolerance and reduce the pain perception during orthodontic treatment. Initially recorded pain decreases in intensity during the advancement of treatment, the use of NiTi thermal archwires has a positive effect, being associated with a lower intensity of pain during orthodontic treatment.

2.3.5. Conclusions

During stomatological or dentoalveolar surgery, the onset of a medical emergency is influenced by the indication of anesthesia. The potential of the local anesthesia represents the most physiological anesthesia method and has the widest indications in stomatology. Anesthetic substances, rather numerous at the moment, have common properties, distinguished only by the swiftness of onset, the power of action, the duration, secondary reactions and the maximal posology of each and every one of them. The purpose of premedication must unconditionally be determined by the anxiolytic and analgesic effect on the improvement of tolerance to pain, and the administered doses must be correlated with the DASS anxiety score, the manner in which it is used, the probable duration of the intervention. The thoroughness lying at the core of the anesthetic practice, most especially the safe gurading of a technical accuracy in the performance of anesthesia, instead of improvisations, the lack of anatomical and stomatological training in general and the resulting inefficiency as such, is the underlying in-depth structuring element of this paper. Topical local anaesthetics are effective for temporary pain relief in dental practice. The dentist must choose wisely the best anesthetic, recommending caution in its administration, the restricted posology, personalized for each patient.

2.4. Dysfunctional syndrome – impact in oral health

2.4.1. Introduction

The dysfunctional syndrome represents a dyshomeostasis, an imbalance of the mechanisms for the adjustment of the functions of the morpho-functional complex represented by the stomatognathic system (Belotte-Laupie et al., 2011). The imbalance may be triggered by a series of factors in some cases, and in others it may be the result of a combination of factors. Due to the complexity of the etiopathogenic action mechanisms, from the triggers to the clinical signs there is a less precise determination, a determination that most of the times may only be suspected. Moreover, the commencement of the disease is usually concealed by complex compensatory phenomena, it may be registered on only one element of the system or on multiple elements and the gravity of the affection of the system in its entirety may not be correlated with the number of elements aimed at by the disease. Passing from normal to pathological is achieved gradually, going through successive stages

the susceptibility dyshomeostasis, then the preclinical, the onset manifested and decompensating, the early diagnostic being difficult and imprecise when one counts only on the clinical instinct (Wang et al., 2013).

Unnoticed, orthodontists may find themselves treating patients suffering from craniomandibular disorders - known as dysfunctional sydrome, who may be adults, but not necessarily. Both young children and adolescents can also have these problems. Orthodontists often fall into the trap of not recognizing these disorders especially when the clinical signs are discreet or even non-existent (Dupas et al., 2008). In order to save orthodontists from encountering unpleasant post-treatment surprises, we present in this paper a review of some of the indications of how to explore the etiology of craniomandibular dysfunction, how to diagnose it, and the importance of its major clinical signs. These could be a great help to them in achieving a keener state of vigilance in this regard throughout orthodontic therapy (Carlsson, 1984).

To assist the dental practitioners in their daily routine to meet the modern standards of best practice, guidelines are explained and accompanied with clinical trials for an evidence-based treatment of patients diagnosed with these disorders (Fricton and Schiffman, 1986).

Considering the multifactorial character, De Boever described the well-known multi-factorial etiological approach for craniomandibular. He distinguished five theories: the mechanical displacement theory, the neuromuscular theory, the psychophysiologic theory, the muscle theory, and the psychological theory. De Boever stated that none of the theories as such give an adequate explanation of the cause and the symptoms of CMD. He concluded that the etiology of functional disturbances is multifactorial and is a combination of dental, psychological, and muscular factors (DeBoever, 1979).

The stomatognathic system represents an extremely complex system and involves the temporomandibular joint, the teeth and the muscles of mastication, and is influenced and controlled via the neuromuscular regulatory circuit. Disorders in one part of the system will frequently have an effect on one or more of the muscles involved in the movements of mastication. A pathological change in the area of the mandible signifies a changed situation for the muscles of mastication, which can, as a result, be subject to painful adaptation processes. Conversely, however, muscle pathology can also lead, for example, to arthropathy of the mandibular joints as a result of a parafunction such as bruxism (teeth grinding). The patient's bite can thus have a causative function and can also experience a change caused by the altered interplay between the joints and the muscles, which is displayed as an occlusion disorder from a dental point of view (Helkimo, 1974).

It is therefore important for the therapist to get an overview of the condition of the whole stomatognathic system in order to provide therapy if necessary for the causative factors, as well as to treat the muscular components, if necessary in cooperation with other disciplines (Jenni and Schürch, 1987).

Changes in the muscles caused by parafunction are predominantly found in the area of the large muscles which close the jaw, as described: the *temporalis muscle* is mostly affected in the anterior and medial segment, while the M. masseter is affected over all parts of the muscle from the origin to the insertion. These muscles are most easily accessible to extraoral palpation and represent the first indicator. The *pterygoideus medialis muscle* is found inside the mouth and forms a loop of muscle with the masseter muscle. Other frequently affected

muscles are the *pterygoideus lateralis*, which is active in laterotrusion and the posterior and anterior parts of the M. *digastricus* and the *mylohyoideus muscle* which belong to the muscles in the floor of the mouth and are accessory muscles of mastication (Irnich, 2013).

The aim of this study was to evaluate the frequence of the dysfunctional syndrome in a heteromorphic group of population represented by the total number of the patients that have come to the Fixed Dental Prosthesis Clinic, of "Grigore T. Popa" University Of Medicine And Pharmacy Iași Clinic, for a period of 5 years (2010-2015).

2.4.2. Materials and methods

The study group included a number of 6507 patients admitted for treatment in Fixed Dental Prosthesis Clinic, of "Grigore T. Popa" University Of Medicine And Pharmacy Iași, between 2010 and 2015. We evaluated mandibular dynamic through Electromyography (EMG) and kinemyography (KMG) tests. The sample and the inclusion and exclusion criteria were established by means of anamneses, clinical exams, and presence signs and symptoms of temporomandibular disorders (TMD) through specific questionnaire (Research Diagnostic Criteria), which provided data regarding the participants' personal information, medical and dental history, any existing parafunctional habits or signs and symptoms of temporomandibular dysfunction.

EMG is a diagnostic procedure that evaluates the health condition of muscles and the nerve cells that control them. These nerve cells are known as motor neurons. They transmit electrical signals that cause muscles to contract and relax. An EMG translates these signals into graphs or numbers, helping doctors to make a diagnosis.

The response of the muscles to electrical stimulation of the nerves can be recorded subjectively (qualitative) or objectively (quantitatively). Quantitative techniques include kinemyography which uses a piezoelectric sensor as a device that uses the piezoelectric effect to measure changes in pressure, acceleration, temperature, strain, or force by converting them to an electrical charge.

In the next stage, we evaluated a lot of 48 young clinically healthy subjects. They agreed to participate in this study after being contacted via telephone. These patients were clinically and paraclinically examined, following the routine of the gnathologically-characteristic examinations. They were EMG and KMG examined with the machines owned by the clinic. Therefore, the masseters and the anterior arc of the bilateral temples were electromyographically examined on the surface at rest, with an isotonic and isometric contraction. The kineses mandibular graphic examination was performed via the analysis of the mandibular trajectories of the dynamic in the testing position and the trajectory towards the testing position, in maximum intercuspation, centric relationship, registration of the movement speed of the mandible and of the frontal lateral deviation.

During the clinical and paraclinical investigations, sick persons with signs of the syndrome were identified. All affected patients have received additional investigations, model study and occlusal analysis or ATM tomographies. Minor gnathological therapies, depending on the case and which would solve the presented dysfunctional problems, were subsequently set up.

2.4.3. Results

From the statistical processing of 6507 consultation sheets, 13,75 % presented the dysfunctional syndrome, out of these, the gender spread certifies that the affection of the female gender is the highest (54,6%) than the male gender (45,4%) (**Figure 31**).

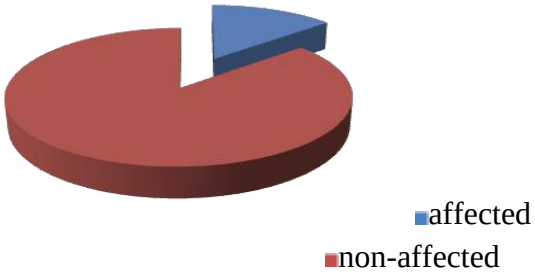


Figure 31 - Patients affectation by the syndrome

The highest incidence of the affection through the dysfunctional syndrome was the proportion of affectation by types of disorders (**figure 32)**. It appears that the most frequent are the occlusal disorders revealed in the youngers, between 25 and 35 years old, as well as the older persons and in descending order, the articular ones, followed by the muscular ones and finally, the periodontal ones (**figure 33**).

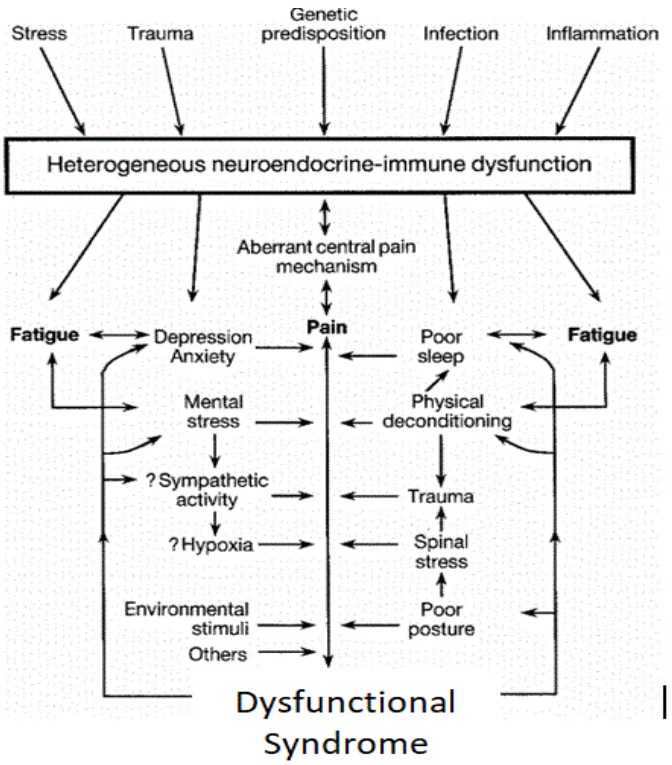


Figure 33 - A schematic representation of the biophysiological mechanisms in Dysfunctional Syndrome; the primary abnormality is in the 'box' (heterogeneous neuroendocrineimmune dysfunction). Various factors, e.g. genetic predisposition, mental stress, trauma, infection and inflammation, may cause such dysfunction. Other possible interacting factors which may amplify the pain syndrome of fibromyalgia are also shown.

Among the mandibular-cranial malrelations, the most frequent are the complex eccentric extra-postural malrelations. All the values are overall higher for the feminine gender.

The lot consisting in the young, healthy patients had the ages between 21 and 27 years, 44% being males with an average age of 25.3 and 56% were females with an average of 23.5. The percentage study of the results of the KMG analysis has revealed a series of important data.

Out of the total number of subjects examined, 81% presented evident changes of mandibular dynamic, changes most often registered being: different routes on the opening-closing trajectory accompanied by an articular skip, accident or frontal lateral deviation, asymmetrical laterality movements as range and angulation, accidents and deviations in propulsion with ascents of the anterior guide heavily modified in terms of orientation and range, discreet dysfunctional deglutition, modified masticatory cycle.

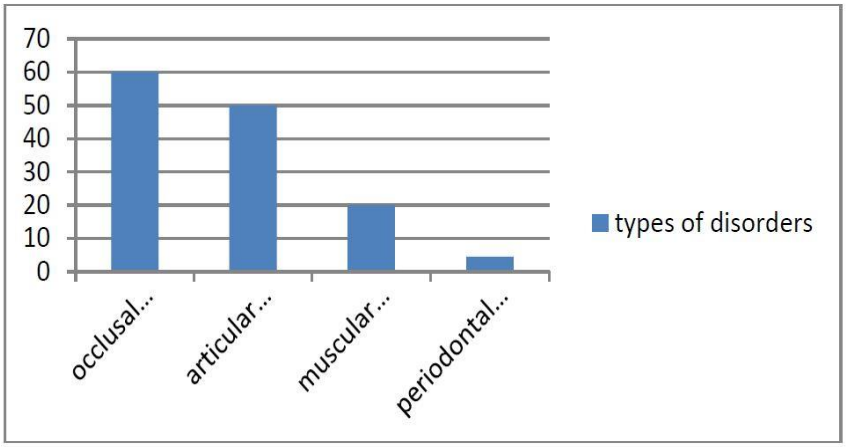


Figure 32 - Types of disorders found

The large majority of the subjects with changes of mandibular dynamic (56%) did not confirm the existence of a clinical symptomatology which falls within the dysfunctional syndrome. Most frequently, we have found: the change of the opening and closing routes with a predisposition for articular skip and laterality in the final stage of the opening, less frequently, we have also found the change of the movement trajectories for right and left laterality maintained by changes of the posterior determinant of the occlusion.

Out the total number of subjects with changes of the dynamic, only 9 of them presented dental- maxillary anomalies materialised through isolated dental malpositions on the level of the frontal dental group and which modified the movement parameters of the anterior guide, but were not accompanied by an alarming clinical symptomatology. To be noted is one case of deep occlusion with tight ower-jet, which presented normal routes of dynamic. At a rate of 25%, the following were also present in addition to evident changes of mandibular dynamic and a characteristic clinical symptomatology: muscle fatigue, condylar subluxation, articular pains, articular noises (**Table 7**).

Table 7 - Clinical features of the patients

Clinical features	Frequency	Percentage %
Pain		
No	1	4.8
Yes	20	95.2
Total	21	100
Habits		
Clenching	1	4.8
Grinding teeth	3	14.3
Gum chewing	3	14.3
Jaw movement	1	4.8
Nil	13	61.9
Total	21	100
Symptoms by Patients		
Clicking	7	35
Headaches/ Clicking	2	10
Headaches/ Locked Jaw	1	5
Pain on opening	10	50
Total	20	100

It is important to point that none of the subjects analysed came for the treatment for the DS, actually ignoring the presented disorders. This subgroup of volunteers has received medication, physiotherapeutic treatment, selective polishing or occlusal interception. The symptomatology has spectacularly ceded in a few days (maximum 8 days).

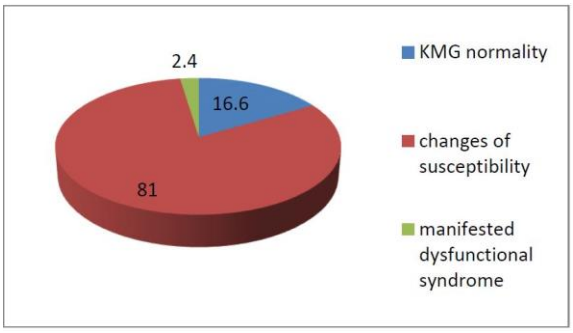


Figure 34 - KMG results

Out of the total number of subjects examined 16,6% presented KMG normality. The prevalence of the changes of mandibular dynamic in the subjects considered to be healthy is high. Thus, 81% presented changes of susceptibility, 16,6% fell within normality and the rest

of them formed a sublot with manifested dysfunctional syndrome and this was valid for a young age of 21-27 years (**Figure 34**).

This highly increased frequency of affectation, certainly minor but existing, given the major disorders it may generate in time, must be a wake-up call for any occlusion-aware doctor. In the initial stage of affectation of the system, when the susceptibility diagnostic is prevalent, extremely simple corrective measures are necessary, easily practicable in mass therapy.

One must not forget the fact that their apparent simplicity efficiently ensures a later balance necessary for normal functionality.

