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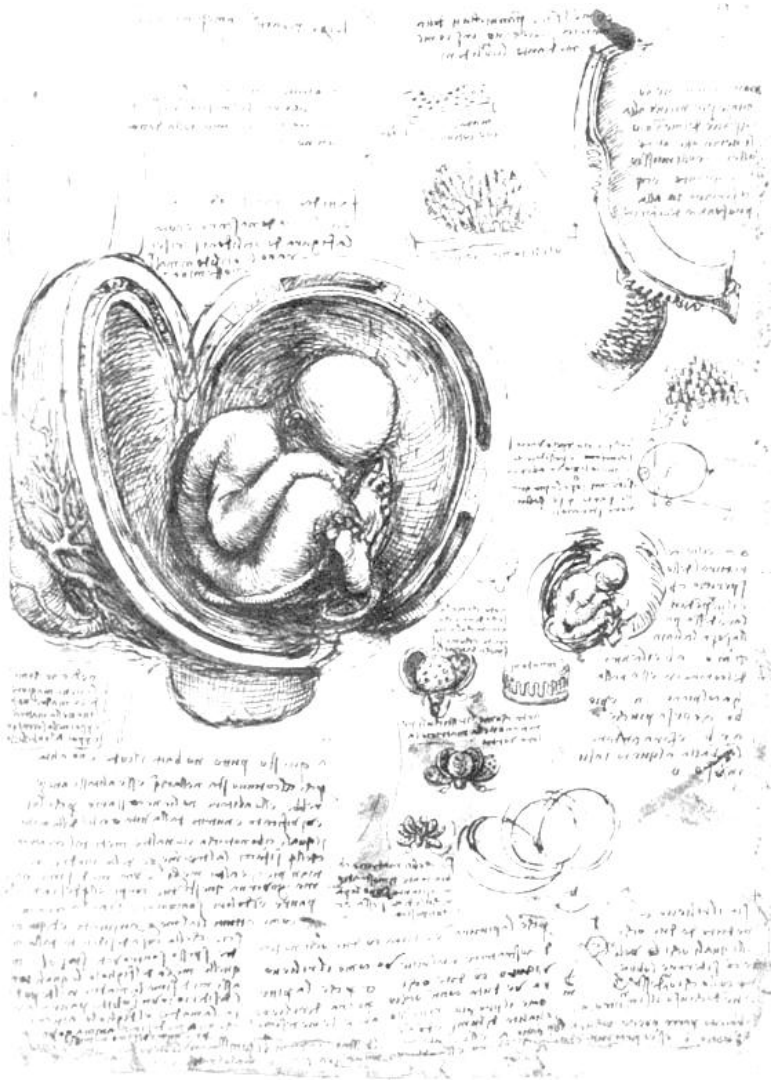

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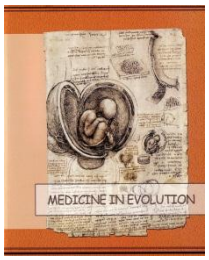
THE SCHIZOPHRENIA PATTERN: ASSOCIATIONS WITH THE SHANK3 GENE – A CASE PRESENTATION



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ABSTRACT

Schizophrenia is defined as a complex disorder that is due to the polygenic effect of several genes in interaction with epigenetic factors. We are presenting the case of a 21 year old female with a psychopathological picture that consisted of: psychomotor agitation, a suicide attempt by defenestration, which was secondary to delusional ideation, auditory and visual hallucinations, anxiety, lack of insight, mixed insomnia. Our case, diagnosed with Schizophrenia, also presents manifestations that are characteristic for Autistic Spectrum Disorders. Based on her academic performances, our patient might have a superior than average intellect. The result of the arrayCGH showed several genomic imbalances, including the SHANK3 gene imbalance. For this case, the overexpression of the SHANK3 gene can be linked with the schizophrenic manifestations and the autistic features, with probably no influence on the intellect. We suggest that the genomic evaluation of patients with schizoaffective manifestations is very useful, in order to elucidate the implication of genomic rearrangements in the complex and heterogenic etiology of the schizophrenic disorder.

Keywords: Schizophrenia, Autistic Spectrum, SHANK3 gene

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INTRODUCTION

Schizophrenia is one of the major illnesses in contemporary psychiatry that manifests clinically through symptoms, such as: bizarre delusional ideas, auditory hallucinations, broadcasting of thought, controlled thought, bizarre behavior, formal thought disorders, affective blunting. Along with these clinical manifestations, cerebral neuro-imaging alterations, as well as genetic variations can be observed. Schizophrenia is defined as a complex disorder that is due to the polygenic effect of several genes in interaction with epigenetic factors.

Regarding the issue of genetic alterations, there are multiple clinical studies that have attempted to identify the changes at a gene or chromosome level [1]. Starting with the studies that identified CNVs as Schizophrenia candidate loci, an overview of the mechanism and genetic pattern of the disorder was brought. The etiopathogeny of Schizophrenia, a neurodevelopmental disorder, includes an important genetic contribution, and, to date, several candidate genes and specific loci were identified. A well-known risk CNV associated with a high probability to develop Schizophrenia is the deletion 22q11.2. Other risk loci where found on different genomic regions, such as 1q21.1, 3q29, 15q11.2, 15q13.3, 16p11.2, 16p13.1, 17q12 [2]. Among the candidate gene an important part are key players in the neurodevelopmental processes and neural plasticity [3, 4].

The SHANK3 gene alterations have been identified in subjects suffering from Schizophrenia and/or Autistic Spectrum Disorders [5]. The Autistic Spectrum is comprised of the Asperger's Syndrome, Heller Syndrome, Rett Syndrome, Atypical Autism, Disorganized Schizophrenia, as well as, although somewhere marginally placed, Schizoid Personality Disorder and Obsessive Compulsive Disorder.

CASE PRESENTATION

We are presenting the case of a 21 year old female that was presented to the psychiatry emergency room in September 2011, having been brought by her family, for psychopathological features that consisted of: psychomotor agitation, a suicide attempt by defenestration, secondary to delusional ideation, bizarre delusional ideas, auditory and visual hallucinations (denied by the patient, observed by the psychiatry consultant), anxiety that was secondary to the delusional experiences, lack of insight, mixed insomnia. We should mention that the data have been gathered by means of clinical interview with the patient, as well as by discussions with family members. The onset of the illness was insidious, 3 years previously to the first admission, with significant cognitive dysfunctions that have led to a reduced educational performance, social withdrawal and anxiety regarding the end of the world in 2012, as well as changes in the Zodiac. Among the clinical elements that have constituted the prodrome, the following should also be noted: lack of personal hygiene, bizarre behavior with selective mutism, unmotivated rictus, and episodes of verbal and physical hetero-aggression.

The first contact with a psychiatric service was established at the beginning of 2011 through the clinical ambulatory service, where antipsychotic treatment was instituted. The clinical evolution under treatment was oscillating, with periods of symptom amelioration, alternating with periods of intensified symptoms, possibly due to the

discontinuity in treatment administration (with a non-adherent patient).

Afterward, the patient had three more admissions with the same diagnostic at short intervals, due to the fact that immediately after discharge she refuses to continue taking the medication.

The biographical history of the patient shows that she comes from a family where the parents were married, both having a high educational status (the age at the birth of the patient was of 29 years for the mother and 30 for the father). From what we gathered from the interview with the parents, it is worth mentioning that there were no issues during pregnancy or birth and that later the neuropsychological development of the child was normal, without any incidents.

Regarding the educational-professional level, the patient has always had superior than average results in school, having won numerous awards in national Olympic competitions. After the first few months at University, she shows a decreased interest in educational activities, with absenteeism and reduced communicational skills, as well as social withdrawal.

As a premorbid personality, the parents have stated that she had always shown an increased need for solitude, interior pliability and contemplative activities, ever since childhood.

The somatic examination did not show any pathological aspects.

The mental examination during admission was difficult to conduct due to increased hostility and suspiciousness of the patient. She presented with persecutory delusional ideas, prejudice delusional ideas, auditory and visual hallucinations, transparence-influence of thoughts phenomena, bodily perceptual disorders, hallucinatory-delusional motivated behavior, delusional memories, delusional ideas of royal descent, self-neglect, lack of interest in any activity, verbal stereotypes, ideo-verbal dissociation.

Generally, in psychiatry, the diagnostic is set on the data obtained from the clinical examination, as well as from data collected by means of heteroanamnesis, while taking into account the current diagnostic systems (in this case, the WHO ICD 10, namely the 10th revision of the International Statistical Classification of Diseases and Related Health Problems, by the World Health Organization). In order to exclude an organic pathology, the patient has been subject to MRI, EEG and laboratory testing. For the appreciation of the evolution of clinical symptoms under treatment, we have applied the BPRS (Brief Psychiatric Rating Scale) weekly (with an average score of 89 points).

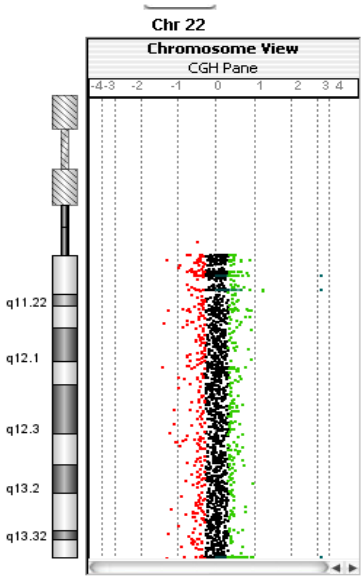
When the genetic evaluation was completed, mild phenotypic features (hyperpneumatization of paranasal sinus, epidermal cysts, and vertebral anomaly) characteristic for Gorlin syndrome were noticed and *PTCH1* gene mutation was taken into account. The gene sequencing analysis did not show any mutation and the arrayCGH was proposed as a comprehensive method of evaluation in this case.

METHOD

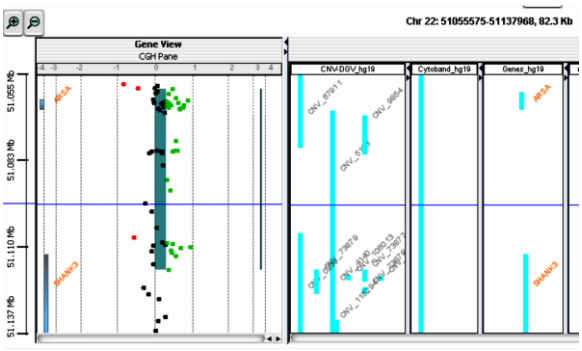
Array comparative genomic hybridization (aCGH) of the proband DNA against a commercial female genomic DNA (Promega) was performed on the SurePrint G3 Unrestricted CGH ISCA v2, 4x180K Agilent oligo array CGH, according to manufacture's instruction and analyzed by Cytogenomics v2.0.6.0 (Agilent Technologies, CA, USA). All genomic positions are based on the February 2009 human reference sequence (GRCh37/hg19)

produced by the Genome Reference Consortium.

The result of the arrayCGH showed genomic imbalances, including several genes. From the list of deleted/duplicated genes, one that was previously reported in patients with psychotic disorders is the SHANK3 gene. ArrayCGH analysis identified a duplication of Chromosome 22q of 82.3 Kb from 51055575-51137968 (fig.1).



a.



b.

Figure 1. Result of arrayCGH showing duplication on Chromosome 22 in the patient described here, using high-density micro-array platforms, 4x180 KAgilent: a. Normal aspect of arrayCGH of Chr. 22; b. Duplication aspect of arrayCGH of Chr. 22 (at 22q11-13 locus, which includes SHANK3).

DISCUSSIONS

The link between the alteration of the SHANK3 gene structure and/or expression was established following and investigating patients with Phelan-McDermid Syndrome that show chromosome 22q13 deletion. In the Phelan-McDermid Syndrome manifestations of the Autistic Spectrum Disorders, associated with language impairment and developmental delays, are present. By analyzing the deleted region it was established that there is a critical region and the hypothesis was raised that the candidate gene for neurobehavioral aspects of the patients with Phelan-McDermid Syndrome could be the SHANK3 gene [6, 7].

The hypothesis was sustained by studies that showed that the disruption of this gene is responsible for the phenotypic spectrum of the Phelan-McDermid Syndrome [8]. The neurobehavioral aspects of the 22q13 deletion syndrome were also found in several cases with SHANK3 gene mutations [9]. The presence of three copies of the SHANK3 gene where reported in association with Autistic Spectrum Disorders in other reports [10]. These findings led to the idea that both haplo-insufficiency, as well as an overexpression of the SHANK3 gene, are associated with manifestations of Autistic Spectrum Disorders and other neurobehavioral abnormalities.

The link between the SHANK3 gene and Schizophrenia was realized after the report of a case of Schizophrenia and borderline mental retardation in a patient with a 22q duplication [11, 12]. Supplementary information for the

correlations between SHANK3 gene mutations and Schizophrenia were brought by the Gauthier et al. report [13]. They studied patients with Schizophrenia and found one familial case harboring the same mutation of the SHANK 3 gene and a case of Schizoaffective Disorder showing a de novo mutation of the SHANK3 gene. This report also brought information about the role of the SHANK3 gene in the area of cognitive development, by showing that all of the studied cases suffered from a degree of mental retardation or borderline intellectual functioning [13].

The SHANK3 gene, located on the 22q13.3 chromosome, encodes a scaffolding protein necessary for the postsynaptic density and dendritic spines of excitatory synapses [14]. The SHANK3 gene expression influences the number and size of dendritic synapses [15]. The studies on mice with disrupted SHANK3 protein showed a reduction of the glutamatergic synaptic transmission and plasticity [16]. An effect on the social and communicational skills was observed, with an obvious deficit of both aspects [16].

Our case, diagnosed with Schizophrenia, also presents manifestations that are characteristic for Autistic Spectrum Disorders. Even if the IQ score couldn't be established, based on her academic performances, our patient might have a superior than average intellect. For this case, the overexpression of the SHANK3 gene can be linked with the schizophrenic manifestations and the autistic features, with probably no influence on the intellect.

CONCLUSIONS

There are serious difficulties in establishing the molecular background of many psychiatric disorders, and in many cases the diagnosis is essentially clinical, with no phenotype-genotype correlation. Based on the previous findings, our main hypothesis was that common SHANK variants would contribute to differential neuropsychological functioning. Previously, it was shown that SHANK3 is sensitive to gene dosage and that duplications and deletions are both implicated in ASD. When genetic counseling is requested, and taking into account the advances in genetic diagnostic methods, new insights for the disorders or for the mechanism underlying these conditions might be revealed. For psychiatric disorders a good cooperation between different medical specialists is crucial.

We suggest that the genomic evaluation of patients suffering with Schizophrenia or presenting schizoaffective manifestations is very useful in order to elucidate the implication of genomic rearrangements in the complex and heterogenic etiology of this psychosis.

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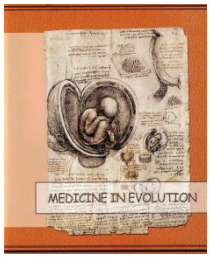
BRONCHOPULMONARY CANCER



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ABSTRACT

Lung cancer is the main cause of death by cancer in the world. The incidence and mortality of this lung pathology has increased dramatically in the last decades, especially in developed countries. The essential mechanism in the etiology of bronchopulmonary cancer consists in the interaction of environmental factors like carcinogens (in the tobacco smoke and pollutant environment) with the genetic factors, so family history plays an important role in the development of this type of pathology. In present, curative surgical treatment is possible in about one-third of the cases, while other patients benefits from chemotherapy and/or radiotherapy. The newest approach in lung cancer treatment is the gene therapy. An oncogene known as K-RAS is currently studied as a promising target for this type therapy, as well as studying different ways to replace mutant p53 gene with a normal one. The new imaging techniques and minimally invasive procedures have led to a new age in the treatment of some tumors that were considered inoperable in the past.

Keywords: lung cancer, treatment, pulmonary resection, chemotherapy, gene therapy.

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GENERAL DATA

Bronchopulmonary cancer is defined as the development of malignant cells in the lungs, starting from bronchial and pulmonary structures. Incidence of bronchopulmonary cancer increased dramatically, particularly in the developed countries. Thus, in Romania bronchopulmonary cancer showed a significant increase in incidence, being nowadays on the first place in men and on the third in women.

Bronchopulmonary cancer is an extremely severe disease, as clinical signs appear late in the course of disease, many cases developing asymptomatically over a long period of time. At the time the diagnosis is made, only 1/3 of the cases are in a manageable therapeutic stage.[2,8,9]

Etiopathogeny

The essential mechanism in the etiology of bronchopulmonary cancer consists in the interaction of environmental factors (carcinogens in the tobacco smoke and pollutant environment) with the autocrine growth factors and their receptors (EGF, CRP), as well as the activity of dominant oncogenes. At these factors are adds by the action of antioncogenes (recessive suppressor genes) and proliferation-inhibiting factors (TGFb).[8,9]

ETIOLOGIC AGENTS

Tobacco smoke. Bronchopulmonary cancer appear at the pacients who are or were smokers in percentage 80%. Correlations were established with the type of tobacco used, type of cigarettes (with or without filter), number of cigarettes, length of smoked cigarette, smoking start age, duration of smoking period, etc. It is thus estimated that 1 of 7 people who smoke more than 2 packs of cigarettes per day will develop bronchopulmonary cancer. So is prove that the prolonged exposure to tobacco smoke leads to the emergence of a succession of histopathological aspects: bronchial epithelium hyperplasia, malpighian metaplasia, then cell dysplasias, *in situ* and invasive carcinoma.[7,27]

Professional exposure

Numerous agents deemed to possibly be the cause of bronchopulmonary cancer were isolated in the work environments and then analysed. The most significant ones are: polycyclic hydrocarbons, N-nitrosamines, ionising radiations, mineral dusts (asbestos, ceramic), metals and metallic compounds (chromium, nickel, cadmium, and arsenic).

Environmental pollution

A possible etiologic factor of bronchopulmonary cancer is considered to be the presence of radioactive substances, aliphatic hydrocarbons, and 3,4-benzpiren.

Pre-existent pulmonary lesions

Pulmonary infarcts and suppurations, as well the scars after old pulmonary TBC lesions seem to be involved in the onset of cancer, and particularly adenocarcinoma.

GENETIC CHANGES

1. Family factor. The possible involvement of a family factor in the onset of bronchopulmonary cancer and chronic obstructive pulmonary diseases has been evidenced. The relationship between the onset of bronchopulmonary cancer and familial predisposition is confirmed. The hypothesis of genetic susceptibility to prolonged exposure to carcinogens has been also advanced. It is known that first-degree relatives of lung cancer patients are at high risk of developing bronchopulmonary cancer. It seems that certain people inherit differences in the ability of metabolising the carcinogens in the tobacco smoke. The specific gene involved has not been identified, but it might be associated with the production of p450 enzymes. While most researchers in the post-war period focused on the environmental etiologic factors, researches in the recent years concentrated on molecular biology studies. These studies began with the identification of chromosomal changes through cytogenetic analysis, the polymorphic analysis of a restriction length fragment polymorphism (RFLP) and the polymorphic analysis of a *single-strand conformation polymorphism* (SSCP).

2. Induced genetic changes. There is some genes directly involved and of the mutations developed at this level has been performed. A series of researches showed the role of human oncogenes involved in the onset of bronchopulmonary cancer. As known, there are two types of oncogenes: dominant and recessive (tumour suppressive genes). Mutations in these genes or in their control elements would be responsible for the initiation of tumour process. There are several dominant oncogenes and tumour suppressive genes involved in different types of bronchopulmonary cancer. (Table 1)

Oncogene dominante
c-myc, N-myc, L-myc, (exprimare dereglata)
K-ras, H-ras, N-ras, (mutatie de activare)
Her-2/neu (exprimare dereglata)
Oncogene recesive (gene "supresoare tumorale")
3p14
3p21
3p24-25
5q (MCC gene cluster)
9q (gene interferonului)
11p15
13q14 (gene retinoblastului, rb)
17q13 (gene p 53)

Table 1. Dominant and recessive oncogenes involved in bronchopulmonary cancer

There are few significant differences between various histopathological types of bronchopulmonary cancer.

- Mutations in ras family: 70% NSCLC (30% of adenocarcinomas), not in SCLC
- myc gene mutation: 80% SCLC, 30% NSCLC
- rb gene mutation: 80% SCLC, 20% NSCLC
- p53 gene mutation: 60% NSCLC, 80% SCLC

In the dominant oncogenes, a single mutation in one of the two copies of the gene is required. There are two possibilities for cancer to develop:

1. Genetic mutation: production of mutant protein – genetic overexpression (ras family: punctiform mutations in the codons, with the alteration of ras proteins fixed in a permanently activated form that discharges continuous signals for cellular growth).

2. Overexpression of a normal gene (myc family: in almost all types of SCLC and many NSCLC).

The cases with tumour cells with ras mutation have a significantly inferior survival. This is evidenced in the studies on operated stage I or II adenocarcinomas. Biochemical researches suggest certain ways to block ras action, therefore a possible future adjuvant therapy. Two different lesions on both chromosomes are needed to trigger the oncogenesis in the recessive oncogenes. These genes are recessive because both copies (maternal and paternal) must have a mutation.

By punctiform mutation (it affects a single amino acid), the second one is massive (deletion, translocation, nonreciprocal). The most researched genes were rb and p53. The mutagen mechanisms seem to be similar in both genes (transverse mutation: guanine – thymine). Experimental studies suggest the reversibility of tumorigenicity may be achieved by correcting p53 mutation.

The p53 proteins are detectable by immune correlations and provide prognostic significance. If the immunostaining is seen, the survival is reduced, if not, the survival is increased. Production of anti p53 antibodies is correlated with increased survival in SCLC.[12,20,22]

RADIOLOGICAL EVALUATION

This is the first and the most important paraclinical investigation in the pulmonary neoplasm. Radiological changes are caused by the tumour itself, by secondary pulmonary changes (atelectasis, infection), and by the neoplastic extension to the other intrathoracic structures. Images vary depending on the site, cell type, and the period of time from the onset of tumour process. For the tumour to be seen on radiograph, it must have at least 7 mm in diameter, but usually when it is less than 10 mm the diagnosis is not made. The first radiological signs, which are critical in establishing the diagnosis, are the following ones:

1. opacity in pulmonary parenchyma, with a peripheral pulmonary nodule aspect
2. cavity inside a solid tumour mass
3. poorly defined segmental opacity
4. segmental infiltration along a blood vessel
5. aspect of segmental infiltration (pneumonitis)
6. apical triangular opacity, extending towards hilum
7. mediastinal mass (more rarely)
8. enlargement of a pulmonary hilum
9. apical segmental or lobar emphysema
10. segmental atelectasis

The classic thoracic radiological evaluation is generally performed by radiographs (postero-anterior and profile), as well as by radioscopic examinations.[25,26]

Computer tomography evaluation

Computer tomography has become a routine examination in patients with bronchopulmonary cancer. It accurately shows the tumour characteristics and structure (cavities, calcifications), its relations with the pulmonary parenchyma, the bronchial wall, and the large vessels, but also the extrapulmonary extension. It evidences the mediastinal adenopathies, it proves the tumour invasion of vertebral bodies and it detects small pleural collections that are not seen at the radiological examination.[25]

Magnetic resonance

This technique has the advantage to be used in cases of intolerance to contrast medium. It offers better images of mediastinal fat, and of blood stream in the mediastinal vessels and in the tumour. It is more useful than the CT in assessing the thoracic wall invasion, as well as the superior thoracic outlet invasion.[25]

Ultrasonography

It is largely used instead of CT in order to assess the hepatic or suprarenal metastases and is considered to be equally useful. Transesophageal endoscopic ultrasonography allows the evaluation of mediastinal lymph nodes in the paraaortic area, as well as in the subcarinal, paraesophageal and pulmonary ligament stations.

Radioisotopic studies

Gallium-67 Pulmonary scintigraphy is useful for the identification of the primary tumour, but also of the metastases in the mediastinal lymph nodes. It is currently replaced by CT. The use of monoclonal antibodies marked with isotopes in order to identify the primary tumour, the regional metastases and the tumour recurrences is still under evaluation. RS5-4H6 monoclonal antibodies, anticarcinoembryonic monoclonal antibody marked with $Indium^{111}$, Po66 monoclonal antibody marked with $Iodine^{131}$ are utilised, the primary tumour being detected in 75% of the cases.

PET (Positron Emission Tomography)

This technique plays an important role in the diagnosis and staging of bronchopulmonary cancer. Cancerous tumour consumes more glucose than normally. The procedure consists in the injection of a glucose analogue radioactively marked with fluorodeoxyglucose (FDG). PET is a functional exploration, unlike the CT, which is a morphological exploration. PET-CT may be used in the evaluation of solitary pulmonary nodule, in the staging of metastatic disease of mediastinal lymph nodes and in the detection of occult metaplasias. Glucose standard uptake value (SUV) of a pulmonary lesion is 2.5. A SUV larger than 2.5 is a positive PET proof. For the malignant pulmonary nodule, PET has more than 96.8% sensitivity and 77.8% specificity. In the case of benign nodule, the sensitivity is 96%, with specificity greater than 88%. False negative results may occur in the bronchoalveolar carcinoma and in the carcinoid – due to the reduced glycolysis. PET-CT false positive results are represented by inflammatory lesions (pneumonia, granulomatosis, and sarcoidosis). The diagnosis rate of metastases in mediastinal lymph nodes, as well as the detection rate of occult metastases in other organs has increased by the aid of PET-CT technique.

Detection of brain metastases by the means of PET is not effective due to the intense glycolysis in the central nervous system. Tc^{99} depreotide SPECT is a new functional exploration method based on the increased incorporation of amino acids in the tumour cells. Tc ^{99} depreotide is a synthetic peptide composed of 6 amino acids, analogue of somatostatin, which is intensely and specifically incorporated by bronchopulmonary cancer.[25]

INVASIVE DIAGNOSTIC PROCEDURES

Bronchoscopy

Bronchoscopy is the method of choice for the direct visualisation of tracheobronchial macroscopic changes. Supplemented by techniques for harvesting specimens in view of cyto-histological examinations (puncture, aspiration, biopsy), it allows to ascertain the malignity diagnosis in BPC. The indications of bronchoscopy have extended nowadays, this method being used routinely in the endobronchial treatment of BPC as well. Bronchoscopy consists in the direct visualisation of the tracheobronchial tree by the aid of rigid or flexible bronchoscope. Fibrobronchoscopy is the most useful diagnostic investigation when the bronchopulmonary cancer is suspected.

We mention the following modern bronchoscopic methods for the detection of bronchopulmonary cancer:[16]

1) Laser Fluorescence: consists in the illumination of bronchopulmonary tissues with laser light after injection of a substance (hematoporphyrin derivative – PHOFIN 2-3 mg/kg BW) that attaches to tumour cell.
2) Autofluorescence (LIFE): uses tissues property of emitting fluorescent radiations when exposed to various light types (violet or blue). The dysplastic tissue has a greatly reduced fluorescence, making it easily detectable. This is an effective, though very expensive method, which allows the high rate diagnosis of *in situ* carcinoma (many European studies are currently conducted, only partial results being available)
3) Echobronchoscopy: it determines intraluminal and intramural extent of the tumour, enabling the analysis of very small (a few mm) tumours. Echobronchoscopy is a very good method for analysing small lesions. Along with the autofluorescence bronchoscopy, it enhanced the specificity (regarding the malignity diagnosis) from 50% to 90%.
4) Magnification Bronchovideoscope: it is a combination of two systems: a video system for a high image magnification and a fibre optic system for orientation of the bronchoscope. This system performs the visualisations of vascular network, enabling the differentiation between dysplasia and preinvasive lesions.
5) Confocal Fluorescence Microscopy (CFM): allows the identification of cellular and subcelullar structures, providing high resolution images.
6) Narrow-Band Imaging (NBI): enables also the examination of the microvascular network in the bronchial mucosa, differentiating between dysplasia and other bronchial lesions.
7) Optical Coherence Tomography (OCT): is a method to acquire real-time, high resolution images. OCT identifies the microscopic features of cilia, glands, crypts, lymphatics and vessels; unlike the echobronchoscopy, the image is depth limited (approximately 2 mm as compared to approximately 5 mm for 30 MHz echobronchoscope).
8) Virtual Bronchoscopy (VB): permits the acquisition of 3D images with the visualisation of tumour distribution and localisation in the tracheobronchial tree and of its relations with the mediastinal structures.

Percutaneous transthoracic needle biopsy

It is useful in pacients with advaced lung bronchopulmonary cancer outdate stadium in terms of surgical or the previous diagnostic method (bronchoscopy, tumour marker) have failed to confirm the diagnosis.[14] It is also useful in patients in wich disease associated miniinvazive not allow surgery or who refuse a diagnostic surgical procedure. It is performed under the control of computer tomography, or ultrasound. CT it is the best option.[15] An amount of tissue is harvested from the lung tumour by two method either fine needle aspiration or tissue fragment with those of biopsy .[19] Mediastinal tumour is limited and has a specificity of approximately 90% - 95%. Data from literature related a mortality between 0,15% and 0,47%. Complication are relativ benign method reprezented especially pneumothorax rate averaged 17% and bleedind intraparenchyma a 2% - 26%.[24]

Table 2. *Bronchopulmonary cancer staging*

Category	Description
T: Primary tumour	
Tx	Primary tumour cannot be assessed or proved through the presence of malignant cells in sputum or bronchial lavage fluid, but not visualised by imagistics or bronchoscopy
T0	No signs of primary tumour
Tis	*In situ* carcinoma
T1	Tumour ≤ 3cm in greatest diameter, surrounded by lung or visceral pleura, without bronchoscopic signs of invasion more proximal than the lobar bronchi **T1a –** tumour < 2cm in greatest diameter **T1b –** tumour > 2cm but < 3cm in greatest diameter
T2	Tumour with one of the following characteristics of size or extent: • larger than 3 cm in diameter • it involves the primary bronchi, ≥ 2 cm distal from carina at bronchoscopy • it invades the visceral pleura • associated with atelectasis or obstructive pneumonia which extends to the hilar region, but it does not involve the entire lung **T2a –** tumour > 3cm but < 5cm in greatest diameter **T2b –** tumor > 5cm but < 7cm in greatest diameter
T3	Tumour > 7cm or one that directly invades one of the following: • thoracic wall (including superior sulcus tumours), diaphragm, mediastinal pleura, parietal pericardium • or tumour in the primary bronchi < 2 cm distal from carina, without invasion of carina
T4	Tumour of any size that invades one of the following: • mediastinum, large vessels, trachea, esophagus, vertebral bodies, carina • tumour with malignant pleural effusion • satellite pulmonary nodules in the lung lobe in which the primary tumour is located
N: lymph nodes invasion	
NO	No metastases in regional lymph nodes
N1	Metastases in peribronchial lymph nodes and/or in ipsilateral hilar lymph nodes and intrapulmonary lymph nodes invaded by direct extension of primary tumour
N2	Metastases in ipsilateral mediastinal lymph nodes and/or in subcarinal lymph nodes
N3	Metastases in contralateral hilar and mediastinal lymph nodes , ipsilateral and contralateral scalene lymph nodes or in supraclavicular lymph nodes
M: distant metastasis	
M0	No distant metastases
M1	Distant metastases present **M1a –** separate tumour nodule in a contralateral lung, tumour with pleural nodules or malignat pleural/ pericard effusion **M1b –** distant metastasis

Table 3. *TNM staging*[18]

Stage	TNM substage
0	*in situ* carcinoma
IA	T1aN0M0 T1bN0M0
IB	T2aN0M0
IIA	T1aN1M0 T1bN1M0 T2aN1M0 T2bN0M0
IIB	T2bN1M0 T3N0M0
IIIA	T1aN2M0 T1bN2M0 T2aN2M0 T2bN2M0 T3N1M0 T3N2M0 T4N0M0 T4N1M0
IIIB	T4N2M0 Any T, N3, M0
IV	Any T, any N, M1a Any T, any N, M1b

BRONCHOPULMONARY CANCER TREATMENT

Primary approach of bronchopulmonary cancer depends on the histological type of the tumour (NSCLC or SCLC) with the evolution stage defined following the TNM classification, on the cardio-respiratory function and on the biological state of the patient.

Stage I. If the tumour is located peripheral and size small segmentectomy or atypical resection is performed and the tumour increased in size, occupying a lung lobe, type of perform is indicated lobectomy.Pneumonectomy is performed in central tumours. Lung resection with the dissection or biopsy of mediastinal lymph nodes to detect lymfatic metastasis is indicated in this stage.[1,11,13]

Stage II. Stage II is divided into IIA and IIB and into T1, T2, and T3 according to tumour dimension. Surgical procedure will be elected (lobectomy, pneumonectomy or bilobectomy) depending on the tumour topography. The presence of N1 peribronchial adenopathy limits the indication for bronchoplastic resection. Mediastinal lymph-node dissection is indicated in stage II. Cytostatic, irradiant and immunological treatment is required after surgery.