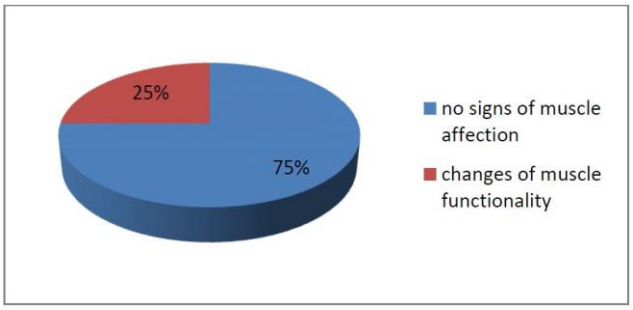


Figure 35 - EMG analysis results

The EMG examination practised for the entire lot of youngsters has materialised the presence of electromyographical changes, traceable on the examined temple muscles and the masseters. For the achievement of the objective of the paper, focused on the investigation of the neuromuscular activity, a global EMG detection was practised, using surface electrodes, in isometric and isotonic contraction.

The results of the EMG analysis certify the lack of signs of muscle affectation traceable at 75% of the cases examined and the presence of changes of the muscle functionality traceable in 25% of the cases (**Figure 35**), changes most frequently materialised through the presence of action biopotentials at rest or EMG with changes in the aspect characteristic for the oboist muscle, right-left contraction asymmetries.

2.4.4. Discussions

The dysfunctional syndrome referes to the occlusal disorders, the articular ones, followed by the muscular ones and finally, the periodontal ones. Temporomandibular disorders (TMD) are the second most common cause (after dental pain) of this syndrome, characterised by pain in the temporomandibular joint area and in the facial muscles. Apart from pain, patients may experience other signs and symptoms, such as clicking of the joint and restricted mouth-opening. Around 5% to 12% of the population have TMD symptoms to some degree, varying by age group and gender.1 One of the most common ways in which dentists, particularly in primary care, manage symptomatic TMD is the provision of oral splints (Aggarwal et al., 2012; Riley et al., 2020).

Splints are also provided to help manage tooth wear caused by bruxism. The prevalence of bruxism ranges from 8% to 31% within the general population,3 and it is estimated globally that sleep bruxism affects 16%, and awake bruxism 24%, of the adult population (Melo et al., 2019).

There is continuing debate about the exact mechanism of action of oral splints. Mechanisms include: muscle relaxation/habit-breaking for patients with increased parafunctional or muscle-tightening habits; protection of teeth and jaws, particularly where teeth clenching and grinding may lead to damage of teeth; normalising periodontal ligament proprioception, by utilising a splint to spread the forces placed on individual teeth; and repositioning of the jaws and condyles into centric relation (Schiffman et al., 2014).

The evidence level for prescribing self- exercises and occlusal splints in the treatment of myalgia is low. Despite the fact that self- exercises and hard acrylic splints presents little or no risk to the patient, both prescriber and patient should be aware that the treatment itself is not supported by satisfactory scientific evidence (Edward et al., 2009).

Thanks to advances in neuroscience, biopsychosocial models for diagnostics and treatment (including physical, psychological, and pharmacological therapies) currently have more clinical support and scientific growth. At present, a conservative treatment approach prevails over surgery, given it is less aggressive and usually results in satisfactory clinical outcomes in mild–moderate temporomandibular disorder (TMD) (Gil-Martínez et al., 2018).

For an extremely heteromorphic group of population, with a majority of 67,13% of cases with ages over 35 years, the most frequent disorders were the occlusal disorders and the mandibular-cranial malrelations.

The question that we have asked ourselves is the one regarding the proportion of the susceptibility affection. Is it a clinical reality and to what extent can it be shown through specific paraclinical examinations? In order to answer to this question, we have practised gnathological routine investigations on a group of healthy youngsters. The lot thus formed, with ages between 21 and 27 years, has proven the presence of the susceptibility syndrome. The high frequency of affectation by the syndrome through the changes of mandibular dynamic represents the entry point in the dysfunctional syndrome.

We believe that it is absolutely necessary from a young age to aim at the early diagnostic of the dysfunctional syndrome, through the accurate analysis of this complex symptomatology. One may prevent the settlement of the syndrome manifested through simple therapeutic measures, available to any occlusion-aware doctor.

Ever since Goodfriend described functional disturbances of the stomatognathic system in the Dental Cosmos of 1932, several terms were used to describe deviations from the optimal and healthy normal status of the stomatognathic system. Currently, the term “temporomandibular disorders” is generally accepted and most frequently used to represent disturbances and dysfunction of the stomatognathic system (Goodfriend, 1932).

Moreover, in 2014, this term became the “golden standard” for the disorder’s diagnostic criteria and taxonomy through a series of workshops and symposia, a panel of clinical and basic science pain experts, reaching a consensus to differentiate them into 5 pain-related temporomandibular disorders: 3 disorders of muscular origin, 1of joint origin, 1 headache-provocated disorder, and 5 intra-articular temporomandibular disorders (Schiffman et al., 2014).

Considering the topic "occlusion" in the dental literature, this term is used for 4 different entities: the anatomic or" orthodontic" jaw relation: the Angle classification, static contact between the teeth of the upper and lower jaws, dynamic contact between the teeth of the upper and lower jaws, for example, cuspid guidance versus group function, articulation, and occlusal interferences, and the prosthetic classifications, the complete/incomplete dentition versus complete dentitions and the presence of fixed/removable prosthetics. It can be concluded that due to the phenomenon of the multiple catch-all or container concepts of both "occlusion" and "temporomandibular dysorders" there are many different options to research (Aggarwal et al., 2011; Manfredini et al., 2011).

In addition, considering the etiology, the cause and effect relation, and vice versa, there are almost inexhaustible possibilities. In summary, research dealing with other topics than exclusively "occlusion" has a consequence of the generally accepted multifactorial and multi-causal character of temporomandibular dysorders, as well as our study does (Donovan et al., 2016).

2.4.5. Conclusions

Our study highlights the importance of the multimodal and multidisciplinary approach in this type of patients to improve clinical outcomes. At present we have innumerable techniques and tools to approach this type of patients from a biopsychosocial model where active and adaptive type treatments are fundamental.

There are various health professions that have competence in the treatment of this condition, however, although in the most complex cases should be treated simultaneously, still too many patients receive unique treatments and only from one point of view.

Temporomandibular dysorders are considered to be the most common orofacial pain conditions of nondental origin, but the frequent concurrent presence of other symptoms, such as earache, headache, neuralgia, and tooth pain, which may be related to the TMD or be present as ancillary findings to be assessed in the differential diagnosis process, makes the assessment of this condition prevalence a complex issue.

2.5. Advantages and limits in dental gypsum model usage

2.5.1. Introduction

The dental disorders are now about to become a public health problem due to its special features: wide spread in the population, with increasing general trend, aesthetic disturbances that may lead to difficulties in social integration of individuals, a complex etiopathogeny, disturbances in the general state of the organism.

To establish a proper treatment is primary required to know the incidence of the different types of dental disorders, the quantitative dimension of the phenomenon, but also the qualitative aspect expressed in the gravity index of the malocclusion.

The need for treatment is correlated with the development of the dentition. In Finland it is found that at the age of 7, 23% of children have a malocclusion requiring immediate

treatment, and 34% require repeated controls to observe the evolution of anomalies (Kaisa 1989). In Iceland, there is a prevalence of dentomaxillary anomalies of 11% in temporary dentition and 52% in permanent dentition (Magnusson et al., 1977).

Establishing an orthodontic diagnosis and treatment strategy involves knowing the characteristics of a dentomaxillary disorder and also the identifying and quantifying changes in the dental and muscular skeleton. The dental arch is defined by size and shape. The interest in knowing this sector of the stomatognathic system is determined by: the relations established between the dental arcade and the cranio-facial structures, the fact that the dental arcade often reacts, compensating for the disequilibrium at the skeletal level, and, importantly, that the dental intra-arch harmony has consequences on dental occlusion (Kanashiro and Vigorito 2000).

Researchers focused on the relationships between the cranio-facial structures and the size of the dental arch among the subjects with malocclusions, finding that the maxillary dental arcade in class II/1 malocclusion is narrower in the dolicocephalus and wider at brahicephalus, while the size and shape of the mandible arch is similar to all three facial types (mesocephalic, brahicephalic, dolicocephalic) (Brezniak et al., 2002). Other authors followed the characteristics of the dental arch by comparison between class II/l and class II/2 malocclusion, in subjects who did not perform orthodontic treatments (Buschang et al., 1994).

While some researchers find intercanines distances in the maxillar and mandible higher than in the class II/2 witness group and lower in class II/1, other researchers (Wallow et al., 2002) find in their studies a lesser intercanine distance compared to the average. Other researches refer to the characteristics of the dental arch in class II malocclusion as compared to the dental arch of children without abnormalities, revealing almost insignificant differences (Isik et al, 2006). In contrast, Staley finds larger intermolar and canine distances in children normally developed than those with Angle second class (Staley et al., 1985).

The large diversity of clinical forms in Angle class II malocclusion explains the interest of researchers and clinicians in identifying changes in the dental arch in subdivisions II/1 and II / 2 as well as the differences that may exist between them.

The aim of the study was to identify the characteristics of the dento-alveolar arch in order to determine the differences between class II/l, II/2 malocclusions for evaluation the changes on both dental and alveolar level.

2.5.2. **Materials and methods**

The study was conducted on gypsum dento-alveolar models of 62 orthodontic untreated patients diagnosed with class II/1 Angle malocclusion, respectively class II/2 Angle, 40 girls (64.5%) and 22 boys (35.5%).

Regarding the frequency, according to the two subdivisions of the 2nd Angle class, the distribution was: 35 subjects with class II / 1 Angle (56.5%) and 27 subjects with class II / 2 Angle, (43.5 %) with an average age of 10.76 class II / 1 and 10.167 class II/2.

The dental-alveolar arcades were made by the same doctor, and the molding and processing of the dental model by the same dental technician.

The measurements were made by two independent examiners, the differences being identified by a third examiner who also determined the average error.

The ideal values for the width and length parameters of the dental arch were calculated and the differences between measured and calculated values were made.

The database was computerized. Statistical processing was done using SPSS 16.0 programm (Statistical Package for Social Sciences).

We used descriptive statistical analysis methods for presenting the two clinical forms, including analysis of the central trend of distribution and variant or dispersion indicators.

In relation to the descriptive statistical analysis of the obtained results, we have previously verified the nature of the distribution of the values of the tested parameters. If the values of the tested parameters followed the normal law, we used the "t" test to analyze the differences between the two subdivisions, and when the measured parameter values did not follow the normal law, we used the non-parametric Mann-Whitney test.

2.5.3. Results

2.6.3.1. Characteristics of the maxillary dental alveolar arch

2.6.3.1.1. Evaluation of the width of the maxillary arch:

- **at the premolar level** - shows an global average of 34.5 with a standard deviation of 2.5429; in the class II/1 subdivision the average value was 34.1034 and 35.1765 in the II/2 Angle subdivision, with a standard deviation of 2.7947 and 1.93602 (**Figure 36**). The difference from the required value (**Figure 37**) showed an average over the whole group of -4.5183 (Figure 4), with a standard deviation of 3.4998; in class II/l, the average difference was -5.1886 and -3.3747 in class II/2 with standard deviations of 3.5269 and 3.2369 (**Figure 38**). In conclusion, the maxillary arcade is narrowed at the premolar level more in II/l than in II/2, but insignificantly statistically.
- **at the molar level.** The global average of this parameter was 45.9828mm (**Figure 39**), with a standard deviation of 2.8376; in class II/1, it was 45.3235mm and 46.9167mm in class II/2, with a standard deviation of 2.5904 and 2.9623 (**Figure 40**).

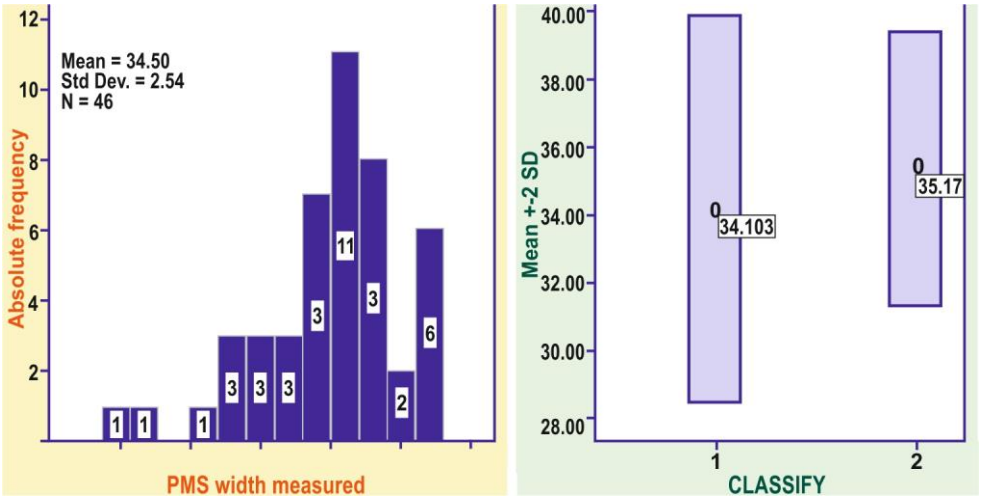


Figure 36 - Width of the maxillary arch at the premolar level

The difference from the value of dento-alveolar equilibrium (**Figure 40**) is -2,6988 global, with a standard deviation of 3,9889 (**Figure 42**); a value that ensures balance in subdivisions II/1 and II/2 is 48,66 and 48,397 respectively, resulting in a difference of -3,3 in the class II/1 and 1,8471 respectively in class II/2, with standard deviations of 3.9541 and 3.9763.

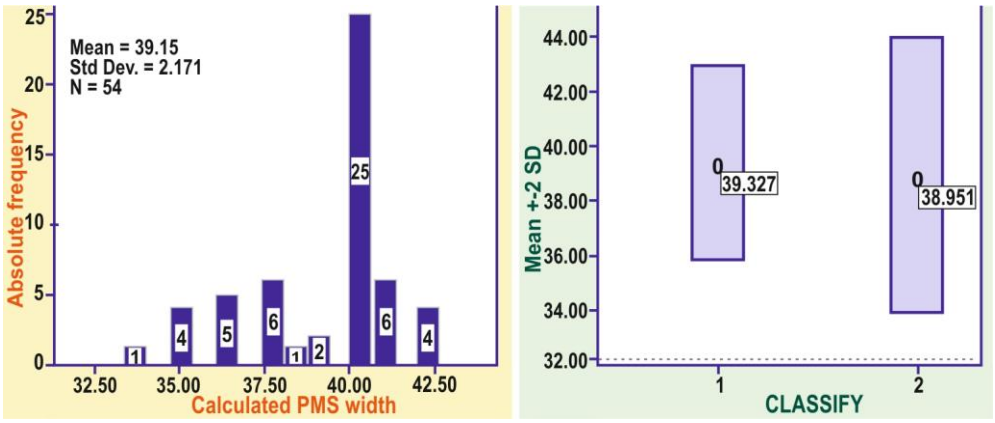


Figure 37 - Required value of dental alveolar arch

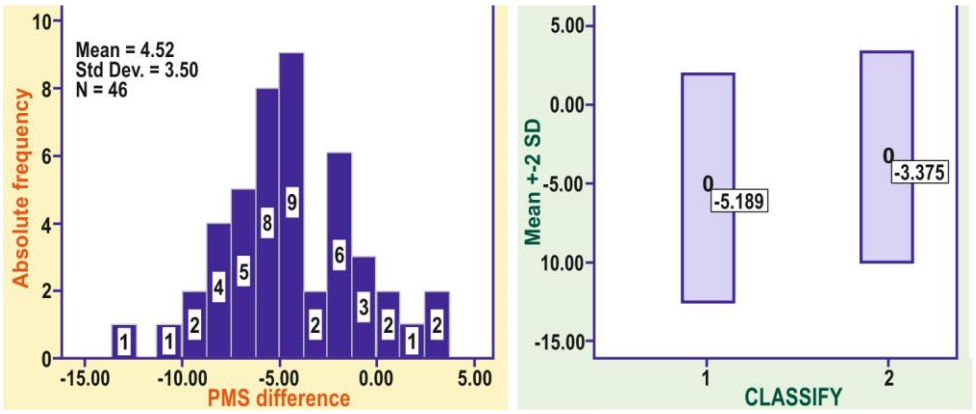


Figure 38 - The difference from the required value of dental alveolar arch

2.6.3.1.2 Evaluation of the <u>length</u> of the maxillary arch:

- **at the premolar level.** The global average of the arcade length was 18.9674mm, with the standard deviation of 2.2494 (**Figure 41**); in class II/l the average was 19.6552 and 17.7941 in class II/2, with standard deviations of 2.0402 and 2.1510 respectively (**Figure 41**). The difference that ensures the equilibrium of the arcade in the premolar area at the global level is 19.75, and the values that ensure the equilibrium in subdivisions II/1, II/2 are 19.64 and 19.86, respectively.