Stage IIIA - of parietal pleura and thoracic wall. Parietectomy is performed when is invaded parietal pleura. Lung resection for peripheral tumours who invading the thoracic wall is lobectomy. Mediastinal lymphadenectomy will be associated. The thoracic wall reconstruction depends on the parietal

defect topography and size. In small defects parietal postero-superior stability is provided by scapula, while in lateral and anterior defects synthetic materials are inserted to stabilise the wall. Reconstruction is mandatory to avoid lung herniation and paradoxical breath.[17] Pericardium invasion – involves pericardiectomy with lobectomy or pneumonectomy. Preservation of phrenic nerve is mandatory in partial lung resection. Pericardium reconstruction is performed by means of synthetic mesh and is required after right pericardiectomy. Invasion of phrenic or vagus nerves – resection of the right vagus nerve may have no serious effects if it is performed under the emergence point of the fibers forming the cardiac plexus. Manipulation of vagus may lead to severe bradycardias up to the cessation of heart during distole. Left vagotomy, above the emergence of recurrent nerve will lead to permanent paralysis of the homonymous vocal cords.[21]

Stage IIIB. Invasion of tracheal carina – it performs pneumonectomy with termino-terminal anastomosis between tracheal and bronchus. Patients who can perform this type of intervention required in advance a series a paraclinical (bronhoscopy with biopsy).[10] Damage and traheo-bronchial lymph paratraheal is a relative contraindication, while metastasis to limph nodes and contralateral superior mediastinum is an absolute contraindication. Postoperative mortality is 0-31%, and the 5-year survival rate is 0-23%.[6] Vertebral body invasion - decision of surgical indication is made in consultation with the neurosurgeon. Extension of tumour invasion is appreciated by a clinical examination, CT and MRI. Survival rate is 10%.[30] Invasion of superior vena cava - consist of the en bloc resection of tumour and vena cava. The presence of invasive N_2 adenopathy contraindicates surgical intervention. 5-year survival rate is 0. Esophagus invasion - it is more rarely encountered in bronchopulmonary cancer. En bloc resection of pulmonary parenchyma and esophagus is performed in the absence of mediastinal and/or systemic lymph nodes metastasis. Heart invasion – it is directly invades by pulmonary tumour or by means of pulmonary vessels. Resection of cardiac wall is rarely possible together with the tumour. Metastasis in N3 lymph nodes represents surgical contraindication.[21]

Stage IV. It must be taken into account the possibility of a single metastasis or a synchronous lung cancer. Regarding the brain, suprarenal or hepatic metastases, from the surgical point of view single metastases are considered.

Other treatment methods in bronchopulmonary cancer

Radiotherapy. Usually not use preoperative radiotherapy. Postoperative radiotherapy is used when metastases in hilar and mediastinal lymph nodes are evidenced. Curative-intention radiotherapy is recommended to patients in stage III, but also to those in stages I and II who could not undergo surgery due to their refusal or because other diseases are associated. The total dose usually prescribed to treat lung cancer is approximately 50-70Gy. The treatment lasts 5 to 8 weeks on average, with one session a day, for 4 or 5 days a week. It is not performed in patients with distant metastases, with the invasion of supraclavicular lymph nodes, pleural effusion, and cardiac invasion. Mean survival time is 1 year and 6% of the patients live for 5 years.[3]

Postoperative and neoadjuvant chemotherapy. The complete clinical regression of tumour occurs in only 5% of the cases. The tumour response to cytostatics determines the survival duration. It is deemed that 30-40% of the patients have an objective, evidentiable response to treatment. A series of researches have studies the effect of preoperative (neoadjuvant) chemotherapy with or without radiotherapy. The most

used chemotherapy drugs in bronchopulmonary cancer are: **etoposide,** cyclophosphamide**, a**driamycin, vincristine, t**opotecan, paclitaxel,** c**isplatin,** c**arboplatin and irinotecan.**

The postoperative therapeutic schemes consist of the administration of 4-6 cycles of adjuvant chemotherapy with:

- Etoposide - 150 mg/m^2 intravenous on days 1-3, Cisplatin - 90 mg/m^2 intravenous on day 1. Treatment is repeated every 3 weeks.
- Etoposide - 120 mg/m^2 intravenous on days 1-3, Carboplatin - 100 mg/m^2 intravenous on days 1-3. Cycles are repeated every 4 weeks. Two other modes are possible in this system: etoposide - 100 mg/m^2 intravenous on days 1-3, carboplatin - 300 mg/m^2 intravenous in the first day of the cycle only. Treatment is repeated every 3 weeks.
- Cisplatin - 50 mg/m^2 intravenous on days 1 and 8 of the cycle, Etoposide - 50 mg/m^2 intravenous, on days 1 and 5 of the cycle and Vincristine - from 1.4 mg/m^2 intravenous on days 1.Treatment is repeated every 4 weeks.
- Cisplatin - 60 mg/m^2 intravenous on day 1, Doxorubicin - 45 mg/m^2 intravenous on day 1, Etoposide - 120 mg/m^2 intravenous on days 1-3 of the cycle. Treatment is repeated every 3 weeks.
- Cisplatin - 40 mg/m^2 intravenous on day 1, Doxorubicin - 40 mg/m^2 intravenous on day 1 and Cyclophosphamide - 400 mg/m^2 intravenous on day 1. Treatment is repeated every 3 weeks.
- Doxorubicin - 60 mg/m^2, Cisplatin - 90 mg/m^2 on day 1 (24-hour perfusion), Vincristine - 2 mg/m^2 intravenous on day 1. Treatment is repeated every 3 weeks.
- Cyclophosphamide - 1000 mg/m^2 intravenous on day 1, Doxorubicin - 40 mg/m^2 on day 1, Etoposide - 150 mg/m^2 intravenous on day 1. Treatment is repeated every 4 weeks.
- Etoposide - 75 mg/m^2 intravenous, on days 1 and 4, Cisplatin - 20 mg/m^2 intravenous on days 1 to 4, Ifosfamide - 1200 mg/m^2 4 days of intravenous perfusion. Treatment is repeated every 4 weeks.
- Carboplatin - 300 mg/m^2 intravenous on day 1, Ifosfamide - 5000 mg/m 2 intravenous on day 1, as 24-hour perfusion, Etoposide - 120 mg/m 2 intravenous on 1and 2 days of treatment and 240 mg/m 2 orally, on third day, Vincristine – 0.5 mg/m 2 intravenous on day 14. Treatment is repeated every 4 weeks
- Cisplatin - 60 mg/m^2 intravenous on day 1, Vincristine - 3 mg/m^2 intravenous on day 1, Etoposide - 120 mg/m^2 intravenous on dat 1. Treatment is repeated every 3 weeks.
- Carboplatin - 300 mg/m^2 intravenous on day 1, Etoposide - 140 mg/m^2 on day 1, Vincristine - from 1.4 mg/m^2 on days 1, 8 and 15. Treatment is repeated every 4 weeks.
- Carboplatin - 300 mg/m^2 intravenous on day 1, Etoposide - 120 mg/m^2 intravenous for 1 and 2 days and 240 mg/m^2 orally, on the third day, Ifosfamide - 5000 mg/m^2 intravenous on day 1, Vincristine – 0.5 mg/m^2 intravenous on day 14. Treatment is repeated every 3 weeks.

New medical treatments, called specific therapies, become available for lung cancer. Unlike the traditional chemotherapy, these treatments target the proteins on cancer cells. This is why they tend to have fewer side effects as compared with the other drugs used in cancer treatment. They are currently used mainly for the stages 3 and 4 of lung cancer, which has not responded to other treatments. Two of the most frequent

specific therapies include: Tarceva (erlotinib) that acts through EGFR and does not allowed to cancer cells to grow and Avastin (bevacizumab - angiogenesis inhibitor) prolongs the survival time of bronchopulmonary cancer patients.[3,4,5]

Adjuvant immunotherapy

Immunotherapeutic techniques, such as tumour specific antigens, BCG, Corynebacterium parvum, and Levamisole are used. Studies with Bestatin, an immunomodulatory agent used in stages I and II of evolution, have been also conducted.

Gene therapy

One approach of gene therapy is to block or deactivate oncogenes in the cancer cells. This stops the growth of cancer cells, allowing them to die naturally. Researchers were able to block oncogenes by inserting a DNA fragment specifically into the cancer cells. Once blocked, the oncogenes cannot contribute anymore to the cancerous process. An oncogene known as K-RAS is currently studied as a promising target for this type of gene therapy in lung cancer. K-RAS is involved in the reproduction and spread of lung cancer cells. In the case of lung cancer an important role in the growth of cancer cells is played by the mutations of a tumour suppressor gene called p5. Researchers are searching in present for different ways to replace mutant p53 gene with a normal one.

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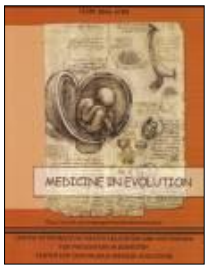
THE VELOCITY OF THE DOPPLER MITRAL INFLOW E WAVE UNDER TREATMENT WITH IVABRADINE VERSUS METOPROLOL



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ABSTRACT

GOALS: Comparing the effects of ivabradine versus metoprolol on the velocities of the E waves of the Doppler pattern of the mitral inflow, in two groups of patients, comparable as age, sex, drug associations and morbide associations, coming from the ambulatory of the Diabetes and metabolic diseases Clinic of the Sibiu County Academic Emergency Hospital, at the initial moment, being under treatment with metoprolol and after three months, the first group continuing the treatment with metoprolol and the second group replacing it with ivabradine.

RESULTS: It appeared that after three months of ivabradine there was a statistically significant enhancement as of the E wave velocity and of the A wave duration as well, showing a better diastolic function, partly better effects than with continuing metoprolol.

Key words: ivabradină, metoprolol, Doppler, waves E

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INTRODUCTION

The maximum velocity of the early diastolic E wave of the mitral inflow Doppler pattern is given by the transmitral early diastolic gradient. Rising values under treatment mean better left ventricular relaxation, provided that they are accompanied by longer late diastolic A wave duration [1].

GOALS

We aimed to study the effect of ivabradine versus metoprolol on the velocity of the early diastolic E wave of the mitral inflow Doppler pattern and to judge by context their influance on the diastolic function of the left ventricle with preserved ejection fraction in diabetic patients [6].

MATERIAL AND METHOD

We studied the evolution of the mitral inflow E wave on two groups, comparable as age, sex, initial Doppler pattern and morbid and drug associations, treated with metoprolol during the last month and continued for three months (the control group, consisting of 40 patients), respectively replacing metoprolol with ivabradine for thre months (the experimental group, consisting of 50 patients). The patients came from the ambulatory of the Diabetes and nutrition diseases of the Sibiu County Academic Emergency Hospital. We used an Acuson Sequoia Doppler ultrasonographer.

The inclusion criteria were: the presence of type II diabetes mellitus, ejection fraction over 50%, no diastolic dysfunction or presence of altered relaxation diastolic dzsfunction type, sinus rythm, stable clinical condition, unchanged treatment during the last month. Exclusion criteria were: giving up initial treatment out of any reason, myocardial infarction more recent than 2 months, haemodynamically significant valvular pathology, pericardial pathology, cardiosurgical hystory, coronary revascularisation, resynchronisation, atrial or ventricular pacemaker, congenital heart disease, stroke during the last month, active myocarditis, bad acoustic window, atrial fibrillation, atrial flutter, sinus bradicardia, sick sinus syndrome, atrioventricular heart block, severe ventricular arrythmias, uncontrolled arterial hypertension (over 180/110mmHg), arterial hypotension (under 85mmHg), non dihydropyridine calcium blockers association, classI and III antiarrythmic drug association, strong cytochrome P450 3a4 blockers association, svere hepatic or renal failure, known anaemia, lack of efficient contraception in fertile females.

We measured the maximum velocity of the E wave of the mitral inflow and the A wave duration [2] at the initial moment of the study and after three months of treatment with ivabradine (the experimental group) versus metoprolol (the control group).

For statistics, we used the SPSS v.10 programme. To compare calitative variables, we used the Crosstabs association table. To compare quantitative variables, we used Independent T test. There was a homogenous distribution of ages (p=0,687), sexes (p= 0,665), also of

morbid and drug association for both groups, permitting comparison.

The limits of the study were the relatively short follow-up interval and the fact that there was only one observer [3].

RESULTS

At the initial examination, the maximum E wave velocity for the experimental group had a mean value of 0,82m/s, with a standard deviation of 0,7m/s and for the control group a medium value of 0,80m/s with a standard deviation of 0,18m/s. This parameter had thus unsignificant differences between the two groups (p=581). The A wave duration had at the initial moment a mean value of 143,3ms for the experimental group, treated with ivabradine, with a standard deviation of 47,22ms and a mean value of 141,03ms, with a standard deviation of 47,02ms for the control group, treated with metoprolol, so that there were no significant differences between the two groups regarding this parameter. There were no partial fusions of the E and A wave, no cardiac arrythmias, no second or third degree heart block, no short PR interval, to determine a difficult evaluation of the A wave [4].

After three months, the mean value of the maximum velocity of the E wave was for the experimental group 0,94m/s, with a standard deviation of 0,21m/s and for the control group 1,03m/s, with a standard deviation of 0,46m/s. Both rises were statistically significant (p= 0,03). There are no significant differences between the two groups, the effects of the two drugs being similar.

The late diastolic A wave duration after three months had a mean value of 177,78ms, with a standard deviation of 54,96ms for the experimental group and a mean value of 146,53ms, with a standard deviation of 44,53ms. The conclusion is that ivabradine significantly prolongues this parameter versus metoprolol (p=0,04) after three months, indicating a superiority of ivabradine from this point of vue and certificating the significance of better left ventricular diastolic function of the E wave velocity rise.

CONCLUSIONS

We can say that ivabradine has similar effects with metoprolol on the maximum velocity of the mitral inflow A wave, after three months of treatment, meaning a better diastolic function only for ivabradine, the fact being sustained only for this drug by the significant prolongation of the late diastolic A wave.

DISCUSSIONS

In literature there do not seem to exist similar finished studies. We started from the benefic effects of ivabradine on the left ventricular systolic function, shown by the SHIFT study [5].

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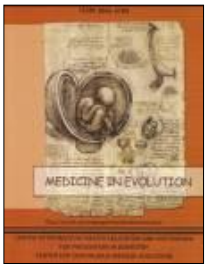
THE ASSESSMENT OF IMATINIB TREATMENT IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA



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ABSTRACT

Chronic myelogenous leukemia (CML) is a disease of the stem hematopoietic cell characterised by the presence of the Philadelphia chromosome (PH) resulting from the translocation between the chromosomes 9 and 22t (9; 22).

The introduction of imatinib, a specific and potent BCR/ABL tyrosine-kynase inhibitor, in the treatment of CML patients dramatically changed the treatment in CML.

In 2006 a group of experts appointed by the European Leukemia Net (ELN) defined the optimal and suboptimal response parameters, as well as those for treatment failure. The Monitoring the tyrosine-kinase inhibtor (TKI) treatment is crucial for CML patient management. ELN recommends the monitoring of the hematologic, cytogenetic and molecular response at 3, 6, 12 and 18 months, respectively.

Patients with a suboptimal response represent a subgroup requiring attentive monitoring and may benefit from alternative treatment.

Key words: imatinib, optimal response, suboptimal response, treatment failure.

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INTRODUCTION

CML is a clonal disease of the stem hematopoietic pluripotent cell characterised by the presence of the Ph chromosome and/or of the BCR/ABL rearrangement with proven pathogenetic role. It is the first malignant disease in which a characteristic cytogenetic abnormality was described and the first disease for which a focused molecular treatment has been applied [1].

Before the introduction of TKI, the average period from the diagnosis of chronic phase (CP) to advanced phases (accelerated –AP – and blastic – BP) of CML was around 3 to 4 years. The complexity of CML management evolved during recent years in parallel with the improvement of diagnostic procedures and with the introduction of TKI in treatment regimens: imatinib, dasatinib, nilotinib[2]. At present, patients with CML may remain in CP for much longer periods of time.

AIM

The assessment of optimal, suboptimal responses, as well as of treatment failure in the case of imatinib treatments in newly diagnosed patients with CML-CP.

MATERIAL AND METHOD

During the period between January 2006 – January 2011 in the Clinic of Hematology of the Clinical Town Hospital in Timisoara, 61 patients were diagnosed with CML of whom 2 in AP, 6 in BP, 53 in CP. Patients diagnosed with CML-CP followed a cytoreductive treatment with hydroxiurea for two to six months; 8 patients continued to be treated with hydroxiurea due to age and reduced compliance to treatment, 7 were treated with nilotinib as part of an ongoing clinical study and 38 were treated with imatinib.

We conducted a prospective, descriptive study for 5 years, from January 2006 to January 2011 which enrolled a group of 38 patients admitted and diagnosed with CML-CP in the Clinic of Hematology of the Clinical Town Hospital in Timisoara and who received imatinib treatment.

The diagnosis of CML was supported by the complete blood count, bone marrow examination and was confirmed by the cytogenetic examination of the bone marrow revealing the presence of the Ph chromosome or by revers-transcriptase polymerase chain reaction (RT-PCR) which showed the BCR/ABL translocation.

The chronic phase was defined by:

- Leukicytosis with left shift
- Normal or increased platelet counts - over $450x10^9/l$
- Less than 10% blast cells in the peripheral blood or in the bone marrow

Response criteria:

- Complete hematologic response (CHR): platelets < $450x10^9/l$, leukocytes < $10^9/l$, lack of immature granulocytes, basophiles < 5%;
- Cytogenetic response (CyR):
  - Complete cytogenetic response (CCyR) – PH positive metaphases = 0
  - Major cytogenetic response (MCyR) – PH positive metaphases ≤ 35%
  - Minor cytogenetic response (mCyR) – 35% < PH positive metaphases ≤ 95%
  - No cytogenetic response – PH positive metaphases > 95%.
- Molecular response (MR):
  - Complete (CMR) – untraceable BCR/ABL transcript
  - Major (MMR) - BCR/ABL transcript < 1%

Definitions of treatment failure, optimal and suboptimal response depending on first line 400 mg imatinib treatment in patients with CML-CP according to ELN[3]					
	3 months	6 months	12 months	18 months	At any moment during treatment
failure	< CHR	No CyR (Ph+ > 95%)	< MCyR (Ph+ > 35%)	< CCyR	Loss of CHR, CCyR, mutations, CCA/Ph+
Suboptimal response	No CR (Ph+ > 95%)	< MCyR (Ph+ > 35%)	MCyR (1% < Ph+ < 35%)	< MMR	Loss of MMR, mutations
Optimal response	≥ mCyR (Ph+ ≤ 65%)	MCyR (Ph+ ≤ 35%)	CCyR	MMR	Stable or improving MMR

Treatment protocol. All patients received a cytoreductive treatment with hydroxiurea for two to four months, followed by the introduction of 400 mg imatinib/day. The blood count was monitored weekly until the achievement of hematologic response and then monthly.

RESULTS

The conducted study monitored optimal, suboptimal responses and treatment failure in the 38 CML-CP patients who received imatinib. Of the 38 patients, 22 were men and 15 were women aged between 22 and 66 years, with a mean age of 54 years.

The most common symptoms at onset in patients with CML-CP were asthenia, abdominal discomfort. Other symptoms were abdominal pain, weight loss and fever. On clinical examination almost all patients had splenomegaly (36), 20 had moderate splenomegaly, 8 presented adenopathy, and 28 had pale teguments.

Characteristics of patients		
Total number of patients		38
Men/Women		22/15
Age upon onset		22-66 years
Mean age		54 years
Clinical symptoms at onset	splenomegaly	36
	hepatomegaly	20
	adenopathies	8

Imatinib treated patients were monitored by weekly blood counts until complete hematologic response was obtained and then blood counts were performed monthly; cytogenetic testing was performed at 6, 12 and 18 months, respectively, while molecular tests were performed at 12 and 18 months, respectively. The treatment targets CHR, CCyR and MR at certain moments in time after treatment initiation.

Hematologic, cytogenetic and molecular monitoring

Period of treatment	3 months	6 months	12 months	18 months
No. patients	38	35	35	34
CHR	36	35	35	32
CCR	-	15	29	28
MCR	-	14	3	-
mCR	-	3	3	-
No CR	-	3	3	3
CMR	-	-	3	5
MMR	-	-	10	12
CCA	-	-	-	1
No MR	-	-	-	11

CCA – clonal chromosomal abnormalities

In the monitored group we obtained the following responses:

- After 3 months - failure in two patients who experienced adverse effects. They continued with IInd line treatment with nilotinib

  - suboptimal and optimal response in 36 patients in whom CHR was achieved

After 6 months - failure in 4 patients, one gave up treatment and in 3 patients CCR was not obtained, with the increase of imatinib dosage in the latter.

- suboptimal response in 3 patients who reached mCR, two of them continued to receive the same imatinib dosage, in one patient the imatinib dose was increased to 600 mg with further MCR at 12 months and CCR at 18 months, respectively.

- optimal response in 29 patients who remained on the same dosage of imatinib

- After 12 months - failure in 3 patients who did not reach CCR, the imatinib dosage is increased

  - suboptimal response in 3 patients who reached MCR and continued with the same imatinib dosage

  - optimal response in 29 patients who obtained CCR

- After 18 months - failure in 6 patients of whom one presents clonal chromosomal abnormalities and in whom IInd line treatment with dasatinib is initiated followed by favourable evolution and CMR, 3 patients did not reach CCR and IInd line treatment with dasatinib is initiated, with one of these patients obtaining CMR and the other two not being yet evaluated ; 2 patients evolved towards the blastic phase, although at 12 months they had CCR but did not have MMR. The BP mechanism in CCR patients is not known

  - suboptimal response in 11 patients who obtained CCR but did not obtain MMR, and who continued with the same imatinib dosage

  - optimal response in 17 patients who obtained CMR (5) and MMR (12)

DISCUSSIONS

CML usually starts with the CP, and the clonal expansion of mature myeloid cells leads to increased leukocyte counts. In the absence of therapeutic interventions, CML-CP will progress to advanced disease stages including AP and BP. CML-AP is characterised by an increased number of immature myeloid cells in the peripheral blood and in bone marrow, as well as by new cytogenetic abnormalities together with the presence of the Ph chromosome. During the BP, imature blood cells prevent the production of normal blood cells. Patients with CML-AP may respond to treatment for several months or even years, while CML-BP is

resistant to treatment, and patients frequently die due to infections and hemorrhagic complications, with survival around 6 months [4].

The general response to first line therapy in CML-CP patients may be defined as optimal, suboptimal or failed. According to the ELN, an optimal response consists of the absence of signs indicating that a treatment change might improve patient survival. Failure involves a low probability of favourable results in a patient requiring a different treatment whenever indicated and whenever this is available [5]. Patients with a suboptimal response may still benefit from a certain treatment on long term, but the chances to obtain optimal results are low. Pateints with suboptimal response are a distinct and heterogeneous category with results less favourable than in optimal response patients. The prognostic of suboptimal response patients may differ depending on the moment of assessment[6].

In the studied group, 2 of the imatinib treated patients had 3rd and 4th degree adverse reactions, low platelet and neutrophile counts and required a change of treatment with IInd generation TKI i.e. nilotinib; one patient quit treatment, one had additional clonal chromosomal abnormalities after 18 months of treatment and further received IInd line treatment i.e. dasatinib leading to a favourable evolution, achieving a CMR, one patient died in a traffic accident after the CCR had been obtained at 12 months, 2 patients evolved towards BP after 12 months of treatment and received type "3+7" chemotherapy with unfavourable evolution and death; in 3 patients the CCR was not obtained at 12 and 18 months, respectively, and IInd line treatment with dasatibin was initiated.

In an analysis of four consecutive clinical studies conducted in the USA on 281 imatinib treated patients, assessed for the detection of treatment response according the ELN criteria, patients with suboptimal response at 6 months had similar long term results with those in whom the treatment did not succeed, as compared to those who had an optimal response (risk rate 3.2 and 3.4, respectively). Similarly, patients with suboptimal response to imatinib at 12 months had a less favourable survival without adverse events as compared to optimal response patients. Those with suboptimal response at 18 months had similar results with optimal response patients. Additionally, survival data with no disease progression at 4 years in patients with no or suboptimal response at 6 months were significantly less favourable than in optimal response patients (60%, 78% vs. 95%)[6].

These data underline the need for early intervention at 6 and 12 months when a suboptimal response is detected because these patients may be eligible for alternative treatment.

The development of TKI represented a revolution in the CML treatment. According to the ELN, the standard treatment in patients with CML in BP consists of 400 mg imatinib, daily. If imatinib resistance is confirmed, the treatment must be replaced as soon as possible in order to avoid the selection of mutant clones which may be resistant to IInd generation TKI [7].

ELN recommends the early and continuous response monitoring by increasingly sensitive molecular techniques such as RT-PCR in order to aid the identification of adequate therapies for suboptimal response patients or for non-responders.

CONCLUSIONS

Imatinib 400 mg/day is the standard treatment for CML in BP patients.

In our study, most patients with CML-CP reached complete cytogenetic response, a large part of the patients who responded to imatinib had a MMR and only in a small CMR was achieved.

Physicians must perform early and continuous monitoring of patient response at 3, 6, 12 and 18 months, as well as at any other moment during the treatment period in order to establish the optimal therapy for better benefits in CML patients.

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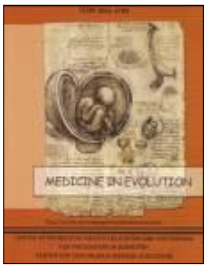
THE INVOLVEMENT OF HUMAN PAPILLOMAVIRUSES IN THE CARCINOGENESIS OF TONSIL MALIGNANT TUMORS



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ABSTRACT

There is already a signifficant amount of data which suggests that human papiliomaviruses with oncogenic risk are associated with a subgroup of squamous cell carcinomas of the head and neck, especially carcinomas from the oropharynx area.

More than 100 types of human papillomaviruses have been identified so far, out of which types 16, 18, 31, and 33 have high oncogenic potential.

The viruses have oncogenic actions by production of oncoproteins coded by genes E6 and E7. E6 binds p53 protein and degrades it, while E7 binds pRb and inactivates its function. Due to the low levels of p53 and pRb, the control of the cellular cycle is altered, and infected cells enter rapidly in G1 phase and begin to synthesize DNA, while apoptosis is blocked.

The diagnosis of the infection with human papillomavirus was confirmed by the identification of the serum titer of anti-HPV16 and anti-HPV18 antibodies through ELISA. Due to the fact that out of the 100 types of human papillomavirus, the types 16 and 18 are the most signifficantly associated with carcinogenesis and with the presence of malignant squamous cell tumors both of the head and neck and of the tonsil, they were the only studied. An antibody titer higher than 8 and 7 ELISA/ml units, was considered a positive sign for ongoing or past infction with human papillomavirus 16 and 18, respectively.

None of the correlations studied had statistical significance, with the exception of that between the anti-HPV18 antibodies titer and the frequency of local pain.

Key-words: human papillomavirus, tonsil carcinoma, pathology

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INTRODUCTION

There is already a signifficant amount of data which suggests that human papiliomaviruses with oncogenic risk are associated with a subgroup of squamous cell carcinomas of the head and neck, especially carcinomas from the oropharynx area[1, 2], and that smoking and human papillomaviruses with high oncogenis risk infection, detected by the presence of anti-human papillomavirus antibodies synergically or aditively increase the risk for squamous cell carcinomas of the head and neck.

More than 100 types of human papillomaviruses have been identified so far, and classified in high oncogenic group (16, 18, 31, and 33), associated with human malignant tumors, out of which the uterine cervix carcinoma is the most studied, and low risk group, associated with benign hyperplasic lesions, such as verucae, condillomas and papillomas. The viral genome is divided into a noncoing region and two coding regions, I and II. The first coding region contains the exons for the formation of proteins E1–E2 and E4–7, which are important for the pathogenesis and transformation, and the second region contains the genes for L1 and L2 proteins of the capsid. The human papillomavirus has been suspected for the first time to have a role in squamous cell carcinoma of the head and neck pathogenesis due to their morphologic alterations[3]. Ever since there ia a continuous accumulation of molecular proves for the presence of the virus in such lesions[2]. The type 16 of the human papillomavirus is present in a signifficant proportion in oropharynx carcinomas[4-9] and has been identified in over 90% of the squamous cell carcinomas of the head and neck positive to human papillomavirus. The human papillomaviruses types 18, 31, and 33 had been identified in the other cases. The viruses have oncogenic actions by production of oncoproteins coded by genes E6 and E7. E6 binds p53 protein and degrades it, while E7 binds pRb and inactivates its function. Due to the low levels of p53 and pRb, the control of the cellular cycle is altered, and infected cells enter rapidly in G1 phase and begin to synthesize DNA, while apoptosis is blocked[10]. Moreover, there is an induced overexpression of other factors involved in the cellular cycle such as $p16^{INK4A;}$ [11]. The fact that the major viral capsid protein, L1, is able to selfassembly and thus form virus-like particles, useful for anti-human papillomatvirus infections vaccination, is also of interest[12].

MATERIAL AND METHOD

The presence of human papillomavirus infection in the patients studied.

The diagnosis of the infection with human papillomavirus was confirmed by the identification of the serum titer of anti-HPV16 and anti-HPV18 antibodies through ELISA. Due to the fact that out of the 100 types of human papillomavirus, the types 16 and 18 are the most signifficantly associated with carcinogenesis and with the presence of malignant squamous cell tumors both of the head and neck and of the tonsil, they were the only studied. An antibody titer higher than 8 and 7 ELISA/ml units, was considered a positive sign for ongoing or past infection with human papillomavirus 16 and 18, respectively. For the identification of all the epidemiologic components of the infection with human papillomavirus, including its roles in the

development of benign lesions and in carcinogenesis, we have correlated the anti-HPV16 and anti-HPV18 antibody titer with the patients age, sex, living environment, histopathologic type of the lesion and with the symptoms from the clinical picture.

RESULTS

The statistic study of type 16 human papillomavirus infection in patients from the study group

The infection with type 16 oncogenic human papillomavirus was detectated by the presence of high titers of anti-HPV antibodies in the patients serum. Out of the 134 patients, 127 (79.41%) were positive to HPV16 (antibodies with titer higher than 8 ELISA units/ml), mainly those with malignant tumors.

The correlation between anti-HPV antibodies titer and the age of the patients

Normally the most exposed patients to human papillomavirus infection are the young, with active sexual life and frequently gather, thus having a higher probability for infection than the aged ones. In addition, the higher reactivity of the immune system in these patients should make this scenario possibile.

The correlation between the two variables is weak, with a correlation coefficient close to 0,2. Mosr probably this behaviour is due to the association between the infection with type 16 and type 18 human papillomavirus, present in most of the patients.

The association of type 16 human papillomavirus infection and the living environment of the patients

Only the association of the two variables was studied by Chi square test, which shown that the hypothesis is not concludent, even if it was expected that the patients from the urban environment, more exposed to infection, to have higher antibodies titers than those from rural areas. The lack of association between the two variables can be explained, the same as the correlation with patients age, by the fact that most of the patiens had presented high titers of anti-HPV18 antibodies.

The study of the association between type 16 human papillomavirus infection and the sex of the patients studied

From this point of view the expected behaviour was that female patients, more exposed to general infection with human papillomavirus, to have higher incidence, with a higher anti-HPV16 antibodies titer. Only the association between the presence of an antibody titer higher than the threshold and the sex of the patients was studied by Chi square test, which, as in the case of the relationship between the high anti-HPV16 antibodies titerand the patients living environment, has shown no statistical signifficant correlation between the two variables.

The study of the correlation between the anti-HPV16 antibodies titer and the histopathologic type of the lesion is a subject of interest especially in the case of the squamous cell carcinomas, which are documentedly associated with human papillomavirus infection, especially types 16 and 18. Although in the patients group was a male patient with flat condilloma, which is caused by human papillomavirus, the anti-HPV16 antibodies titer in his case was rather small.

The correlation between the two variables is not signifficant, with a small correlation coefficient. Thus, it seems that the high anti-HPV16 antibodies titer is not necessary associated with a decrease incidence of the squamous cell carcinoma.

The study of the correlation between anti-HPV16 antibody titer and the frequency of local pain.

As the local pain is the main symptom in the studied patients, it was

also the first studied. The patients with lower antibodies titers than the infection threshold were also considered. There is no correlation between the anti-HPV16 antibody titer and the frequency of local pain. Taking into account the fact that usually the human papillomavirus infection is painless until the appearance of specific lesions which cause pain by local compression or inflammation/ulceration, this result was somehow expected.

The study of the correlation between anti-HPV16 antibodies titer and the frequency of foreign body sensation

The correlation was studied by both methods, Pearson and non-parametric correlations, but none of them was statistically significant. The correlation coefficients were extremely small, almost neglectable.

The study of the correlation between anti-HPV16 antibodies titer and the frequency of disphagia.

Disphagia was a quite rare symptom, due mostly to local inflammation and less to the presence of a locally advanced tumor. The study of this correlation aims to ellucidate an eventual cause-effect relationship between the inflammation followed by disphagia and the presence of the human papillomavirus. The correlation is not significant, although stronger than the later one.

The study of the relationship between anti-HPV16 antibody titer and the frequency of odinophagia.

Odinophagia was rarely present, associated in 2 cases out of 3 with disphagia. From this point of view, it is expected that in this case the correlation was not statistically significant. Taking into account the fact that odinophagia was present in only 3 cases, the results must be assessed acordingly. This is the reason why the other rare symptoms, such as disphonia and irradiated ear pain, which we can assume that were not caused by an eventual infection with human papillomavirus have been investigated by Chi square test only. Both in disphonia, and in irradiated ear pain, the Chi square test show there is no association between the antibody titer higher than the threshold and the frequency of these symptoms.