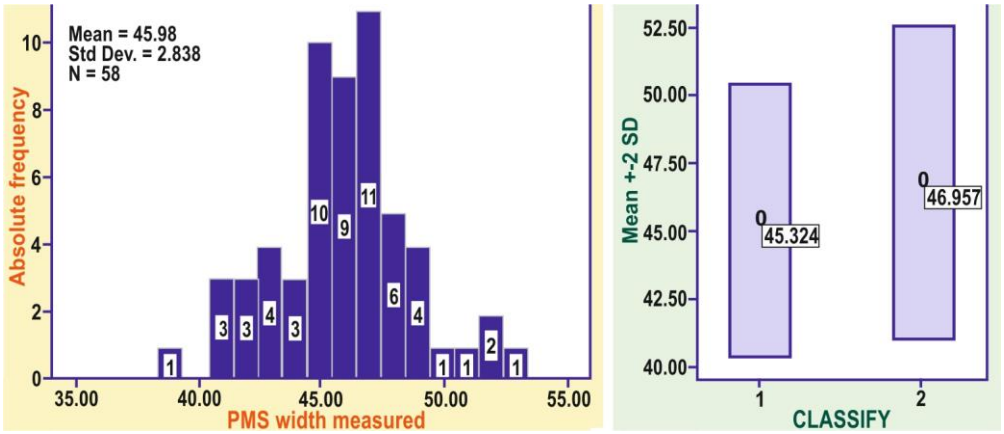


Figure 39 - The arcade width at the molar level

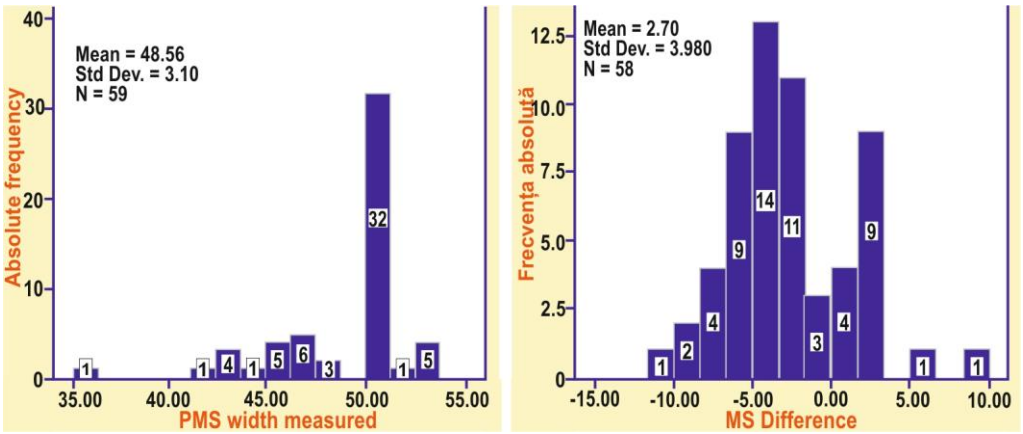


Figure 40 - The difference from the required value at the molar level

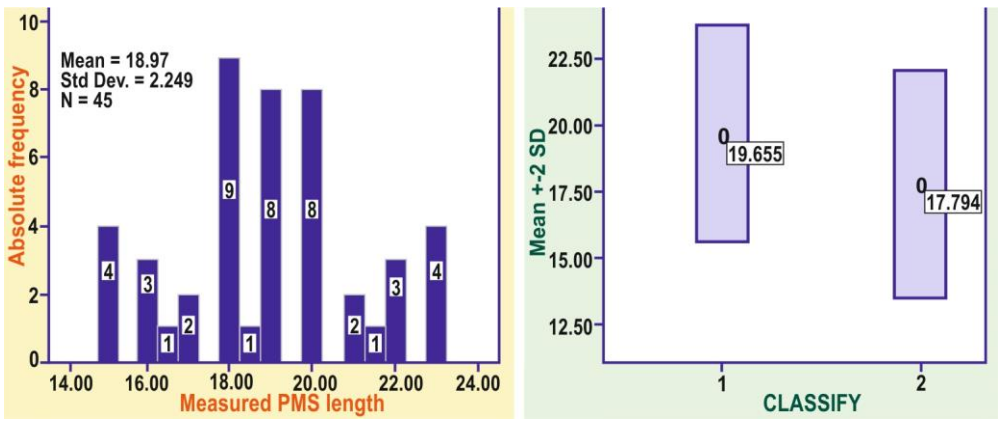


Figure 41 – Evaluation length of the maxillary arch at the premolars level

The difference from the ideal norm shows an global average of 0.7598, with a standard deviation of 2.2390 in subdivision II/1, the difference was 0.0031 and -2.0506 in class II/2.

- **at the molar level.** The global average of the group at the level of the upper molar was 25.84, with a standard deviation of 1.76. In subdivisions the average was 26.006

in II/1 and 25.625 in II/2, with standard deviations of 2,6777 and 1,6151. The difference that ensures the equilibrium of the maxillary arcade at the global level is 28.71 (**Figure 42**), and the values that ensures the equilibrium in subdivisions II/l, II/2 are 29.74 and 27.25, respectively (**Figure 42**).

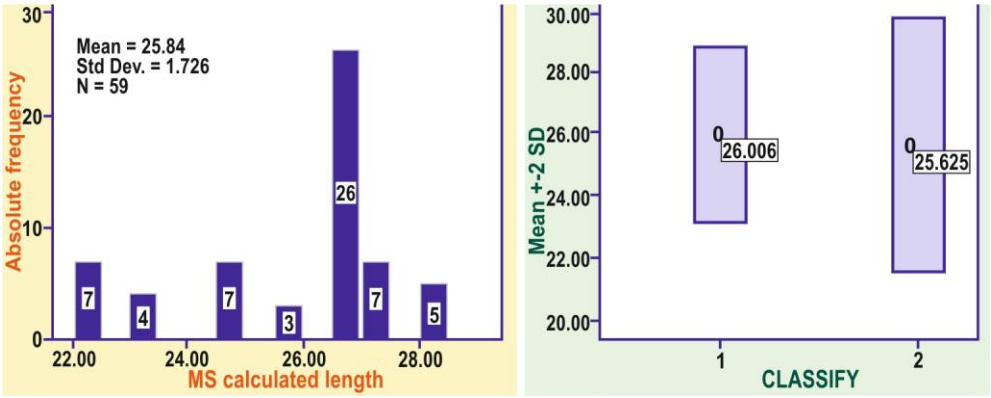


Figure 42 – The evaluation of length of the arcade at the molar level

The difference from the ideal norm is 2.7805, with a standard deviation of 2.7134. In subdivisions the average was 3.5671 and 1.663 in subdivision II/2, with standard deviations of 2.7399 and 2.2945.

There are statistically significant differences between Class II/1 and Class II/2 Angles in the molar width (p = 0.034); the arcade is narrowed in class II/1.

There are statistically significant differences between subdivisions of class II/1 and class II/2 Angles in arcade length at both premolar (p = 0.005) and molar (p = 0.000): in class II / l the arcade is longer.

2.6.3.2. Characteristics of the mandibular dental alveolar arch

2.6.3.2.1. Evaluation of the width of the mandibular arch:

- **at the premolar level.** The global average of the batch at the lower premolar level was 37.77, with a standard deviation of 3.3318. The average in subdivisions were 38.92 in II/1 and 35.9688 in II/2, with standard deviations of 2.8419 and 3.3189 (**Figure 43**). The difference that ensures the equilibrium of the mandibular arch at the global level in premolar area is 39.16, and the equilibrium values in subdivisions II/1, II/2 are 39.475 and 38. 847, respectively. In the class II/l subdivision was -0.955, in class II/2 being -2,342, with standard deviations of 1.6699 and 2.4871. The arcade width at the mandibular premolar level is statistically significant p = 0.004, lower in class II/2.

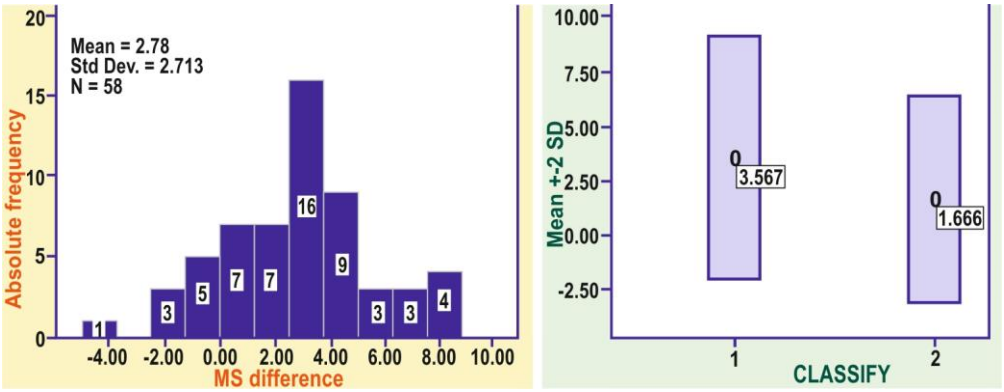


Figure 43 - The width of the dental arch at the premolar level

- **at the molar level.** The difference from the ideal value indicates an average of 1.9186, with a standard deviation of 4.4781. In class II/1 the average difference is -2.2562 and in Class II / 2 it is -1.4290 with standard deviations of 4.6772 and 4.2423.

2.6.3.2.2. Evaluation of the <u>length</u> of the mandibular arch:

- **at the premolar level.** The global average of the batch at the lower premolar level was 16.90, with a standard deviation of 1.655 (**Figure 44**). In subdivisions the average was 17.12 in II / l and 16.562 in II/2, with standard deviations of 1.6411 and 1.6720 (**Figure 44**).

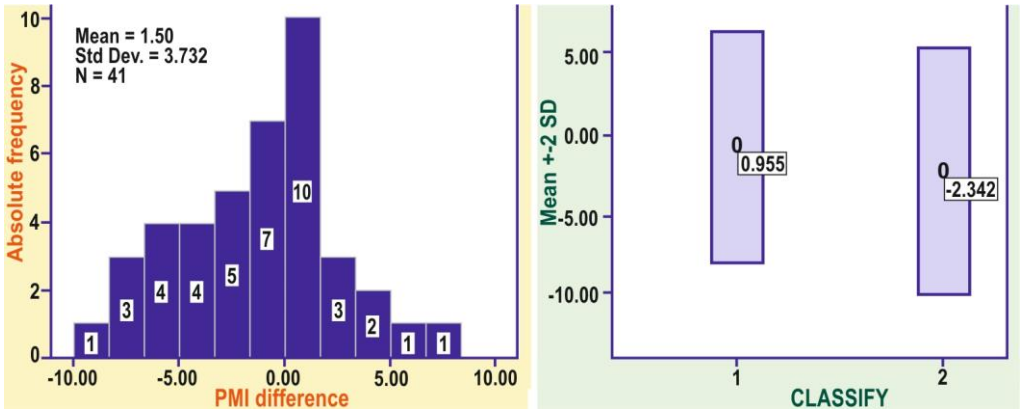


Figure 44 - The length of the mandibular arch at the premolar level

The difference that ensures the equilibrium of the maxillary arcade in the lower premolar area on a global level is 17.77, with a standard deviation of 1.477, and the equilibrium values in subdivisions II /1, II/2 are 17.73 and 17.79, respectively.

The difference from ideal values is -0.7751 on the global lot. In the class II/l the difference is -0.697 and -0.897 in II/2, with standard deviations of 1.7884 and 2.1888.

The differences are smaller between II/1 and II/2, with a discreet shortening in II/2 at the premolar level.

- **at the molar level.** The difference from the ideal value is 2.0841 on the whole lot, with a standard deviation of 2.6358. In subdivisions II/l the difference was 1.6172 and 2.6995 in subdivision II/2, with a standard deviation of 2.6559 and 2.5374.

The depth of the palatine veil indicates an average of 8.1441 in the whole study group, with a standard deviation of 3.2043. In subdivision II/1, the average of palatine veil depth was 8.7941 and 7.2600 in II/2, with a standard deviation of 3.3555 and 2.8141 respectively.

From the point of view of the depth of the palatine veil, no significant statistic differences exist between Class II/1, II/2.

2.5.4. Discussions

The results obtained by us reveals a statistically significant differentiation in the dental arcade, the group investigated by us reveals a narrowed maxillary arch at the molar level and elongated at premolar and molar level, in subdivision II/l. Our data are consistent with the results of literature (Moorrees et al., 1969; Wallow et al., 2002). At the same time it confirms McNamara's opinion, who believes that in class II/l malocclusion ”there is a transversal component, which will also influence the treatment algorithm (Mc Namara, 2000).

From the therapeutical point of view, the conclusion regarding the narrowing of the maxillary arch in class II/1 agree with the relation of jaw expansion/disjunction, in order to harmonize the dental springs for obtaining an eugnate occlusion. As far as the mandible arch is concerned, it shows more stability compared to the maxilla, which is highlighted in the specialized literature (Kanashiro, 2000).

There is a decrease in the width and, significantly, in the premolar length, as evidenced by Pancherz's studies (Pancherz, 1984). The shortening of the mandibular arcade in the canine-premolar region is considered a consequence of the high degree of overcoat, which produces the inferior retroalveolodention, in class II/2 malocclusion (Lapatki et al., 2002).

There are 5 types of gypsum products: type I is impression plaster, type II is the dental plaster, type III reffers to dental stone (medium strength stone), type IV is the improved stone (high strength stone) (die stone) and type V is the high strength/high expansion stone (Nagasawa et al., 2019).

Application of these gypsum models include:

- impression plaster,
- mounting the casts to the articulation,
- form casts and dies,
- used as a binder for silica,
- used as a mold for processing dental polymers,
- used for bite registration (record centric jaw relation) (Duseja et al., 2014).

Research are still looking for one product to fulfill the properties of an ideal model material (gypsum products):

- dimensional stability, no expansion or contraction during or after setting,
- high compressive strength to withstand the force applied on it,

- hardness, soft material can be easily scratched,
- reproduce the fine details,
- produce smooth surface,
- reasonable setting time,
- compatible with the impression material,
- **c**an be disinfected without damaging the surface **(**Aragón et al., 2016).

Intraoral dental arch impressions are used in different areas of dentistry that require records of dental anatomy, alveolar bone, gingival shape, and other oral structures, for diagnostic and/or research purposes.

This well-established procedure in dental practice, obtaining a dental model is a critical procedure and should be performed with attention to details, in addition to following biosafety norms for disinfection, to accurately and reliably reproduce the oral structures (Ivanovski et al., 1995; Jagger et al., 2007). It is noteworthy that plaster models need space and favourable long-term storage conditions to prevent plaster chipping or breakage, which could generate substantial loss of patient data (Abizadeh et al., 2012).

Impression-free models have been generated from different techniques that may have some advantages over conventional plaster models. Intraoral scanners were developed to offer the advantage of obtaining digital dental models directly from the patient without the need for dental impressions, thus a possibility of reducing the required time between patient examination and full diagnosis. Transfer of the impression to laboratory plus model pouring and detailing could be eliminated (Nedelcu and Persson, 2014). However, the time required for intraoral scanning is still longer than the conventional impression methods. It could be argued that once the involved laboratory time is added, the difference may actually be in favour of the direct intraoral scanning. In addition, scanning times and detail quality have continuously improved with newer versions of the intraoral scanners (Patzelt et al., 2014).

Recent studies suggested that measurements from scanned digital models are as reliable as the measurements from conventional dental plaster models. The authors suggested, based on strong evidence, that laser scanning of existing plaster models is a suitable technology to preserve the original data (de Luca et al., 2015).

Not only tooth size and arch dimensions should be analyzed when evaluating effectiveness of dental impressions. Procedure duration, patient comfort, and associated costs should also be accounted when analyzing the efficacy of clinical procedures. Chair side time required to perform alginate impressions is clearly shorter than the time to produce digital models with intraoral scans (Grünheid et al., 2014). However, in a recent study, the time required to pour orthodontic stone was not considered. It could be argued that once the involved laboratory time is added the difference may actually be in favour of the direct intraoral scanning. This could be a deciding factor that could easily level out the time required for both techniques. Future studies are suggested to take a deeper look into these variables to provide more conclusive clinical relevant evidence (Wiranto et al., 2013).

The associated costs are also an imperative variable to be studied. Most offices will probably have just one scanner, due to its high cost. Therefore, only one scan could be performed at time, so an additional 5–10 minutes to disinfect the scanner and the dental chair will increase each procedure duration. In contrast, several alginate impressions can be taken simultaneously by staff members, improving practice's efficiency. In addition, scanning

times and detail quality are continuously improved with newer interactions of the intraoral scanners. It has to be noted that the included intraoral scanners do not necessarily represent their latest versions (Wiranto et al., 2013).

Another factor to be considered is the fact that nowadays technology is advancing rapidly. Manufacturers continuously release new intraoral scanners claiming reduction in clinical time to perform oral scanning, combined with more compact devices, to provide more comfort to patient. However, studies considering these new versions are needed to confirm these claimed advantages (Nicolatou-Galitis et al., 2020).

Digital models produced by intraoral scan eliminate the need of impressions materials; however, currently, longer time is needed to take the digital images. In addition, the size of the tip of the intraoral scans makes it difficult to access some intraoral areas, potentially interfering with the image quality. The relatively scarcity of studies performed in vivo or intraorally is another limitation. To execute an intraoral scanning in a dry skull, or in a plaster model, implies no difficulties in comparison to those found intraorally in daily practice. Clinically, there are some restrictions as individual tolerance of patients, little work space in mouth, or interferences by tongue movement or orthodontic appliances. Flügge et al. even suggest that the intraoral conditions can influence the inaccuracy of a scan (Flügge et al., 2013).

2.5.5. Conclusions

There is limited evidence suggesting that intra- and inter-arch measurements obtained from digital dental models generated from intraoral scanners are at least equivalent when compared to dental plaster models or digital images generated from conventional dental impressions. Associated costs, time required, and patient's preferences are not variables that have been adequately considered so far in the available literature.

Dento-maxillary anomaly can have a major impact on the population, due to the damages of the dento-alveolar apparatus, which reflects on the general health status of the population. On the other hand, it is necessary to know the index of addressability of the population towards the dental care services, in general, and towards the orthodontics and dental-facial orthopedics, in particular. Treatment complexity index and treatment priorities can be established taking into account important data, like: the identification of the clinical manifestations of the anomaly, the etiological factors and the treatment needs.

Changes in class II malocclusion demonstrate that alters both dental and alveolar level. The maxillary dental arch is narrowed and elongated in subdivision II/l. The mandibular dental arch is narrowed and shortened in the anterior section of the premolar region. The knowledge of dental arch features serves to develop a correct and complete diagnosis and also to reach the therapeutic goals and to evaluate post-treatment response in short, medium and long term.

CHAPTER 3. COGNIZANCE IN GERIATRIC DENTISTRY

3.1. State of the Art

Oral health is gaining global attention because it is closely linked to general health and the quality of life. As the global population ages, healthcare services for elderly people have been further developed to improve their health and quality of life (Chalmers and Pearson, 2005). Among adults 65 years old or older, missing natural teeth and chronic oral diseases, such as dental caries, periodontal diseases, oral infections, oral mucosal lesions and temporomandibular disorders, are common (Tsakos, 2011; De Oliveira et al., 2013: Pradhan et al., 2016).

Older people often suffer from chronic illnesses for which daily medications are needed. One common oral side effect of medications is hyposalivation. Caries or mucosal infections dramatically increase with the impairment of the saliva function, giving rise to various oral health complications. These problems make the fulfilment of basic daily needs (e.g., chewing and communication) more difficult, leading to consequential physical health problems, such as nutritional inadequacy and psychosocial distress (Bekiroglu et al., 2012). The institutionalized elderly population is a vulnerable subpopulation and is often care-dependent with poor oral health. Numerous studies were published profiling the oral health and the oral health-related quality of life of the institutionalized elderly population, using validated instruments or approaches (Handelman et al., 1986; Razak et al., 2014; Jokanovic et al., 2015; Backer et al., 2020).

Shared common risk factors drive the development and worsening of poor oral health and non-communicable diseases, which eventually lead to self-care inability (Wong et al., 2019).

This particular elder segment of the population comprises vulnerable people who have poor oral health because they are relatively weak and dependent. The identified non-modifiable and modifiable factors associated with vulnerable oral health among this underprivileged group could be categorized as individual or environmental factors. Individual factors are most likely due to limited physical functioning and/or cognitive impairment, leading to a limited self-care ability (Frenkel and Harvey 2000; Zenthöfer et al., 2017).

Based on the evidence-based findings of the relevant included studies over the past decade, the institutionalized elderly population is at increased odds to have poor oral health with various degrees of dental, periodontal, oral mucosal and TMJ problems and poor functions due to missing teeth, inadequate replacement or poor dentures. Chronic oral distress and poor chewing function could affect general health, such as through the increased risk of malnourishment. The outcomes of this review are comparable to those of the previous findings (Hopcraft et al., 2012).

The most common dental problems include large amounts of plaque, debris and calculus and moderate to advanced periodontitis. Periodontitis is a common oral problem in middle age or elderly populations, particularly in institutional residents. All residents are found to have plaque and more than 70% of residents had plaque covering one index tooth. Poor oral hygiene indicates a higher possibility of gingivitis, periodontitis, periodontal

attachment loss and tooth loss (Montal et al., 2006; Xavier et al., 2020). In the elderly population suffering from hyposalivation due to ageing or concurrent medications, caries of crown and roots, tooth loss and so on are common. Dental problems also increase the risk of oral health complications and general health problems (Zimmerman et al., 2017).

The number of natural teeth and, hence, the presence of proper, well-maintained oral rehabilitation are important in maintaining oral function and oral health. This is because they facilitate masticatory performance that promotes chewing, enables a variety of food selection and processing and promotes the enjoyment of eating and, ultimately, a balanced diet. Such oral function is important in maintaining physio-psychosocial well-being. Physically, adequate teeth or full denture units/full tooth units maintain masticatory performance, which is important for satisfactory nutrition intake, normal social interactions and enhanced psychosocial satisfaction (Sheiham et al., 2001). However, when dentate residents have untreated dental problems, they can experience discomfort while eating, increased sensitivity to extreme temperatures in foods and chronic or acute pain or ulcers in the mouth, ultimately causing difficulty in eating, limited choice of food, and, sometimes, difficulty speaking. Subsequently, they experience more psychosocial concerns, such as embarrassment and discomfort, when they eat in front of others. As a result, they take in a limited amount of food, leading to nutritional problems as well (Hassel et al., 2006).

Strategies for the prevention of tooth loss and for maintaining adequate oral hygiene and preventing oral diseases at an early stage among institutionalized residents are key for increasing the oral health of residents. Even a basic twice-a-day tooth-brushing habit could be difficult to achieve, as more than 70% of the institutionalized residents undertook tooth brushing or cleaning only once per day or even less and they did not have regular yearly dental visits (Mesas et al., 2008). More than 90% of the residents did not attend a dental visit more than once per year. The elderly population typically visits a dentist only in a symptom-driven mode (e.g., due to pain or discomfort), rather than attending regular recalls/check-ups for disease prevention and health maintenance. Uncomfortable or painful treatments may lead to the refusal of future dental consultations and worsening oral health status (Cornejo et al., 2013).

Wearing dentures, including complete or partial dentures, can help improve physical functions, such as chewing, communication, speaking and smiling. Dentures that do not fit can reduce chewing efficacy, leading to an impaired OHRQoL. However, the dentures require adequate daily oral hygiene and aftercare to maintain good oral function and health (Portal et al., 2015).

Both male and female residents have dental and/or periodontal problems and structural alterations. Regarding the effect of age on oral health, the results showed that increasing age generally increases the risk of a poorer OHRQoL (Piuvezam and de Lima, 2013).

In general, approximately 20% to 50% of elderly people aged 65 or older have lost all of their natural teeth and around 15% were without complete dentures. For those who had complete dentures, many are ill-fitting or needed repair. Together those affected have multiple functional problems and an impaired OHRQoL. An estimated 10% of the elderly population had natural functional dentition and the rest (est. 50%) are partially dentate and required prosthetic rehabilitation. Older residents who have inadequate natural teeth are also

susceptible to poor oral health, such as periodontitis, tooth crown or root caries and so on. In fact, the association between various systemic conditions and poor oral health was well recognized (Kshetrimayum et al., 2013).

Among the top five systemic diseases/conditions of the older residents are also readily observable among dementia/MCI residents for incidence, risk of dementia, depression/anxiety and teeth lost, which perhaps is related to poor self-care and oral hygiene. Also the two-way relationship between diabetes mellitus and poor periodontal health was among the most well established. There is reported that dentated Diabetes Mellitus (DM) residents had more missing teeth (Loesche et al., 1995; Loesche et al., 1998). Associations between atherosclerosis, cerebral vascular accidents and poor oral/periodontal health were reported, probably related to poor oral care and, perhaps, to the direct or indirect effects of periodontopathic bacteria. Meanwhile, hypertension and increased subgingival colonization of periodontal bacteria and, hence, periodontitis risk were considered mechanisms underpinning the corresponding poor general health (Shay 2002; Meurman et al., 2004; Stein et al., 2007; Desvarieux et al., 2010; Preshaw et al., 2012).

Oral health affects physical and psychosocial well-being and can lead to malnutrition, a serious physical problem that is more prevalent in residents with poor oral health and a poorer OHRQoL (Hugo et al., 2016).

This research direction has been materialized by publishing the following articles:

1. Surdu A, Bobu L, Topoliceanu C, Filip C, Goriuc A, **Scutariu MM**, Murariu A. Study regarding the relationship between nutritional status and oral health in the elderly patients. Rev Chim (Bucharest) 2019; 70(7):3032-3036.
2. **Scutariu MM**, Untu I, Surdu A, Ciurcanu OE, Bolos A. Particularities of oral health problems in patients with Alzheimer`s dementia. Rev Med Chir Soc Med Nat Iaşi 2018; 122(2): 381-387.
3. **Scutariu MM,** Ciupilan C, Sălceanu M, Melian A, Forna DA, Sioustis I, Ciurcanu O. Incidence of dento-periodontal pathology in geriatric patients. Romanian Journal of Oral Rehabilitation 2018; 10(1): 128-132.

3.2. Nutritional status and oral health in geriatric patients

3.2.1. Introduction

The increase of the proportion of aged people in total population is a global phenomenon, with an increment from only 8% in 1950, to 10% in 2010, being estimated to reach 21% in 2050. In Romania, due to demographic changes (migration of young adults) and low birth rate, the old population (> 60) reached 21%. This percent is similar to other developed countries like Japan (26%), Italy (21%), Germany (21%), Sweden (20%), UK (18%) (D'albis 2013).

Elderly people are a population with unsatisfactory oral health and high risk of poor nutritional status, due to physical, physiological, and psychosocial factors. The diet of old people is characterized by low energy and nutrient content, as well as deficiencies in calcium, zinc, magnesium, iron, vitamin D, vitamin B6, vitamin B12, vitamin E, thiamin, retinol, carotenes, and folic acid. The supply of proper oral health care to old people is deficient due to inadequate resources and time, the lack of motivation and interest for oral health care of seniors or their relatives, changed eating habits or saliva quality and composition, and behavioral changes in terms of oral hygiene, and decrease income as well (Montgomery et al., 2014; Gil-Montoya et al., 2015).

Despite the evidences that suggest the implications of the unsatisfactory oral health for general systemic health, the association between oral health and nutritional status is still debatable (Hugo et al., 2016).

The aim of this study was to investigate the relationship between oral health and nutritional status in a population of elderly patients with systemic chronic or acute pathology.

3.2.2. Materials and methods

The research consisted of a prospective, cross-sectional clinical investigation of 115 aged patients (> 60yo; mean age 66.2) from Clinical Foundation, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy of Iasi. The inclusion criteria were as follows: patients without dialysis, cancer, malabsorption syndrome, able to understand and communicate. The demographic data of the study group were collected, the information regarding the systemic status of the study group being either recorded. Nutritional input along with data related to the oral health of the study group were also collected. In the second stage of the study, a questionnaire was used to record demographic and socioeconomic data of the subjects (such as age, gender, life conditions, education level, income), as well as data regarding systemic status. The medical data included the type and evolution of systemic pathology (diabetes, cardiovascular pathology, respiratory diseases, rheumatoid arthritis), as well as cure and medication. The required nutritional informations were related to the possibility to prepare the meals, the foods quantity and texture. Moreover, weight and height were recorded for a proper BMI (body mass index) estimation.

In the third stage of the study, the nutritional status was evaluated by using Mini-Nutritional Assessment questionnaire (MNA). The questionnaire included anthropometric evaluation, diet features, environment data, as well as self-assessment of the systemic health and nutritional status. The final score allowed the classification of subjects in three categories: proper nutritional status (>23.5); malnutrition risk (17-23.5); malnutrition (<17).

In the fourth stage of the study, the oral health status was determined, by using clinical and radiographical exams. Also, it was used geriatric oral health assessment index (GOHAI) to determine subjects' self-perception of the oral health status and need for oral care. The oral health recorded parameters were as follows:

- number of the remaining teeth (≥20, <20, total edentation);
- type and status of dentures (well-fit, mal-fit);
- pain during mastication;

- xerostomia.

The GOHAI questionnaire (12 questions) was used to evaluate the self-perception of the patients for their own oral health-related issues and the need for oral health care, regarding:

- oral functions;
- pain and discomfort due to the oral pathology; -psychological and social aspects related to the oral pathology.
- the need for dental treatment (considered for patients with GOHAI ≥14).

A standard statistical procedure involves the collection of data leading to test of the relationship between two statistical data sets, or a data set and synthetic data drawn from an idealized model. Descriptive statistics we used are most often concerned with two sets of properties of a distribution (sample or population): central tendency (or location) seeks to characterize the distribution's central or typical value, while dispersion (or variability) characterizes the extent to which members of the distribution depart from its center and each other. Inferences on mathematical statistics are made under the framework of probability theory, which deals with the analysis of random phenomena.

3.2.3. Results

The demographic data of the study group are presented in the **table 8**, while data regarding the systemic status of the study group are presented in the **table 9**. Referring to the nutritional status of the study group the results are depicted in **table 10**.

Table 8 - Demographic data in the studied group

Study group data	%
Age groups:	
60	8.7%
61-70	45.2%
71-80	27%
>80	19.1%
*Gende*r:	
Females	51.3%
Males	48.7%
Life conditions:	
Married	56.5%
Single	44.4%
Education level:	
Primary school	48.7%
Highschool	33.9%
Faculty	17.4%

The main collected data related to the oral health of the study group are presented in **table 11**, while main results concerning the GOHAI score for all the subjects included in the study are included in **table 12**.

Table 9 - Systemic status data

Study group data	%
Number of chronic diseases:	
0	9.5%
1	22.6%
2	32.2%
3	35.7%
Treatment with at least 3 drugs:	
Yes	54.8%
No	45.2%

In the first stage of the study, the patients were submitted to a mini-test (MMSE) for the assessment of the mental status (patient able to understand and communicate) (**Table 12**). Only subjects with scores 24-30, with acceptable cognitive status were included in study.