The correlation between anti-HPV16 titer and the frequency of halitosis

Halitosis may be caused by initial viral infection with an overlying bacterial infection, especially in ulcerated tumors. From this point of view, he study of the correlation between the anti-HPV16 antibodies titer and the frequency of halitosis might prove useful. The correlation between the two variables is not significant, with the smaller correlation coefficient of all the relationships studied. It is obvious that halitosis is due most probably to bacterial or fungal suprainfection of an ulcerated tumor.

The Correlation between the anti-HPV16 antibodies titer and the frequency of local haemorrhage

Local haemorrhage was most frequently associated with tumor ulceration and not necessary with a local bacterial or viral infection.

The correlation between the two variables is weak, without statistical significance, which was expected because haemorrhage was directely associated with tumor ulceration, especially in the case of malignant tumors.

The study of the type 18 human papillomavirus infection in patients with tonsil tumors

The type 18 of the human papillomavirus is the other type with high oncogenic potential besides type 16, which causes increased proliferation of keratinocytes producing squamous cell carcinomas and proliferation and migration of lymphocytes, with the apparition of lymphoepithelial carcinomas. Due to the lack of association between viral infection and the parameters such as the living environment, sex and rare symptoms such as odinophagia, disphonia and irradiated ear pain, as shown by the anti-HPV16 antibody titer correlation study, the correlation between anti-HPV18

antibody titer and this parameters was not investigated.

The correlation between anti-HPV18 antibodies titer and the patients age

The number of the patiens with antibody titer higher than the 7 ELISA units/ml threshold was 130 (88,23%). Most of the patients with high anti-HPV18 titer didn't have the same high titer of anti-HPV16 antibodies.

The correlation between anti-HPV18 antibodies titer and the age of the patients

On the model of the correlation between the age and anti-HPV16 antibodies, the anti-HPV18 antibodies was expected to be higher in young patients, more exposed to infection, due to agglomeration in colectivities and to active sexual life. The correlation between the two variables, although not statistically significant, is inverse, which shows that younger patients tend to have a higher anti-HPV18 antibodies titer, expected not only due to significant exposure of the young to infection, and to higher reactivity of their immune system.

The study of the correlation between anti-HPV18 antibodies titer and the histopathologic type of the lesion

From the histopathologic type point of view, the patients with malignant tumors would be expected to have a higher viral load, triggering a stronger immune response, which normally should lead to the detection of higher antiviral antibodies titer. The correlation between the two variables is not statistically significant.

Completing this corelation study, we have investigated the anti-HPV18 antibody titer in benign and malignant lesions patients separately, emphasizing mostly on squamous cell carcinomas, which were most frequent. We have first identified the statistical descriptors for each series.

The two series are sufficiently close as dispersion and average value, which shows that the anti-HPV18 antibodies titer is not significantly different in patients with malignant tumors as compared to those with benigne lesions.

The correlation betwen the anti-HPV18 antibody titer and the frequency of local pain

The correlation between the two variables is statistically significant, with p low under the significance threshold.

The correlation between anti-HPV18 antibodies titer and halitosis

As we have shown before, the halitosis was not due to viral infection but mostly to fungal or bacterial suprainfection on an ulcerated tumor. The correlation is extremely weak.

The correlation between anti-HPV18 antibody titer and the frequency of local haemorrhage

The last studied aspect was the eventual relationship between anti-HPV18 antibody titer and the ulceration of the local lesions followed by haemorrhage from ulcerations. The anti-HPV18 antibody titer is not correlated with the frequency of local haemorrhage. This is anyway difficult to identify and is most probably due to tumor ulceration followed or accompanied by necrosis and then by suprainfection most frequently bacterial, with or without associated fungal infection.

Globally, the infection with type 18 human papilloma virus is associated significantly only with local pain. It is remarkable that the mere presence of active infection is not necessary to trigger the pain, but the existence of a high anti-HPV18 antibodies titer, probably correlated with inflammation and with local oedema which produce such symptoms. None of the other signs and symptoms of the studied patients was correlated significantly with anti-HPV18 antibodies titer. In addition, the statistical nalysis results of the anti-HPV18 antibodies titer were not comparable with those of anti-HPV16 antibodies titer. The distribution of anti-HPV18 antibody titer is a little different, and this fact can explain the differences in statistical analysis.

DISCUSSIONS AND CONCLUSIONS

The study published by Thavraj[13], on 142 patients with squamous cell carcinoma of the tonsil, show the role of human papillomavirus infection by the study of its prevalence in tumor tissues, especially through the immunohistochemical study of p16 expression and by the study of the presence of the viral DNA by in situ hybridization. Most patients were positive to human papillomavirus, the same as in our study, in which anti-HPV antibodies were present in most of the cases, either anti-HPV16, or anti-HPV18 antibodies. From the age point of view, it was studied as risk factor in a study on a high number of patients (5538), establishing the threshold of 50[14]. As in our study, the age was not correlated with the increase in the incidence of tonsil carcinoma, even if in combination with infection with human papillomavirus seemed to have a more significant role. As in our study, the age was not associated with the presence of the human papillomavirus, even if younger patients had a higher incidence of HPV infection.

The study of Nguyen documented strictly the presence of the virus, with the identification of viral DNA and immunohistochemical expression of p16, unlike our study, which identified the immune reaction to the presence of the virus by the measurement of the antibody titer. Young age was associated in Nguyen study with a favorable prognosis, due to the higher incidence of well differentiated carcinoma associated with type 16 and 18 human papillomavirus infection. From the histopathologic point of view, besides the differentiation grade, which seems to correlate an increasing incidence of well differentiated tumors associated with human papillomavirus infection, the presence of cystic lymph node metastases seems to be a halmark of human papillomavirus positive tumors[15]. In our study, we have identified a single patient with cystic lymph node metastases, with G_2 keratinized squamous cell carcinoma, with high titer of anti-HPV16 antibodies and a slightly lower titer of anti-HPV18 antibodies, both over the infection threshold established (8 and 7 ELISA units/ml, respectively). The presence of human papillomavirus in tumor tissue is already a fact in a high percent of squamous cell carcinomas, with significant increase of the percentage of positive cases in tonsil carcinomas. However, the prototype of squamous cell carcinoma positive to human papillomavirus is exactly the tonsil squamous cell carcinoma. The identification of the virus presence in tissue fragments is possible either by gene analysis, with the identification of the presence of viral DNA fragments in tumor cells, with a percent which varies signific ntly from a study to another; the general opinion is that the human papillomavirus genome is present in more than 60% of the cases and considers especially types 16 and 18, which have high oncogenic risk.

The study of Glombitza et al[16] correlates the presence of the viral genome of HPV16 in primary tumors and in lymph node metastases of head and neck squamous cell carcinomas and find the higher presence rate in tonsil carcinomas, manifested also by a higher concordance between the positivity of the primary tumor and that of lymph node metastases than in other localizations. This study documents the highest positivity rate of tumor cells at viral DNA fragments, of 83% globally. The study of Rotnáglová et al[17] has identified the presence of the virus in 65.1% of the cases, closer to the average of the other studies; the same authors have identified anti-E6/E7 antibodies as a surogate marker for the presence of the virus in tumor cells, same as the immunohistochemical identification of p16 in tumor specimens.

The concordance between the presence of viral genome fragments and these markers was so good that their use is highly recommended. The factthat the study is one of the most representative as number of patients (108) which has investigated the relationship of surogate markers with the presence of viral genome fragments in tumor specimens validates the method that we have used in the identification of human papillomavirus infection, the presence of antiviral antibodies, even if the immunohistochemistry for $p16^{INK}$ is widely used for the identification of the infection with human papillomavirus besides the identification of mutant p53 protein by immunohistochemistry.

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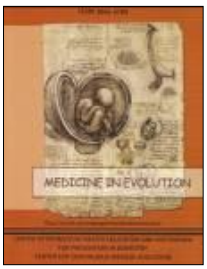
LAPAROSCOPIC TREATMENT OF THE HYDATID CYST OF THE LIVER



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ABSTRACT

Introduction: *In the 1990's, the hydatid cyst of the liver, an affection caused by the larval stages of Taenia echinococcus, started to be treated by laparoscopic surgery, given the advantages of this procedure.*

Objectives*: To assess the results of the laparoscopic treatment of the hydatid cyst liver.*

Material and method*: A number of 46 patients with hydatid cyst liver who underwent laparoscopic surgery in April 1996 – December 2011 were studied; 93.47% of these were patients with Lagrot partial pericystectomy.*

Results: *Lagrot partial pericystectomy was performed in 43 cases (93.47%) and total cystectomy only in 3 cases. Patients with biliary lithiasis (17.39%) associated with the hydatid cyst underwent laparoscopic cholecystectomy. Mortality was nil and morbidity was 8.69%. The average operating time was 85 minutes, the average hospital stay was 6 days and only one patient relapsed.*

Conclusions*: The laparoscopic treatment of the hydatid cyst of the liver is recommended if the selection criteria are met. Laparoscopy has several advantages: reduced postoperative complications, lower costs and quick social and professional reintegration.*

Key words: *Taenia echinococcus, hydatid cyst of the liver, laparoscopic treatment.*

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INTRODUCTION

Echinococcus granulosus, the parasitic agent that causes cystic echinococcosis, is part of the smallest worms of the *Taenia* family whose larval stage causes zoonosis in men. [1]

The treatment of the hydatid cyst of the liver includes treatment with albendazol or mebendazol, plastic surgery or laparoscopy and PAIR (puncture, aspiration, injection, reaspiration [2]

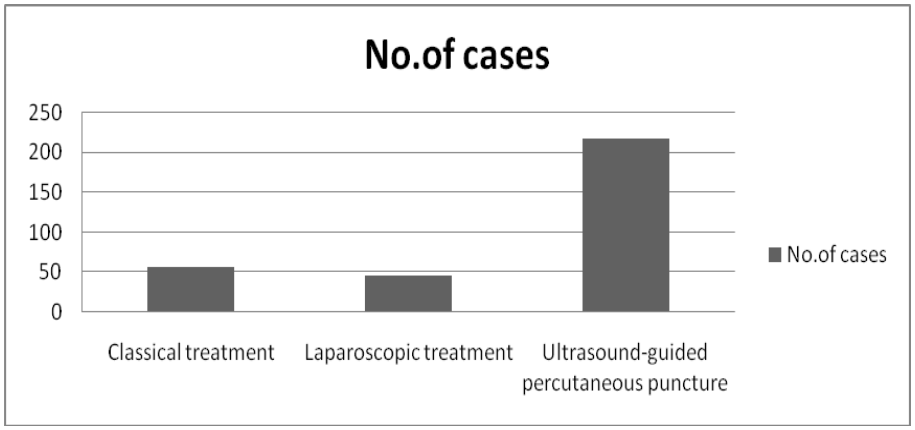
Laparoscopy is recommended in accessible cysts. It provides very good visibility, safe haemostasis and shorter hospital stay, while open surgery is more useful especially in cysts that are difficult to access, in giant cysts and in the presence of adhesions and involves a higher rate of complications and longer hospital stay [3, 4, 5].

Currently, the laparoscopic treatment of the hydatid disease is considered a feasible and safe solution in selected patients. Laparoscopy has low morbidity and mortality rate, complies with the principles of open surgery and has the advantages of minimally invasive surgery: postoperative comfort, minimal pain, short hospital stay, rapid social reintegration [6]. In 1993, Bickel A. & colab. (Israel) and Khoury G. & colab. (Lebanon) reported the first cases of laparoscopic approach in hydatid disease at the Laparoscopic Surgery Symposium in Athens [7]. In Timisoara, the first laparoscopic surgery of a hydatid cyst took place at Surgical Clinic II, in 1998.

The aim of the present paper is to assess the experience gained in the laparoscopic treatment of the hydatid cyst disease in Surgical Clinic II Timisoara.

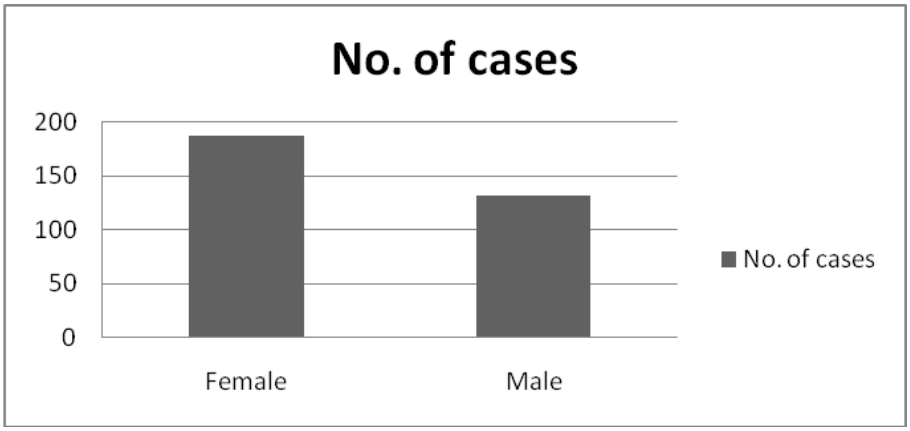
MATERIAL AND METHOD

In April 1996 – December 2011, 319 patients with hydatid disease were admitted to hospital and underwent surgery in Surgical Clinic II of County Emergency Clinical Hospital in Timisoara. Of these, 46 underwent laparoscopic surgery and 56 classic surgery. Ultrasound-guided transcutaneous puncture was performed in 217 cases (graphic 1).



Graphic 1: Case distribution according to type of surgery

Of the 46 patients who underwent laparoscopic surgery, 31 were women (67.39%) and 15 men (32.60%). The patients were between 16 and 67 years of age and the highest incidence occurred in the 31-40 age groups.



Graphic 2: Case distribution according to sex

The patients showed one or several of the following symptoms or signs: pain in the right hypochondrium, biliary dyspeptic syndrome, hepatomegaly. In 5 cases, a renitent tumour was felt by palpation in the right hypochondrium.

As a method to detect hydatid cysts, all patients underwent abdominal ultrasonography. Sonography was followed by CT scan only in 6 cases. The liver cysts were classified according to Gharbi and Niron's criteria [8, 9].

Patient selection is essential for the proper performance of the procedure. A number of 46 patients were selected for laparoscopic treatment according to the following criteria:

- cysts in the anterior and lateral segments - II, III, IV, V, VI [10, 11]
- cysts with thin walls and daughter vesicles, not recommended for the PAIR procedure
- cysts at least 7 cm in diameter
- totally or partially calcified cysts; at present, these are accepted, as most of the time they are inactive ad the risk of secondary dissemination is almost nonexistent [12, 13, 14].

Laparoscopy was not recommended in the following situations:

- infected cysts or liver abscesses
- cyst located in the superior and posterior segments – I, VII or VIII [15]
- more than three cysts
- intrabiliary rupture of cysts or open cysts detected before surgery
- patients with cirrhosis
- patients who had undergone previous superior abdominal surgery
- cysts located near the vascular elements of the liver
- heart and lung affections that do not allow pneumoperitoneum

The therapeutic tactics has the following objectives:

- ultrasonography and CT scan of cyst topography and cyst classification
- complete biological and functional preoperative assessment; diagnosis of associated affections
- an anthelmintic will be administered 24 hours before surgery and after surgery, for three monthly cycles, with a two-week break between them. This additional measure prevents secondary echinococcosis in the event of peritoneal contamination during cyst puncture
- the same objectives will be followed as during the classic surgery: to inactivate the parasite, to prevent contamination, to empty the cyst (and to extract the endocyst), to manage the residual cavity [16]

RESULTS

In the 46 patients who underwent hydatid cyst laparoscopic surgery, the lesions were located in the left hemiliver in 12 cases (26.08%), in the right hemiliver in 32 cases (69.56%) and in both hemilivers in 2 cases.

All patients underwent preoperative ultrasound and radiology imaging and in several cases also CT scans. The patients included in the study consented to this approach.

Table I. Number of cysts and their location

No of cysts/patient	Patients (no)	Patients (%)
1 cyst	41	89.13
2 cysts	5	10.87
Cyst location		
Right hemiliver	32	69.56
Left hemilivers	12	26.08
Both hemilivers	2	4.34

Table II. Clinical parameters of the study group*

Clinical symptomatology	Patients (no)	Patients (%)
Abdominal pain	34	73.91
Hepatomegaly	10	21.74
Dyspeptic syndrome	14	30.43
Asymptomatic	9	19.56

*The patients showed one or several parameters

Cyst dimensions varied from small (7 cm) to huge cysts (15 cm). The average diameter was 9.2 cm. Most cases were acephalocysts (71.74%), but 7 cysts had daughter vesicles and 2 were calcified.

In 43 cases (93.47%), Lagrot partial pericystectomy was followed by extraction of the proligerous membrane, the pericyst and the possible daughter vesicles in a small plastic bag. Next the residual cavity was drained.

In 3 cases, total cystectomy was performed. Two were calcified hydatid cysts and one had daughter vesicles. Total cystectomy was carried out after the inactivation and evacuation of the parasite.

In 8 cases (17.39%), biliary lithiasis associated with hydatid disease also required laparoscopic cholecystectomy.

The mortality was nil and the morbidity rate was 8.69%, - 4 cases, 2 residual cavity abscesses drained by laparoscopy and 2 biliary fistulas managed by endoscopic sphincterotomy. Two patients (4.34%) had preoperative allergic reactions and received hydrocortisone hemisuccinate and antiallergic drugs. One patient (2.17%) relapsed after surgery.

The average operating time was 85 minutes, with limits between 60 and 170. The average hospital stay was 6 days, with limits between 3 to 18 days.

Complicated cases required longer hopital stay.

None of the patients developed postoperative wound infections or eventration. Bowel transit restarted in 48 hours and oral diet started in the first day after surgery. Patient follow-up was possible in all cases, with an average duration of 18 months (10-36 months).

DISCUSSIONS

In laparoscopic treatment, location, dimension and cyst number, the possible biliary cystic fistula and the cyst wall thickness are factors must be taken into consideration in the patient selection process [6].

Lagrot pericystectomy is the preferred surgical procedure. Although it does not involve the risk of cyst dissemination in the peritoneal cavity [15], it is performed in 75-90% of cases [17]. In our group, it was used in most cases (93,47%).

In superficial or marginal cyst locations, extensive pericyst resection can be performed in the cavity. In deep intrahepatic forms, minimal pericystectomy is preferred, which is enough only for the extraction of the proligerous membrane.

During puncture, fluid leakage in the peritoneal cavity can cause anaphylaxis, besides secondary dissemination [18]. For prevention purposes, pads filled with hypertonic solution were introduced through trocars, as recommended by several authors [6,7,15,16,19]. However, the lavage of the peritoneal cavity with this solution must be avoided to reduce the risk of hyperosmolar syndrome. One can also use formalin 2%, which can cause sclerosing cholangitis in case of biliary fistula [19], or oxygenated water, which can increase pressure in the cyst and cause its rupture in the peritoneal cavity; in case of biliary fistula, it may also cause gas embolism [19]. Some surgeons use a combination of Cetrimide 0.5% and Chlorhexidine 0.05% as scolicidal agents; however, Cetrimide may cause methemoglobinemia with cyanosis and metabolic acidosis [20].

Various surgical instruments have been created to avoid intraperitoneal contamination and to prevent secondary echinococcosis. D. Sabău, for instance, has created a coaxial double- lumen device for aspiration that adheres to the pericyst owing to the vacuum in the exterior channel. Cyst puncture and aspiration are performed through the interior channel. Some models have clips to avoid accidental detachment of the aspirating device [21]. Bickel and Eitan introduce a 12-mm transparent trocar with negative inside pressure; the trocar is attached to the pericyst like a suction cup and prevents dissemination during puncture [22]. Yucel uses a 5 or 10-mm trocar with an umbrella-type blocking mechanism in the cavity; the mechanism opens after the trocar is introduced in the cyst [23].

The cyst is emptied and the proligerous membrane is removed and put in a sack. This is an easy procedure when the hydatid cysts are recent. Generally, the proligerous membrane is detached from the pericyst and pressed within the cystic cavity because of the pneumoperitoneum pressure.

In older hydatid cysts with thicker, calcified membranes and biliary fistula, the treatment requires laparoscopic biliary drainage. Residual cavity inspection is required to detect possible complications, especially biliary fistulas that are mentioned in the literature of the field in 12-17% of cases [24,25]. These can be sutured with atraumatic thread, as clips cannot be used. If bile keeps leaking

through the drain tube, endoscopic sphincterotomy and prolonged drainage are recommended. In our cases, most drain tubes were removed after 48-72 hours.

The literature of the field specifies that the average operating time in laparoscopic approach is 40-180 minutes and postoperative complications occur in 8-25% of cases [13,25,26,29,30]. In our study, the conversion rate was 0%, as it was in the study presented by Manterola et al [27]. The average hospital stay is 2-5,6 days, mortality is nil and relapses occur in 0-3,6% of cases [25,26,27,30]. Our results are comparable to these findings.

CONCLUSIONS

Experience in the field of laparoscopic surgery is not very rich. The studies published so far deal with small groups of patients and the number of prospective, randomized studies on the laparoscopic approach is small as well.

Consequently, we believe that conventional surgery can be performed also laparoscopically and the surgical treatment demands can be respected. This works well in selected patients with superficial and smaller hydatid cysts located in anterior segments. In their case, percutaneous puncture is also effective.

Laparoscopy has several significant advantages: it is a minimally invasive procedure involving minimal pain, low infection rate, short hospital stay, therefore, low costs and quick social and professional reintegration.

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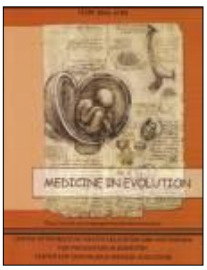
JUVENILE NASOPHARYNGEAL ANGIOFIBROMA - ENT CLINIC TIMISOARA EXPERIENCE



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ABSTRACT

Objectives:*Juvenile nasopharyngeal angiofibroma is a rare, histologically benign tumor, very aggressive locally, affecting male adolescents or those at puberty (around 15 years of age). The aggressiveness and high vascularization make surgery very difficult, and surgical biopsy is not recommended.*

Patients and method: *This is a retrospective study including 31 patients diagnosed and treated at the ENT Clinic Timisoara between 1981-2012. Surgery is the main method of treatment used, especially for early stages, while for advanced stage cases and having intracranial extension, radiation remains the chosen therapeutic option.*

Rezults: *All patients were males. The surgical approach varied, as well as the technique, and technical, but the most frequently used technique was Denker-Rouge. The relapse rate was 16.13%.*

Dicussions: *In order to establish a preoperative diagnosis and staging, we use CT scan imaging or MRI with and without contrast material as well as angiography. The patients were classified using the Sessions staging system for juvenile nasopharyngeal angiofibroma.*

Conclusions: *The nasopharynx tumor size is not necessarily in accordance with the real tumor extension and size. Extranasopharyngeal extension is very common in all cases - the most common being the nasal extension.*

Keywords *Juvenile nasopharyngeal angiofibroma, benign, tumor extension, surgical approach, relapse*

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INTRODUCTION

Juvenile nasopharyngeal angiofibroma is a rare, histological benign tumor of the nasopharynx, affecting almost exclusively male teenagers or at puberty, being typically diagnosed in 14-25 old boys. It is a highly vascularized tumor with high tendency towards local and loco-regional invasion; it has a capsule, with scattering within the submucosa. For this reason it's a destructive tumor. From a histological point of view, it's a mesenchymal tumor, vascular, having high cells, composed of fibrous connective tissue and abundant vascular endothelium lined spaces. There are various theories regarding the formation of angiofibroma, but the most accepted theory is the theory of angiogenesis and histogenesis. This describes angiofibroma as a pure vascular tumor which proliferates in a hemangiomatous manner and in which all other components, including fibrous connective tissue, are derived from non-differentiated vasoformator mesenchymal. The tumor origin can be found on the posterolateral wall of the nasopharynx at the upper edge of the sphenopalatine foramen, which is also a prop for the sphenopalatine artery, a branch of the internal maxillary artery. More exactly, it is located where the sphenoid process of the jaw bone meets the horizontal wing of the vomer and pterygoid foot of the sphenoid. The juvenile nasopharyngeal angiofibroma represents 0.5% of head and neck neoplasm and is considered the most common benign neoplasm of the nasopharynx. Despite its benign nature, the local growth and destruction method can cause bone remodeling. Although it does not produce bone erosion, the tumor has a high potential of life threatening complications such as: epistaxis, intracranial extension and massive intraoperative bleeding.

Classic signs of juvenile nasopharyngeal angiofibroma are progressive nasal obstruction and / or epistaxis and rhinorrhea, together with other symptoms that are dependent on the direction and extent of the tumor (5). The tumor extension in adjacent areas can cause "swelling facial" type deformities as well as proptosis.

Diagnosis is based on history, clinical examination and radiological and imaging results, knowing that biopsy is not recommended. Angiography helps demarcate the tumor's blood supply and identify the vessels that feed it. However its real use is still debatable. Surgical excision was the main angiofibroma treatment and the approach normally depends on the tumor extension. An important role in this regard is played by the CT scan and appropriate staging.

In this study we try to evaluate various clinical signs of the tumor, as well as the different surgical approaches, used depending on the tumor extension and staging.

PATIENTS AND METHOD

This study is a retrospective study of all patients diagnosed and treated at the ENT Clinic in Timisoara between 1981-2012 and who received histologic confirmation from the of surgically extracted piece. The study includes a total of 31 patients, all men aged between 11 and 20. Patient data was studied

retrospectively from observation sheets related to age, sex, signs and symptoms, duration of symptoms, moment of diagnosis, tumor location, imaging evaluation, staging, treatment modality and surgical technique, relapse rate, last assessment and its status. All patients were followed between 1 and 2 years. No patients underwent preoperative embolization as we were trying to control intraoperative bleeding through hypotensive anesthesia. One single patient underwent radiotherapy treatment for relapse. Primary staging included for all cases CT scan or MRI evaluation. All patients were evaluated according to Sessions classification for juvenile nasopharyngeal angiofibroma:

Fig.1. Sessions classification

Stage		**Localization**
I	A	Tumor limited to nose and / or nasopharynx
	B	Tumor extended into one or more paranasal sinuses
II	A	Tumor with minimal extension to pterygopalatine fossa
	B	Tumor occupying entirely the pterygopalatine fossa with or without orbital apex erosion
	C	Tumor involving the infratemporal fossa with or without extension to the throat
III		Tumor with intracranial extension

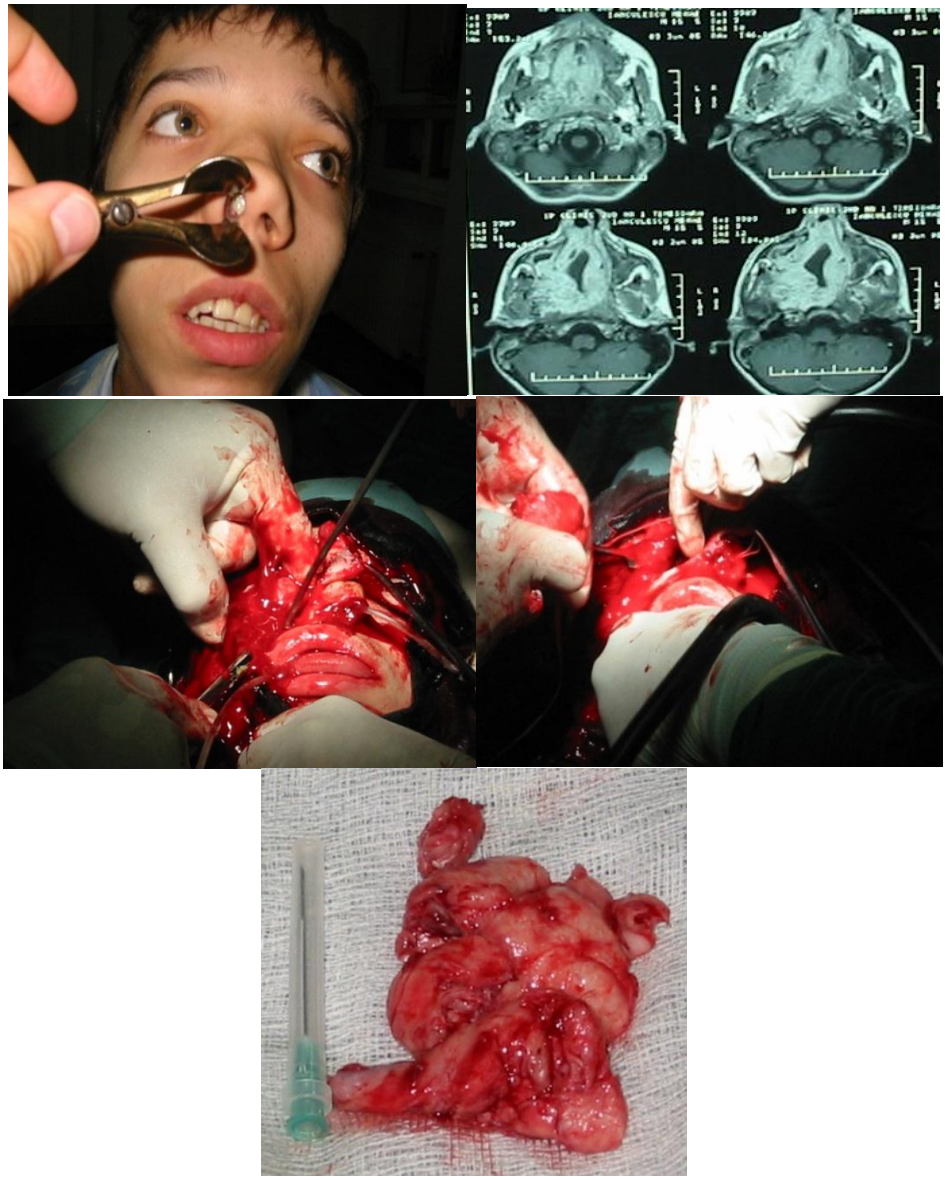


Fig.2. Name: I.M. Skull MRI: voluminous expansive process that fully engages the rinopharynx, the bilateral nasal cavities, the ethmoid and sphenoid sinuses.
Status: IIB. Technique:Paralateronasal -3 recurrent

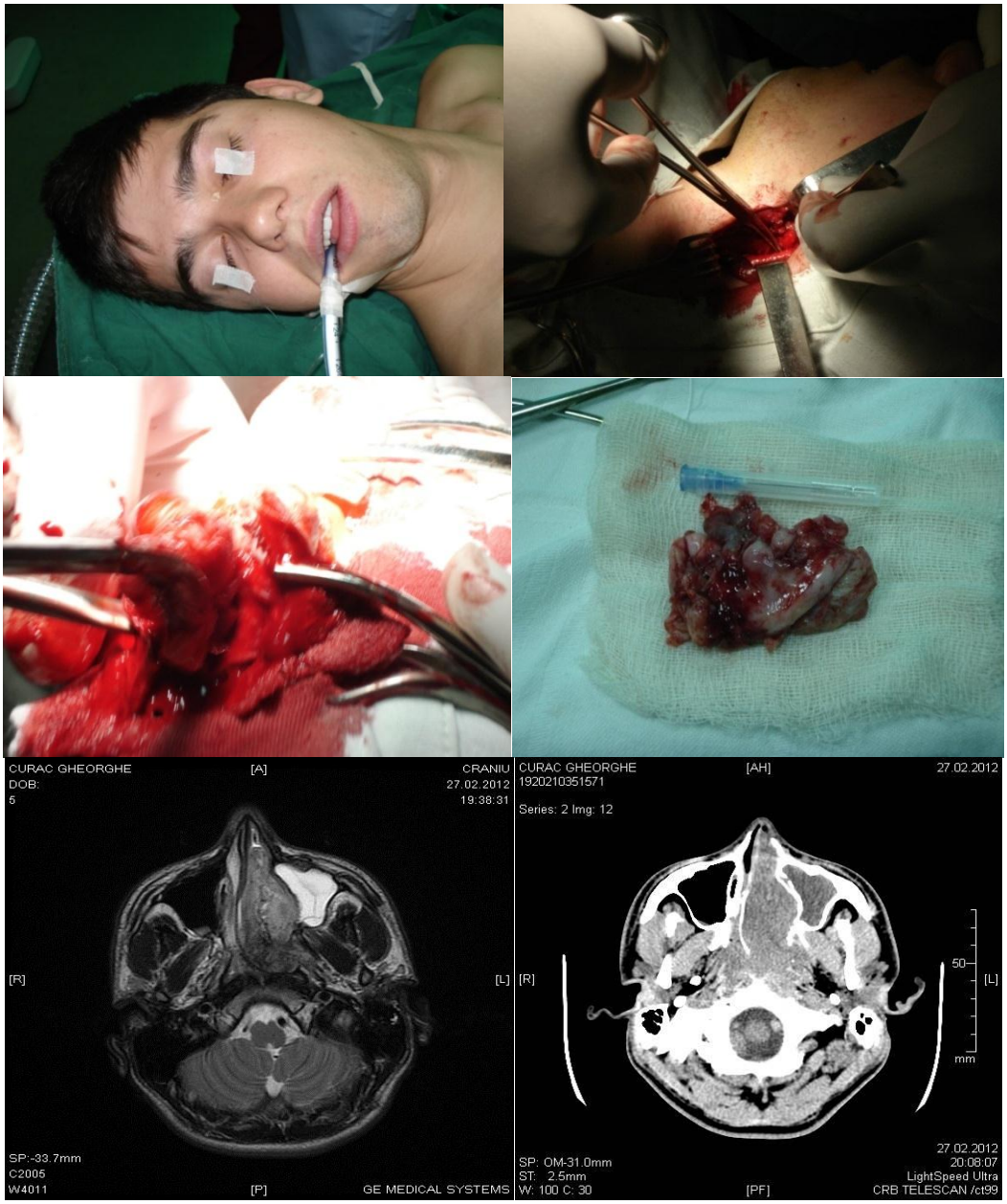


Fig.3.Name: C.G. Status: IIC Ligation of the venous trunk and waiting thread on the left carotid artery. Paralateronasal approach. Skull MRI axial section highlighting tumoral extension in the left nasal fossa, nasopharynx, and infratemporal pterygopalatine left fossa. Skull CT scan axial section with visualization of the tumoral formation in the left nasal cavity, nasopharynx, pterygopalatine fossa and left infratemporal fossa.