Table 10- Nutritional data

Study group data	%
Able to buy food:	
Yes	60.9%
Sometimes	15.6%
No	23.5%
Able to cook:	
Yes	55.6%
Sometimes	19.4%
No	23.5%
Eating:	
Family	60.9%
Single	39.1%
Trouble with firm food:	
Yes	49.6%
No	50.4%

Table 11 - Oral health status in the studied group

Study group data	%
Number of remaining teeth:	
20	5.2%
<20	25.2%
ET	69.6%
Edentation:	
Yes	69.9%
No	30.4%
Prostheses:	
Yes, well-fitted	59.6%
Yes, ill-fitted	19.2%
No	21.1%
Xerostomia:	
Yes	69.6%
No	30.4%

Table 12 - GOHAI score

Study group data	**%**
Need for treatment:	
Yes	55.6%
No	44.4%
GOHAI score:	
1	39.1%
2	14.8%
3	22.6%
4	23.5%

The relationship between the nutritional status and demographic data is given **in table 14**. The results related to the relationship between the nutritional status and the systemic status are presented in the **table 15**. The results related to the relationship between the nutritional status and the nutritional subjects' features are presented in the **table 16**.

The results related to the relationship between the nutritional status and the oral health are presented in the **table 17**, while main results concerning the relationship between the nutritional status and GOHAI score are depicted in **table 18**. The nutritional data showed that 25% of subjects were unable to prepare meals, and 23.5% were unable to buy foods. 6% were severely malnourished, and 37.4% presented malnutrition risk. 49.6% of the old subjects evaluated presented difficulties in eating firm foods.

Regarding the systemic status, 35.7% of subjects were diagnosed with at least 3 chronic diseases (most frequently, diabetes with hypertension and osteoporosis), and 54.8% administered more than three drugs. In what concerns the oral status, only 5.2% of subjects presented at least 20 natural teeth, 55.6% required dental care for decayed or broken teeth, 69.6% being affected by xerostomia. 21% of the completely or partial edentulous people were not treated with removable dentures, 25% of the edentulous patients complaining of poorly-fitted removable dentures.

This study describes the status of oral health and nutrition for a group of aged patients requiring oral health care. The study uses validated assessment tools and qualified dental practitioners which practice daily on elderly people in the Clinical Foundation, Faculty of Dental Medicine, University of Medicine and Pharmacy of Iasi.

Table 13 - MMSE score (mental status)

MMSE Score	Stage	Features
30	Normal	Absent symptoms
24-27	Early cognitive disorders	Amnesiac symptoms
21-23	Mild phase	Mild cognitive deficiency
18-20	Moderate phase	Clear cognitive deficiency
15-17	Moderate-severe phase	Cognitive and psychiatric symptoms Assistance required
12-16	Severe phase	Assistance required for daily activities
0-11	Worse Phase	Institutionalized assistance

Table 14 - Demographic data *vs* nutritional status

Study group data	Good nutritional data	Poor nutritional status
Age groups:		
60	60%	40%
61-70	61.5%	38.5%
71-80	51.6%	48.4%
>80	50%	50%
Gender:		
Females	59.3%	40.7%
Males	53.6%	46.4%
Life conditions:		
Married	59.3%	40.7%
Single	53.6%	46.4%
Education level:		
Primary school	42.9%	57.1%
Highschool	71.8%	28.2%
Faculty	65%	35%

Table 15 - Systemic status data *vs* nutritional status

Study group data	Good nutritional status	Poor nutritional status
Number of chronic diseases		
0		
1	63.6%	36.4%
2	65.4%	34.6%
3	64.9%	35.1%
	58.5%	41.5%
Treatment with at least 3 drugs:		
Yes		
No	42.9%	57.1%
	73.1%	26.9%

Table 16 - Nutritional data *vs* nutritional status

Study group data	Good nutritional status	Poor nutritional status
Able to buy food:		
Yes	77.1%	22.9%
Sometimes	22.2%	77.8%
No	25.9%	74.1%
Able to cook:		
Yes	80%	20%
Sometimes	35.7%	64.7%
No	27.8%	72.2%
Meals:		
Family	60%	40%
Single	54.3%	45.7%
Difficulties with firm food:		
Yes		
No	43.9%	56.1%
	69%	31%

Table 17 - Oral status in the studied group

Study group data	Good nutritional status	Poor nutritional status
Number of remaining teeth:		
20	66.7%	33.3%
<20	58.9%	41.1%
ET	55%	45%
Edentation:		
Yes	55%	45%
No	60%	40%
Prostheses:		
Yes, well-fitted	63.1%	36.9%
Yes, ill-fitted	52.4%	47.6%
No	39.1%	59.9%
Xerostomia:		
Yes	43%	57%
No	56%	44%

Table 18 - GOHAI score

Study group data	Good nutritional status	Poor nutritional status
Need for treatment:		
Yes	46.9%	53.1%
No	68.6%	31.4%
GOHAI score:		
1	68.8%	31.1%
2	64.7%	35.3%
3	50%	50%
4	37%	63%

Our study reveals a low proportion of severe malnourished old people (6%), and moderate proportion of the old subjects with risk of malnutrition (37.4%), despite relative high proportion of elderly with difficulties in eating firm foods (49.6%). Considering the high percentage of subjects affected by xerostomia (69.6%), decayed and broken teeth (55.6%), total or partial extended edentation (94.8%), as well as the proportion of subjects untreated or wearing unfitted removable dentures (21%), we concluded that risk of malnutrition can be influenced by the oral pathology. Cofounding factors must be considered as well, as all subjects were affected by chronic or acute systemic disorders.

3.2.4. Discussions

Other studies on the same area found different results upon the nutritional status: 62% were malnourished (55% moderately malnourished, 7% severely malnourished), 82% needed a dental referral. More people who had decayed or broken natural teeth (77%) encountered difficulties eating firm foods than those who had healthy natural teeth (23%).

Significant statistical differences were found between old people with normal diet texture and those with hardship eating firm foods related to their oral health status. 58% of the subjects with a normal diet texture had unhealthy changes to their teeth compared to 100% of subjects who had a modified texture diet. 68% of the subjects with good nutritional status reported never having deficiencies eating hard foods, compared to only 30% of moderately malnourished subjects. The conclusion of this study was that malnutrition was strongly associated with poor life quality and modified diet texture was associated with poor nutritional status. The differences are related to the pool of old people as this study was performed on aged care residents with higher degree of dependency and lower levels of health care than patients from our study group (Hugo et al., 2016).

During our research, a low level of perception of oral health (highest GOHAI score) was associated with elevated risk of nutritional deficiency (MNA <24), result similar with other study that reported a 3.41 higher nutritional risk for elderly people with poor perception of their oral health (Mesas et al., 2010).

Only a few studies were focused on the relation between oral health, nutrition and quality of life are scarce (Cousson et al., 2012; Palmer 2003). A systematic review of the relationship between poor nutrition and oral health status in the elderly found lack of methodological robustness and the use of validated assessments. Of the 16 considered studies, only 3 of them used a validated assessment tool. In this context, interpretation of the literature data is challenging, due to the absence of universally agreed tests and the multifactorial nature of both, poor oral health and malnutrition along with a range of confounding factors (Van Lancker et al., 2012). Interpretation of the results reported by studies must consider that poor oral health and xerostomia have previously been associated with reduced chewing function and avoidance of firm foods by elderly people. Also, the poor dental status and the decrease of the life quality can influence meals time and loneliness which will negatively impact dietary intake (Budtz-Jørgensen et al., 2001; Aynaud-Simon and Lesourd 2000).

Oral pathology can lead to improper nutritional status, by pain and discomfort related to dental caries, periodontal disease and ill-fitted fixed dentures or removable dentures. Also, even treated with removable dentures, the extended partial edentation or total edentation affect severely the digestive function and the nutritional status (Ouger-Decker and Mobley 2007).

This category of researches highlights the role of maintaining a proper oral health as improper health status can lead to underweight, that increases the risk of infections and mortality (WHO 1995).

Our results differ from those published by Guigoz in 2006 in a systematic review of studies that included 30,000 elderly patients from different countries worldwide, especially in Europe and the United States, in which the proportion of malnutrition and risk of malnutrition were 23% and 46%, respectively, in hospitalized elderly individuals. This lower prevalence could be explained by the diversity and the high nutritional density of the basic Lebanese diet which includes more nutritious foods with semisolid or liquid textures, compared to the mostly “meat-based” diet in European countries (Guigoz Y. 2006). The discrepancy in results could also be explained by a difference in the patients' dependency level, health status, and especially by the exclusion of patients with cognitive impairment.

The bivariate analysis revealed that lack of education, use of more than three drugs daily, physical inability to shop and prepare meals, and the exclusion of certain solid foods from the diet are significantly associated with a nutritional deficit. These results are consistent with the results reported in the literature (Guralink and Simonsick, 1993; Bouteloup C. 2005). With regard to the association between oral health and nutritional status, more than 50% of patients needed dental care. The need for dental care was associated with the risk of nutritional deficit. Similar results were observed elsewhere in which the GOHAI, which is a categorical variable, showed a significant association with the MNA results (Gil-Montoya, 2008).

In addition, when the GOHAI was categorized into quartiles, it showed a significant association with the risk of nutritional deficit. The nutritional deficit was higher for the fourth quartile than for the first quartile. In the regression analysis after adjusting for age, sex, educational level, and pre-existing chronic diseases the association persisted between nutritional status (assessed by the MNA) and the GOHAI score categorized into quartiles, regardless of the dental status. However, this association was not significant because of this small sample size. A poor perception of oral health, defined by the highest GOHAI quartile, is conversely associated with a 2.84 times increased risk of nutritional deficit.

This finding is consistent with the findings of Mesas et al. who reported that the nutritional risk is increased by 3.41 times. The nutritional risk was higher in completely edentulous patients and in patients not wearing dentures or wearing ill-fitting dentures (Masas et al., 2010). These results are similar to those of other studies addressing the relationship between oral health and nutritional status in the elderly (Sawa et al., 2020).

Considering these data, the management of the elderly requires a multidisciplinary, biomedical, psychological, social, socio-demographic, administrative, legal, economic, ethical and affective approach.

The interpretation of the results reported by various researches is difficult due to the heterogeneity of populations in anthropometric and nutritional parameters and the use of different assessment tools related to oral health status and nutritional status.

Further studies are required to investigate if regular oral health assessments and therapeutic plans in elderly can improve or reduce the decline of the nutritional status. Also, specific researches performed on large populations can better establish the relation between the growth of the nutritional status and the improvement of oral health status and quality of life. Recommendations for future practice include increasing training and education of RACFs in terms of oral health care and its connection with nutritional status and quality of life, increased access to dental professionals in RACFs and the importance of concurrent referrals to dietitians when dental issues are identified.

The paucity of education, treatment with over 3 drugs daily, physical inability to buy foods or to prepare meals, as well as the difficulties in eating firm foods are strongly associated with poor nutritional status. *Moreover, our study empowers for the need for dental care for these subjects, as the shortfall of the oral status was strongly associated to nutritional deficiencies.*

3.2.5. Conclusions

Not withstanding the limitations represented by the sample size and a bias towards the lower socioeconomic data, our study demonstrated an association between oral health and nutritional status in elderly individuals. In spite of the high prevalence of oral health problems, the prevalence of nutritional disorders remained relatively low. These findings open up a new question about spontaneous compensatory patterns in the diet patterns of elderly individuals that need further investigation. The study findings suggest the importance of dental care among the elderly population as an integral part of their medical care, of facilitating access to dental care, and of the necessity of their receiving proper oral care (starting at an early age) to reduce the risk of nutritional deficit later in life and to improve the oral health-related quality of life.

3.3. Oral health in Alzheimer's patients

3.3.1. Introduction

Dementia is a progressive and irreversible neurodegenerative disorder characterized by a variety of symptoms: cognitive impairment, memory disorders, progressive deterioration of daily exercise capacity, and a range of neuropsychiatric symptoms. According to the World Alzheimer Report from 2015, regarding "The Global Impact of Dementia", the number of people diagnosed with dementia will increase from 46 million, in the present, to 131,500,000 by 2050. Statistical data show that about 9.9 million new cases of dementia will be diagnosed this year around the world. Thus, a new case is diagnosed every three sec- onds. According to the report, about 94% of people living with dementia in low-income and middle-income countries are home-grown. In many of these countries there is no developed nursing and specialized care network for people with dementia (The World Alzheimer Report 2015).

The world population is experiencing an aging process, the number of people over 60`s years old suffered an increase of 450 million in only 40 years. The structure of the elderly population has changed to more than 50% in developed countries, the most aging being from the European area. Dementia caused by Alzheimer's disease is the most common type, representing approximately 60% of all dementia patients. As for the link between age and illness, it is estimated that 5% of persons of 65 years old have dementia in Alzheimer's disease, and at the age of 85, the rate goes up to 25% (Knopman et al., 2001).

Oral health problems may adversely affect the quality of life of patients with Alzheimer's dementia. However, as the patient suffering from Alzheimer's dementia requires a wide range of care, often oral health care is not a priority. Increasing the quality of life and improving the general condition of the elderly is closely related to the maintenance of natural teeth and the development of well-adapted prostheses (McDowell et al., 2001). Given that cognitive functions and motor skills are progressively altered in patients with Alzheimer's dementia, this circumstance can lead to a level inadequate control of biofilm and

oral hygiene. Alzheimer's dementia is a complex pathology that, beyond the loss of cognitive function, leads to alteration of the ability to perform common daily activities (Pasinetti 2001).

The spectrum of adverse effects attributed to the medication used to control the symptomatology specific to dementia also reaches the oral sphere, emphasizing the issues already exposed. On the onehand, anti-dementia medication (anti-acetylcholinesterase) causes hypersalivaion, which makes it more difficult to maintain a dry field during dental treatment and causes difficulties in maintaining a mobilizable prosthesis (Cerutti-Kopplin et al., 2016). On the other hand, xerostomia is a common side effect associated with adjuvant medication used in dementia, such as antidepressants and benzodiazepines. Insufficient salivation reduces the lubrication of mucosal tissues, increasing the potential for mucosal ulceration, and increasing the difficulty of maintaining a mobilizable prosthesis. At the same time, xerostomia leads to alteration of normal oral flora, causing gingival plaque formation, predisposing to periodontal disease, dental caries and increased risk of infection (Naorungroj et al., 2015; Takeuchi et al., 2017).

With the aging of the population, a higher prevalence of dementia and an increase in oral health problems can be expected. It is of interest to have an overview of the prevalence of oral problems in people with dementia.

The aim of our study was to evaluate the dental status of older people with dementia who have multiple oral health problems related to oral soft tissues, such as gingival bleeding, periodontal pockets, mucosal lesions, and reduced salivary flow. The oral health and hygiene of older people with dementia is not sufficient and could be improved with oral care education of formal and informal caregivers and regular professional dental care to people with dementia.

3.3.2. Materials and methods

The study group included 50 patients admitted to acute department III, V and VII in "Socola" Psychiatry Institute Iasi, during the year 2017. The study group consists of roughly equal numbers of males and females with a slight superiority of female gender (56% female and 44% male). Clinical and anamnestic data were extracted from the observation sheets of these patients. The dental evaluation was conducted within the dental office of the Institute. The criteria for inclusion in the study were: confirmed diagnosis of Alzheimer's dementia, both with early onset and with late onset and edentation.

Alzheimer's dementia diagnosis was established according to ICD 10 criteria (17). Dementia in Alzheimer's disease with early onset: The criteria for dementia in Alzheimer's disease must be met, and the age at onset being under 65 years; In addition, at least one of the following requirements must be met:(a) evidence of a relatively rapid onset and progression; (b) in addition to memory impairment, there is aphasia (amnesic or sensory), agraphia, alexia, acalculia, or apraxia (indicating the presence of temporal, parietal and/or frontal lobe involvement).

We considered the following as criteria for dementia in Alzheimer's disease with late onset:

- the age at onset must be 65 or more.

- at least one of the following requirements must be met:(a) evidence of a very slow, gradual onset and progression (the rate of the latter may be known only retrospectively after a course of three years or more); (b) predominance of memory impairment over intellectual impairment (see general criteria for dementia).

Once the patient is diagnosed, the progression of the disease is clinically and anamnestically assessed, also with the help of the Mini Mental State Examination, a nonspecific instrument that has no diagnostic but merely indicative role. At the same time, scores on the Hamilton Depression Rating Scale of patients with Alzheimer's dementia showing negative hypertimic symptoms are also recorded (Takeuchi et al., 2017).