RESULTS

The age distribution of the 31 patients diagnosed with juvenile nasopharyngeal angiofibroma was as follows: 8 patients (25.80%) aged 11-12 years, 5 patients (16.13%) aged 13-14 years, 10 patients (32.26%) between 15-16 years old, 7 patients (22.58%) between 17-18 years and 1 patient (3.23%) between 19-20 years old.

The most common symptoms were: progressive nasal obstruction

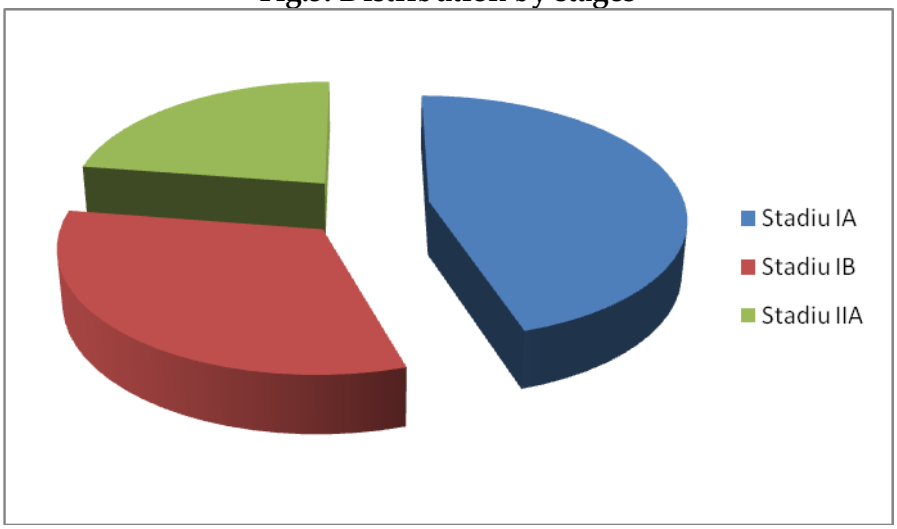
appeared in all patients (100%), massive and recurrent epistaxis in 23 patients (74.19%), rhinolalia in 16 patients (51.61%), nasal obstruction associated with epistaxis in 16 patients (51.61%). Other symptoms seen in these patients were: serious medium otitis and hearing loss in 19 cases (61.29%), headache occurred in 4 cases (12.90%), rhinorrhea in 3 patients (9.68%) and facial swelling in 1 patient (3.23%). For 11 patients (35.48%), these symptoms occurred less than a year before.

Fig.2. Frequency of symptoms

Symptoms	No. of Patients	%
Progressive nasal obstruction	31	100
Massive and recurrent epistaxis	23	74.19
Closed rhinolalia	16	51.61
Nasal obstruction + Epistaxis	16	51.61
Serious medium otitis + Hearing loss	19	61.29
Headache	4	12.90
Rhinorrea	3	9.68
Facial swelling	1	3.23

Patients were staged by tumor size and location according to the Sessions classification, as follows: stage IA 14 patients (45.16%), stage IB 10 patients (32.26%), stage IIA in 7 patients (22.58%). In this study we found 5 cases of relapse (16.13%), classified 2 (40%) in stage IIA and 3 (60%) in stage IIB.

Fig.3. Distribution by stages

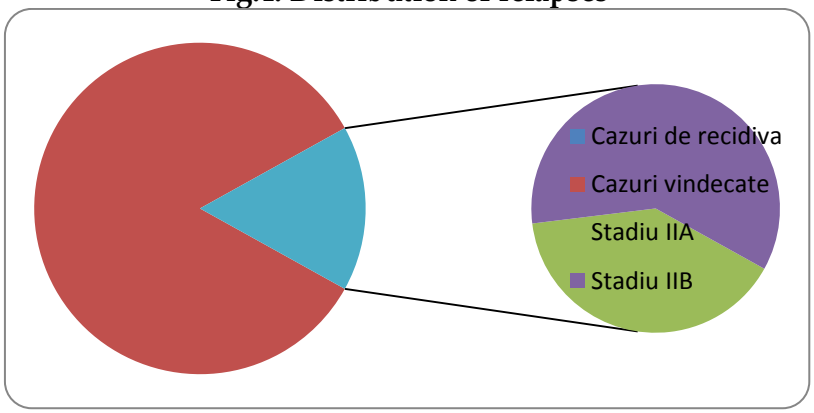


All patients were treated surgically, and the technique chosen was justified by the tumor extension and staging: retrovelopalatine technique used in 5

patients (16.13%), Denker-Rouge technique in 13 patients (41.93%), the transpalatine technique - 5 patients (16.13%), and for 8 patients (25.80%) the paralateronasal technique was used. There has been no pre-surgery embolization. There were no recorded cases of post-surgery mortality either related to the procedure or other major complications.

Cases of relapse were treated surgically. In no case of relapse was the surgical endoscopic technique used. All 5 cases of relapse have been addressed by the paralateronasal technique. In relapses classified IIB, ie with intracranial extension or incompletely extracted tumor, external radiotherapy was used, which practically healed the area. Postoperative follow-up lasted between 1 and 2 years.

Fig.4. Distribution of relapses



DISCUSSIONS

Tumor incidence is rare, being found predominantly in the developing males. The occurrence age in this study varies between 11-20 years with an average of 14 years.

Diagnosis is made mainly on clinical manifestations of the tumor. Most of the times, the present of the triad: progressive nasal obstruction, epistaxis and nasopharynx tumor in young men suggests the diagnosis of juvenile nasopharyngeal angiofibroma. In some studies the appearance of anterior radiological bulging of the posterior wall maxillary sinus (antral sign) is a pathognomonic sign. An essential role in preoperative diagnosis of the angiofibroma is played by endoscopic evaluation of the patient, and imaging: CT scan and MRI, which apply to all patients evaluated in our clinic. It is well known that preoperative biopsy should be avoided because of the risk of subsequent massive hemorrhage. Therefore preoperative diagnosis is a stage diagnosis, because the safety diagnosis is established only postoperatively by histopathological examination from the surgically extracted piece.

There were 5 (16.13%) complete recurrences encountered post-surgery in our clinic. Fagan et al. reported a total of 16 patients relapsed to 37.5%, which is far from what Radkowski et al. had reported: 22% from a total of 23 cases, of which one third were recurrent stage III. Most recurrences appeared in stage IIB and a surgical re-intervention took place, and 3 cases (9.68%) underwent radiotherapy. Undervaluation of staging may be one of the explanations leading to this result. Recurrences must be taken into consideration when large tumors were probably not fully extracted. Routine usage of imaging evaluation in postoperative follow-up period shows

that some tumor remnants detected in asymptomatic patients may or may not require additional intervention. This suggests that extensive surgery meant to remove the entire tumor may not be necessary. In this study recurrences were found on average 9 months post-surgery.

There is a correlation between tumor stage and the time period between initiation of symptoms and diagnosis. It was found that this decreases in advanced stages. Paris et al. revealed this relationship by showing comparable results that tumors in high states are more aggressive than those in small stages.

The choice of surgical technique is still debatable, but surgery remains the main treatment that completely removes the tumor. Traditional techniques used in surgical treatment of juvenile nasopharyngeal angiofibroma are: transpalatal technique, transpharyngean, Denker-Rouge, medial maxilectomy, lateral transfacial rhinotomy, mediofacial degloving and Le Fort I osteotomy, as well as infratemporal or subtemporal lateral approach. In this study the most widely used technique was Denker-Rouge as initial intervention. For relapses only the paralateronasal technique was used.

Transpalatal approach technique is using U-shaped palace incision, but its disadvantage is the increased risk of relapse in large tumors due to poor exposure and risk of palatal fistulas. One of the patients treated by this technique showed recurrence at 16 months post-surgery. Lateral rhinotomia provides good exposure for removing a large tumor. Currently the mediofacial degloving is an often technique used due to good exposure and avoiding postoperative scarring. In large tumors in intracranial or orbital fossa, Le Fort I osteotomy technique is the best approach.

External radiation therapy is reserved for patients with unresectable intracranial lesions or those who refuse surgery for various reasons. Risks of radiation include radiologically induced malignancies, radiologically induced cataracts as those described by Cummings. Some studies show that of all patients treated primarily with radiotherapy, 80% had good tumor control, although recent studies cannot confirm this. Recently a study introduced three-dimensional congruent radiotherapy, which has demonstrated superiority over conventional radiation therapy on extensive forms of juvenile nasopharyngeal angiofibroma. In this study all three relapsing patients (9.68%) classified as stage IIB were treated with conventional radiotherapy and had good tumor control.

CONCLUSIONS

Juvenile nasopharyngeal angiofibroma is a benign, rare tumor of the nasopharynx, which, due to its strategical anatomical position can affect several vital structures of the skull base. The tumor almost exclusively affects male teenagers. The triad: progressive nasal obstruction, epistaxis and tumor mass in the nasopharynx leads to the clinical diagnosis of juvenile nasopharyngeal angiofibroma. The nasopharynx tumor size is not necessarily in accordance with the real tumor extension and size, it can be only the "tip of the iceberg". Extranasopharyngeal extension is very common in all cases - the most common being the nasal extension.

A CTscan with contrast material is pathognomonic for the diagnosis of juvenile nasopharyngeal angiofibroma and allows accurate staging of the tumor, which is very necessary for choosing the surgical technique, estimating prognosis and reporting results.

Radio therapy must be reserved for inoperable cases, for recurrences or for patients who refuse surgery because of various reasons.

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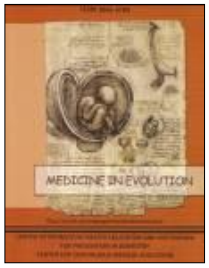
COMORBIDITY BETWEEN BIPOLAR AFFECTIVE DISORDER AND CHRONIC MEDICAL DISEASES – EPIDEMIOLOGICAL AND PUTATIVE ETIOPATHOGENETIC CONSIDERATIONS



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ABSTRACT

Scholarly literature and clinical practice have both shown that the comorbidity of bipolar affective disorder with other chronic medical diseases is rather a rule than an exception. On the one hand, the medical comorbidity profile of patients diagnosed with bipolar disorder may display a certain parallelism to the medical profile of the general population; the difference between the two profiles lies in the higher sensitivity displayed by the psychiatric subjects. On the other hand, the latest studies in the field indicate that there is a peculiar association between bipolar affective disorder and some chronic medical diseases.

For example, there has been noted a significantly higher prevalence of diseases such as migraine and obesity in bipolar individuals as compared with the general population. The proper evaluation of and the ethics of promoting greater awareness of this clinical reality are all for the more important as it can be the source of therapeutic resistance during psychiatric treatment and, subsequently, it may influence for the worse the history of the psychiatric illness.

Recent research has detected new molecular and genetic mechanisms which could argue for the existence of specific links between bipolar affective disorder and certain chronic medical diseases. On the one hand, the paper offers an exhaustive review of the main directions in scholarly literature on the issue of comorbidity between bipolar affective disorder and chronic medical diseases. On the other hand, by starting from scholarly data and by applying an analytical perspective, we seek to point out certain putative links that might explain comorbidity between these two distinct nosological entities.

Keywords: bipolar affective disorder, comorbidity, chronic medical disease

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INTRODUCTION

Considerations on comorbidity between bipolar affective disorder and chronic medical diseases

The awareness of the link between mental illness and medical disease goes as far back as the Antiquity; the Hippocratic Corpus already mentions the connection between depression and epilepsy by means of the following statement: 'melancholics ordinarily become epileptics, and epileptics melancholics: what determines the preference is the direction the malady takes; if it bears upon the body, epilepsy, if upon the intelligence, melancholy'.[1] More recently, Alvin Feinstein has introduced the notion of 'comorbidity' in medical sciences in order to define those cases when "a distinct additional clinical entity" occurs during the clinical course of some other disease. The latter's onset clearly preceded the start of the new clinical entity.[2]

Somewhat expectedly, a higher prevalence of chronic medical conditions has been recorded among individuals with mental disorders than among healthy individuals. Thus, an important epidemiologic survey carried out in the US between 2001 and 2003 by Kessler et al. (National Comorbidity Survey Replication, NCS-R) clearly showed that 25% of the American population had been diagnosed with at least one mental disorder and that an overwhelming majority of those who suffered from psychiatric disorder had also at least one medical comorbid diseases.[3]

In the case of bipolar patients, comorbidity of medical diseases with mental illness is rather a norm than an exception. Medical conditions such as obesity and cardiovascular diseases generate a high mortality ratio among this particular category of mentally-ill patients.[4]

In a meta-analysis which comprised a review of scholarly literature from 1970 to 2005, Krishnan pointed out that migraine, overweight, obesity, diabetes mellitus type 2 and hypothyroidism are among the most frequent medical diseases associated with bipolar disorder. The author has particularly emphasized that, at least in part, it is rather difficult to ascertain whether the medical diseases were truly comorbid with the mental illness or whether the former were the direct consequence of the medication used to treat bipolar disorder.[5]

A vast research study carried out in the UK has pointed out that mortality rate is significantly higher for mentally-ill patients as opposed to the general population. The main goal of the research was to assess the mortality rates for patients diagnosed with severe mental diseases, such as schizophrenia, bipolar affective disorder and persistent delusional disorder .The main causes of deaths were coronary heart disease, strokes and bronco pulmonary cancer. These mortality rates remained significantly high, irrespective of smoking, maladaptative social behavior or of factors connected to antipsychotic medication.[6]

Comorbidity of bipolar affective disorder with chronic diseases – putative etiopathogenetic connections

Comorbidity between medical diseases and bipolar disorder has to be approached from different angles. One scholarly perspective takes into consideration the temporal plane. Thus, it is important to assess whether the bipolar disorder preceded the medical disease or vice versa. However, it is an open question whether the two chronic diseases are interdependent. In such a case, the notion of 'comorbidity' is somewhat misused, but at least theoretically, the amelioration of either of the two pathological conditions should implicitly lead to the amelioration of the other. Hence, any affective disorder caused by a subjacent primary disease or by a systemic disease which has a secondary impact upon the central nervous system cannot be cured unless the main or the initial disease is completely treated; one fact which can clearly demonstrate the causal link between the two. In this particular situation, the diagnostic manuals of mental disorders stipulate a separate category which is distinct from the mood disorders not caused by medical diseases or by substance use. Another possibility is that the second disease underlines an iatrogenic determinism, in the sense that the medication used in the treatment of the first disease may constitute a major cause for the occurrence of the second disease but this does not happen directly but rather through the long-term effects of medication. And there are eloquent examples in this respect. On the one hand there is the mood disorder triggered by the use of corticosteroids or of interferon alpha in C viral chronic hepatitis. On the other hand, the occurrence of the metabolic syndrome with all its negative effects upon the physical health of patients treated with the latest antipsychotic medication (Figure 1).

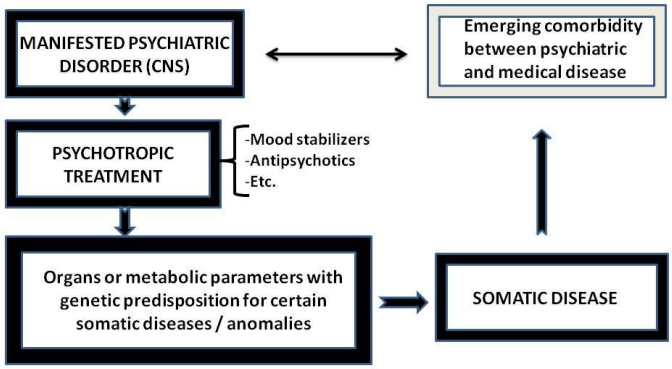

Figure 1. Iatrogenic perspective of comorbidity between bipolar affective disorder and chronic medical diseases

Comorbidity between mental disorders and medical diseases can also be analyzed through the dichotomist type of the classic bidirectional approach, which can illuminate the interdependency - psychosomatic vs. somatopsychic - of the two diseases. The notion of somatic-psychic implies that the mental disorder is either the result of a series of peculiarities or of biological dysfunctions - caused by genetic determinism - or the result of a plurality of factors at the biological level. The latter usually extend over a long period of time in the individual's lifetime.

In fact, it is very debatable if this type of approach is truly pragmatic because as it is already known even in reactive psychopathological conditions (such as depressive reaction or anxiety reaction) the medication acting on biological level had proved clinical efficient. In the same way, the psychotherapeutically approaches of mood disorder (e.g. major depressive episode) even though it act on psychological level, in some situations had proved as being at least as efficient as psychotropic medication. Hence, psychosomatic versus somatopsychic dichotomous perspective on psychiatric disorders must be regarded as having merely theoretical value.

According to the first case, we could argue that the genetic predisposition - both for the medical disease and for the mental disease – is kindled by certain alterations performed at the genotype level and very closely related but which can be expressed phenotypically different. Thus, genetic studies, such as 'heterogeneity-based genome-scan meta-analysis' (HEGESMA) indicate the fact that there might be a certain overlapping in *loci* which contributes towards assessing both the genetic predisposition for bipolar affective disorder and the genetic determinism for the hereditary characteristics of pulse pressure, which can subsequently cause cardiovascular diseases.[7] (Figure 2)

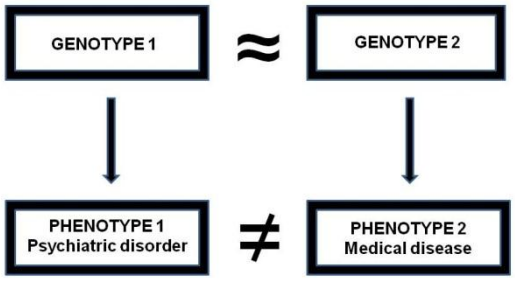

Figure 2. Putative genetic substrates underlying the comorbidity between bipolar affective disorder with chronic medical diseases

More recently, epigenetics, which comes from molecular biology, offers a new perspective upon the disease mechanism, and it may also be successfully applied in the field of therapy. According to epigenetics, the disease is not the result of anomalies found at the level of the genes (the structure of the DNA remains the same) but of alterations within the genetic profile triggered by environmental factors which bear a negative influence on the individual's health. The three fundamental mechanisms attributed to epigenetics are the DNA methylation, and the alteration of histones and dysfunctions of the micro RNA. Hence, under the pressure of environmental factors, the expression of certain genes, which carry a positive or protective role for an individual's health, may be inhibited whereas the expression of genes having a negative or pathogenic role may be activated. Therefore, the disease is nothing more than the result of the interaction between genes and environmental factors and logically following, alterations brought in the latter will lead to the reversibility of the disease producing mechanism (for example, one's lifestyle, or daily physical exercise can diminish the rate of cardiovascular

diseases). Bipolar affective disorder might display fundamentally similar epigenetic mechanisms with some chronic medical diseases. Moreover, a research study which has mathematically molded the metabolic function has pointed out that at the basis of the association between various diseases known for their frequent comorbidity there might be an alleged metabolic link determined by the increased co-expression of genes which codify the enzymatic activity engaged in the pathogenesis of both diseases.[8, 9] (Figure 3)

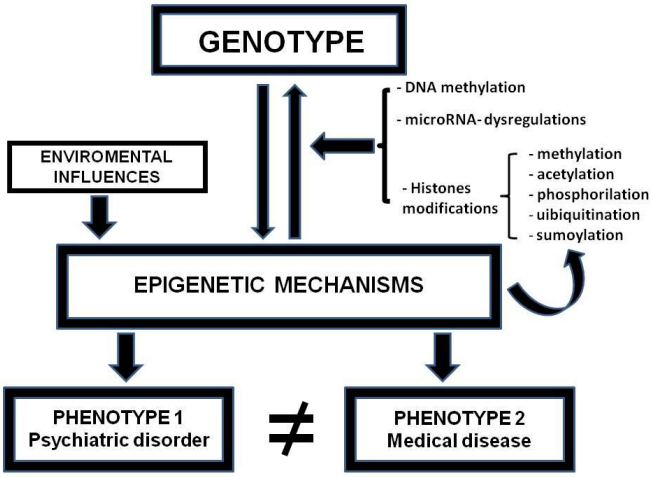

Figure 3. Presumed epigenetic mechanisms that supports the comorbidity between bipolar affective disorder and chronic medical diseases

From the second case we can infer that within the etiopathogenetic mechanism of both diseases – medical and psychiatric – there is a certain overlapping of the biological markers which bring their contribution to the mechanism. The latest neurobiological research on depression comes to support this hypothesis by clearly indicating that proinflammatory cytokines, endothelial dysfunction, the activation of sanguine platelets, and the dysfunction of hypothalamic-pituitary-adrenal axis, etc all play an important part in the pathogenesis of depression.[10-15] However, at the same time, these anomalies can also determine the occurrence of a wide range of medical diseases. (Figure 4)

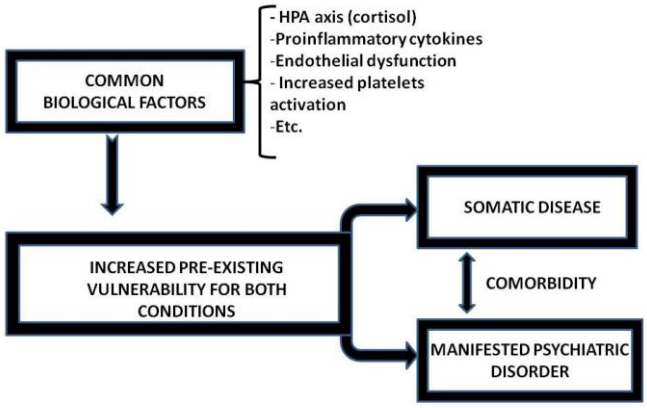

Figure 4. Somatopsychic perspective on comorbidity between bipolar affective disorder and chronic medical disease

Reversely, if we take the psychosomatic perspective as the possible explanation for the high rate of comorbidity between bipolar affective disorder and medical

diseases, we must then assert that both the mental disorder and the medical disease may be caused by mutual psychological, cognitive, emotional and behavioral factors. These factors act synergistically upon the organs more vulnerable to various anomalies resulting in medical diseases; the combined factors work either through an adjustment mechanism - general and unspecified - or directly upon a targeted organ which formerly displayed a higher vulnerability.[16] (Figure 5)

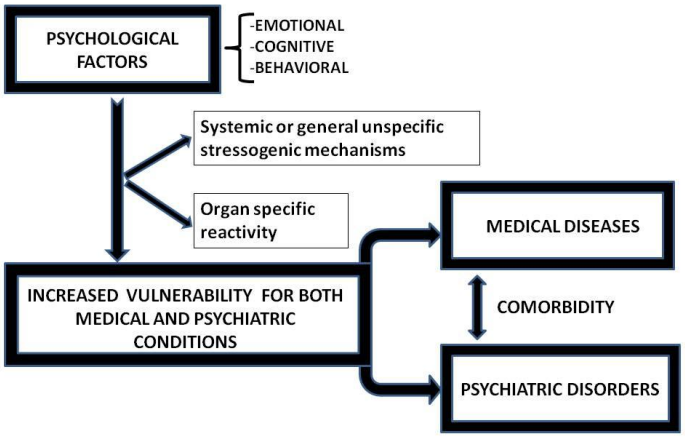

Figure 5. Psychosomatic perspective on comorbidity between bipolar affective disorder and chronic medical disease

A more recent concept has been put forward by Grande et al. using the perspective of allostatic load, what is the multisystem cumulating of physiological efforts that are needed to adapt to external factors. He stated that at a given moment, due to the action of different stressors, systemic allostatic overload will occur and this will result in a systemic disease. Hence, bipolar affective disorder together with cognitive dysfunctions, other psychiatric comorbidities but especially with the medical diseases – are nothing more than parts of larger systemic disease.[17] (Figure 6)

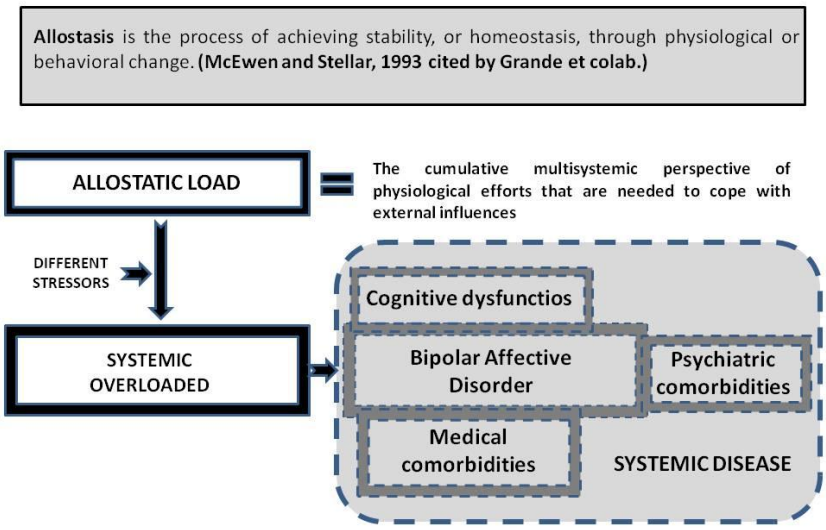

Figure 6. Systemic perspective of comorbidity between bipolar affective disorder and chronic medical diseases

Taking into consideration all these hypotheses which might explain the high comorbidity rate between bipolar affective disorder and medical diseases, we can draw as a half-way conclusion that the relationship between these two medical conditions – although manifested in different planes, body versus psychic - is bidirectional and having negative effects not only therapeutically but also in the long-term evolution of the medical conditions. Under these circumstances, there is all for the more the necessity of a tripartite approach (clinical, diagnostic and therapeutic), that is, of a holistic point of view applied in the treatment of the patients.

Chronic medical diseases more frequently associated with bipolar affective disorder

Bipolar affective disorder and cardiovascular diseases

Comorbidity between bipolar affective disorder and cardiovascular diseases remains a hotly debated topic as it still remains to be analyzed the extent to which these two afflictions are actual comorbid or whether the latter conditions are the result of metabolic side effects of the psychotropic medication used in the treatment of psychiatric disorder.

Despite all these, an important epidemiologic survey carried out in the United States, which made use of the diagnostics of cardiovascular diseases and arterial hypertension registered in primary care setting showed the fact that, after the proper adjustment to age, sex, and race, the prevalence of cardiovascular diseases has been considerably higher in the case of patients suffering from bipolar affective disorder type I [odds ratio (OR) = 4.95, 95% confidence interval (CI): 4.27–5.75], as compared with the rest of the population and versus the subjects with major depressive disorder [(MDD); OR =1.80, 95% CI: 1.52–2.14]. As regards the prevalence of arterial hypertension among patients suffering from bipolar disorder I, the following applies (OR = 2.38, 95% CI: 2.16-2.62 versus controls, OR = 1.44, 95% CI: 1.30-1.61 versus MDD; $p < 0.0001$ for all). An additional check of other categories, such as marital status, education level, income level, obesity, smoking, anxiety disorders, and substance use disorders did not substantially alter these findings. The mean age of bipolar affective disorder type I subjects with cardiovascular diseases and hypertension was 14 and 13 years younger, respectively, than controls with cardiovascular diseases and hypertension. These results prove that subjects suffering from bipolar affective disorder type I are more prone to both medical conditions, and their mortality rate is a decade higher than in the case of individuals who do not suffer from bipolarity.[18]

Bipolar affective disorder and migraine

Population and clinical studies have pointed out that there is significantly high comorbidity of bipolar affective disorder with migraine. On the one hand, patients suffering from migraine present a higher rate of mental diseases and of committing suicide as compared to the rest of the population. [19-22] On the other hand, migraine is far more frequent in the case of patients who suffer from bipolar disorder, especially from type II.[23-25] The association of the two diseases could be explained in a number of ways. Both migraine and bipolar affective disorder are considered to be polygenic multifactorial diseases which have both genetic and environmental causes. Both disorders have been associated with dysfunctions of the serotoninergic, dopaminergic and glutamate neurotransmission.[25-29] A vast linkage type study on the comorbidity of migraine with bipolar disorder has shown the overlapping susceptibility of the two diseases.[30] Furthermore, studies on migraine and bipolarity have dealt with the dysfunctions found at the level of the membrane ionic channels of calcium and of sodium; up to a point, they are both considered as channelopathies.[31,32]

Bipolar affective disorder and obesity

According to the most representative US epidemiological study based upon the data collected from 9125 individuals responding to the NCS-R survey (National Comorbidity Survey - Replication), Kessler et al found that there is a significantly high association between obesity (defined as body mass index BMI ≥ 30) and the prevalence of bipolar affective disorder not only over the past 12 months (the odds ratio of 1.61 [1.07-2.43]) but also during the individual's lifetime - odds ratio of 1.47 [1.12-1.93]). The comorbid level – as indicated by the probability rate of the prevalence over the last 12 months or during the individual's lifetime - surpassed not only the episodes marked by major depressive disorder but also by anxiety disorders and it further on proved to be significantly high for the young age category and for white non-Hispanics. The study did not cover distinctions related to gender.[33]

Bipolar affective disorder and type II diabetes mellitus

A research study on hospitalized patients who had been diagnosed with type I bipolar disorder and schizoaffective disorder (26% and 50%) showed a high prevalence of type II diabetes mellitus. The psychiatric diagnostic and the body mass index of the patients hospitalized and suffering from type I bipolar affective disorder and schizoid-affective disorder have represented predictable factors of the risk of diabetes mellitus; these factors are stronger than the ones associated with atypical antipsychotics.[34]

When considering the biological causality underlying the coexistence of bipolar affective disorder with diabetes mellitus, hypercortisolemia, which is usually associated with bipolar disorder, has allegedly been responsible for the drop in the sensitivity of insulin receptors through mechanisms yet unknown.[35] Recent molecular studies hypothetically associate bipolar affective disorder with diabetes mellitus on the basis of transducing the intracellular signal which implies the synthetizes kinaza-3beta (GSK-3β) glycogen enzyme.[36]

On the one hand, anomalies in the well-functioning of GSK-3β certainly play a role in determining the insulin resistance. The inhibition of GSK-3β, facilitated by insulin will also lead to an increased use of glucose and of glycogen production.[36] On the other hand, another important aspect is that lithium bears an impact upon GSK-3β, through the considerable reduction of this enzyme found at the cerebral level to the therapeutic levels of lithium.[37] Besides its role in keeping glycaemia under control, activated GSK-3β facilitates neuronal apoptosis whereas the inhibition of GSK-3β lowers the neuronal apoptosis and hence it has a brain-protective role.[38]

Bipolar affective disorder and the thyroid pathology

There are scientific studies which have revealed that a mere variation in the normal levels of thyroidal hormones can bear a considerable influence upon mood swings.

More than that, the insufficient treatment of thyroid dysfunction is an important impediment in the remission of bipolar depression.[39,40]

Comorbidity with thyroidal pathology might be far more frequent in the case of bipolar women than in the case of bipolar men, a fact which has been revealed by STEP-BD. The study pointed out that the occurrence of thyroidal affections in bipolar women stands at 26.9% as compared to 5.7% in bipolar men.[41] Although, the thyroidal disease has predominantly been associated with depression symptoms, there have been studies which have associated hyperthyroidism with manic symptoms.[42]

CONCLUSIONS

Bipolar affective disorder, which is classified among the major mental diseases, poses a real challenge as regards its probability of being comorbid with other medical diseases. This has to be taken into consideration both in the diagnosing of the patient and during therapy. The former has to be approached in all its multi-axial complexity while the latter can reveal the association of bipolarity with other medical diseases by means of a therapeutic barrier and of an unfavorable prognosis.

When considering the medical diseases comorbid with bipolar affective disorder, bidirectional determinism can intervene. Hence, it becomes difficult to ascertain which the initial disorder was. The interdependency between the two diseases may lie in their similar molecular anomalies. However, either disease has a distinct phenotypic development; one has a strong biological expression whereas the other one has a mental and behavioral expression.

Classically, the main explanation that was offered in order to connect mental illness with medical diseases was based on hypothalamic-pituitary-adrenal axis dysfunction. More recently, once with the progresses made in genetics, immunology and endocrinology, new evidences has been taking into consideration. Among these new putative biological factors it must be outlined the roles of proinflammatory cytokines, endothelial dysfunction, platelet activation, epigenetic changes etc. The most important consequence of these findings should not be limited to the merely description of certain putative ethiopatogenetic substrates in bipolar disorder but it should be a start point in developing of new classes of medication for the treatment of psychiatric condition.