In terms of edentation, patients were in one of the following classes: partial edentation, when at least three consecutive teeth in the lateral area or four front teeth are missing; subtotal edentation, when the arcade remains at most three teeth; total edentation, lack of all teeth.

Oral Health Indicators used in this study were the OHI index and the CAO-D index, both of which are internationally recognized by dental scientific organizations. The OHI is the sum of the soft deposit index and the tartar index (Takeuchi et al., 2017).

The soft deposit index:

- 0-absence of soft deposits;
- 1-soft deposits covering up to 1/3 of the surface of the teeth;
- 2-soft deposits covering 1/3-2/3 of the surface of the teeth;
- 3-soft deposits covering more than 2/3 of the surface of the teeth (Okamotoa et al., 2015).

Tartar index:

- 0-the absence of tartar;
- 1- supragingival tartar covering not more than one-third of the surface of the tooth;
- 2- supragingival tartar that covers between 1/3 and 2/3 of the exposed tooth surface;
- 3- surgingival tartar covering more than 2/3 of the tooth surface (Okamotoa et al., 2015).

3.3.3. Results

The results obtained by correlating the degree of dental affection with the degree of psycho-cognitive impairment and the presence of depression symptoms, apathy and serious difficulty in targeting, indicate the existence of a direct relationship between these variables. Thus, most patients with dementia have associated oral pathology, the most significant, with an increased impact on the patient's outstanding function being the edentation of different degrees. It is noticeable that in most cases we face subtotal edentation, followed by the total edentation, present in a significant percentage.

Distribution of cases by age reveals a predisposition for the eighth decade. It should be noted that patients are not newly diagnosed cases and clinical and anamnestic information

in the observation sheets reveals that most of them were originally dementia Alzheimer type, with early onset (**Table 19, Figure 45**).

Patients with dementia develop depressive symptoms of varying degrees of intensity, the group of those with overweight depressed mood associated with 92% of total and subtotal edentation (**Figure 46**).

Table 19 - The distribution of cases by age and gender

Age	Male		Female		Total	
	Number of patients	%	Number of patients	%	Number of patients	%
50-59	1	2	2	4	3	6
60-69	4	8	6	12	10	20
70-79	11	22	15	30	26	52
80-89	6	12	5	10	11	22
Total	22	44	28	56	50	100

The distribution of the CAO-D index in patients in the study group is the following:

- number of caries-1, 12 teeth;
- number of absent teeth - 4.48;
- number of teeth closed - 0.32.

Regarding the oral hygiene index:

- 84% of the patients obtained a weak index,
- 14% moderate
- only 2% of the patients a good hygiene index.

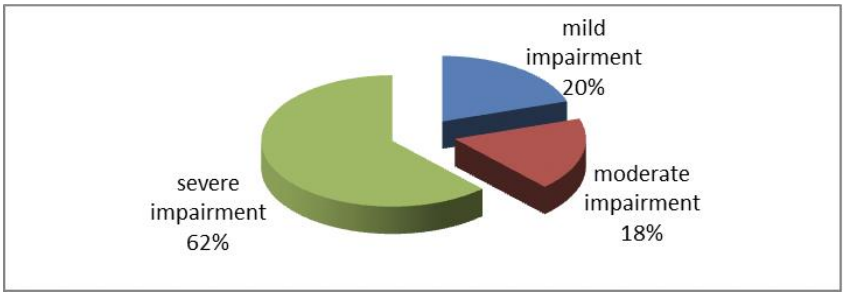


Figure 45 - Degree of cognitive impairment

Of all patients included in the present research, it is noted that there are none that do not show a degree of edentation (**Figure 47**). In addition, 78% of patients with moderate dementia had partial and total edentation and 56% of patients with severe dementia had partial edentation and 21% had total edentation.

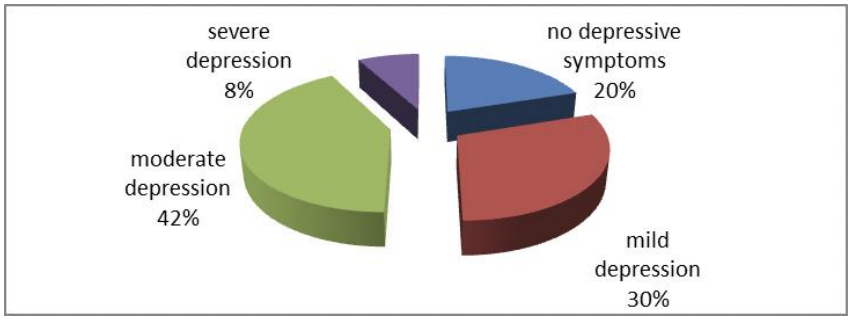


Figure 46 - Depressive symptoms in patients with Alzheimer`s disease

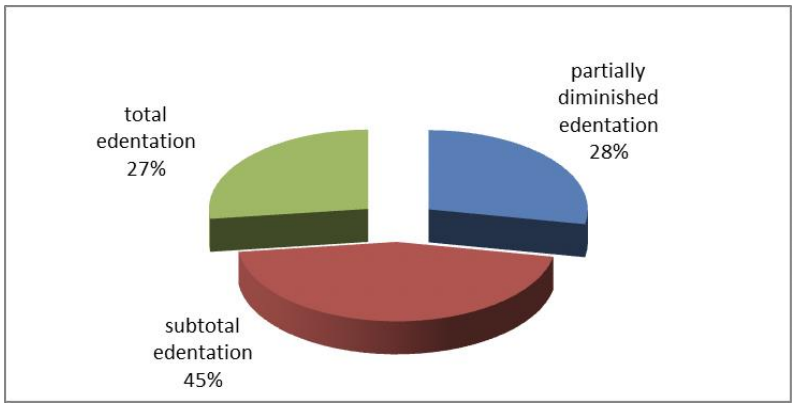


Figure 47 - Edentation in patients with Alzheimer`s disease

3.3.4. Discussions

In 2014, the General Assembly of the World Federation of Public Health Associations issued a resolution on the importance of oral health of patients with dementia (Harding et al., 2017). The prevalence of dementia is growing globally. Patients with dementia gradually install serious communication difficulties with those responsible for their care. Recent studies show that the precarious oral condition of people with dementia is directly correlated with socio- economic status, but also with demographic variables. Thus, the results of this research are linked to the results obtained in the literature, emphasizing the direct relationship of a precarious socio-economic status and a greater severity of the pathology in the oral sphere in the patients diagnosed with dementia (Okamotoa et al., 2015).

In addition, as the present research shows, patients with dementia receive psychotropic treatment, especially for controlling behavioral and psychotic symptoms, but also for controlling depressive symptoms that can cause xerostomia and dry mouth syndrome, favoring candida infections, but also the appearance of dental caries and, on the other hand, sialorrhea. In addition to this, patients with dementia either forget or lose their ability to ensure daily oral hygiene. Thus, the persons responsible for these patients (family members or medical staff) must ensure this process.

Depending on the evolutionary state of dementia, oral health becomes a growing problem, being totally dependent on the care of others. Multiple studies have directly

correlated the degree of cognitive impairment with the precariousness of oral health (Popovac et al., 2016). In the same vein, the present research shows that most cases of severe edentation (subtotal and even total) occur in patients with moderate to severe dementia, who are, moreover, frequently hospitalized in psychiatric or institutionalized services.

A study conducted in Sweden between the years 2007 and 2015 (Fereshtehnejad et al., 2018) shows that as dementia progresses, addressability for dental services decreases significantly. *At the same time, the MMSE score has been shown to be a remarkable predictor of edentation, in the context of increasingly poor dental hygiene and the appearance and evolution of dental caries. These elements are also found in the results of this research. In addition, the same study is the first longitudinal research that examines oral health in premorbid stages and after diagnosis of dementia. Unlike our study, it reports the results to the population with cognitive impairment before dementia.* This study only indicates the prevalence of oral pathology among patients with already diagnosed dementia and especially among those with moderate-severe dementia (Ghezzi and Ship, 2000; Fereshtehnejad et al., 2018;).

In 2017, a study realized by Campos shows that the masticatory efficacy in Alzheimer's dementia remains below the control level, as indicated by our study, which highlights the severity of the masticatory and swallowing disorders in direct proportion to the evolutionary stage of dementia (Campos et al., 2017).

Cognitive decline, apathy and apraxia, symptoms that characterize the average progression of dementia are responsible for the lack of interest and inability to perform oral hygiene techniques (Fereshtehnejad et al., 2018). In advanced stages, Alzheimer's disease leads to total disability, augmented by the associated general health problems and by the diverse oral pathology resulting in even greater dysfunctionality. Loss of teeth reduces masticatory capacity, increasing the risk for serious swallowing and denaturing disorders (Ghezzi and Ship, 2000).

The results of this research relate to the results of international general statistics, revealing a remarkable share of depressive symptoms in dementia, which correlates with specific progressive deterioration, aggravates apatoabulic phenomena, as well as apraxia itself, that correlates directly with the appearance and evolution of oral pathology, reaching to the edentation.

This study could be the premise of a program designed to increase the quality of life of patients with dementia, including oral health care among all the other fundamental problem of these patients. The results of our study show once more that beyond the psychiatric symptomatology, the patient with dementia associates a series of general health problems that hinder the evolution of the underlying disease. Through the correct, multidimensional approach of the patient with dementia, by managing all psychiatric symptoms such as depression, general health problems, including oral pathology and the risk of edentation, can be avoided or alleviated.

Recent studies that compared gingival and periodontal disease of participants with and without dementia showed significantly more (severe) periodontal disease in older people with dementia (Chapman and Shaw, 1991). De Souza Rolim reported significantly more periodontal infection in participants with dementia (58.6%) than in participants without dementia (26.7%). Zenthöfer found significantly more periodontitis in participants with

dementia (100.0%) than in participants without dementia (73.9%). Furthermore, the community periodontal index of treatment needs was significantly higher in participants with dementia (3.1–3.4) than without dementia (2.7–2.8) (Zenthöfer et al., 2014).

When specifically looking at nursing home residents, a significantly higher percentage of participants with dementia had a high amount of calculus, plaque, or gingival bleeding (40.4%), when compared to people without dementia (26.2%). A study by Warren et al. found that the Gingival Index was significantly higher in people with Alzheimer's disease (1.1) than in people without dementia (0.7), while people with dementia other than Alzheimer's disease scored not significantly different (0.9) from people with Alzheimer's disease and people without dementia (Warren et al., 1997).

Another studies show the oral hygiene measures in percentages and means in older people with dementia, compared with older people without dementia. Studies including the Plaque Index by O'Leary found a mean percentage of 63.4 to 90.1% in participants with dementia (Zenthöfer et al., 2014; Zenthöfer et al., 2015).

Studies using the indices by Greene and Vermillion found a Debris Index of 2.1, a Calculus Index of 2.0, and an Oral Hygiene Index of 4.5 in participants with dementia (Adam and Preston 2006; Fjeld et al., 2014). The Plaque Index by Silness and Loe was 0.7 in a study by Chalmers et al. and 2.5 in a study by Gil Montoya et al. Sumi et al. reported a Plaque Index by Quigley and Hein (modified by Turesky) of 1.6 in people with dementia (Chalmers et al., 2003; Sumi et al., 2012).

Factors influencing oral health, the ability to self care, routine access to, and provision of, oral care include: the severity and stage of the dementia, the individual's level of cognitive impairment, physical disability, lack of personal perception of oral health care problems, previous dental history, including oral health care and dental attendance, ability to receive oral hygiene care from carers and/or the dental team, impact of medication on the oral cavity, especially xerostomia (dry mouth), motivation and behaviour, capacity to consent to oral health care and knowledge of, and attitudes towards, oral care of health and social care workers and carers (Marchini et al., 2019).

3.3.5. Conclusions

Our results associates as predisposing factors the lack of information on how to access dental services, dental team's attitudes to, and awareness of, ageing and dementia, the lack of training and understanding by dental professionals in oral health care and strategic, long-term, treatment planning for people with dementia, dental personnel unwilling or unable to provide appropriate care and the site of oral care provision, e.g. dental surgery, day centre, at home.

3.4. Dental lesions in geriatric patients

3.4.1. Introduction

The geriatric patient is defined by age over 65 years. With increasing age, the risk of dental disease, such as caries, periodontal lesions, mucosal lesions and edentations increases. The main causes of dental affections in this group of patients are related to the medication they take for chronic illnesses and also because of changing eating habits, changing the quality and composition of saliva, and behavioral change in terms of oral hygiene (Kay et al., 2003; Wildiers et al., 2014; Batchelor 2015).

All these peculiarities of the geriatric patient lead to the elaboration of treatment principles, methods and therapeutic techniques that are distinct from the pathology of the adult.

In the context of global demographic aging by increasing life expectancy, the problem of the elderly dental patient has changed. At the same time, there is an improvement in dental hygiene, which allows old-aged patients to preserve their teeth. The geriatric dental patient is characterized by polypathology, multidiagnosis and plurimedication.

Management of this particular patient category is thus fundamentally different from adult patients and requires a multidisciplinary, biomedical, psychological, social, socio-demographic, administrative, legal, economic, ethical and affective approach.

The principles of modern treatment for this category of patients respect the minimally invasive *vs* maximum preventive concept, which requires minimal biological sacrifice. At the same time, consideration should be given, as much as possible, to the emotional and social comfort of these patients, by restoring the quality of mastication, phonation and orofacial aspect (Frincu et al., 2013).

The aim of this study was to highlight the oral health of elderly patients in our area, the possibilities of early detection of the factors favoring the dental pathology. Prophylactic treatment remains the key to "active aging" from a stomatologic point of view.

3.4.2. Materials and methods

The study was conducted on a number of 50 subjects, ages 55-85, who presented themselves for various dental symptoms or just a routine check. The study method consisted in corroborating thorough clinical examination with a specific anamnesis of the group of geriatric patients. We evaluated associated comorbidities together with caries frequency index, caries intensities index, grade of dental abrasion in order to establish peculiarities of dental caries in the elderly. We applied mathematical formulas in order to calculate caries frequency index (CFI), as follows: (number of patients with caries) x100 / (number of examined subjects) and the caries intensities index by formula IIC = (number of decayet teeth) x100 / number of examined subjects.

3.4.3. Results

The gender distribution of subjects in the study group is 52% female and 48% male. Of these, 24% are between 55-65 years of age, 42% between 66-75, 32% between 76-85 and 2% over 85.

We have customized the patients' medical history by grouping them into various types of diseases: cardiovascular, locomotor, hematopoietic, digestive, neuropsychic, respiratory, metabolic, endocrine and genito-urinary. Any of these comorbidities have been proven by a letter from the GP (**Figure 48**).

We also quantified the number of patients with one or more chronic diseases. Thus, 8% had only one chronic condition, 28% had 2 chronic conditions, and 64% had 3 or more chronic conditions. For all these associated pathologies, patients have already received a medication. We emphasize that all patients in the study group had at least one chronic condition. We took into account the calculation of the caries frequency index (CFI), which we calculated as follows: (number of patients with caries) x100 / (number of examined subjects) (**Figure 49**).

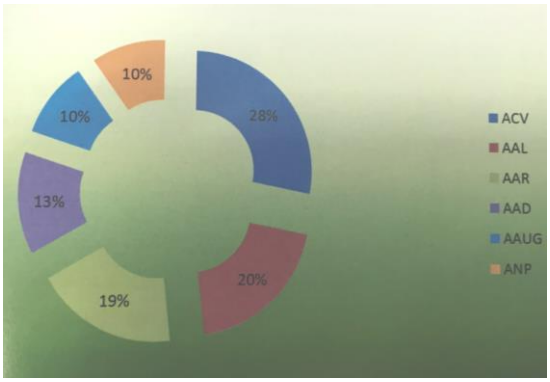


Figure 48 - ACV = cardiovascular disease, AAL = locomotor system disorders, AAR = respiratory diseases, AAD = digestive system disorders, AAUG = urogenital disorders, ANP = neuropsychiatric disorders

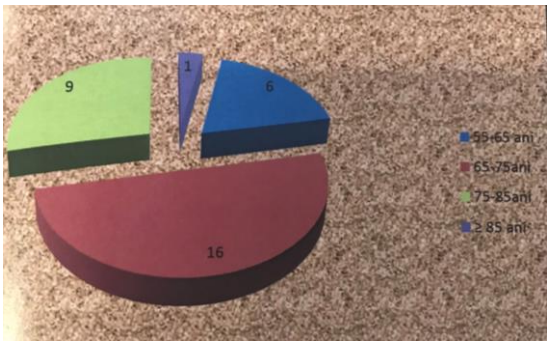


Figure 49 - Calculation of the caries frequency index

We calculated the caries intensities index by formula IIC = (number of decayet teeth) x100 / number of examined subjects (**Figure 50**).