Moreover, the medical disease may have preceded the mental disorder in an underlying latent form and the mental disorder, either per se or due to its medication could have triggered the complete clinical profile of the disease. The role of psychosocial factors may as well create a heightened vulnerability for the occurrence of both bipolar affective disorder and chronic medical diseases, and both could work in synchronicity. Concepts such as unhealthy behaviors together with dysfunctional coping styles or cognitive distortions should be considered as cornerstones for psychological perspective of the comorbidity between bipolar disorder and certain chronic medical diseases. A holistic approach could make us conclude that bipolar affective disorder, as well as other chronic medical diseases, stands not for two distinct pathologic conditions, which are comorbid, but rather for two components with different clinical profiles and which fall under the same systemic disease.

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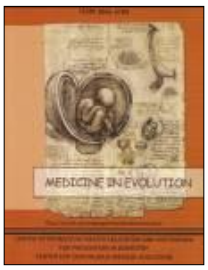
TRANSLOCATION ENCOUNTERED IN LEUKEMIAS ACCORDING TO CLASSIC AND MOLECULAR (FISH) CYTOGENETIC



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ABSTRACT

The translocation is an equilibrated structural chromosomal anomaly, no genetic material being lost or added. Within the leukemias the reciprocal translocation, which involves the removal and the exchange of genetic material between the non-homologous chromosomes, is the most frequent translocation encountered.

Chromosomal abnormalities in neoplastic marrow cells often correlate closely with specific clinical and biologic characteristics of the disease and serve as a tool to predict the clinical outcome and develop effective therapeutic approaches.

Fluorescence In Situ Hybridisation (FISH) using locus-specific probes which are capable of defining these stereotypic structural rearrangements has now become a routine diagnostic test in the clinical laboratory. The technique has thus been shown to be useful in the management of cancer patients.

Regarding this article there are presented below the following translocation: t(9;22), t(8;21); t(15;17); t(4;11); t(14;14), these anomalies were found by using the classic and molecular cytogenetic methods between 2010 and 2012 within the Classic and Molecular Laboratory of the Oncohematology Department.

***Keywords**: translocation, classic cytogenetic, FISH, leukemias*

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INTRODUCTION

Leukemia is a malignant disease, the site of its occurrence and further development is the hematopoetic cell from the bone marrow. Nowadays they are widely regarded as malignant diseases of the hematopoetic tissue, their source being an abnormal stem cell

The AML1 (CBFA2 or RUNX1) gene is the most frequent target of chromosomal rearrangements observed in human acute leukemia.The most common rearrangements are the TEL/AML and AML/ETO fusions. The TEL(ETV6)/AML1 fusion is brought about by the t(12;21)(p13;q22) translocation which is observed in 21% of childhood B-ALL cases(1) whilst the AML/ETO fusion is the result of the t(8;21)(q22;q22) translocation observed in ~40% of AML M2 and 7% of AML cases overall. Using FISH, gene amplifications have also been found essentially in childhood ALL(2,3). Amplification of the gene has further been shown to be associated with poorer outcome in ALL(4). One mechanism for this is thought to be because of the overexpression of RUNX1 (AML1)(5).

Philadelphia chromosome is characteristic of Chronic Myeloid Leukaemia (CML), found in around 90% of cases but represents a significant abnormality in 30% of adult
and 2 to 10% of childhood(6) Acute Lymphoblastic Leukaemia (ALL) cases(7,8,9). This rearrangement is also seen in rare cases of acute myelogenous leukemia (AML)(10).
In ALL, the rearrangement is associated with an extremely poor outcome with an event-free survival (EFS) of 15% or less at 5 years in adult and childhood patients treated with chemotherapy alone(11,12). There are no reports of long-term survivors(13). Allogeneic bone marrow transplantation is the only curative therapy for these patients. Children with this rearrangement are treated on a high-risk protocol and adults are recommended for immediate bone marrow
transplantation. Philadelphia (Ph) chromosome positive acute myeloid leukaemia (AML) is characterized by its resistance to conventional standard chemotherapy and poor prognosis so accurate and rapid identification of this chromosomal abnormality is vital. In a small number of cases of ALL, the translocation does not result in a cytogenetically visible Philadelphia chromosome. In these cases, FISH is essential for highlighting the fusion gene(14).

In ALL, IGH is most notably involved in rearrangements involving the cMYC oncogene as a result of the t(8;14) translocation(15). However, less common rearrangements of the IGH gene are most often seen in T-ALL but can also be found
in B-ALL. In T-ALL for example, IGH is observed in the t(14;14)(q11;q32) translocation
(or inv(14)(q11q32) rearrangement)(16) and is found in T-cell leukaemia associated with ataxia-telangiectasia (AT). However rare reports have indicated that this abnormality also occurs in B-ALL.

Rearrangement of the MLL gene at chromosome band 11q23 can be detected in the leukaemia cells of approximately 85% of infants with B-ALL(17,18,19). Translocations involving the MLL (11q23) gene are generally associated with increased risk for treatment failure(20). The most frequently observed of these translocations is the t(4;11) translocation involving the MLL gene and the AF4 gene on chromosome 4 (20,21,22). A poor outcome for infants with ALL is strongly associated with the presence of this rearrangement in particular.

A structural rearrangement involving chromosomes 15 and 17 in acute promyelocytic leukemia was first recognized in1977. The fusion gene PML/RARα is created by the t(15;17)(q22;q21) translocation found in

98% of AML M3 Acute hypergranular Promyelocytic Leukaemia and 9% of AML overall (23,24). The annual incidence is 1/106 and the disease may occur at any age although often young adults are affected. The breakpoint in PML is variable between intron 3 and exon 7a which is in contrast to the RARα breakpoint which remains constant in intron 2.

MATERIAL AND METHOD

During a period from 2010 to 2012, in the Classic and Molecular Genetic Laboratory of the Oncohematology Department from the Timisoara Louis Turcanu Children Hospital, 204 cytogenetic and 115 molecular analyses were carried out within the patients with suspicion of acute lymphoblastic leukemia (ALL), acute myelocitic leukemia (AML) and chronic myeloid leukemia (CML). Following the results there were emphasized 5 translocations (9;22), 1 translocation (8;21), 1 translocation (15;17), 2 translocation (4;11) and 2 translocations (14;14).

The both methods, classic cytogenetic and molecular FISH, led to the diagnostic of the aforementioned translocations.

Moreover the FISH interphase and metaphase analysis brought to us the percent of the cells carrying hybrid genes as the result of translocation with further role in leukemogenesis.

In order to perform there were used locus genetic sondes from Metasystem: XL BCR/ABL dual fusion, XL AML1/ETO dual fusion, XL PML/RARA dual fusion, XL MLL break apart, XL IGH break apart.

The patients are represented by children and adults.

The samples were obtained from the bone marrow using the direct method (without cellular cultures) and the indirect method, this method implying cellular cultures with the length of 24, 48, 72 hours.

For specimen collection, 1-2 ml of marrow are aspirated aseptically into a syringe coated with sodium heparin and transferred to a sterile 15 ml centrifuge tube containing 5 ml culture medium (RPMI 1640).

For blood specimens, 5 ml are drawn aseptically by venipuncture into a syringe coated with sodium heparin. Specimen should be maintained at room temperature and transported in culture medium.

To prepare metaphase cells, the sample is exposed sequentially to mitotic inhibitors to accumulate cells in mitosis, hypotonic KCl (0.075M) to swell the cells, and fixative (absolute methanol: glacial acetic acid, 3:1).

Slides are prepared by dropping the cell suspension onto precleaned glass microscope slides, and the slides are air dried.

The most popular chromosomal banding techniques is trypsin-Giemsa banding.

Using this technique, a consistent chromosome banding pattern is induced by exposing cells to a dilute trypsin solution (0,1-0,25 percent), followed by staining in phosphate-buffered Giemsa stain.

The assessed metaphases, 30 for each patient, had a proper quality due to the adherence to the protocol. It also was noticed a better dispersion of the chromosomes from the cells with normal karyotype.

For confirmation was used FISH technique. Slides were immersed in +37 ^{o}C 2xSSC for 30 min and then dehydrate in alcohol 70% and 100% 2 min each.

Slides were denaturated at +73^{o}C (in a pre-warmed water bath) for 2 min and rinsed in ice-cold series of alcohol 70-80-100% 2 min each.

Slides were put on a slide warmer (40°C) and applied 10ul probe (7µl hybridization buffer, 1µl probe, 2µl distillate water), overnight were left at 37 °C.

The slides were analyzed through a fluorescent microscope.

The best images were captured using the camera mounted on the microscope attached to a computer with karyotyping and FISH software.

RESULTS AND DISCUSSION

As for the patients diagnosed with translocations we reached to the following conclusions: t(9;22) – 5 patients: 2 with ALL, 1 with AML, 2 with CML (figure 1); t(8;21) – 1 patient with AML type M2 (figure 2); t(15;17) – 1 patient with AML type M3 (figure 3); t(4;11) – 2 patients with ALL subtype B (figure 4); t (14;14) – 2 patients with ALL (figure 5). The encountered translocations were associated to the morphological subtypes as in the medical literature.

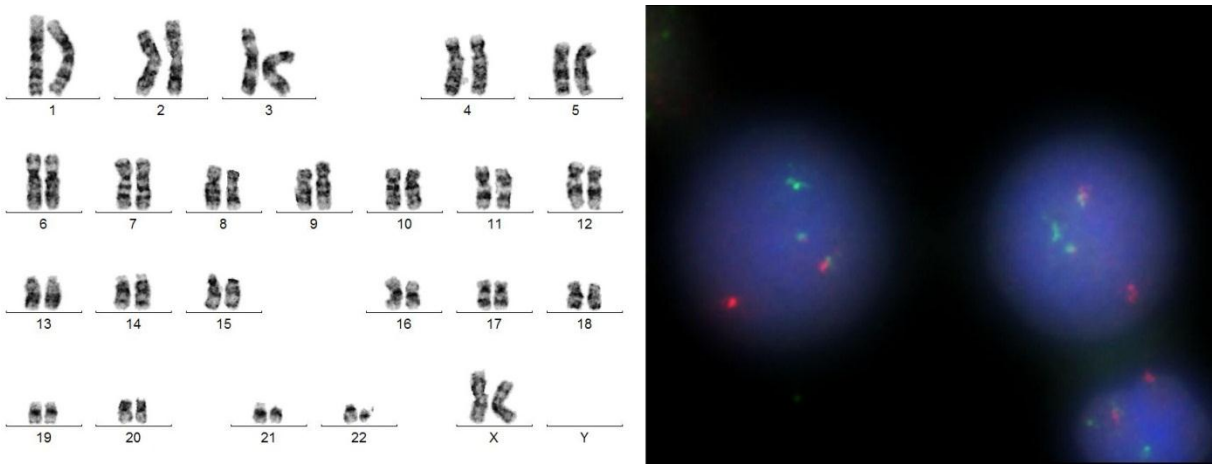

Figure 1 – Karyogram with translocation (9;22)(q34;q11) and FISH with dual fusion BCR/ABL

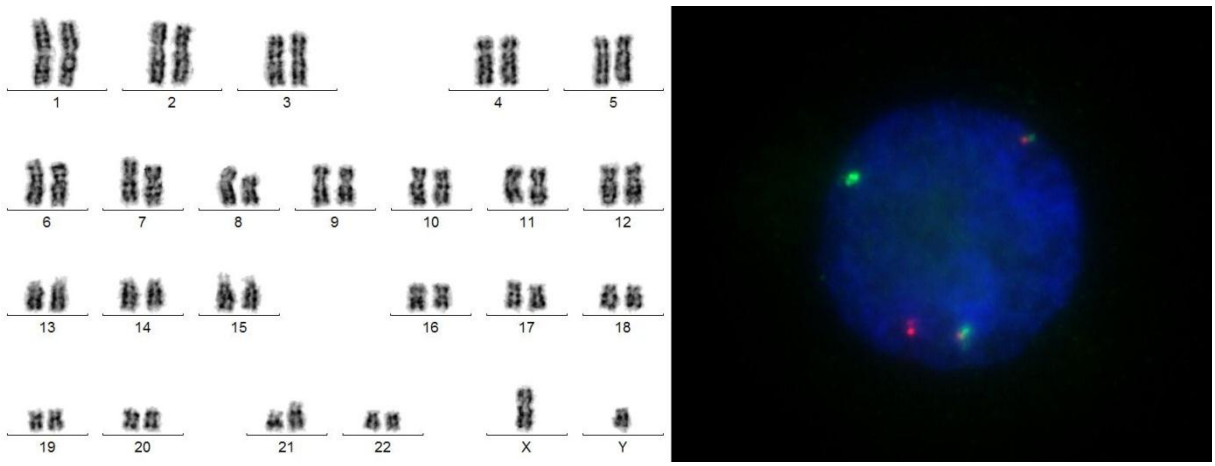

Figure 2 –– Karyogram with translocation (8;21)(q22;q22) and FISH with dual fusion AML1/ETO

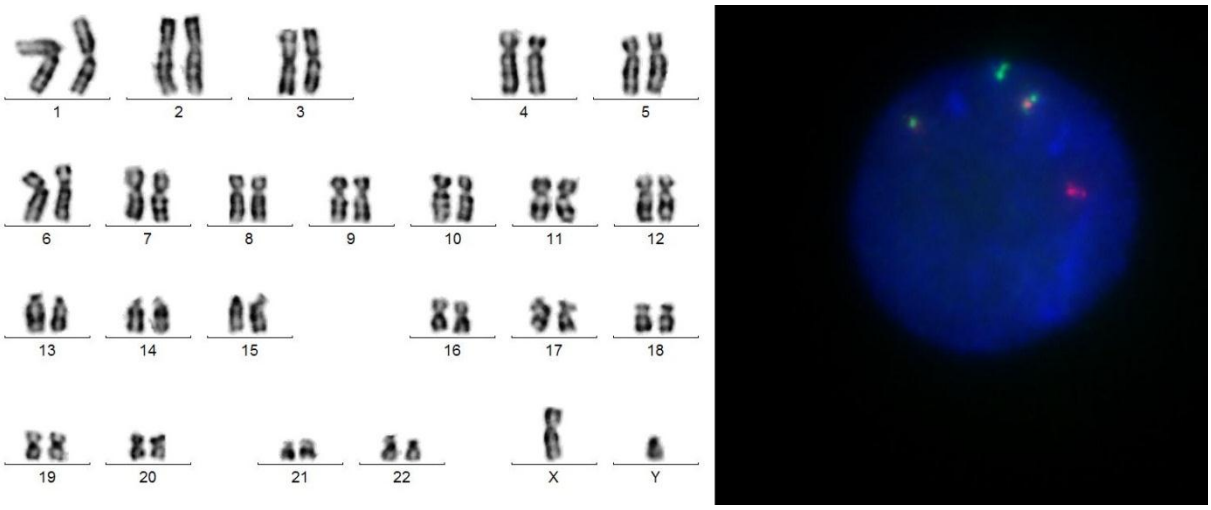

Figure 3 –– Karyogram normal and FISH with dual fusion PML/RARA

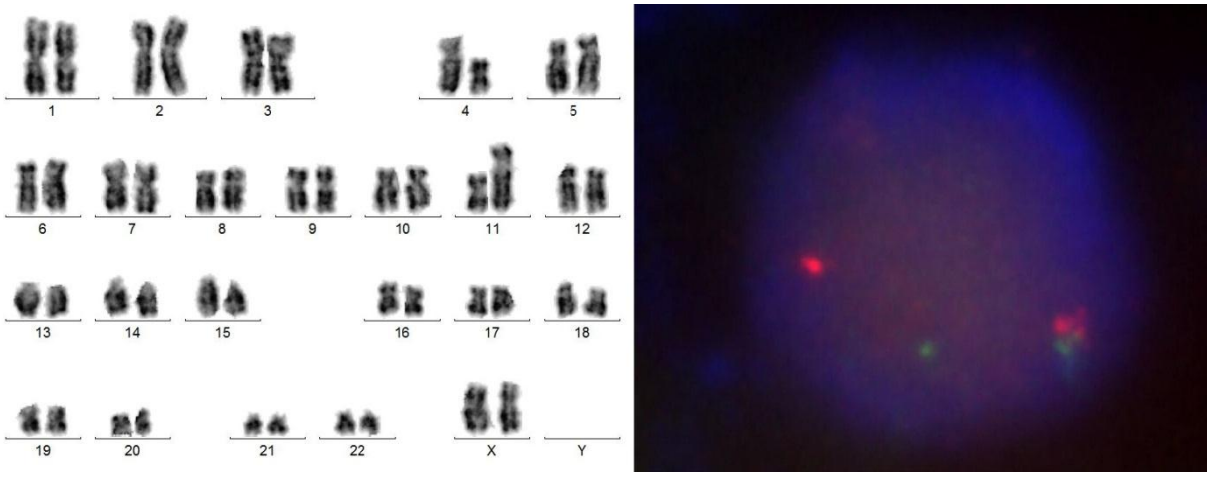

Figure 4 –– Karyogram with translocation (4;11)(q21;q23) and FISH with MLL break apart

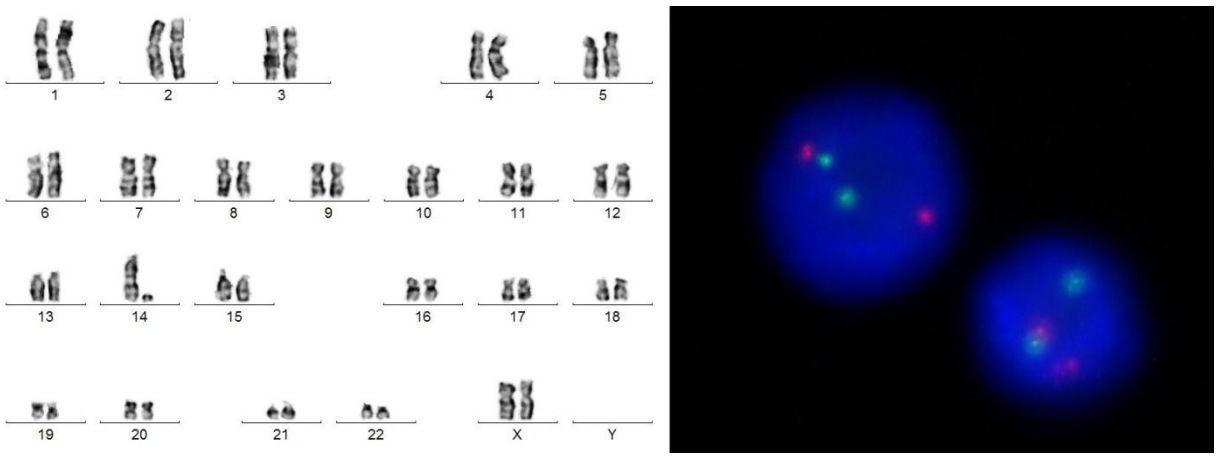

Figure 5 –– Karyogram with translocation (14;14)(q11;q32) and FISHwith IGH break apart

The translocation t(8;21)(q22;q22) is the characteristic anomaly in acute myeloblastic leukemia subtype M2, where myeloblast with maturation features are present. AML1/ETO is the hybrid gene with role in leukemogenesis, its detection required the use of the locus genetic sonde Metasystem XL AML1/ETO dual fusion.

The Philadelphia chromosome, outcome of the reciprocal translocation t(9;22)(q34;q11), is the anomaly currently present in the chronic myeloid leukemia but it could be also encountered in the acute myeloblastic leukemia and acute lymphoblastic leukemia.

The fusion gene with a key role in leukemogenesis is BCR/ABL, the FISH technique with locus genetic sonde Metasystem XL BCR/ABL was used to diagnose it.

This genetic anomaly is deemed to be associated with a poor prognostic and a fast evolution, the remission period being shorter than the remission period of the patient with normal karyotype.

Concerning the translocation t(15;17)(q22;q21) it is the specific anomaly in acute promyelocytic leukemia (AML M3), in the leukemogenesis the fusion gene PML/RARA is involved, the prognostic of the patient is better owing to the treatment with all-trans-retinoic-acid (ATRA). The locus genetic sonde Metasystem XL PML/RARA dual fusion is used.

Another translocation emphasized was t(4;11)(q21;q23), which is specific in ALL with B precursor.

A part in leukemogenesis is the hybrid gene AF4/MLL, its presence was achieved by using the locus genetic sonde Metasystem XL MLL break apart.

The aforementioned translocation was encountered in 1 patient with interphase FISH technique, while in the other patient the translocation was diagnosed by using both classic cytogenetic and FISH technique.

The last translocation was t(14;14)(q11;q32), it is mostly present in ALL with B or T precursors.

Within our tests t(14;14) was found in 2 cases, in one case as a single anomaly, in the other one as part of a hyperdiploid karyotype.

We pointed out the new genetic anomalies after the following methods were undertaken, classical cytogenetic and also FISH interphasic and metaphasic technique with locus genetic sonde Metasystem XL IGH break apart.

CONCLUSIONS

Nowadays the classic cytogenetic turns out to be still important because of the useful information with regard to diagnostic, prognostic and treatment of hematopoetic malignancies.

Nevertheless this technique is used usually along with FISH technique, which is performant, expensive and laborious, therefore bringing efficiency in diagnostic and oncologic treatment.

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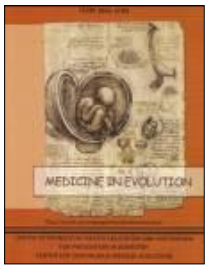
EARLY DETECTION OF THE HEARING LOSS AT THE NEWBORN BABIES WITH RISK



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ABSTRACT

***Objectives**. Conducting the screening for hearing loss to all newborns motherhood in Oradea, the analysis of the risk factors for hearing loss and the telephoning screening to those with risk factors, which promote the first stage of the universal screening of the newborn.*

***Material and methods**. Using the appliance Echo Screen (TEOAE and AABR). Questionnaire with questions for evaluating the hearing and language.*

***Results**. Of those 18336 newborns undergoing to the screening, 178 were detected REFER and 11 infants were diagnosed with sensorineural hearing loss. 4600 neonates with risk factors detected "PASS" to the universal screening were subjected to the telephone screening, of which 3 infants were evaluated by hearing aid, of which 2 were diagnosed with sensorineural hearing loss.*

Conclusion. The correct application of the protocol of screening and telephone monitoring of the infants with risk factors for a hearing loss early identify the existence of a congenital hearing loss.

***Keywords**: universal screening of newborn, evoked transient oto acoustic-emissions (TEOAE), automatic auditory brainstem response (AABR), telephonic screening, risk factors for hearing loss, sensorineural hearing loss.*

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INTRODUCTION

The sensorineural hearing loss (SNHL) is the most common congenital disease, with an incidence of 1-3 cases/1000 newborns, or ~ 3 times the frequency of Down's[1,2,3,4] syndrome, and about 2-4/1000 newborns in the Neonatal Intensive Care Unit[5]. The risk factors for the neonatal hearing loss are grouped in the Declaration of 2007 Joint Committee of Infant Hearing (JCIH) in the U.S.[6] Because it is a deficiency that is not seen, newborns with hearing loss look and behave like the same as those with normal hearing.

Undetected at the time, this will be discovered at 12-24 months, when the parents notice the lack development of the language to the child. In the absence of the acoustic stimulation, the bilateral congenital hearing aid decreases the volume of the white substance, by reducing myelination of the auditory aids.

Undiagnosed and untreated or detected late will have a severe impact on the development of linguistic, cognitive, psychosocial and professional, leading to the isolation of the individual by society. JCIH issued a statement of principles and guidelines to support the implementation of the screening and intervention programs for SNHL to the newborns.

This statement implies carrying out the neonatal screening shortly after birth, before the external of the hospital, or before the age of 1 month, diagnosing the hearing loss by auditory and medical evaluation before 3 months and therapeutic intervention before the age of 6 months[7].

OBJECTIVES

Starting from these premises, the objectives of this study consist in making the screening for hearing loss to all newborns of the Neonatology and Neonatal Intensive Care Unit of the Emergency County Clinical Hospital Oradea, using as a method of screening the auditory testing by evoked transient acoustic oto-emissions (TEOAE) , automated auditory brainstem response (AABR) and in parallel with it the analysis of risk factors for hearing loss at all newborns undergoing to the screening, as well as the telephonic screening of the newborns with risk factors for hearing loss, promoting the first stage of the universal screening of the newborn.

MATERIAL AND METHODS

This observational study, prospective, of cohort was presented in the Clinic of Neonatology and Neonatal Intensive Care Unit of the Emergency County Clinical Hospital Oradea, in the period from January 2008 to June 2012 on a number of 18336 newborns with the gestational age between 27 and 42 weeks of gestation, 2171 premature and 16165 newborns at term.

The device used for testing hearing was an Echo-Screen type ES/TA produced by Fisher-Zoth Mack Medizintechnick Germany (Figure 2). It combines two methods of auditory testing: oto-acoustic emissions transient evoked (TEOAE), able to detect even mild hearing loss and evoked potentials (AABR) that have as a result in the auditory brain stem response.

Figure no. 2 Echo-Screen

It is tested all newborns premature and mature babies by the oto acoustic emissions method, starting with the third day of life, subject to the following two conditions: calmness and newborn baby asleep. If the new-born infant promotes the test, on the device screen appears the word PASS, if he does not promote the test the word REFER (Figure No. 4) appears. Apply the protocol of the screening of hearing loss for:

A. all mature healthy newborns:

- PASS – good result
- REFER – repeat test at 3-4 weeks
- If at the second testing the answer is the same REFER, perform a test AABR and if the answer is REFER is suspected the existence of a hearing impairments and the patient is directed to consult a ENT specialist.

B. For newborns at high risk, apply the AABR test:

- PASS – good result
- REFER – repeat test at 3-4 weeks
- If at the second testing the answer is the same REFER, perform a test AABR and if the answer is REFER is suspected the existence of a hearing impairments and the patient is directed to consult a ENT specialist.

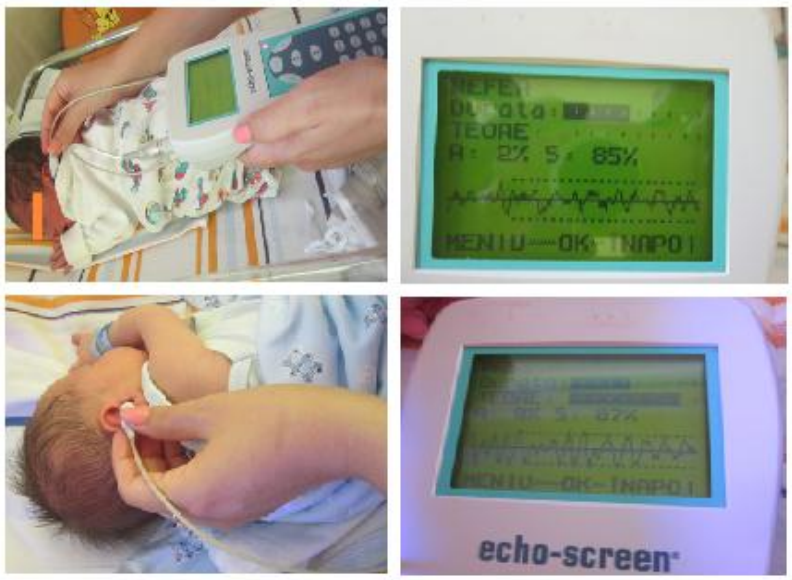

Figure No. 4 Newborn Testing Using the TEOAE Technique

The newborns with risk factors that have passed the first test were monitored using a telephone questionnaire to assess the hearing and language during the period between 1 month and 12 months. For each age, the questionnaire contains questions related to hearing and language development at the newborn. There are only two possible answers, "yes" or "no", so in cases where the answer "sometimes" was obtained, it was considered as "no". We considered "no" also if the answer "no" was obtained from at least one question, what would determine the need of an immediate auditory assessment. In cases where the telephone contact was not possible because of the non-existent numbers or lack of response to calls after five attempts, at various times, a letter was sent requesting that the family to contact the Neonatology Clinic to communicate a phone number to allow the implementation of the screening by phone. In cases where there was no contact of the family after 20 days a second letter was sent in strengthening the demand.

All the data were stored and processed using Microsoft ® Excel ® 2010 (Microsoft ® Corporation, USA), the database of statistical study. As methods of statistical analysis Chi-square were used – square, Mann-Whitney and calculating the relative risk using the MedCalc® medical statistics version 12.2.1.0 (MedCalc ® Software, Mariakerke, Belgium).

RESULTS AND DISCUSSION

This observational study, prospective, of cohort, was presented on a number of 18336 newborns with gestational age between 27 and 42 weeks, of which 2171 premature and 16165 newborns at term.

From the analysis of the data collected for each patient, we obtained the following results highlighted in the following table:

Table no. 2 Demographic, clinic features and risk factors

Total of newborns	**18336**	$p^{\dagger}$
Sex M/F	8824/9512	***p < 0,0001****
Premature newborns	2171	***p < 0,0001****
Mature newborns	16165	
Gestational age (weeks of gestation)	Average (27 – 42)	***p < 0,0001***
Caesarian operation	11000	***p < 0,0001****
Natural birth	7336	
Newborns with risk	6133	***p < 0,0001****
Newborns without risk	12203	
Risk factors for neuron-sensory hypo auditory hearing		
Birth weight under 1500 g		1447
TORCH		110
Craniofacial anomalies or syndromes associated with hearing loss		82

Postnatal infections associated with sensorineural hearing loss (e.g. bacterial meningitis)	147
Fan support over 5 days	497
Ototoxic medication	1280
Severe asphyxia, hypoxia (Apgar 0-4 at 1 min. 0 – 6 to 5 min.)	1580
Hyperbilirubinemia > 20 mg/dl	988
Family history for hearing deficiency	2
p†	***p < 0,0001****

** Chi-square test*
***Mann-Whitney test*
† p < 0.05 proves a difference statistically significant between the studied lots

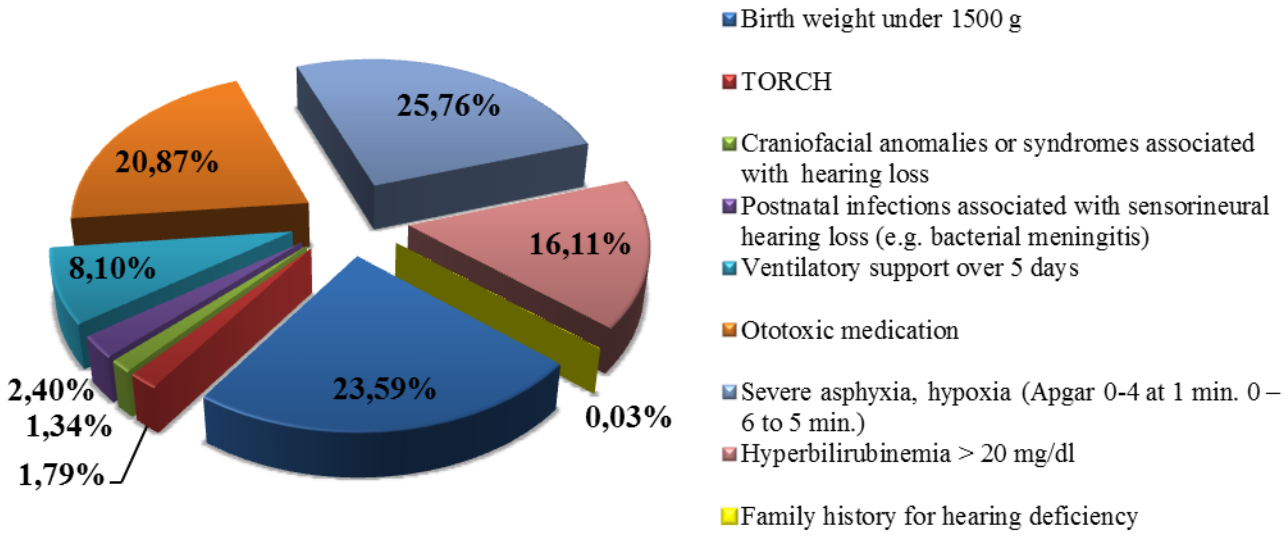

Chart No. 4 Distribution of newborns undergoing to the screening hearing loss depending on the risk factors

Analyzing the screening data for hearing loss in the newborn babies taken into study, we came to the following results (see Figure 5)

In January 2008 – June 2012 were test 18336 newborns. Of these, 6133 reported risk factors for hearing loss. Following the test through AABR and TEOAE, precisely so as not to omit the existence of auditory neuropathy to that TEOAE is normal[8], 25% of the cases have not passed the test. They were recalling a month later for retesting. Unfortunately, 75% of the cases abandoned and their valuation could not be ascertained. Only 25% were presented at the second test, with negative results in 9% of cases. These infants were sent to ENT specialist counseling, in which six infants were diagnosed with auditory deficiency.

The newborns without risk factors for hearing loss were in number of 12203. Of these, 92% were promoted through the TEOAE test, and for 8% the result was REFER. They were recalling a month later for retesting. In addition, this time the abandonment was raised, 830 (85%) cases, this could not be traced later. Only 15% of cases were retested by TEOAE and AABR, and only 11% of them have passed the test, the rest being suspected of auditory deficiency. These infants were sent to ENT

specialist counseling, in which 5 infants were diagnosed with auditory deficiency.

The 4600 newborns with risk factors for hearing loss who promoted the first screening test through AABR and TEOAE were undergoing to the screening by phone. Of these, only at 3450 (75%) they could apply the telephone questionnaire, for the rest they could not obtain a telephone contact after the second letter sent to its request. Following the hearing and calling language testing, 0,11% (4) of the cases did not promote to the test and were recommended specialty exam ENT. Out of the 4 patients, one dropped out and 3 have been assessed audiological, 2 were diagnosed with sensorineural hearing loss.

By calculating the relative risk for the two groups of patients, the neonates with risk factors and those without risk factors (table no. 3), we have found that there is not a statistically significant risk in any of the groups, coinciding with the literature of specialty that says that out of 10 infants with congenital hearing loss, 9 come from parents who do not have hearing problems [9,10].

Tabel no. 3 The relative risk of hearing loss in newborns with / without risk factors

6/6133	5/12203	Relative risk	0,9994
		95% CI	0,9986 to 1,0003
		z statistic	1,294
		Significance level	P = 0,1958
		NNT (Harm)	-1761,213
		95% CI	758,484 (Harm) to ∞ to 5469,317 (Benefit)

Thus, the neonatal auditory screening is aimed at all newborns, regardless of the presence or absence of the risk factors for hearing loss.

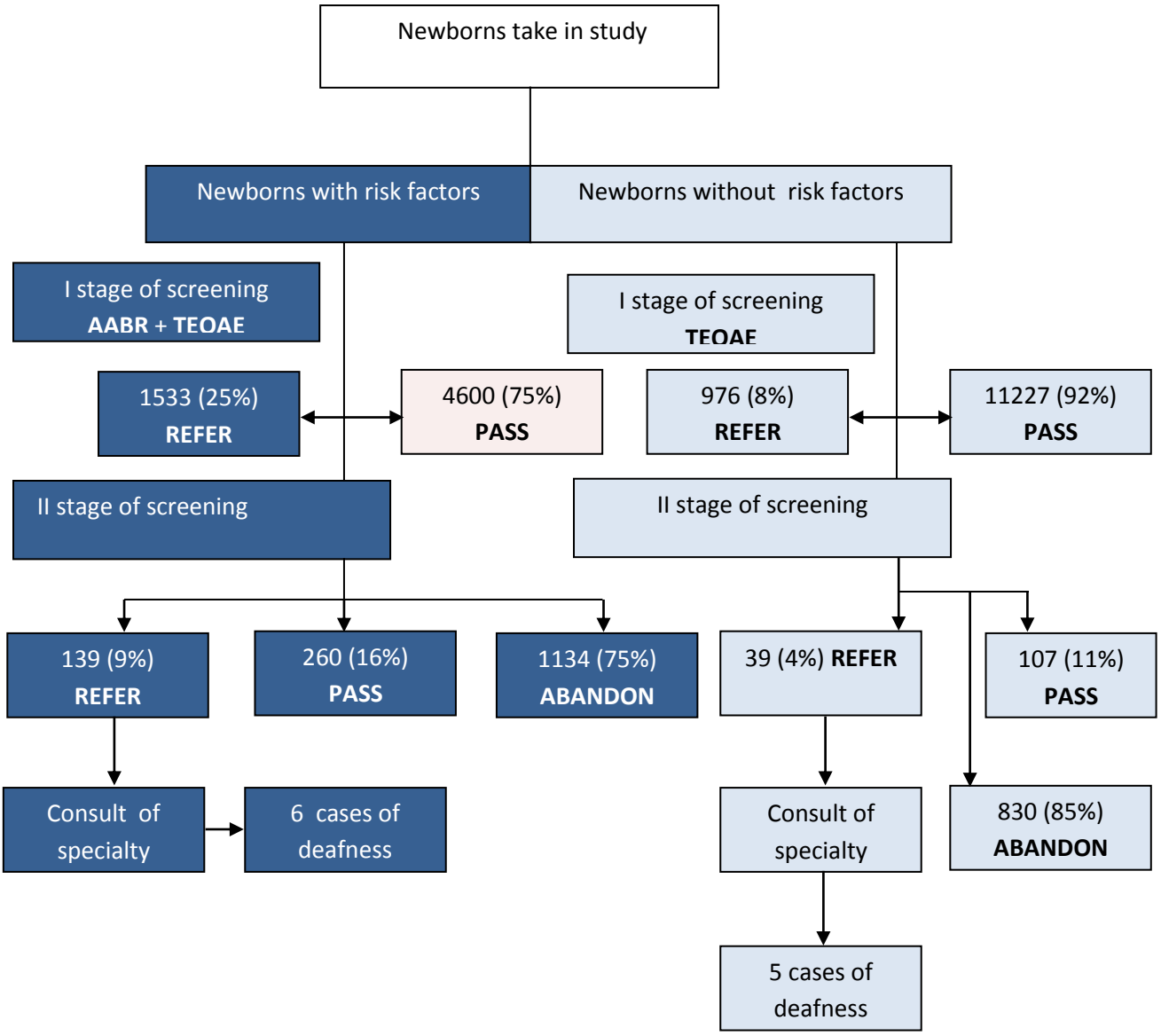

Figure No. 5 The Screening Results for the Newborns Hypo Auditory Hearing

CONCLUSIONS OF THE STUDY

1. Analyzing the demographic and clinical data, we found that there are statistically significant differences in terms of sex, gestational age, and way of birth and the presence of risk factors among the patients undergoing the screening for hearing loss.

2. The risk factors for hearing loss, confirm statistically are: birth weight under 1,500 g, smother severe hypoxia (Apgar 0-4 to 1 minute, 0 – 6 to 5 minutes), ototoxic medication, hyperbilirubinemia, fan support over 5 days, the presence of postnatal infections, TORCH, craniofacial anomalies or syndromes associated with hearing loss and heredo-colaterale antecedents presence.

3. Calculating the relative risk for the two groups of patients, neonates with risk factors and those without risk factors, it was not found a statistically

significant risk in any of the groups, so that the neonatal auditory screening is aimed at all newborns, regardless of the presence or absence of the risk factors for hearing loss.

4. The large number of abandonment gives the difficulty in follow-up of the newborns undergoing the screening for hearing loss from the second stage of retesting those who have not passed the first test.

5. Following the universal screening of the newborn for hearing loss and auditory assessment 6 neonates with risk factors have been diagnosed with sensorineural hearing loss, two of whom come from families where one or both parents are deaf and dumb. From the lot of the newborns without risk factors 5 were diagnosed with sensorineural hearing loss, these coming from healthy families.

6. The monitoring of infants showing risk factors for hearing deficiency using the questionnaire over the telephone proved to be viable, given that 2 patients were diagnosed with sensorineural hearing loss.

7. Therefore, we consider that the proper application of the protocol of screening and monitoring of infants with risk factors for hearing loss, using the questionnaire by telephone up to one year of age shall identify early the existence of a congenital hearing loss.

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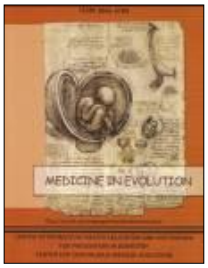
CONSIDERATIONS ON USING PERSUASION IN DENTAL MEDICINE



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ABSTRACT

Daily exposure to thousands of persuasive messages is a feature of postmodernism and it represents an area that dental art practitioners can not ignore. The strategies, the techniques and the principles of changing attitude and behaviour towards dentistry are used in private practice but also in health education campaigns or in the promotions of dental care products. The doctor and the patient are at the same time, recipients and actors of the persuasion processes.

The present work presents six basic categories of persuasion, formulated by Robert B. Cialdini (reciprocation, commitment and consistency, social proof, authority, liking and scarcity) and stresses the need for dentists to be familiar with the strategies and techniques of persuasion and to decide, according to the principles of professional ethics, which is the limit of their applicability in the therapeutic relationship, in order to respect the Hippocratic credo "primum non nocere"

***Key words**: persuasion, health-directed behavior, professional ethics*

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INTRODUCTION

Changing attitudes and behavior towards oral health is a permanent objective of dental practitioners, of specialists in public health, but also of companies that sell dental care products. The World Health Organization considers that health promotion in places where people live, work, learn or play is the most efficient way to improve their quality of life. Currently, preventive programs are moving towards the development of individual responsibility regarding their own health and a lifestyle change. And last but not least, manufacturers and distributors of dental care products intent to change consumption behavior and increase sales. At this point, the interests of the doctors and health educators coincide with those of the companies, as a higher consumption of toothpaste and a toothbrushes sales growth indicate that the target audience has health-oriented skills. Apart from the fact that dental care products advertising has an educational role, these companies make or sponsor educational campaigns in schools and socially disadvantaged communities, aiming to shape and stabilize health-oriented attitudes and behaviors.

Jean-Noel Kapferer (1978/2002, 78) defines persuasion as "changing attitudes and behavior by exposure to messages". Commenting on this definition, Septimiu Chelcea (2002, 138) emphasizes two elements of persuasion: the person exposed to the messages must be aware that the communication intends change their attitudes and behavior, and the message transmitter must have this change as purpose. In the case of manipulative communication, the victim is not aware about the differences between his goals and the manipulator's goals, while in a persuasive process the individual knows that the approach aims at changing his opinions, attitudes and behavior. Manipulation has negative consequences both for individual and society, being ethically reprehensible, and damaging, most often, to human dignity (Chelcea, 2002, 146).

This article presents some of the principles used for attitudinal changes, examining how these principles are used in dentistry, both in private practice and in health education campaigns or in the promotion of dental care products, starting with the ideea that the oral health has an important impact on the quality of life, even in children (Dumitrache, Comes *et al*, 2009, 76). This impact is physical, psychological and has an important influence on the way people develop, look, speak, eat and also on their social relations (Dumitrache, Sfeatcu *et al*, 2009, 5).

PERSUASION PRINCIPLES APPLIED IN DENTAL PRACTICE

Robert B. Cialdini (1984/2004) formulated six basic categories – reciprocity, commitment / consistency (or constancy), social proof, authority, liking and scarcity - each governed by a fundamental psychological principle that leads the human behavior and to which these categories due the power. The author notices that humans, just like animals, have "complex preprogrammed behavior" or fixed action patterns, where the sequences appear always in the same way and in the same order. They are activated by an almost mechanical process, a so-called "trigger feature" a minor detail in the entire ensemble. Moreover, principles of persuasion lend their power to the person that use them, so "victims" consider their act of accepting as rather normal and natural than determined by the plans and actions of the person who take benefits of them.

Therefore it is necessary a discussion about professional ethics, regarding the manner in which the doctor uses principles of persuasion in his relationship with the patient. Dentistry is a liberal profession, but a humanistic one. The dentist is obliged to

have theoretical knowledge and practical ability in order to use them to improve and maintain the patient's health and only secondarily to the welfare of his business.

1. Reciprocity

The rule of reciprocity says that we should reward in a similar manner the gesture of another person, valid rule in any society. This rule acquires a great power and often favors dishonest exchanges. A small initial favor can trigger a sense of obligation with an unpleasant character, so that the manipulator can achieve a bigger favor as response. For example a marketing strategy is to give free dental care products samples, which are, in fact gifts, retaining the appearance of having just an informative purpose. By accepting the gift, the subject will feel compelled to buy that product later.

Manipulation technique metaphorically called "door in the face", conceptualized by Robert B. Cialdini in 1975, is based on reciprocity. If we start by asking an extreme favor, which most likely will be refused and will continue with a less important demand, the individual may feel a normative pressure to answer our concession (in fact, an appearance) with another concession. For example, if a dentist presents an extremely expensive treatment option and then presents the most commonly used one, the latter will be accepted and considered rather cheaper than if it were presented from the beginning.

The rule of reciprocity is extremely strong and with a great social validation. To withstand its pressure, we must accept the initial desirable offers just realizing what they essentially are, which are their qualities, beyond the form in which they are presented to us.

2. Commitement and consistency

The need for consistency is, for Robert B. Cialdini (1984/2004, 83), a central motivational force of human behavior. Cognitive consistency theories are based on the observation that people are motivated to appear in the eyes of others as being consistent with what they say and what they do. Consistency is associated with a strong personality and intellect, while inconsistency is seen as a sign of flippancy, duplicity or mental impairment.

The manipulation technique "foot in the door", as proposed and verified by Jonathan L. Freedman and Scott C. Fraser (1966, *apud* Chelcea, 2006, 232), starts with a tiny request in order to gain at the end the approval for the initial request, which normally would have been denied. The fact that the patient goes to a dental office for a consultation or for a dental cleaning is a first step in getting commitment to complex treatments, discovered on the occasion of that first visit.

The public commitment tends to be durable because the individual has the need to appear consistent in the eyes of the others. This observation can be used successfully in cases as quitting unhealthy habits such as smoking, or adopting new habits, such as oral hygiene. Robert B. Cialdini (1984/2004, 112) published a letter of a lady who managed to give up smoking by writing on business cards her decision and handing them to the people whose respect was important for her. As well, parents can receive a weekly calendar on which kids have to draw two stars every day, morning and evening, after having washed their teeth, this being a public commitment.

Being responsible for a commitment, even in the absence of external pressures, is very important for changes. The tactic of manipulation called "low ball" procedure "bait and switch", conceptualized by Robert B. Cialdini and his colleagues in 1978 (*apud* Chelcea, 2006, 238) assumes that the original reason for a particular choice may even disappear without the individual to reconsider the decision he made, just because he built another foundation of his choice. Children may receive small gifts without material value or just praises and, when external incentives are withdrawn, they persist in that kind of behavior due to developing internal motivations.

To identify dishonest attempts to change a behavior by getting a

commitment, Robert B. Cialdini (1984/2004, 137) proposes the question: "knowing what I know now, would I make the same choice?". If your first sensation is a wrench, then the reasons are not real, but built in order to justify the choice.

3. Social proof

The principle of social proof (or social validation) leads to the idea that one of the means that we can use to ascertain what can be considered fair is to find out what people think it is right to adopt their conduct. The higher their number the more appreciated the idea.

The principle of "social proof" is found in the learning process of healthy-oriented behavior or in the familiarization with the dental office, especially for children. The little patient, by in-vivo or filmed observational modeling (Ollendick and King, 1998, 156-167), notices that other children exhibit situation management skills (deep breathing, relaxation) during dental intervention and so they will be able to develop and use their own skills in a similar situation. Catherine Do (2004, 133) has two researches that demonstrate the usefulness of the method. In the first study, children aged between 5 and 10 years who had never been to the dentist watched a 13 minutes movie whose protagonist was a boy in the dental office. They showed a much higher compliance than the control group, composed of children who watched a movie unrelated to dentistry (Melamed *et al*., 1975). Another study showed that children's exposure to positive images related to dental offices and dental treatments reduced on short term, the anticipatory dental anxiety (Fox and Newton, 2006, 455-459).

There are also situations when the principle of social proof works automatically, reflexively, in response to incomplete or false evidences. To avoid the sometimes dramatic effects of social validation, we need to identify the incorrect data, intentionally falsified. Robert B. Cialdini (1984/2004, 200) even recommends a rejection attitude of the products promoted in this way. So, to face persuasion through social proof, we need information and certainty, avoiding the situation of looking to others the most accepted behavior.

4. Liking

Another "shortcut" that we use mechanically is to say "yes" to someone we know and sympathize. There are several factors that make a person to like another, factors which "induce" sympathy and can be used in the relation with patients: attractiveness, similarity, familiarity and compliments.

Beauty induces a *halo effect*, being related to traits as sociability, independence, dominance, success, femininity, masculinity (Ilut, 2009, 242), intelligence, kindness and talent (Cialdini, 1984/2004, 212). The second factor that induces feeling of liking somebody is the physical or psychological similarity – regarding the systems of values/attitudes and the personality traits (Ilut, 2009, 244). The similarity lies in the evocation of a common past and similar experiences in areas such as age, religion, political preferences or health-related habits (for example, a smoker will quickly become friend with another smoker, "while taking a cigarette break"). Making compliments is the third way that can determine us to give our agreement to a particular matter and the familiarity, by establishing some connections is the fourth way to win approval, our attitude being influenced by the frequency with which in the past, we experienced similar situations.

Liking may be, however, used by professionals as a disloyal manner of persuasion in order to gain unfair advantages. Therefore, Robert B. Cialdini (1984/2004, 251) recommends us to become aware of our feelings of liking our interlocutor and then to separate these feelings from the product we want to buy or the service we choose to use, trying to appreciate their real qualities.

5. Authority

The respect for authority is deeply rooted in human psyche, because the social multilayered organization provides society many advantages: it allows the

development of production systems, trade, defense, expansion and social control, while a life in anarchy is quite impossible.

The authority has such a big influence that often people accept messages without passing them through the filter of reason. As highlighted also at the "social proof" principle, the individual has to endure the pressure of the majority. Stanley Milgram's experiments (1974) related to obedience came from the general belief that we can withstand any pressure, when life or health of others is in danger. The researcher has shown that most people can not follow at the same time a group or an authority and their own conscience (*apud* Drozda-Senkowska 1999/2000, 81).

Professional titles are normally acquired in time and by collecting knowledge and experience. Clothes are a symbol of authority very easy to counterfeit. From the uniforms worn by various professionals (doctors, priests, soldiers or police officers) to the businessman suit, clothes make people to show respect and obey. The commercials for dental hygiene products also use this "dress code", resorting to actors or doctors in white who present us the benefits of toothpaste.

In order to avoid persuasion through the principle of authority, Robert B. Cialdini (1984/2004, 279-280) proposes two questions: "Does this person have real authority?" and "How reliable is the authority in this situation?" In this way, the information or the request will be analyzed objectively and the decision will be made according an assessment, not using the obedience to authority shorthand.

6. Scarcity

The idea of a possible loss is an important factor in human decision making, says Robert B. Cialdini (1984/2004, 289). The principle of scarcity says that opportunities are more valuable when they are available in a limited quantity. People seem more interested in something that could lose than in something of a similar value they could earn.

The most direct use of the scarcity principle in sales promotion campaigns is the technique of "limited number" products, the customer committing to buy one of these objects suddenly so attractive and desirable. The "deadline" strategy sets a date by which the offer is valid, while the buyer is pressured to hurry, the "now or never" formula working for many people.

Resistance to the principle of scarcity is based also on its identification. However, along with the knowledge about the used tactics, there is also an irrational need to have that product, the solution consisting not in using that product, but just in having it. This requires, therefore, a list of qualities that define that product or service: if these are real and we need that object, then we can find a similar one, as useful as the other one and not limited stock.

CONCLUSIONS

The speed in which changes occur, the large amount of information that the individual collects, stores, accesses daily, as a result of technological development make impossible for us to know and analyze all the different areas of our life. Building this complex world, the ability to process information is exceeded and, in this context, it appears, on the one hand, the temptation, and on the other hand, the danger that these shortcuts to be used by various specialists on personal persuasion.

The use of persuasion in doctor-patient relationship is justified only as an educational approach to facilitate people's change of attitudes and behavior towards oral health. Dentist's counsels can be an educative approach, as long as they comply with scientific truth, don't confine to one aspect of the problem, but expose it in all its complexity, avoiding to plead for a particular product or treatment as the only solution. A dental office is actually a business which must assure the doctor the right benefit out of the investment he made.

We believe that the promotion and the business development can be done, however, in two different ways: the classical one, the emphasis being on the products and services "sold" on a market that needs them, and the modern one, that focuses on the consumer and on his needs. The doctor may use theories and techniques of persuasion to make the patient to want more, better, more expensive, to quit his bad, not-healthy oriented habits, to acquire new skills or as long as there is always a balance in the complicated relationship between the doctor - manager of a dental office and the patient - the consumer of medical services.

Advertising and promotional campaigns of dental care products abound with examples of efficient use of the persuasion's principles, but it should be noticed that in this situation, the interests of the manufacturers, distributors and clinicians coincide. Increased sales of toothbrushes and toothpaste, and also of oro-dental h*ygiene* aids represent a quantitative measure of behaviors change with impact on health. However, the doctor is obliged to know and to communicate the real qualities of these products and recommend them according to the needs of his patients, providing all the options existent on the market at that time.

Given the changes of the contemporary society, this work stresses the need for dentists to be up-to -date with the strategies and techniques of persuasion, to be trained regarding their use, or, if necessary to unmask them and to decide, knowing the principles of professional ethics, which is the limit of their applicability in the therapeutic relationship, according the Hippocratic credo "primum non nocere. "

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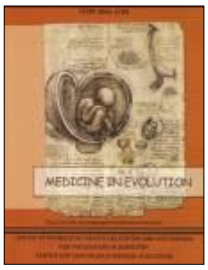
ANALYSIS OF THE BONE-IMPLANT INTERFACE DURING THE INSERTION AND REMOVAL OF IMPLANT OVERDENTURES USING NON-INVASIVE METHODS



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ABSTRACT

This paper addresses an area of great interest for many specialists: the therapy of complete mandibular edentulism using implant supported prosthetic restorations. The quality of the evaluation of implant insertion could be investigated by implant bone interface analysis. In this study the numerical simulation, tensional stamps as a noninvasive method were used in order to evaluate these interfaces. The results obtained point out the existence of gaps between the implant and the bone. In conclusion the optical coherence tomography could be used for implant bone interface investigation. After elucidating the clinical and technological issues which until recently were being questioned, a clinical strain measurement study is attempted by making implant supported overdentures with different types of mesostructures: conjunction bars with or without distal extensions, ball abutments, and also, following the simulations and the data obtained, the study is meant to determine the stress area that occurs along the bone-implant interface during the insertion and removal of the superstructure from the mesostructure.

The long time success of the implant supported overdenture therapy is closely related to selecting the appropriate retention system and to maintaining good oral hygiene; monitoring the patient and periodic optimization of the overdenture is mandatory.

Keywords: dental implant, overdenture, implant interface, numerical simulation, tensional stamps, transducer

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INTRODUCTION

The prosthetic restoration of the fully edentulous mandible is generally a challenge for any practitioner. The small size of the prosthetic field (7-12 cm^2), the perpendicular insertion of many muscles, the mobility of the oral floor, the presence of the tongue and the gradual obliteration of the alveolar artery contributing to the accelerated atrophy of the mandibular ridge, all together represent insurmountable obstacles. Even if they are made to the highest quality standards, complete mandibular dentures finally show instability in all three spatial dimensions.

So whenever the financial aspect allows it, all failures of the complete mandibular prosthesis can be solved by stabilizing the dentures using 2-3 interforaminal implants. If the remaining bone is sufficient, success is guaranteed generating patients` satisfaction. Implants stabilize the denture, increasing its retention.

Today, in many European countries, the complete mandibular prosthesis can be hardly dissociated from inserting some intraforaminal implants (see Sweden and Germany, where from approximately 74 000 implants inserted in 1990,10% were inserted interforaminally in cases of complete mandibular edentulism).

Also, the decisions taken by practitioners worldwide have led to the development of the McGill Consensus Statement on overdentures (May 2002 in Montreal) which states that the mandibular two-implant denture should be considered as a first standard of care for the edentulous patient.

Also, nowadays the treatment plans using implants as means of additional retention have evolved, the healing time being reduced to the placement of provisional prosthesis immediately after the surgical insertion of the implants.

So, in terms of patients` satisfaction, the implant supported complete overdentures are obviously a better and more accepted option (Kent, 1992, Bouma et al, 1997, Locker, 1998, Raghoebar et al , 2000, Stellingsma et al, 2003).

Prosthetic restorations of the totally edentulous jaw

Nowadays there are many solutions to restoring the completely edentulous mandible. According to **Rignon Bret**`s concept, there are four different treatments for the complete mandibular edentulism:

a) The traditional complete denture

b) Fixed-prosthetic implant restoration (Spiekermann IV concept)

c) Overdentures supported by ball abutments implants

d) Overdentures supported by implants with conjunction bars (short or long)

Generally, for patients with a complete edentulous mandible, the endosseous implants are inserted interforaminally.

Both stage I and stage II implants can be used, the final results not being influenced by the types of implants. For some practitioners, 2 implants are enough (1). If the implants are shorter than 10 mm, their number must be increased (2). Other practitioners use three or more implants. If the implants conjunction bars offer increased retention, the ball-abutments are more simple alternatives, better tolerated and easier to clean. When we decide to use conjunction bars, a minimum distance of 8 to 10 mm must be maintained between the two implant pillars, or better said 20 mm between the centers of the implant pillars in order to allow the placement of a bar which measures at least 8-10 mm in length but which is not longer than 15 mm so there is no risk of bending deformation under the influence of occlusal forces.

Motivation for the study

This paper addresses an area of great interest for many specialists: the therapy of complete mandibular edentulism using implant supported prosthetic restorations.

After elucidating the clinical and technological issues which until recently were being questioned, a clinical strain measurement study is attempted by making implant supported overdentures with different types of mesostructures: conjunction bars with or without distal extensions, ball abutments, and also, following the simulations and the data obtained, the study is meant to determine the stress area that occurs along the bone-implant interface during the insertion and removal of the superstructure from the mesostructure.

The long time success of the implant supported overdenture therapy is closely related to selecting the appropriate retention system and to maintaining good oral hygiene; monitoring the patient and periodic optimization of the overdenture is mandatory.

Electrical strain measurement, general aspects

Electrical strain measuerment is a method measuring deformations and elongations of an object by means of transducers transforming the variation of mechanical deformation into electric signal variations.

Transducers can be classified based on the electrical parameter to which the mechanical quantity is converted (resistive transducers, capacitive transducers, inductive transducers) or based on the mechanical parameter which is being measured (displacement transducers, speed transducers, acceleration transducers, transducers for specific strains). Of the above, resistive transducers are most widely used.

The resistive transducer used in strain measurement is made up of one or more conductors, mostly metallic, connected in series, having a very small diameter (0.015-0.02 mm) and having an electrical resistance with values usually between 50 and 1000 ohms. This resistor is either attached or embedded in a paper or synthetic body. Due to its real shape and size, the resistive transducer is also called strain gauge.

The working principle of the resistive transducer is that when mounted on the surface that we wish to investigate, closely following its deformations, the resistance of the transducer changes due to strain. It was found that the changes in the transducer`s resistance are, within certain limits, practically proportional to the specific strain it suffers.

Installing a transducer is the most delicate phase of a strain measurement process, requiring speed and precision.

Immediately after mounting, the transducers must be protected against humidity using the technology and materials recommended by the supplier of the adhesive. The cables are connected to the transducers exclusively by soldering.

Performing several measurements in the laboratory with transducers under static loading poses no particular problem. Multiple trials are needed in order to obtain enough data and to eliminate measurement errors. For the tests carried outside, several measures must be taken to avoid or to reduce temperature variations. That is why the charging/discharging operations must be performed as fast as possible. For the dynamic measurements, i.e. the time-depending strains measured by transducers, the measurement devices are very important: the bridge measurement system and the recorder are chosen taking into account first of all the frequency of the object and then the possibilities of processing and interpretation of results. The range of equipments used today for dynamic measurements varies, selecting the devices used for dynamic measurements, performing such measurements and processing the results are tasks performed by personnel specifically trained for this purpose.

METHODS AND MATERIALS

In order to analyze the deformations occurring in the mandible we used CEA-06-032 strain gauges (having a 120 ohms electrical resistance) supplied by Vishay Micro-Measurements(fig 1,2).The strain gauges were placed on the vestibular side and on the lingual side corresponding to the implantation areas. The data acquisition and processing system used was ESAM TRAVELLER I, using the ESAM-Vishay software.

Fig 1.Strain gauges used in the study.

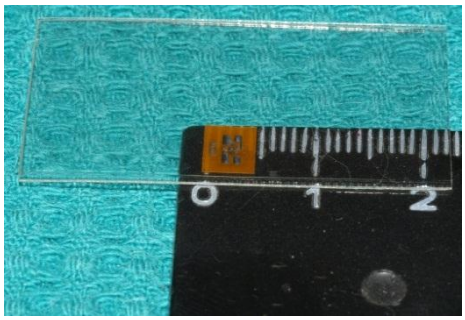

Fig 2. Dimensions of a strain gauge

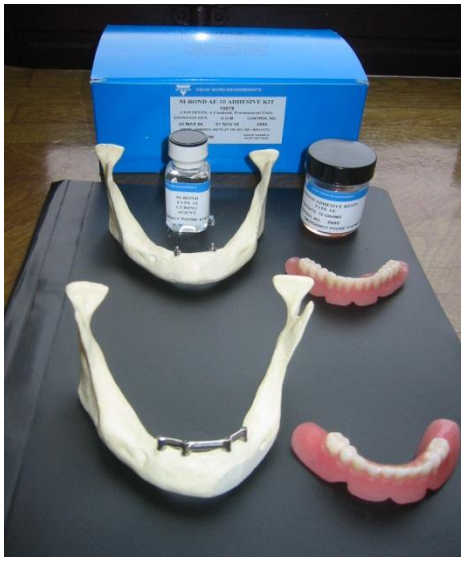

Fig 3.The structures and the adhesives used

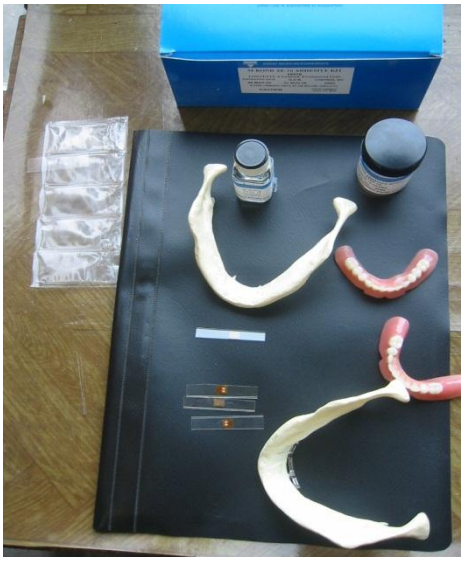

Fig 4.The structures after mechanical cleaning; the measurements will be done on these structures.

Fig 5.Applying the adhesive along the marked area.

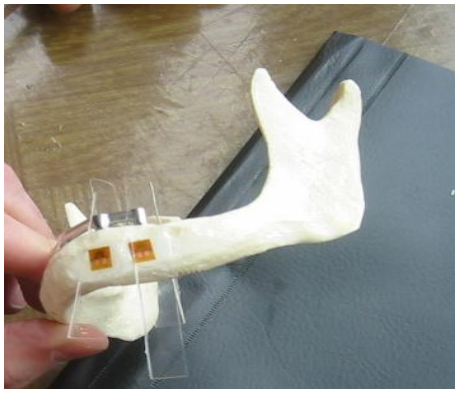

Fig 6.Applying the strain gauges.

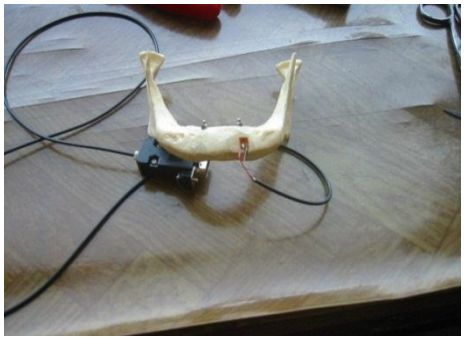

Fig 7.Cable connected to the transducer.

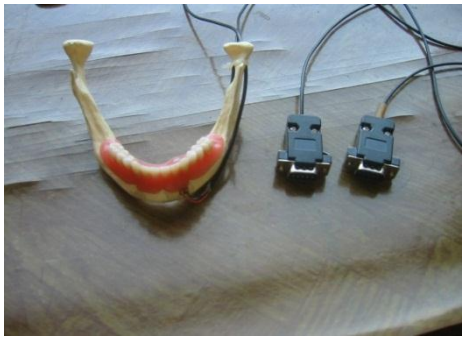

Fig 8.The structure ready for measurements

Fig9.Connecting the cables

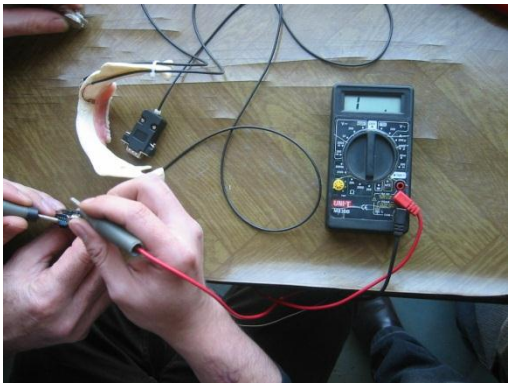

Fig10.Testing electrical continuity.

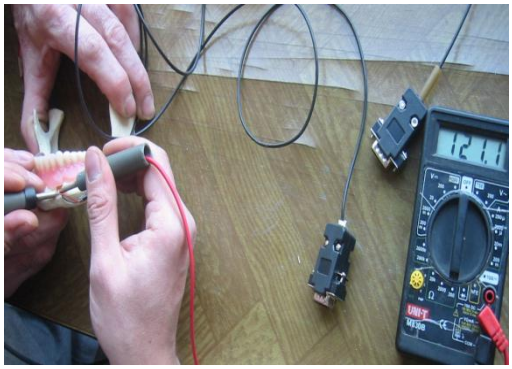

Fig11The value obtained with the transducer.