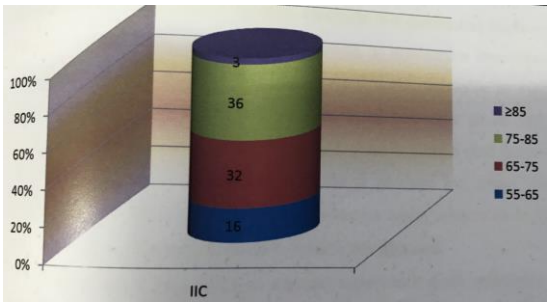


Figure 50 - Calculation of the caries intensities index

Dental caries have peculiarities in the elderly: it appears as a deep cavity, frequently with the opening of the pulp chamber due to a low level of pulp innervation. Of the types of caries discovered, the root ones are more in number than the coronary ones (75.86%).

In the studied group, the most commonly encountered abrasion rate was II, 42%, followed by Grade III, 36%. 18% of patients had a degree of abrasion I, but associated with severe forms of marginal parodontopathy.

4% of patients had advanced, non-uniform and age-related abrasion with old, partial, untreated edentations or incorrect prosthetics, due to the association of 3-4 chronic diseases with systemic involvement and history of neoplastic digestive tract (**Figure 51**).

Figure 51 - A - Grade 3 dental abrasion; B - severe dental abrasion

Grade III and advanced abrasives primarily affect the front, maxillary and mandibular dental groups and lead to exposure of dentin islands.

All subjects with remaining teeth exhibited inflammation of the marginal paradon after the increased of retention spaces, due to multiple chronic systemic disorders. 87% of patients had gingival retraction on the background of involutive tissue changes maintained by overloading the reduced number of restant dento-periodontal units (**Figure 52**).

Figure 52 - A - severe abrasion of mandibular frontal teeth; B - Inflammation of the marginal periodontium; C - exposure of dentine islands

Patients in the first age group (55-65) had arcades with partial edentation (42%), subtotal (2%) and total (4%). 66-75 year old patients had partial edentation (50%), subtotal (18%) and total (10%). Subjects aged between 76-95 had partial edentation (44%), subtotal (10%) and total (10%). Patients over the age of 85 were 4% with partial edentation.

We found that subtotal edentation is more common at the maxillar level and the total one, at the mandibular level.

3.4.4. Discussions

The dental care, either simple or complicated treatments, bring patient in a dependent status and inspire feelings of fear. The patients with positive experiences in dental treatments will come with optimism and confidence, while patients with negative experiences will be stressed and cautious.

The main fear of the patient is pain. He will be concerned about the method, technique and quality of anaesthesia and surgery procedure. He will ask also about possible defficiencies regarding the onset and intraoperatory period under anaesthesia that could favourise pain during the surgical intervention or during the postoperatory period. Also, the dentists meet less confident patients due both to actual „colder" relation with their dentists and various bureaucratic procedures related to practical guides, anamnesis, interpretation of the clinical and paraclinical data, analyses and imagistic tests.

To avoid the increase of the psychologic stress for the patients, the dentists must reduce and adapt all these stages to reduce times and emotional concern. Any proposal for a dental treatment is recorded as an event loaded with unknowable situations and risks. The dentist must explain with patience, by giving positive examples and creating a professional and calm atmosphere. It must be established the psychological status of the patient and his feeling related to the dentist and the proposed therapy.

The dentist must combine his theoretical and practical knowledges with a visible interest for the patient as a human being with feelings and needs of communication and

affection. The clinician must use his ability to understand and react based on intuition, to be able to focus on the psychological features of every patient.

We find that the data obtained in this study is partly the same with the data described by the literature. We report similarities in the prevalence of root caries in the elderly, which in the study group ranges between 50-80% of the literature (Gershen et al., 1991; Steele et al., 2001). This is explained by the decrease of the incidence of root caries with the age and the rate of edentation, by the tendency to preserve as long as possible the dento-periodontal units. For this reason, the number of exposed root surfaces will increase as a result of the gingival retraction and risk factors (Ryan et al., 1998; Laguna et al., 2016).

Congenital disorders that alter the masticatory function have repercussions on the dental device. One of the examined subjects, who had advanced dental abrasion and strong gingival retraction, suffered from left facial nerve atresia. In this context, the aforementioned dental conditions were at a much advanced stage on the homoleteral side of the congenital pathology.

Also, the high frequency of root caries in elderly coupled with dental abrasion and paradontal disease in patients in our study group is similary to that in the literature (Ashwini et al., 2018).

We noticed a higher frequency of maxilar subtotal and mandibular total edentation in patients in our study group, compared to the literature, that shows that the last teeth that are lost are the lower frontal ones.

The frequency of tooth loss is the following: mandibular molars, followed by maxillary molars, then maxillary and mandibular premolars, incisors, superior canines, and then inferior incisors and canines.

At this point, it has been shown that caries are an extremely complex process with multifactorial and heterogeneous determinants, but with peculiarities that depend on patient age and that need to be known. Among the known factors to promote dental decay are: food (carbohydrates and sweets), oral hygiene, salivary disease, bacterial biofilm, socioeconomic standard, hereditary factors and geographical area.

Three of the patients aged 66-75 had a history of colorectal neoplasia. In these cases, we noticed subtotal (2 cases) and total (one case) edentations, with significant gingival retraction. This demonstrates systemic damage of paraneoplastic syndrome and adjuvant oncology treatment (Hînganu et al., 2016).

3.4.5. Conclusions

The systemic characteristics of the pathology of the elderly personalize the oral pathology by changing the local terrain on which they intervene (Britton et al., 2016). Careful observations of elderly patients show structural, functional and behavioral differences compared to those of youth and mature age.

Our study shows that dental disease has an increased incidence among elderly patients. Changes in dental structures are factors that increase the frequency and intensity of carious processes in these patients. Caries lesions are less painful, but they evolve much faster. The aging of the marginal periodontium favors the occurrence of periodontitis. Edentation causes dental migrations and malocclusions.

SECTION II - PLANS FOR FEATURE RESEARCH

The proportion of older people continues to grow worldwide, especially in developing countries. Non-communicable diseases are fast becoming the leading causes of disability and mortality, and in coming decades health and social policy-makers will face tremendous challenges posed by the rapidly changing burden of chronic diseases in old age. Chronic disease and most oral diseases share common risk factors. Globally, poor oral health amongst older people has been particularly evident in high levels of tooth loss, dental caries experience, and the prevalence rates of periodontal disease, xerostomia and oral precancer/cancer. The negative impact of poor oral conditions on the quality of life of older adults is an important public healthissue, which must be addressed by policy-makers.

The means for strengthening oral health programme implementation are available; the major challenge is therefore to translate knowledge into action programmes for the oral health of older people. The World Health Organization recommends that countries adopt certain strategies for improving the oral health of the elderly. National health authorities should develop policies and measurable goals and targets for oral health. National public health programmes should incorporate oral health promotion and disease prevention based on the common risk factors approach. Control of oral disease and illness in older adults should be strengthened through organization of affordable oral health services, which meet their needs.

The needs for care are highest among disadvantaged, vulnerable groups in both developed and developing countries. In developing countries the challenges to provision of primary oral health care are particularly high because of a shortage of dental manpower. In developed countries reorientation of oral health services towards prevention should consider oral care needs of older people. Education and continuous training must ensure that oral health care providers have skills in and a profound understanding of the biomedical and psychosocial aspects of care for older people. Research for better oral health should not just focus on the biomedical and clinical aspects of oral health care; public health research needs to be strengthened particularly in developing countries.

II.1. Predisposing systemic factors affecting oral health in elderly

Operational research and efforts to translate science into practice are to be encouraged. What roles do individual senior citizens play in your life? How do you relate to and interact with older people? What role do they play in neighborhoods and communities, in cities and in states? *From these considerations and from my personal experience I propose to develop two major directions of retrospective research and perspective.* ***The first*** *of these refers to establishing the degree of* ***risk of geriatric patients from the perspective of extrinsic and comorbidities factors****. Thus, I intend to conduct a study on demographic criteria on the topic of social integration or reintegration of geriatric patients in the dental field. The study will be multicentric and will have to run for a minimum of 5 years. We will correlate the results of this study with the evaluation of the impact of* ***diabetes mellitus*** *on these patients..* ***The second research direction*** *is convergent to the first and refers to the a protocol for the*

evaluation and treatment of geriatric dental patients that correlates the most common comorbidities with their sex, age and general condition. In this research direction we will start with the study of ***chronic diseases acquired in the field of otorhinolaryngology*** *following dental treatment maneuvers.*

II.1.1. Diabetes mellitus scoring in elderly dental patients

Diabetes mellitus (DM) is a disease caused by absolute or relative insulin insuficiency; as such it represents a disease that, although not directly treated by the dentist must be known to him in all its inherent factors. Diabetes mellitus is one of the most prevalent diseases in the world, causing severe, sometimes disabling complications. Diabetes mellitus is considered a public health issue, due to the high mortality rate exhibited by affected patients. It is of chronic evolution and inevitable complication, with irreversible lesions of organs vital to life, it causes high mortality rates and affects patient's average survival rates. Diabetes can be found in two types: type I is insulin-dependent and more frequently found in young patients, type II is non-insulin dependent, it is a chronic disease characterized by exhibiting high glucose levels in the blood, cell resistance to insulin action or by a decrecient insulin secretion. DM2 is more common than DM1 and represents 85 to 90% of all diagnosed cases. It is related to risk factors that can be modified by the subject, such as obesity or overweight, physical inactivity, poor oral hygiene habits as well as low nutritional value and hypercaloric feeding habits.

Within diabetes most relevant oral and dental alterations we find signs such as unexplained xerostomy, intense or chronic fungus-related oral candidiasis, burning sensation in the tongue, extensive caries, multiple and recurrent abscesses, gingivitis, rapid progressing periodontitis, canker sores, mean rhomboidal glossitis, increase of salivary glands, persistent oral ulcers, lichen planus, dry alveolitis after extraction, delayed post extraction or surgery healing.

Along the course of history it has been suggested that certain clinical manifestations are more frequent in diabetic patients, nevertheless, results obtained in different studies are contradictory. Presence of these oral manifestations can, in some cases hinder one of the most important proposed achievements which is improving quality of life. This is due to the fact that oral and dental alterations can entail oral pain and/or discomfort, preventing suitable feeding; furthermore they can worsen the diagnosis of the main disease since they frequently alter, reverberate and aggravate some processes affecting certain organs.

Conversely, knowledge of these manifestations can alert the dentist on the existence of a non-diagnosed diabetic patient, and thus help him to achieve suitable diagnosis and oral treatment plan, without fearing unexpected complications. Once the disease has been identified (based on questionnaire, signs, symptoms observation and a blood glucose test) dentists must refer patients to a physician to be evaluated. In the present institution, classification is made according to whether it be type I (insulin dependent) or type II (non insulin dependent). Type I diabetes mellitus is a chronic, autoimmune entity associated to the selective destruction of beta cells in the Langerhans islets, patients thus affected require insulin in order to survive. Type II diabetes mellitus, frequently associated to obesity, is the most common cause for hypoglycaemia in adults, with resistance to ketoacidosis and does not

require insulin administration for survival, although it could be administered in order to better control blood glucose.

Gestational diabetes is characterized by glucose intolerance during pregnancy. The World Health Organization (WHO) estimates there are over 220 million diabetic subjects in the world. Almost 80% of all deaths caused by this disease occur in countries with low or middle income. In 2000, there were already 165 million people afflicted with diabetes, 239 million were predicted for 2010 and 300 million for 2025.11 Out of the 165 million diabetic subjects, 35 million lived in the American Continent, out of these, 19 million resided in Latin America and the Caribbean.

Do Langerhans cells behave similarly in elderly and younger patients with chronic periodontitis?

This research direction represents a team and multicenter effort. We aim to obtain the necessary funds to carry out this project, in order to co-opt as many research centers as possible. The research team will be mixed, consisting of nutrition and metabolism doctors, dentists and family doctors. Thus, we aim to identify the dental problems of the population that is evident with this pathology and to follow them for a period of at least 5 years through periodic dental checks. The final goal of the study is to develop a standardized protocol and a scoring system for monitoring and controlling dental diseases in the elderly population.

II.1.2. Odontogenic sinusites as dental procedure accident in elderly patients

The aetiological relationship between dental disease and procedures and mucosal disease within the maxillary sinus has received extensive attention in the recent otolaryngological and dental literature. In contrast, the concept of an odontogenic cause for sinusitis is not well appreciated by radiologists. Review of the maxillary dentition, the alveolar process, and the relationship of the tooth roots to the floor of the maxillary sinus should be an integral part of interpretation of imaging of the paranasal sinuses. The pathogenesis, clinical presentation, and imaging features of rhinogenic and odontogenic sinusitis are discussed and compared.

Clinical definitions of rhinosinusitis are explained and the huge impact on healthcare of this disease is briefly discussed, especially for elderly patients. Periapical inflammatory lesions, post-extraction oroantral communication, and procedures used to augment the alveolar process prior to placement of dental implants are the commonest causes of odontogenic sinusitis. Current estimates are that an odontogenic cause for maxillary sinusitis is present in 25-40% of cases. The incidence of odontogenic sinusitis is rising, extension outside the maxillary sinus is common, and the diagnosis is often delayed, resulting in inappropriate and failed treatment. Differentiation of rhinological and odontogenic causes of sinusitis is usually difficult on clinical grounds and imaging plays a key role in the distinction.

This research direction is oriented towards the realization of a close collaboration between us and the local implantology centers. Most otorhinolaryngological problems due to dental implant occur as a result of dental implant maneuvers. We propose that, together with colleagues specializing in otorhinolaryngology and oromaxillofacial surgery, we develop a protocol for the preoperative evaluation of elderly patients who need and are suitable for

dental implants. This protocol must be performed based on criteria related to the general condition of the patients' health condition but also from the point of view of the quality and quantity of the basic bone material.

II.2. Dental implications of common movement disorders

II.2.1.Dyskinesias in geriatric patients

Movement disorders – or dyskinesias – are characterized by involuntary movements. Despite the major role for medical specialists in the diagnosis and treatment of dyskinesias, dentists are confronted with such disorders as well. Unfortunately, the literature regarding the dental implications of movement disorders is still scarce. This concise review describes the dental implications of some common dyskinesias, viz., Gilles de la Tourette's syndrome, Huntington's disease, idiopathic torsion dystonia, oral dyskinesias, and Parkinson's disease.

It was concluded that these dyskinesias may have profound dental implications. Not only do generalized dyskinesias have focal manifestations in the orofacial region, but there are also dyskinesias that exclusively affect the orofacial area. The oral manifestations of dyskinesias are in part directly related to the disorder, and in part medicine-related. Dentists should be able to recognize the oral manifestations and, when properly trained, to manage them adequately. In most instances, a multidisciplinary approach upon referral is necessary, including the medical specialists involved. Unfortunately, the level of evidence of the selected papers was generally low. In our rapidly ageing population, it is a challenge for all of us to improve the quality of this emerging field, for the sake of this sometimes heavily infirmed category of patients.

We intend to run this reaserch in order to assess the frequency and the degree of involuntary dental movements. I want to focus especially on edentulous dyskinesias and oro-mandibular dystonia. In most instances, a multidisciplinary approach is necessary, including the medical specialists involved. We intend to perform clinical observational trials on elderly patients reffered to teeth extractions. Thus, we will monitor patients every 6 months for a period of 2 years postinterventional in order to establish the frequency and clinical forms in which this pathology occurs.