Fig12The workbench.

Fig13.Detail of the measuring workbench.

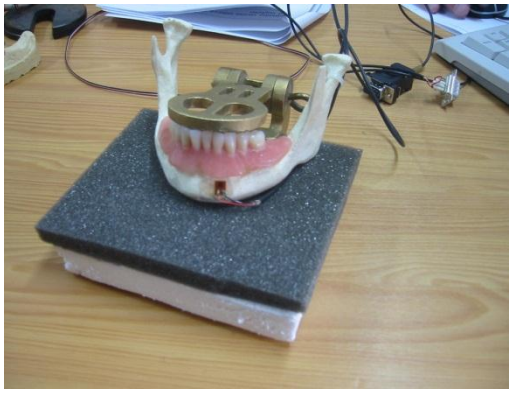

Fig14.Detail of the measuring workbench: uniform load using a simple hinge articulator

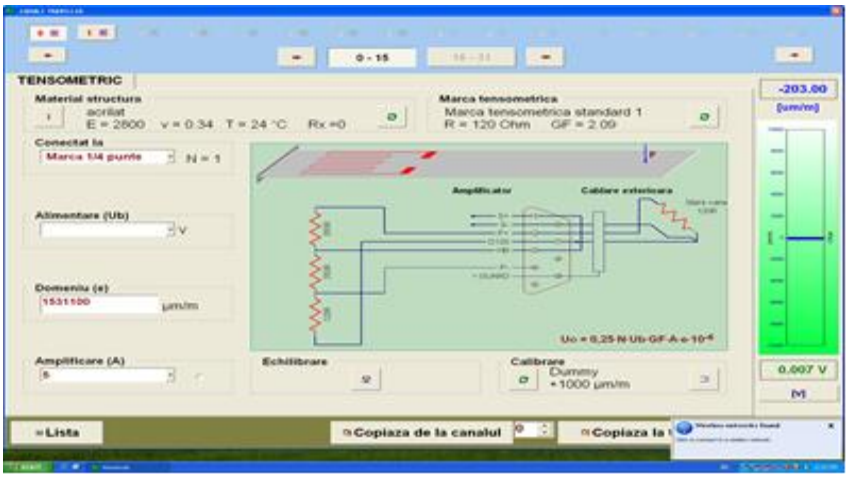

Fig15.Balancing the strain analysis system-performed in the software

The accuracy class of the system is 0.03, with a total of 8 channels. Resistive transducers can be connected to this system. The following connections are allowed: full bridge, half bridge and quarter bridge. The maximum reading speed is 250 000 readings/second. Temperature range is between 0-70°C. Power is 1 V with a consumption of 17 W.

The mechanical loading was performed using weights from 0 to 4.2 kg, in 0.4 kg increments. The load was applied on top of a simple hinge articulator adapted to evenly distribute the force unto the occlusal surface of the overstructure. In order to obtain a uniform stress distribution in the mandible without being influenced by the irregular inferior ridge of the mandible (contact stress, contact strains) we used a special pad made of foam and polystyrene (fig 14).The technique followed the directions of the manufacturer. Thus, the mandibles were prepared for mechanical and chemical cleaning in the vestibular and lingual areas corresponding to the insertion of the implants (fig 4). Because of symmetry, strain gauges were applied only on one side of the mandibular bodies. The adhesive from the kit (fig 5) was activated by depositing the catalyst using a pipette in the pot containing the base. The mixing was performed on a vibrating table in order to avoid air inclusions. We then proceeded to apply the adhesive on the mandibular body, followed by the application of the strain gauge without interacting with the marked area (fig 6).The application of the strain gauge must be done firmly against the mandibular body in order to get a uniform coverage by the transducer. Failure to comply with this indication leads to insufficient adhesion of the strain gauge to the structure thus obtaining erroneous information during the measurement. Afterwards the cables are connected to the transducers by soldering (fig 7). It is very important to obtain an accurate and uniform soldering which will allow us to take information from the transducers. The evaluation of the interconnected structures is made so far by measuring the terminal resistance (121.1 ohms fig 10-11).The assembly is connected to the ESAM TRAVELLER I data acquisition system (fig 13).

The features of the strain gauges used in the experiment along with the type of network achieved is then introduced in the ESAM Vishay software (fig 12). Afterwards, the system is equilibrated using the software. The system is then loaded with weights ranging from 0.4 kg to 4.2 kg and measurements for each situation are made. The data is then processed by the software and a measurement report is obtained.

RESULTS AND DISCUSSION

Figure 16 shows the strain obtained for the 1st case (mesostructure represented by balls-red, mesostructure represented by two implant bars-black).
The results obtained indicate a significant increase of mandibular deformation in the case of ball type as compared to beam type overstructures:

-for the verstibular surface, at maximum load, there was a strain variation of about 450%

-for the lingual surface, at maximum load, there was a strain variation of about 115%

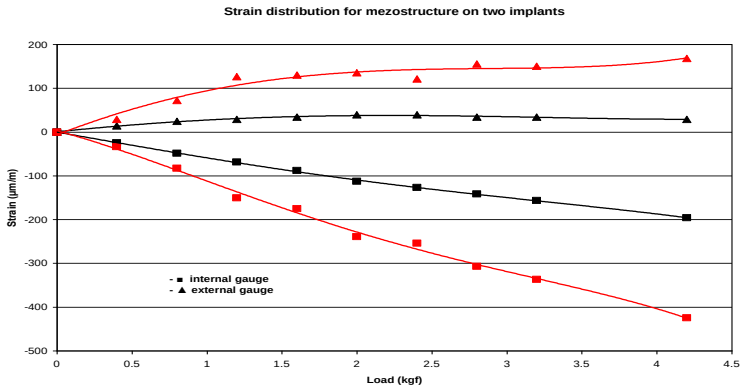

Fig 16.Strain distribution recorder for the case of two implants inserted in the mandible with mesostructures represented by balls (red) and bars (black)

Considering the 4 implants inserted in the mandible (fig 17), the mesostructure represented by a simple bar (black) or extension bar (red), the 2 mesostructures behave as following:

-for the 1st position of the implants (the position closest to the median line of the mandible), the overstructure represented by a simple bar induces higher strain as compared to the strain induced by the extension bar (at full load there is a variation of about 40%)

-for the 2nd position of the the implants, the overstructure represented by the extension bar induced higher strains than those induced by the simple bar overstructure (about 60% higher) for load levels of up to 3.2 kg; for higher values of the specified load, the strain induced by the simple bar mesostructure exceeds the strain corresponding to the extension bar mesostructure.

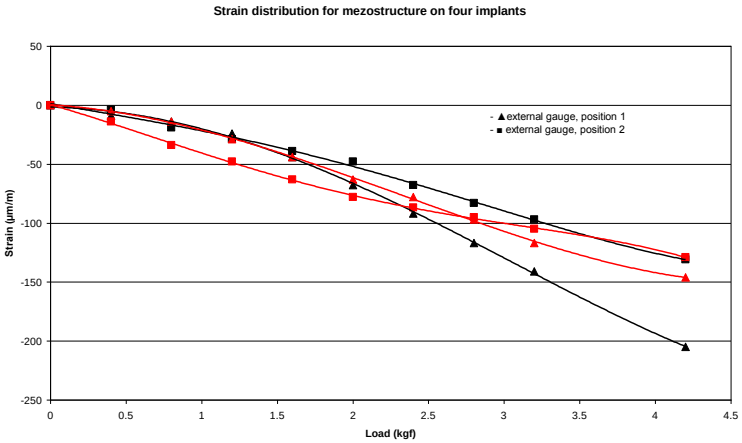

Fig 17.The distribution of the strain recorded in the case of four implants inserted in the mandible with mesostructures represented by a simple bar (black) and an extension bar (red)

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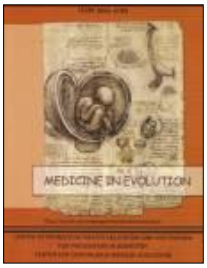
DENTAL MEDICAL EDUCATION IN PUPILS AGED BETWEEN 6 AND 18 YEARS LEARNING IN ARAD SCHOOLS



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ABSTRACT

The very high morbidity by dental caries recorded during recent years in children confronts dentists with a series of prevention and treatment challenges. The present management of dental caries has three major components: prevention, control and treatment.

For this reason we consider dental prevention to be the most effective cario-prophylactic method. By this study we emphasise the special attention dentists and all factors directly involved in the education of pupils need to pay to health education, using all available means (practical demonstrations during education classes, hygiene related lectures, audio-visual means).

In order to obtain a social-hygienic effect, education programmes must be designed aiming at implementing dental prevention programmes in schools and kindergartens. The present paper presents the preliminary results of a study started in several schools in the municipality of Arad with the purpose to check the incidence of carious lesions in pupils aged between 6 and 18 years following dental education activities.

Key words: Dental prevention, dental-maxillary apparatus, oral-dental hygiene

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INTRODUCTION

The programme "Medical dental education in Arad schools" monitored the way dental education, information and detection of oral-dental diseases and dental-maxillary abnormalities in primary school and gymnasium pupils were performed.
The educational approach meant to build up a harmonious individual requires a complete relation between body and spirit, purpose which also tends to be reached by health education with dental education playing the main part. The advocacy for this type of education starts from the concept of health being an essential aspect of human life. Physical shape represents the health level of an individual, while phychological status reflects the spiritual integrity. "If the entire body, except a small part which is hurt or painful, is healthy, we do not feel the health of the body but attention is only directed to the pain in the harmed place and the good spirit of the entire vital sense is lost" (A. SCHOPENHAUER). How right he is and who of those having experienced at least a slight dental discomfort can contradict him.

Historically, we are past antiquity, when a perfect body was the ultimate expression of beauty, and when people were not aware that neglecting body hygiene represented, first of all, a source of diseases. We are witnessing extensive changes, true chances for physical and moral development. Our children must understand they are part of the community, that each of us is responsable for his or her own health, and together we have the duty to equally protect the health of others. Both school and family are important examples of adequate and healthy behaviours. The task of health education, without neglecting dental Elath, belongs primarily to parents, they having the obligation to survey and guide the physical and spiritual development of the child starting from the very moment of birth.
Promoting and maintaining health are achieved by health education. For this, special programmes are required in schools and communities in order to shape positive values towards health, constructive beliefs and attitudes with an important role in the prevention of all dental-maxillary diseases.

OBJECTIVES

The major objectives of dental health education are:

- Information on risk and protective behaviours for the dental-maxillary apparatus
- Shaping acceptance attitudes towards protective behaviours and rejection of risk behaviours
- Exercising behaviours of promoting and maintaining health and avoiding the risk of disease
- Strengthening healthy behaviours.
- Sharing healthy lifestyles in the community and through the mass media.
- Starting from the deep and equal understanding of these pro-health behaviours and risk factors, of health as a lifestyle, various specialists (school physicians, psychologists, educators, teachers, etc.) must create a reliable system of values for children by:
- Promoting and maintaining the general and oral-dental health
- Preventing and treating dental diseases
- Identifying causes and correlatives for health and diseases
- Improving the health system

Teachers may aquire this by organising education classes during which to discuss health topics. Sometimes pupils have more information on a given theme, taken from biology classes, TV shows, clubs and other extra-school activities they attend, and then the discussion becomes more coherent and interesting. Also, teachers may compose various homework topics on several objectives:

- To define hygiene notions
- To use natural resources in order to keep oral-dental health
- To identify the signs of the main dental diseases
- To present their personal dental hygiene rules
- To describe the main anatomo-physiological transformations of the dental-maxillary apparatus.

The school dentist has the possibility and also the professional obligation to observe the dental-maxillary development and healing processes of children in collectivities by

preventive and currative dental assistance i.e.

- Applying measures for oral-dental hygiene, dental caries and oral-dental diseases prevention.
- Actively detecting and monitoring oral-dental diseases.
- Observing the harmonious development of the dental-maxillary apparatus by conserving and restoring dental morphology.
- Ensuring the functional prophylaxis for the conditioning of harmful habits.
- Examining the periodontium in order to detect clinical signs for the onset of chronic diseases.
- Examining, diagnosing and treating dental caries.
- Co-monitoring pupils with dental-maxillary abnormalities and periodontal diseases in collaboration with periodontology specialists, applying treatments these specialists recommend.
- Performing the education of preschool children and pupils in order to achieve the prevention of dental caries and of dental-maxillary abnormalities.
- Cooperating with the school physician and with the family doctor or with other specialists in order to prevent oral-dental diseases which might represent manifestations of certain chronic diseases.
- Early detecting pre-neoplasic lesions and oral cancers and reffer the patient to relevant medical services.
- Performing an oral-dental health summary report at the end of each teaching cycle to accompany each child and adolescent into the subsequent cycle of study.

DISCUSSIONS AND CONCLUSIONS

Starting from these elements we conducted a study between the years 2008-2009 on the morbidity by dental caries in 300 pupils aged between 6 and 18 years in order to check the application and promotion of dental health education in Arad schools. For this purpose we requested the support of the Arad School Inspectorate and of the Arad District Board of Dentists.

One of the objectives of this study was to detect early carious lesions and to determin the prevalence and carious experience in 6-7 and 12-18 year olds, respectively, together with the determination of socio-demographic factors, caries risk groups, by application of bacterial plaque revealing tests, oral hygiene lessons, detection of dental-maxillary abnormalities and harmful habits.

The pupils in our study, being adequately medically selected upon their registration for the new teaching year, were healthy, did not present pathological histories nor did they have evident organic diseases and they were distributed into relatively homogenous groups regarding age, intelectual level and living environment.

Under these circumstances, given the constant improvement of living standards and accessibility to dental assistance, morbidity by dental caries in these pupils would have been expected not to vary significantly but to decrease continuously.

Nevertheless, the variations recorded by us were rather obvious and morbidity increased yearly to levels between 70% and 85.5% in the year 2008 (tab. 1-2).

TABLE 1

Year when study was conducted	Normal	1-2 C.R.E	3-5 C.R.E	TOTAL C.R.E	Morbidity increase
2008	24	184	92	276	----------
2009	16	201	83	284	12

C- caries R-crown restoration E-dental extraction

TABLE 2

School	Year	Normal	1-2 C.R.E	3-5 C.R.E	TOTAL C.R.E
ELEMENTARY SCHOOL no. 22	2008	6	54	20	74
	2009	3	46	31	77
ELEMENTARY SCHOOL no. 13	2008	2	38	40	78
	2009	4	41	35	76
"VASILE GOLDIŞ" HIGH SCHOOL	2008	4	29	47	76
	2009	1	25	54	79

No significant differences were observed between the number of dental lesions in 6-18 year old children in peripheric or rural schools as compared to central urban ones, meaning that the socio-economic status did not have an influence.

This increased morbidity was also observed by other physicians and, as such, it did not represent a characteristic in the schools in our study.

As we could not explain regional variations of morbidity by important differences in the nutrition of pupils among whom the analysed subjects were recruited, we explained this situation by the lifestyle, hygiene standard and lack of dental education and prevention both at home and in school.

As a series of researchers (as well as the encouraging results of prevention by education) proved the importance of this activity, we primarily looked for the dental medical education in schools and kindergartens, enquiring about its existence and about the persons giving it. For this purpose we also included students from the „VASILE GOLDIS" Faculty of Dental Medicine and the Colledge of Dental Technology in the same university as investigators. Following the study we aimed at collecting data for a detailed analysis with the final purpose to elaborate a prevention educational programme allowing decreases in caries, orthodontic treatment need indicators, aquisition of dental knowledge, improving individual behaviours and the acceptance of treatment and the increase of addressability among 6-7 and 12-18 year olds, respectively.

It is estimated that under 20% of the studied subjects perform adequate dental brushing.

Moreover, an important number of persons do not own a dental

brush, and some families still share the same dental brush. The result of this situation is a low level of oral hygiene and a continuous increase in the frequency and intensity of dental caries.

Education must include dental brushing as early as kindergarten and primary school, during the age period when behaviours are being shaped. This may be achieved by practical lessons on the way to use the dental brush as well as by various visual prevention materials. A correct dental brushing reduces the frequency of dental caries by 40% leading to the logical conclusion that brushing must be learnt as early in life as possible in order to fully benefit from its prophylactic effect.

Starting from these premises, we studied the hygiene of the oral cavity in a group of 250 pre-school children. The working method was as follows:

We asked each child to bring his or her toothbrush (and checked its existence and wear degree) and a glass. Then bacterial plaque was revealed by staining with a disclosing solution.

On this occasion, children were shown intensly coloured areas, with emphasis on their significance. Pupils then exercised dental brushing assisted by specialists until removal of the staining agent was obtained and they were explained that the duration of dental brushing must be the same on all dental groups until the staining agent disappears. After several days we presented the recorded images to the pupils together with a health education material regarding oral-dental hygiene.

The overall oral-dental hygiene in children aged between 6 and 18 years is poor, with only 4.9% presenting a favourable state. This is due both to the lack of preoccupation from the family-school-child component, as well as to incorrect brushing techniques.

In order to verify the results, in one group of children brushing was performed before staining and without previous training; no significant difference was observed as compared to the control group who did not practice dental brushing. In another group, the correct brushing technique was taught by practical and educational classes several weeks before staining which led to a better oral-dental hygiene status.

By revealing soft dental deposits, children realise the shortcomings and ineffectiveness of their previously practiced dental brushing, but they especially learn the needed duration and intensity for a correct brushing.

Together with this individual educational character, the collective effect becomes manifest: the mutual example, the wish to imitate and to achieve self-control.

In conclusion, we may state that health education with all available means is imperative: practical demonstrations during education classes, lectures on hygiene or visual means.

In order to obtain a social-hygienic effect, as many and varied as possible oral hygiene activities must be organised in collectivities of pupils:

Encouraging inter-group contests on the „Cleanest teeth" topic, placing mirrors in restroom areas at height levels matching the age of the pupils, improving self-control by periodic bacterial plaque tests, as well as other measures to develop correct oral-dental hygiene behaviours in pupils.

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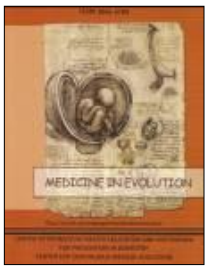
EXTRACTION OF IMPACTED LOWER THIRD MOLARS: POSTOPERATIVE LINGUAL NERVE PARESTHESIA



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ABSTRACT

Several complications are associated with extraction of impacted lower third molars, one of the uncommon being paresthesia of the lingual nerve. Several factors have been associated with this complications, including type of impaction and the surgical technique used. The purpose of this prospective study was to evaluate the incidence of lingual nerve injuries associated with the odontectomy of impacted mandibular third molars. An attempt was also made to determine the risk factors associated with this complication.

Keywords: *third molar, lingual nerve,* surgery

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INTRODUCTION

Among the most severe postoperative complications that arise from lower third molar surgery is lingual nerve injury [1]. In the presence of injury, spontaneous recovery is less common [2].

The aim of our clinical prospective study was to determine the clinical incidence of lingual nerve injury following lower third molar odontectomy and to analyze possible risk factors for the lingual nerve paresthesia.

MATERIALS AND METHODS

We selected thirty consecutive patients who reported to UMF „Carol Davila" OMFS Department from December 2011 to January 2012 for the surgical removal of impacted mandibular third molar.

Preoperative factors such as depth of impaction, tooth position and state of eruption were considered using orthopantomograph.

Surgical procedure was performed under local anesthesia.

Sensory disturbance was evaluated on first postoperative day and one month after surgery. Any complaint concerning sensory disturbance of the tongue was recorded.

Assessment of postoperative deficit was carried out by pain awareness, using light pressure from a sharp probe.

Patient with any complaint concerning sensory disturbance on postoperative evaluation were advised for regular follow up at the interval of one month and observed up to 6 months, if paraesthesia persisted.

RESULTS

Out of 30 patients, two patients (6.67%) were diagnosed with lingual nerve paraesthesia on first postoperative day and one month evaluation [Table 1].

In one patient paraesthesia persisted even after 6 months of follow up in spite of therapy of Supradyn (*Bayer* ®) 1 caplet daily (Figure 1).

Fig.1. Close-up image of an impacted 38. Note the horizontal impaction.

Other patient with paraesthesia recovered within 6 months of observation (Figure 2).

Fig.2. Close-up image of an impacted 48. Note the distoangular *impaction.*

Patient	Tooth	Depth of impaction (*Peterson*)	Tooth position	Path of exit	Time of resolution
1	38	Class B	horizontal	mesial and distal cusps covered	no resolution up to 6 months
2	48	Class C	distoangular	distal cusps covered	3 months

Table 1: Patients with paraesthesia

The small number of paraesthesia precluded statistical analysis.

However when factors possibly contributing to lingual nerve paraesthesia was analyzed separately and combined, it revealed that paraesthesia were generally associated with horizontal and distoangulation of impaction, deep oclusal depth level and medium oclusal depth level, state of eruption and duration of surgery.

RESULTS

The figure of 6.67% for the lingual nerve injury is higher than expected from clinical experience [3]. However it is same as that reported by other reports, denotation a possible association between paraesthesia and complete bone cover, position of impaction and state of eruption [4].

Deep lingual bite while suturing, which might be implicated in lingual nerve injury [5] was not examined as it was considered difficult to evaluate.

Various study reported that the incidence of nerve injury is more frequent with deep bone impaction [6,7]. In our study class B and class C are more significant for paraesthesia.

We also observed the relation of tooth position and incidence of paraesthesia and found more paraesthesia with distoangular and horizontal-impacted lower third molar.

Our study observed that more lingual nerve paraesthesia is significantly related with surgical removal of complete bone cover lower third molar.

In our study paraesthesia in all but one patients resolved within 1to 6 months and paraesthesia in one patient seemed to be permanent.

In this patient, the wisdom tooth was placed horizontal and incomplete covered with bone.

In our study the incidence of permanent nerve injury was 3.33% which is higher to other studies [8,9].

CONCLUSSIONS

From our study and review of literature, it can be concluded that lingual nerve injuries can occur in spite of all the measures taken to protect it. It may be contributed to the fact of anatomical variations of lingual nerve.

Thus, the most effective method of managing lingual nerve injuries remains preoperative assessment of xrays and discussion with patients about the possibility of injury.

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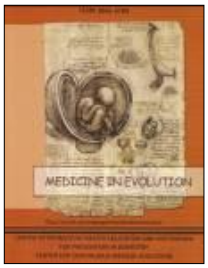
SYSTEMIC CONDITIONS AND TREATMENTS AS RISKS FOR DENTO-ALVEOLAR SURGERY



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ABSTRACT

Dentist should be familiarized with associated serious systemic disease *when providing dental treatment.*

A retrospective study was made to evaluate the type of ambulatory surgery performed under locoregional anesthesia, taking into account the presence of systemic illnesses, the type of anesthesia used, relating these parameters to the development of systemic and/or local complications. Only mild complications were recorded and no significant relation was observed between the development of complications and the drugs used by these patients. Only diabetes was associated with postoperative local complications.

Keywords: systemic dissease, postoperative complications, medication

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INTRODUCTION

Patients with dento-alveolar surgical pathology often present multiple systemic pathologies. In order to treat these problems, patients are usually prescribed different types of medication.

The present study was conducted to determine the most common types of surgical treatment in patients seen in an ambulatory oro-maxillo-facial surgical clinic, using locoregional anesthesia, with an evaluation of the prevalent systemic disorders and correlating the presence of associated systemic disease to the development of postoperative complications.

MATERIAL AND METHODS

Our present retrospective study comprised 50 consecutive patients treated under locoregional anesthesia in the Ambulatory and Compartment Presentation Emergency (CPU), "Dan Theodorescu" Hospital, Bucharest over a five-days period.

The following data were evaluated from the individual case histories: age, sex, the presence and type of background systemic pathology, medication, the cause of consultation, treatment provided, perioperative complications.

All variables were subjected to a descriptive statistical analysis. The association between the development of complication and preoperative variables was analized. The SPSS statistical package under MS Windows XP was used throughout. Statistical significance was considered for $p<0.05$.

RESULTS

A number of 43 patients (86%) presented some associated systemic disorder. The most frequent systemic illness was arterial hypertension (23 patients - 46% of all subjects with physical disabilities), followed by heart disease (16 patients - 32%).

Diabetes mellitus was documented in 6 cases (12%). Type 2 diabetes was considerably more common (5 cases) than type 1 diabetes (1 case).

Other pathologies were recorded in 3 patients (6%).

The most commonly used medication were antiplatelet drugs, antihypertensive agents, analgesics, oral antidiabetic drugs, etc.

It was noted that a number of 12 patients (24%) used no medication treatment.

Thirty-seven patients (74%) presented for teeth extractions and/or bone cyst
resection, while 26% presented for soft tissue treatment (extirpations of oral mucosal lesions).

Most of the 50 operations (33 cases - 66%) were performed under 2% lidocaine (5% of all operations). Some patients presenting cardiovascular disease or some other condition contraindicating the vasoconstrictor received 3% mepivacaine without vasoconstrictor (4 cases - 8%).

Also we used 4% articaine anesthesia with epinephrine 1:100,000 (9 cases – 69.23%) or 1:200,000 (4 cases – 30.76%).

Of the 50 operations, complications were only observed in 5 cases (10%), and were essentially of a local nature:

postoperative hemorrhage (3 case), postoperative hematoma (one case).

Only one systemic complication in the form of a postoperative hypotensive episode requiring specialised treatment in the surgical intensive care unit.

No correlation ($p>0.05$) was found in our study between the evelopment of complications either during or after surgery and the use of associated medication, or as regards the surgical technique employed.

As to the presence of associated systemic pathology and the development of complications, a significant correlation was only found with diabetes mellitus ($p<0.05$). In this context, four of the five patients who presented complications suffered diabetes.

DISCUSSION

Medical history as complete as possible, together with adequate clinical examination and paraclinical tests may reveal the existence of certain associated systemic illness that require special consideration before providing dento-alveolar surgery [1], as in the present retrospective study.

Most of the subjects in our study (86%) presented some systemic dissease requiring the use of various medication.

In our study an important prevalence of arterial hypertension was observed (46% of the patients), together with heart disease (32%) and diabetes mellitus (12%).

Other studies have reported similar data. [2,3]

In this sense, the literature shows cardiovascular disorders such as arterial hypertension and heart failure to be the most common systemic problems (48.8%) (22).

Cardiovascular diseases must be carefully assessed, since they constitute the first cause of death [4].

The associated systemic pathology may lead to increased morbidity and mortality, and this surgical risk is in turn dependent upon systemic conditions, the anesthesia type and the surgery performed.

Systemic effects are to be expected following locoregional anesthesia, as a result of the action of the vasoconstrictor that accompanies the anesthetic substance. These effects are a result of beta-receptor stimulation, which increases heart rate [5].

Ambulatory dento-alveolar surgery is not contraindicated in patients with slight arterial hypertension, adequately controlled [6].

When a blood-free surgical field is required, epinephine a concentration of 1:200,000 is advisable.

Accordingly, in our study we used 4% articaine with epinephrine 1:100,000 in 9 cases, while surgery in patients with cardiovascular disease or any other contraindication for vasoconstrictors was performed administering 3% mepivacaine without vasoconstrictor (four patients).

In our study no significant relation was observed between the presence of diabetes mellitus and inflammatory complications was recorded among these patients. Moreover, these patients are not more susceptible to hemorrhagic accidents than non-diabetics.

In our series there were three cases of bleeding complication, though none of the affected patients were diabetics. The reason for this bleeding were related to the antiplatelet agents used for their cardiovascular pathology.

No association was found between the existence of cardiovascular disease and the appearance of complications in relation to the surgical technique employed ($p>0.05$).

The adoption of suitable measures considerably reduces the risk of complications – particularly those of a systemic nature.

CONCLUSSIONS

A medical history as detailed as possible is essential for the surgical treatment of ambulatory treated patients. Establishing the general condition of the patient and adoption of the required preventive measures should suffice to control any turmoil that may happen.

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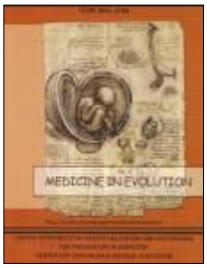
ROUTINE APPLICATION OF AN RETRACTOR THE LINGUAL NERVE DURING ODONTECTOMY OF LOWER WISDOM TOOTH



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ABSTRACT

The incidence of reported postoperative dysaesthesia of the lingual nerve varies widely in literature. There is currently a trend not to raise a lingual flap, in an attempt to reduce the incidence of temporary sensory tongue deficit. A prospective randomised study was designed to evaluate the efficacy of protecting the lingual nerve by insertion of a in 100 patients. In one group, the lower third molar was removed with protection of the lingual nerve and in another group, without protection. The results indicate an incidence of postoperative lingual nerve dysaesthesia lower in comparison with other studies.

Keywords: wisdom tooth, pstoperative complications, lingual nerve

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INTRODUCTION

Odontectomy of lower wisdom tooth is one of the most common surgical procedure.

Routine protection of the lingual nerve by subperiosteal insertion of a retractor during surgery has been subject to controversy [1,2].

The incidence of reported postoperative dysaesthesia of the lingual nerve varies widely in published studies [3,4].

This prospective, randomised study was designed to evaluate the efficacy of
retracting the lingual flap in order to prevent sensory tongue deficit after removal of lower wisdom tooth.

MATERIAL AND METHODS

The study group comprised a total of 100 consecutive patients.

Technique consisted of surgical removal of the lower wisdom tooth, with protection of the lingual nerve by subperiosteal insertion of a retractor (Figure 1).

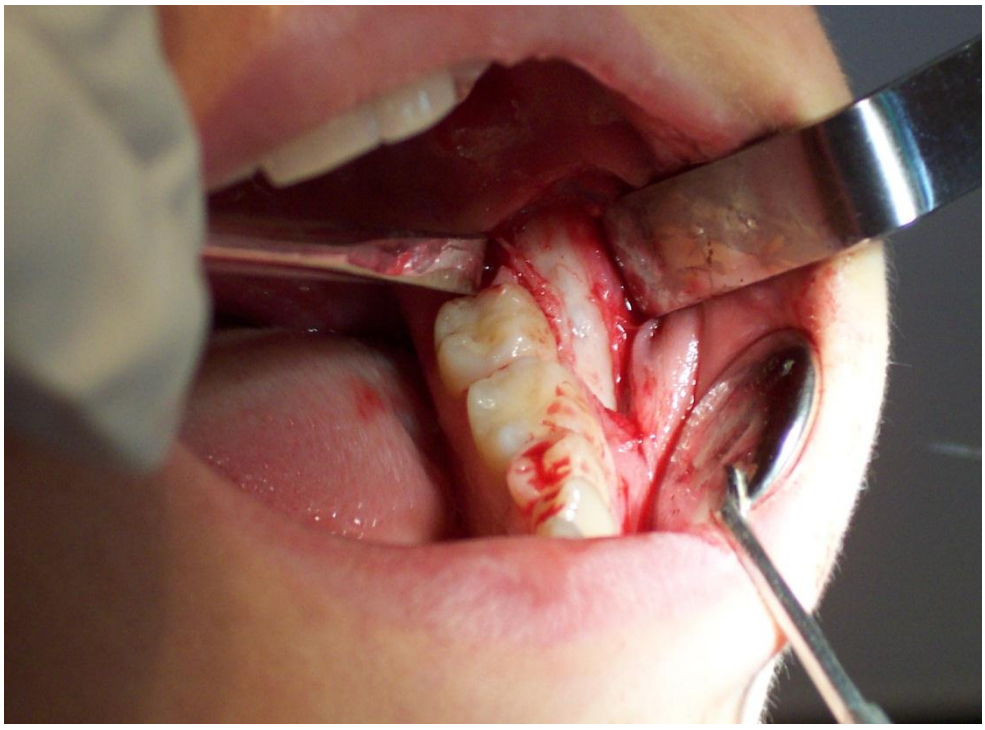

Fig. 1. Odontectomy of impacted 3.8. with protection of the lingual nerve

Alternative technique consisted of removal of the lower wisdom tooth without protection.

Patients were strictly randomised.

All surgeries were done on local anaesthesia.

The degree of inclusion and position of the wisdom tooth and patient gender and age were recorded.

The following measures were performed from orthopantomographic xrays:

1. measurement of the space available distal to the 2nd molar, using the

classification based on the relationship between the impacted lower third molar to the ramus of the mandible and the second lower molar (*Pell & Gregory*)
2. measurement of the angle of impaction, using the inclination of the impacted lower wisdom tooth to the long axis of the second lower molar (*Winter).*

According to these classifications, the *difficulty* degrees of difficulty of the surgical intervention can be described:
- easy (0-3)
- moderate (4-6)
- difficult (7-10).

Degrees of difficulty for *Pell & Gregory's* classification [5]:
a. Vertical space:
Class A: the highest portion of impacted wisdom tooth is on a level with or above the occlusal plane – degree 1
Class B: the highest portion of impacted wisdom tooth is below the occlusal plane but above the cervical line of the of lower second molar – degree 2
Class C: the highest portion of impacted wisdom tooth is below the cervical line of the of lower second molar – degree 3.
b. Horizontal space.
Class 1: sufficient space available between the anterior border of the mandible ramus and the distal aspect of the lower second molar – degree 1
Class 2: the distal portion of the lower wisdom tooth crown is covered by bone of the ascending ramus – degree 2
Class 3: the lower wisdom tooth is totally embedded in the bone of the ascending ramus – degree 3.