II.2.2. Age-induced changes in the teeth and their attachment apparatus

Both the hard substances and the soft tissues of the teeth and their attachment apparatus are subject to constant change. This begins immediately after eruption and continues throughout life. An exact dividing line between changes which are physiological and pathological cannot always be drawn. Enamel undergoes attrition, and in addition its mechanical characteristics alter, owing probably to changes in diffusion conditions. The age-induced changes occurring in dentine are much more obvious, the biological properties of this hard substance being fundamentally altered. The dentine of older people is characterized by the continuous narrowing of the lumen of the dentinal tubule, increasing calcification, reduction in the amount of peritubular fluid and reduced sensitivity. In this process, dentine becomes able to assume the function of enamel as it wears. With age cementum undergoes

continuous deposition, mainly functionally induced. It is evident, even macroscopically, that the volume of the pulp declines owing to the deposition of secondary dentine or of amorphous dentine with age. Histologically, young pulp differs fundamentally from that of the pulp of an older person. Regressive processes commence immediately after tooth eruption. The number, nature, properties and capabilities of the cells change, but the pulp does not suffer any appreciable loss of vitality. Circulation in the pulp is affected by deposition of hard substance in the apical part of the root canal. These processes are important in endodontics, and because of them different treatment methods have to be used for patients of different ages. The tooth supporting tissues are also subject to constant rearrangements, the physiological occlusal and mesial movements of the teeth being relevant here. All these structural and biological differences must be allowed for when therapy is being considered. They have not hitherto been taken sufficiently into account.

Within this research direction we want to highlight the phenomena of tooth repositioning that appear with age. We aim to perform 3D-CBCT examinations on elderly patients, with the help of which we can perform accurate topographic measurements based on fixed reference criteria (spine, hard palate, mandible condyle). Following these values over time, we can conclude how aging affects dentition and, implicitly, mastication.

Biting force and tongue muscle strength as useful indicators for eating and swallowing capability assessment among elderly patients. They could be measured with specific equipment we intend to purchase.

II.3. Didactic challenge

Internationally, the inclusion of geriatrics within dental curricula has been the subject of consideration since the 1970s. The current evidence indicates that geriatrics/gerodontology is not a significant component of dental curricula. Given the projected age distribution in many countries, the need for implementation of dental curriculum content in the area of geriatrics/gerodontology is evident. Dentistry teaching has sought to connect with the national curricular guidelines. However, the simple insertion of a geriatric dentistry module in the curriculum is not sufficient to promote a teaching-learning process that allows the student to develop skills to provide better care for the elderly.

My experience of over 19 years in dental geriatrics allows me to conclude that this field has and demands special professional and educational features. The curricular adoption of dental gerontology must be correlated adapted to the professional training of specialists in this field, from the faculty benches. This requires both in-depth general medical knowledge and psychological and psychopedagogical skills.

Throughout my career I have deepened my knowledge in related fields such as the demographic, sociological and psychological expertise of the elderly dental patient. It is from these perspectives that I want to open up research directions in these fields at the moment, which will allow us to take academic and curricular research to a higher level.

II.3.1. General objectives - SocioDemographic Research Direction

What roles do individual senior citizens play in your life? How do you relate to and interact with older people? What role do they play in neighborhoods and communities, in cities and in states? Sociologists are interested in exploring the answers to questions such as these through three different perspectives: functionalism, symbolic interactionism, and conflict theory.

II.3.1.1. Functionalism

Functionalists analyze how the parts of society work together. Functionalists gauge how society's parts are working together to keep society running smoothly. How does this perspective address aging? The elderly, as a group, are one of society's vital parts. Functionalists find that people with better resources who stay active in other roles adjust better to old age. Three social theories within the functional perspective were developed to explain how older people might deal with later-life experiences. An elderly man and woman are shown from behind sitting on a bench. The man is shown wrapping his arm around the woman's shoulders. Does being old mean disengaging from the world?

The earliest gerontological theory in the functionalist perspective is disengagement theory - Cummings and Henry 1961 - which suggests that withdrawing from society and social relationships is a natural part of growing old. There are several main points to the theory. First, because everyone expects to die one day, and because we experience physical and mental decline as we approach death, it is natural to withdraw from individuals and society. Second, as the elderly withdraw, they receive less reinforcement to conform to social norms. Therefore, this withdrawal allows a greater freedom from the pressure to conform. Finally, social withdrawal is gendered, meaning it is experienced differently by men and women. Because men focus on work and women focus on marriage and family, when they withdraw they will be unhappy and directionless until they adopt a role to replace their accustomed role that is compatible with the disengaged state.

The suggestion that old age was a distinct state in the life course, characterized by a distinct change in roles and activities, was groundbreaking when it was first introduced. However, the theory is no longer accepted in its classic form. Criticisms typically focus on the application of the idea that seniors universally naturally withdraw from society as they age, and that it does not allow for a wide variation in the way people experience aging.

The social withdrawal and its notion that elderly people need to find replacement roles for those they've lost, is addressed anew in activity theory. According to this theory, activity levels and social involvement are key to this process, and key to happiness - Havinghurst 1961; Neugarten 1964; Havinghurst, Neugarten, and Tobin 1968. According to this theory, the more active and involved an elderly person is, the happier he or she will be. Critics of this theory point out that access to social opportunities and activity are not equally available to all. Moreover, not everyone finds fulfillment in the presence of others or participation in activities. Reformulations of this theory suggest that participation in informal activities, such as hobbies, are what most effect later life satisfaction.

According to continuity theory, the elderly make specific choices to maintain consistency in internal (personality structure, beliefs) and external structures (relationships), remaining active and involved throughout their elder years. This is an attempt to maintain

social equilibrium and stability by making future decisions on the basis of already developed social roles. One criticism of this theory is its emphasis on so-called "normal" aging, which marginalizes those with chronic diseases such as Alzheimer's.

II.3.1.2. The three classic theories of aging

Modernization theory (Cowgill and Holmes 1972) suggests that the primary cause of the elderly losing power and influence in society are the parallel forces of industrialization and modernization. As societies modernize, the status of elders decreases, and they are increasingly likely to experience social exclusion. Before industrialization, strong social norms bound the younger generation to care for the older. Now, as societies industrialize, the nuclear family replaces the extended family. Societies become increasingly individualistic, and norms regarding the care of older people change. In an individualistic industrial society, caring for an elderly relative is seen as a voluntary obligation that may be ignored without fear of social censure.

The central reasoning of modernization theory is that as long as the extended family is the standard family, as in preindustrial economies, elders will have a place in society and a clearly defined role. As societies modernize, the elderly, unable to work outside of the home, have less to offer economically and are seen as a burden. This model may be applied to both the developed and the developing world, and it suggests that as people age they will be abandoned and lose much of their familial support since they become a nonproductive economic burden.

Another theory in the conflict perspective is age stratification theory (Riley, Johnson, and Foner 1972). Though it may seem obvious now, with our awareness of ageism, age stratification theorists were the first to suggest that members of society might be stratified by age, just as they are stratified by race, class, and gender. Because age serves as a basis of social control, different age groups will have varying access to social resources such as political and economic power. Within societies, behavioral age norms, including norms about roles and appropriate behavior, dictate what members of age cohorts may reasonably do. For example, it might be considered deviant for an elderly woman to wear a bikini because it violates norms denying the sexuality of older females. These norms are specific to each age strata, developing from culturally based ideas about how people should "act their age."

Thanks to amendments to the Age Discrimination in Employment Act (ADEA), which drew attention to some of the ways in which our society is stratified based on age, U.S. workers no longer must retire upon reaching a specified age. As first passed in 1967, the ADEA provided protection against a broad range of age discrimination and specifically addressed termination of employment due to age, age specific layoffs, advertised positions specifying age limits or preferences, and denial of healthcare benefits to those over sixty-five years old (U.S. EEOC 2012).

Age stratification theory has been criticized for its broadness and its inattention to other sources of stratification and how these might intersect with age. For example, one might argue that an older white male occupies a more powerful role, and is far less limited in his choices, compared to an older white female based on his historical access to political and economic power.

Finally, exchange theory (Dowd 1975), a rational choice approach, suggests we experience an increased dependence as we age and must increasingly submit to the will of others because we have fewer ways of compelling others to submit to us. Indeed, inasmuch as relationships are based on mutual exchanges, as the elderly become less able to exchange resources, they will see their social circles diminish. In this model, the only means to avoid being discarded is to engage in resource management, like maintaining a large inheritance or participating in social exchange systems via child care. In fact, the theory may depend too much on the assumption that individuals are calculating. It is often criticized for affording too much emphasis to material exchange and devaluing nonmaterial assets such as love and friendship.

II.3.2. Dental Gerontology Perspectives

Changing demography due to the increasing population of elderly persons the world over has raised new challenges in every sphere of life. The greatest challenge is to provide affordable, accessible, and equitable health care to this population. Oral health is an integral part of general health and affects physical and mental well-being and quality of life of elderly persons. To provide quality oral health care to the elderly, it is important to focus on education in geriatric dentistry, since it is known that education is closely linked to health care provision. It has been found that education in geriatric dentistry has wide variations in different parts of the world. Also, it is being taught at different levels: the predoctoral curriculum, postdoctoral certificate/diploma courses of varying duration by direct or distance mode using computer-assisted learning, or continuing education programs.

II.3.2.1 Academic educational perspectives - Geriatric Dentistry Teaching Research Direction

Implementing and varying concepts and methods of teaching, learning, and assessment in dental education are compulsory and urgent. This study direction acts as a response to the World Health Organization "Call for Public Health Action" on the oral health of older people and the need of adapting our syllabus to the international ones.

Evidence-based health care educational interventions include knowledge, critical appraisal skills, attitudes, behaviors, or performance related to the practice of evidence-based oral health care and patient health outcomes. However, the gold standard for health care, randomized clinical trials (RCTs), has limited relevance in the education context, given the numerous variables that cannot be controlled or explained. It is also critical that evidence-based education is not interpreted too narrowly with a focus on “what works” when, in fact, research of practice only provides knowledge of “what worked.”

Competency-based dental education introduced in 1993 is based upon early course planning of clearly specified outcomes of learn-ing, as against the traditional approach, which was mainly discipline-based. A competency describes the skills, understanding, and professional values of an individual ready for beginning independent health care practice.19 It is important to emphasize that it is the responsibility of the learner

to constantly update his or her competence by reflection on experiences, attending continuing education programs, and post-doctoral courses.

Outcome-based education is similar to competency-based education. It focuses on learning outcomes and not on learning objectives. It was described as learning outcomes for the dentist, emphasizing the need to keep them unambiguous and manageable without overwhelming details. The learning outcomes are described in a simplified model of three circles: what the doctor is able to do, how the doctor approaches his or her practice, and the doctor as a professional, which is adapted to dentistry.

In inquiry-based teaching-learning (IBTL), inquiry is the umbrella concept partnered with teaching and learning. This may reflect a greater emphasis at school level on the process of learning and development of, for example, sciences as inquiry. At the university level, especially in medical education, the problem-based rather than inquiry-based concept is more popular (such as solving a problem), visible, and influential. It seems to be a tightly structured, problem-solving tutorial process in a resource-rich environment for a smaller, high-achieving, and motivated group of students. As against this, the IBTL tutorial process was less tightly structured for a larger group of students with average academic achievements in a resource-poor environment.

Use of Newer Technology in Teaching and Learning: information and communication technology (ICT), interactive multimedia, also known as "rich media" (which combine text, illustrations, videos, etc. with feedback), Virtual learning environments (VLE) or virtual classrooms and virtual reality.

II.3.3.2. Regional and nationwide evaluation systems in challenging the developing of geriatric dental patient national status

The status of geriatric dental education between health workers and population is essentially to be registered and monitored, in the context of increasing the life expectancy. This research direction means the formation of a working group composed of experts in this field from the country, who will work together to establish a national database of geriatric dental patients. Based on the data collected, the correlation will be made with the data collected by colleagues from abroad and the establishment of general and demographic protocols for the management of these patients. Thus we intend to establish "The Guidelines for the Geriatric Dental Patient in Romania" and to reassess it periodically.

III. The two research directions are interdependent and convergent to a third - Curricular and multicenter research direction in establishing the basis of departure in a new medical specialty in Romania - Dental Gerontology

The Harvard School of Dental Medicine has offered a geriatric dentistry program since 1986. The program offers a certificate in geriatric dentistry (two years in length), a Master of Medical Sciences degree (MMSc—three years, or two years if the candidate holds a prior MPH or equivalent degree), and a Doctor of Medical Sciences degree (DMSc—four years).

Following their program entitled Advanced Graduated Education Program in Geriatric Dentistry (HSDM) as a guideline, a two-year Multidisciplinary Geriatric Program in Dentistry, Medicine, and Mental/Behavioral Health, can be applied to train a dental fellow in the care of older adults, with a focus on underserved populations. The interdisciplinary

program will consists of four main components: coursework, clinical training, teaching/administrative training, and research. This program will prepare a specialist in geriatric dentistry to acquire the skills, experience, and knowledge to both treat patients in an institutional, community, and private setting and contribute to the academic and professional development of geriatrics.

We will start a research project with internal or external funding in this direction, through which we will create a pilot program to evaluate the need and adapt the way of preparation of a dental specialist in geriatric dental medicine.

II.3.2.3. *Academic education in geriatric dentistry - Preparing for the Future*

A multipronged approach is required to establish education in geriatric dentistry:

Sensitization of students during their predoctoral education. All preclinical and clinical subjects should gradually introduce the subject to the students.

Postgraduate diploma/certificate courses. Since at present, there is a dearth of trained faculty in the specialty, e-learning and distance learning may be a solution. In this venture, there is a vast potential for utilizing international expertise and developing collaborations with universities, where these programs are well established.

Continuing dental education. This mode of training for professional development of interested dental surgeons, hygienists, dental nurses, and chair-side assistants can be an effective tool to improve oral health care delivery to the elderly patients.

Dental gerontologists view aging in four distinct processes:

- Chronological aging - is the definition of aging on the bases of a person's years from birth. It is not necessary related to biologic or physical age.
- Biological aging - physical changes that reduce the efficiency of the systems. It is caused by the decline of the cell replications and the loss of certain types of cells that do not replicate.
- Social aging - individual's changing of role and relationships with family, friends and within organizations.

As stakeholders of dental gerontology we face important issues and obstacles:

- the current oral health services and access to oral health services for dependent older people;
- Oral Health Problems - preventing, diagnosing and treating;
- Caregivers' stressful conditions;
- Barriers associated with accessibility;
- Lack of dental health professionals;
- Low awareness of the importance of oral health;
- Dental beliefs and negative attitudes;
- Lack of oral care knowledge and practical skills;
- Insufficient and inappropriate oral care instruments.

The best option to serve the residents would be "home dentistry or domiciliary dental care", however it is yet an infrequent practice in Romania. Surveys should be conducted in this sector very routinely to spot the residents in the need of oral care circumscribing nursing homes, old age homes, ashrams, secure units, and community households.

This research direction requires more than any other joint effort at local and national level. We intend to create a database of patients addressed to us, with the opinion of the

research ethics commission and after obtaining the informed consent of them or their affiliates. Starting from this we can develop as a pilot program the direction of research in evidence based medicine, as a pilot program, extracurricular in the first phase.

Depending on the results obtained in the first phase of the project we will be able to move to the second one, namely the collaboration with the state and private clinics in our region, in order to establish protocols for students to have access to their clinical, paraclinical and radiological examination, both in emergency and elective. In the third phase of the project we have to implement the EMSA (European Medical Students Association) recommendations related to the motivational criterion applied to both students and teachers. This third stage is, practically one of training and motivation of the existing academic body, in the direction of teaching Dental Gerontology and of recruiting new colleagues.

II.3.2.4. National guideline and protocol research

We plan to extend our academic connections throughout Romania and to establish a national protocol for diagnosis and care of this particular segment of dental patients. For this we consider as gold standard the already existing protocols and guidelines in EU, UK and US. This research has a start up point the elderly dental patient status in Romania. Using advanced statistical techniques we will draw a parallel between the status of the dental geriatric patient in our country and the protocols outside the country.

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