Degrees of difficulty for *Winter's* classification [5]:

- Mesio-Angular – degree 1
- Horizontal – degree 2
- Vertical – degree 3
- Disto-angular – degree 4.

The surgical techniques were based on the commonly used methods including envelope or bayonet incision, elevation of a mucoperiosteal flap, bone removal and coronectomy with a surgical burr, elevation of the tooth, wound cleaning and closure with non-reabsorbable suture.

Lingual nerve sensory deficit was tested at ten days in the course of postoperative assessment, using light pressure from a sharp probe.

The data recorded on the study forms were tabulated for each factor and analysed for significant effects (SPSS® statistical package for MS Windows®). The χ^2 test was applied for individual factors. A supplementary logical analysis was used to identify influencing factors.

RESULTS

The records were completed for 100 consecutive patients: 47 men (47%) and 53 women (53%).

The mean patient age ranges from 17 to 34 years.

A total of 100 lower wisdom tooth were removed: 43 from the right side and 57 from the left side.

A number of 24 teeth (24%) were complete impacted in bone and 84 (86%) were partially impacted.

A total of 53 teeth were partially erupted and 47 were unerupted.

The horizontal position of the wisdom tooth is shown in Table 1.

	N (%)
Class I	**9**
Class II	**77**
Class III	**14**

Table 1 Lower wisdom teeth distribution according to horizontal position

The vertical position of the wisdom tooth is shown in Table 2.

	N (%)
Class A	**15**
Class B	**67**
Class C	**18**

Table 2 Lower wisdom teeth distribution according to vertical position

The angle of wisdom teeth impaction is shown in Table 3.

	N (%)
Mesioangular	**28**
Horizontal	**49**
Vertical	**21**
Distoangular	**2**

Table 3 Lower wisdom teeth distribution according to classification of Winter

In Table 4, the wisdom tooth distribution according to the degree of surgical difficulty is represented.

	N (%)
Easy	*0*
Moderate	*67*
Difficult	*33*

Table 4 Lower wisdom teeth distribution according to degree of surgical difficult

A total of 49 lower wisdom teeth were removed with retraction of the lingual flap and 51 were removed without protection.

Ten days after surgery only one patient showed sensory tongue deficit.

Surgical removal of the lower wisdom tooth on that patient was performed with protection of the lingual nerve by subperiosteal insertion of a retractor.

The tooth was moderately difficult to remove.

The lingual disturbances have resoluted after 28 days, with complete sensory recovery after 40 days.

The χ^2 test of the difference in proportions showed no significant results.

No significant association of sensory tongue deficit with the degree of difficulty of odontectomy could be established.

DISCUSSION

In this study there appeared to be little difference between surgical removal of lower wisdom molars, using lingual flap retraction and odontectomy with no protection of the lingual nerve.

Due to the small sample size and the low proportion of sensory disturbances, accurate conclusions cannot be established, due the lack of statistical significance.

A number of variables were considered in the aetiology of lingual nerve damages, including the surgical approach, surgical technique and the skills of the surgeon.

Some studies reported incidences of sensory disturbances when lingual flap retrac-
tion was used, ranging from 0.2% to 1.4%, quite similar to our results [6,7,8].

We consider that lingual flap retraction could not be considered as a major factor to reduce incidence of sensory distrubances.

Of course, the technique used seems to be an important factor, but depends of technical skills [9].

All odontectomies included in our work were performed on local anaesthesia.

Rood reported a higher incidence of sensory disturbances when wisdom tooth removal was performed under general anaesthesia [10].

A larger study, however, comprising a larger number of cases, would be necessary to unequivocally prove that lingual nerve protection by retracting a lingual reduced sensory tongue deficit.

CONCLUSSIONS

Routine application of an retractor the lingual nerve during odontectomy of lower wisdom tooth has been subject to controversy.

Our results indicate a very low incidence of temporary dysaesthesia after odontectomies.

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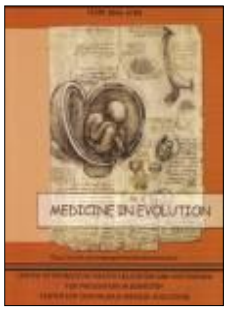
CT-SCAN ASSESMENT AND DETAILED EVALUATION OF ORAL CAVITY AND SUPRAHYOID NECK PRIMARY SQUAMOUS CELL CARCINOMAS WITH LYMPHATIC SPREAD CERVICAL LYMPHADENOPATHIES



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ABSTRACT

The aim of this paper is to point out the value of CT-examination in diagnostic assessment of oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas with lymphatic spread cervical lymphadenopathies

We used a statistic retrospective method to evaluate the results obtained after CT-scan exams of 85 cases of clinically suspected and 19 cases of clinically unknown oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas with lymphatic spread cervical lymphadenopathies clinically, biologically and histopathological assessed at Oral and Maxilo-Facial Surgery Clinic Timisoara.

In all cases the CT examinations detected and clearly delineated the primarily tumoral site and extension toghether with cervical lymph nodes metastases mapping, beeing of great help for TNM classification in order to an adequate treatment application.

In oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas where it is mandatory to detect by imaging methods tumour, lynphatic nodes and metastasis stages, CT - scan are of great importance value in order to offer to the clinicians subtle and no doubt datas for precise diagnostic and clearly cuantificated treatment.

Keywords: Oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas, Cervical lymphadenopathies, CT-scan

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INTRODUCTION

The suprahyoid neck is a very important region which contains the facial fine structures that abut the skull base at the cranial end, the hyoid bone caudally and the oral cavity anteriorly with complex interaction and a huge variety of pathology, very difficult to detect, evaluate and characterize due to such complexity encompass in a small area (1).

Examination and diagnosis of oral cavity and suprahyoid neck pathology by radiological and imaging modalities is the immediate of choice algorithm after clinical evaluation. Not only with the teeth and their surrounding bone the oral radiology concern, but also with the entire oral and maxillofacial complex and associated structures of the head and neck (2, 5).

Among the imaging techniques of dental radiology the most common are panoramic and periapical radiographies together with the classical projections used in the diagnosis of maxilla and mandible pathology (3, 4).

Due to the ability of assessment of both the bone structures and the soft tissue with additional possibility of high-quality multiplanar reconstructions CT exam has proved to be an excellent procedure for characterizing the anatomy and related abnormalities of all oral and neck regions (4, 5).

The purpose of this study is to point out the value of CT exam in the diagnosis algorithms of oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas with lymphatic spread cervical lymphadenopathies

From the malignant morbid entities, oral cavity suprahyoid neck Squamous Cell Carcinoma has by far the most frequent tumoural impact on dento-maxillary structures, in detailed detected by CT-scan due to specific abilities in bone tissue evaluation. In fact, in case of oral cavity squamous cell carcinoma CT exam has an extended value in cervical lymphadenopathies detection which is mandatory, because their presences affect the treatement and reduce the 5-year survival following the rule of 50%. In a case of oral cavity squamous cell carcinoma the presence of a single cervical lymphadenopathy reduces the 5-year survival by 50%. If cervical lymphadenopathies are bilateral, 5-year survival is reduced by another 50%. If there is clinically determined cervical lymphadenopathy fixation or imaging data of extranodal extension expected survival goes down yet another 50% (1, 2)

Due to fine abilities in calcification detection and low density characterization of necrosis or cystic degeneration, CT-scan has proved to be the method of choice in daily practice for diagnosing, evaluation and staging of cervical lymphadenopathies.

AIM AND OBJECTIVES

The aim of this paperwork is to underlie the value of CT-scan in detect and characterize oral cavity and suprahyoid neck Squamous Cell Carcinoma toghether with regional cervical lymph nodes metastases and to reveal the ability of this method, in daily practice, for diagnosing, evaluation and staging in order to a precise and adequate treatment assessment.

MATERIALS AND METHODS

We retrospectively reviewed the CT imaging studies of 85 patients with surgical biopsy proved cervical lymph nodes metastases from regional oral cavity and suprahyoid neck Squamous Cell Carcinoma, clinically, biologically and histopathologically assessed.

The CT-scan examinations were performed in order to evaluate/detect and evaluate the presence and extension of Primary Squamous Cell Carcinoma toghether with cervical lymph nodes metastases mapping. The technique of computed tomography scans used a dedicated contigous thin slices (1-3 mm) protocol in the axial plane from the base of the skull to the clavicles pre and post non-ionic iodinated contrast i.v. injection for the initial assessment of the lesions, with their examination in soft tissue and bone windows.

In all cases of oral cavity and suprahyoid neck Squamous Cell Carcinomas we used TNM classification in order to precise imaging tumoural, lynphatic nodes and metastasis staging.

Imaging CT criteria for cervical lymphadenopathies diagnostic assessment used were based not only on size but also on internal architecture, any cervical lymph node of normal dimensions but with internal calcification, necrosis or cystic degeneration being considered pathologic until proven otherwise.

The size criteria used depends on cervical lymph nodes location in one of the seven levels that they are anatomically classified. Level I nodes are the submental and submandibular nodes. Level II, III and IV are the nodes in the internal jugular chain, with level II nodes located from the base of the skull to the hyoid bone, level III nodes located between the hyoid bone and cricoid cartilage and level IV nodes located below the cricoid cartilage. Level V nodes are posterior to the sternocleidomastoid muscle. Level VI nodes are in the deep visceral chain and level VII nodes are in the superior mediastinum. Level II-VII nodes are considered pathologic in size if they are greater than 1 cm. Level I and the jugulodigastric nodes are considered pathologic in size if they are greater than 1, 5 cm

RESULTS AND DISCUSSIONS

We evaluated by CT-scan 85 cases of clinically suspected and 19 cases of clinically unknown oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas with lymphatic spread cervical lymphadenopathies clinically, biologically and histopathological assessed at Cranio-Maxilo-Facial Surgery Clinic Timisoara.

In all 85 cases the CT examinations clearly delineated the primarily tumoral site and extension toghether with cervical lymph nodes metastases mapping, beeing of great help for TNM classification in order to an adequate treatment application.

In 42 cases cervical lymphadenopathies were oral cavity Primary Squamous Cell Carcinomas metastasis and in 36 cases the cervical lymph nodes metastsis were due from suprahyoid neck visceral spaces Primary Squamous Cell Carcinomas as followed: 11 cases in oropharynx, 13 cases in nasopharynx, 5 cases in

hypopharynx and 7 cases in larynx. Maxillary sinus was primarily site of Squamous Cell Carcinomas with lymphatic spread cervical lymphadenopathies in 7 cases.

We diagnostic assessed cervical lymphadenopathies according to size and architecture imaging criteria and we found in almost all cases nodal diameters between 1 and 6 cm corresponding to TNM stage N1 and N2.

All cases of cervical lymph nodes with internal calcification, necrosis or cystic degeneration CT detected were histopathologically proved as Squamous Cell Carcinoma metastasis.

Table 1. The oral cavity and suprahyoid neck sites of 85 clinically suspected Primary Squamous Cell Carcinomas associated with cervical lymphadenopathies CT-scan evaluated and TNM classified

Oral cavity and suprahyoid neck sites	Number of cases
- Oral cavity:	42
- floor of the mouth	14
T2N2b	8
T4N2c	6
- retromolar trigone	8
T2N2c	5
T4N2c	3
- lower alveolar ridge	7
T4N2c	7
- upper alveolar ridge	6
T4N2c	6
- hard palate	4
T4N2c	4
- oral tongue	3
T1N2b	2
T2N2b	1
- Oropharynx:	11
- base of tongue	4
T2N2b	2
T4N2b	2
- palatine tonsils	3
T4N2b	3
- posterior wall	2
T4N2c	2
- inferior soft palate	2
T4N2c	2
- Nasopharynx:	13
- lateral wall	7
T4N2c	7
- postero-superior wall	4
T4N2c	4
- anterior wall	2
T4N2c	2
- Hypopharynx:	5
- pyriform sinus	3
T2N2b	3

- posterior pharyngeal wall	2
T3N2c	2
- Larynx:	7
- supraglottis	3
T2N2b	3
- glottis	2
T3N2b	2
- subglottis	2
T2N2b	2
- Maxillary sinus:	7
- antero-inferior	4
T2N2b	4
- supero-posterior	3
T4N2b	3

From the 42 cases of oral cavity Primary Squamous Cell Carcinomas with cervical lymphadenopathies, 14 cases were detected in the floor of the mouth with tumoural size between 2-4 cm in greatest dimension and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension in 8 cases, being classified as T2N2b. In 6 cases we found tumours in the floor of the mouth with more than 4 cm in greatest dimension which invade mandible cortical bone and regional adjacent structures with bilateral cervical lymphadenopathies less than 6 cm in greatest dimension classified as T4N2c.

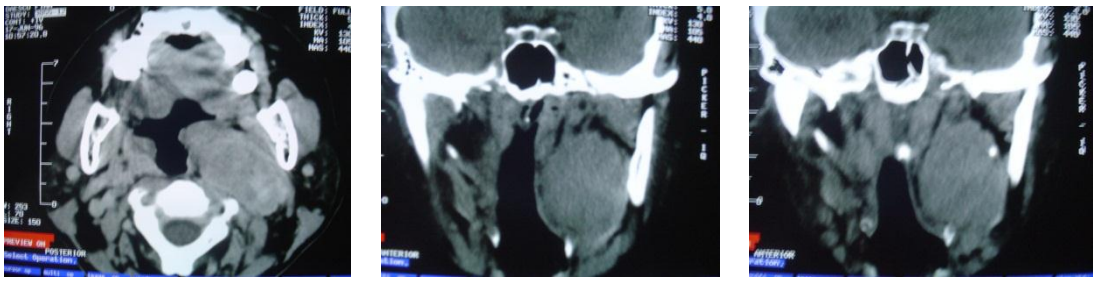

Figure 1. CT axial and coronal scans of a T4N2c floor of the mouth Primary Squamous Cell Carcinomas with bilateral cervical lymphadenopathies.

The retromolar trigone tumoural localization was found in 8 cases, with primarly tumour less than 4 cm in greatest dimension in 5 cases and the size more tnan 4 cm in 3 cases but both with bilateral cervical lymphadenopathies less than 6 cm classified as T2N2c and T4N2c.

From the 7 cases of lower alveolar ridge Primary Squamous Cell Carcinomas, tumoural size were more than 4 cm in greatest dimension with development through mandible cortical bone in regional adjacent structures and skin and bilateral cervical lymphadenopathies less than 6 cm being classified as T4N2c.

In the 6 cases of upper alveolar ridge tumoural size were also more than 4 cm in greatest dimension with hard palate and maxillary sinus invasion in 5 cases and the skin invasion in 1 case together with bilateral cervical lymphadenopathies less than 6 cm in all cases TNM classified as T4N2c.

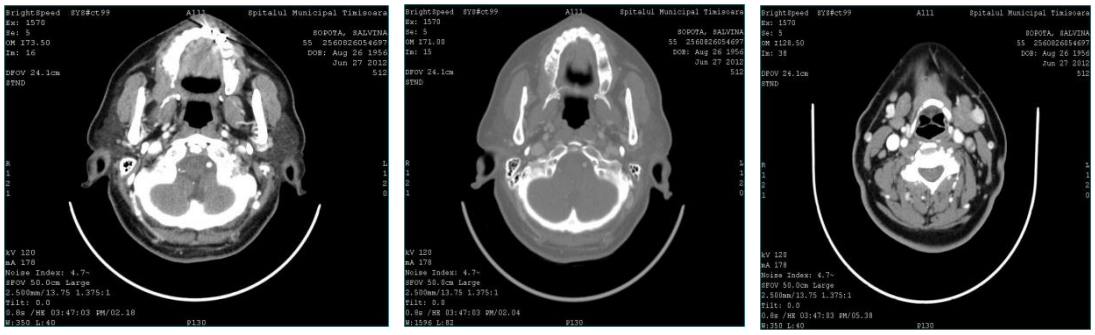

Figure 2. CT axial scans of a T4N2c upper alveolar ridge Primary Squamous Cell Carcinomas with bilateral cervical lymphadenopathies and through cortical bone extension.

Hard palate was detected as the primarly tumoural site in 4 cases with more than 4 cm in greatest dimension and through cortical bone extension in maxillary sinus and middle nasal meatus all with bilateral cervical lymphadenopathies less than 6 cm being classified as T4N2c. In the oral tongue 3 cases of tumoural detection we found masses with less than 2 cm in greatest dimension in 2 cases and in 1 case with less than 4 cm, all with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension TNM classified as T1N2b and T2N2b.

The 11 cases of oropharynx Primary Squamous Cell Carcinomas had base of tongue localization in 4 cases with less than 4 cm in greatest dimension in 2 cases and with the tumoural size more than 4 cm extended to pterygoid muscles and deep muscle of tongue in 2 cases all with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T2N2b and T4N2b.

In the palatine tonsils 3 cases we detected tumours of more than 4 cm in greatest dimension with tonsillar pillars and base of tongue invasion with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension in TNM stage T4N2b.

The 2 cases of oropharynx posterior wall localization of primary tumour we found masses more than 4 cm in greatest dimension with endolumenal protrusion and retropharyngeal space extension together with bilateral cervical lymphadenopathies less than 6 cm in all cases TNM classified as T4N2c.

In 2 cases we found tumours from the inferior soft palate with less than 4 cm in greatest dimension but which invade hard palate cortical bone with bilateral cervical lymphadenopathies less than 6 cm also classified as T4N2c.

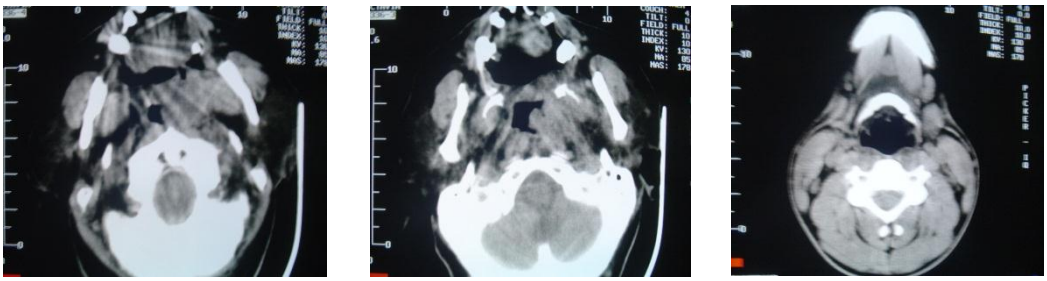

Figure 3. CT axial scans of a T4N2c oropharynx Primary Squamous Cell Carcinomas with bilateral cervical lymphadenopathies, endolumenal protrusion and retropharyngeal space extension.

The nasopharynx localization of Primary Squamous Cell Carcinomas was detected in 13 cases, all with bilateral cervical lymphadenopathies less than 6 cm, TNM classified as T4N2c, with lateral wall as the most frequent affected region, primarily tumour being found in 7 cases with extension beyond the Rosenmüller fossa in ethmoid sinuses and nasal fossa. The postero-superior wall of nasopharynx primarily tumoural site was found in 4 cases all with base of the skull invasion and anterior wall localization in 2 cases with tumoral extension in hard and soft palate.

Figure 4. CT axial and post contrast sagital scans of a T4N2c postero-superior wall nasopharynx Primary Squamous Cell Carcinomas with bilateral cervical lymphadenopathies, and endolumenal protrusion.

In the hypopharynx Primary Squamous Cell Carcinomas was detected in 5 cases with pyriform sinus localization in 3 cases in which tumour size was less than 4 cm in greatest dimension, extended in aryepiglottic and metastasized in ipsilateral cervical lymph nodes wuth less than 6 cm in greatest dimension and classified as T2N2b.

The posterior pharyngeal wall of hypopharynx was involved in 2 cases with retropharyngeal space extension, tumoural size of more than 4cm, together with bilateral cervical lymphadenopathies less than 6 cm, TNM classified as T3N2c.

In supraglottis larynx Primary Squamous Cell Carcinomas we detected 3 cases with involvement of the false cord and the epiglottis with

ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T2N2b.

In glottis larynx we found 2 cases of vocal cord tumours limited to the larynx with fixation and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension classified as T3N2b.

The subglottis larynx was the site of primarily tumour in 2 cases with extension to vocal cords and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension classified as T2N2b.

Maxillary sinus was the site of Primary Squamous Cell Carcinomas in 7 cases, 4 with antero-inferior localization, extended through the cortical bone in regional hard palate and middle nasal meatus with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T2N2b.

Supero-posterior maxillary sinus primarily tumour was detected in 3 cases with invasion through the cortical bone in regional ethmoid and sphenoid sinuses and orbit with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T4N2b.

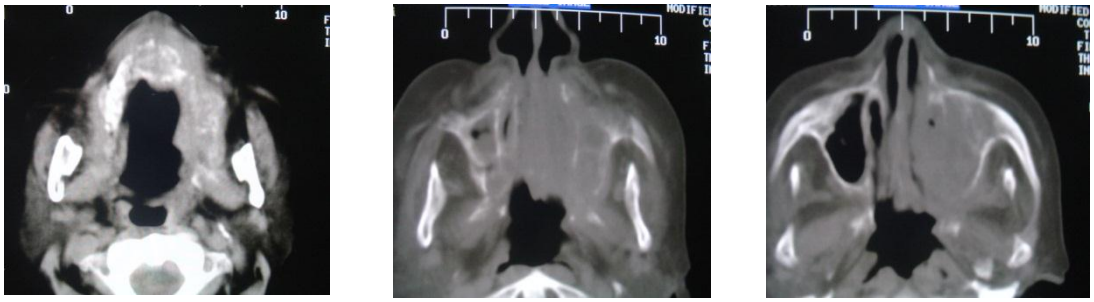

Figure 5. CT axial scans of a T4N2c left maxillary sinus Primary Squamous Cell Carcinomas with bilateral cervical lymphadenopathies, extended through the cortical bone in regional hard palate and middle nasal meatus.

In 19 cases of clinically unknown primary tumoral site but with Squamous Cell Carcinomas cervical lymph nodes biopsy proved metastases CT-scans clearly detected the primarily tumoral localization and helped in extension evaluation according TNM classification offering precise data for treatment and in time evolution appreciation. The clinically unknown Primary Squamous Cell Carcinomas associated with biopsy proved cervical lymphadenopathies were CT detected in submucosal spaces of oral cavity in 4 cases, in palatine tonsils and inferior soft palate of oropharynx in 3 cases, in nasopharynx walls in 6 cases, in pyriform sinus and posterior pharyngeal wall of hypopharynx in 3 cases, in subglottis larynx in 1 case and in 2 cases of maxillary sinus localisation.

Table 2. CT detected sites and TNM classification of 19 clinically unknown primary Squamous Cell Carcinomas associated with biopsy proved cervical lymphadenopathies

CT detected sites	Number of cases
- Oral cavity:	4
- retromolar trigone	2
T4N2b	2
- upper alveolar ridge	1
T4N2b	1
- hard palate	1
T4N2b	1
- Oropharynx:	3
- palatine tonsils	2
T1N2b	2
- inferior soft palate	1
T1N2b	1
T1N2b	
- Nasopharynx:	6
- lateral wall	4
T4N2b	4
- postero-superior wall	1
T4N2b	1
- anterior wall	1
T4N2b	1
- Hypopharynx:	3
- pyriform sinus	2
T2N2b	2
- posterior wall	1
T2N2c	1
- Larynx:	1
- subglottis	1
T2N2b	1
- Maxillary sinus:	2
- antero-inferior	1
T2N2b	1
- supero-posterior	1
T4N2b	1

In the 4 cases of clinically unknown oral cavity submucosal spaces Primary Squamous Cell Carcinomas, CT-scan detected tumoural masses less than 2 cm in greatest dimension, with adjacent cortical bone erosion and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension, located in retromolar trigone in 2 cases, in upper alveolar ridge in 1 case and in hard palate in another one, TNM classified as T4N2b.

The 3 cases of primarily oropharynx unknown tumours were CT detected as small inhomogeneous nodules, less than 2 cm in greatest dimension, 2 in palatine tonsils and 1 in inferior soft palate, with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension, TNM classified as T1N2b.

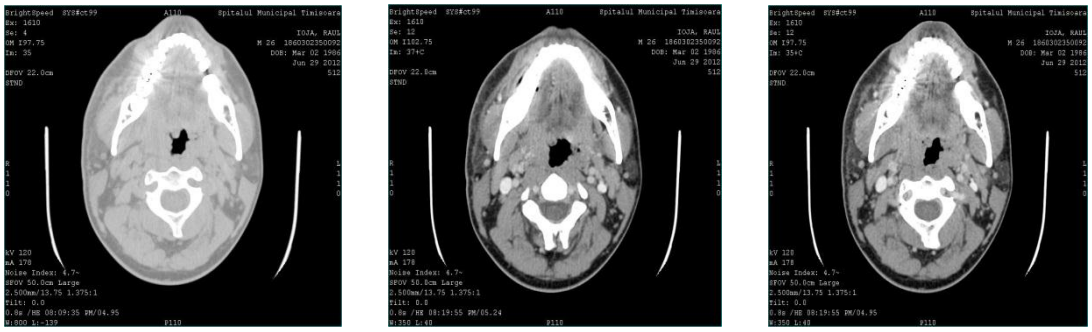

Figure 6. Axial scans of a T1N2b clinically unknown right palatine tonsil Primary Squamous Cell Carcinomas with ipsilateral biopsy proved cervical lymphadenopathies CT detected.

The nasopharynx walls were the most frequent unknown Primary Squamous Cell Carcinomas localisation, primarily tumour being CT detected in 6 cases with extension beyond the lateral wall in ethmoid sinuses and nasal fossa in 4 cases, in postero-superior wall with base of the skull invasion in 1 case and in anterior wall with tumoral extension in hard and soft palate in 1 case, all with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T4N2b.

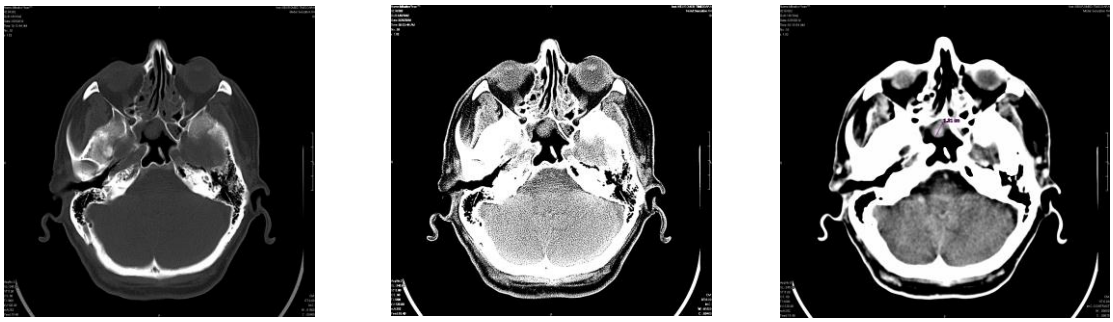

Figure 7. Axial scans of a T4N2b clinically unknown nasopharynx anterior wall Primary Squamous Cell Carcinomas with ipsilateral biopsy proved cervical lymphadenopathies CT detected.

In of hypopharynx CT-scan detected unknown tumoural masses less than 4 cm in greatest dimension in 3 cases: 2 cases in pyriform sinus with aryepiglottic fold involvement and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension, classified as T2N2b and 1 case in posterior pharyngeal wall of hypopharynx with bilateral cervical lymphadenopathies less than 6 cm, TNM classified as T2N2c.

CT-scan also detected in 1 case an unknown tumoural mass in subglottis larynx with ipsilateral vocal cord extension and ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension classified as T2N2b.

In maxillary sinuses CT detected 2 unknown primarily tumours: in 1 case the mass was located antero-inferiorly and caused bone erosion with extension in hard

palate and middle-nasal meatus toghether with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension being classified as T2N2b and in another case the tumour was found supero-posterior with invasion through the cortical bone in regional ethmoid and sphenoid sinuses with ipsilateral cervical lymphadenopathies less than 6 cm in greatest dimension and classified as T4N2b.

CONCLUSIONS

The CT - exam offer a detailed evaluation of the hard and soft tissues of the entire oral cavity and suprahyoid neck being the elected method in accurate assessment of the patient's dento-maxillary region together with the accompanying vital structures: the floor of the sinus, the floor of the nose, the roof of the mandibular canal and the position of the mental neurovascular bundle.

In oral cavity and suprahyoid neck Primary Squamous Cell Carcinomas where it is mandatory to detect by imaging methods tumoural, lynphatic nodes and metastasis stages, CT - scan are of great importance value in order to offer to the clinicians subtle and no doubt datas for precise diagnostic and clearly cuantificated treatment.

CT examination of oral cavity and neck can accurately detect and characterize cervical lymphadenopathies by fine identification of morphologic and volume changes in
local lymph nodes as a common result of any related anatomic pathologies.

The size and architecture criteria for cervical lymphadenopathies imaging diagnostic assessment are the best used by CT - scan to detect, stage and topographic localize their pathological involvement together with the presence of internal calcification cystic necrosis or extra capsular spread.

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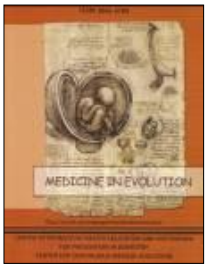
IMPORTANCE OF PROPER PREPARATION OF PRGF PRODUCTS IN MAXILLOFACIAL SURGERY



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ABSTRACT

*PRGF (*Plasma Rich in Growth Factors) *is an autologus product used in accelerating tissue repair and regeneration.*

The product is obtained from patient's own blood.

The present study is based on the experience of clinic CMF Timisoara in PRGF preparation, trying to comply preparation protocol, and follows the formation of the fibrin membrane and plasma rich in growth factors.

The article presents the conclusions on the causes of successes and failures in the preparation of PRGF.

***Key words**: PRGF products, preparation, maxillo-facial surgery*

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INTRODUCTION

PRGF (Plasma Rich in Growth Factors) is an autologus product used in accelerating tissue repair and regeneration.

The product is obtained from patient's own blood and based on: a) possibility of obtaining fibrin membrane from plasma; b) possibility of obtaining a high concentration of platelets in a small amount of plasma.

Platelets contain proteins known as growth factors, such as: PDGF platelet derived growth factor; TGF-ß transforming growth factor-ß1; bFGF basic fibroblast growth factor; VEGF vascular endothelial growth factor; EGF epidermal growth factor; IGF-I insulin-like growth factor type I; HGF hepatocyte growth factor, all involved in the process of tissue repair and regeneration.

PRGF technology can be used in diverse medical areas such as Dentistry, Periodontal Surgery, Maxillofacial Surgery, Orthopaedics, Arthroscopic Surgery Sports Medicine, Aesthetic Medicine, Ulcer treatment, Ophthalmology and Peripheral Nerve Repair.

Product advantages are: preparation takes between 15 and 20 min; it can be prepared in an office environment; no antigenic effect; the system and protocol is simple, user-friendly and affordable. In the studied material were not encountered disadvantages, contraindications or adverse reactions.

AIM AND OBJECTIVES

The present study is based on the experience of clinic CMF Timisoara in PRGF preparation, trying to comply preparation protocol, and follows the formation of the fibrin membrane and plasma rich in growth factors. During PRGF preparation, several situations appeared, some of them bringing benefits, others failures. All of them are presented in this study.

MATERIALS AND METHODS

The study was undertaken at the clinic of Oral and Maxillofacial Surgery, Timisoara. 11 patients (4 women and 7 man) between 45 and 70 years old were used in this study. Patient selection was made without considering any general diseases.

Used materials:

- angular centrifuge (fig. 1)
- 5 ml blood collection tubes with sodium citrate 3,8% (fig. 2)
- calcium chloride (PRGF – activator) (fig.3)
- pipette (fig.4)
- tubes (fig.5)
- Petri vessel (fig.6)

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

PRGF was preparated from 5 cmc of blood collected from the peripheral vein, using sodium citrate as an anticoagulant (fig.7).

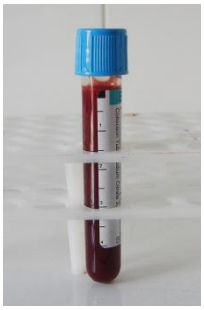


Fig. 7 Colected blood

After a 8 min centrifugation using a angular centrifuge (fig.8),

Fig. 8 Centrifugated blood

the obtained plasma is divided, by carefully pipetting into separate fractions (fig.9):

- F1 fraction: first layer of plasma (1ml).
- F2 fraction: second layer of plasma (≈0,5ml plasma)
- F3 fraction: third layer of plasma (0,5ml), immediately above the red blood cells and white blood cells layers; is rich in growth factors

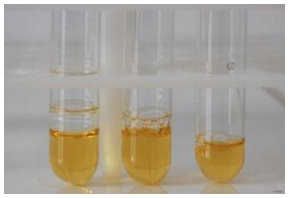


Fig. 9 Plasma fractions F1, F2, F3

The next step was to put the fractions into Petri vessel and apply a small amount of CaCl2 in each of the three fractions, so that plasma activation can take place resulting in a plasma clot.

Trying to follow this algorithm of preparation, several situations occurred; all have been exposed, especially for drawing attention to errors that can occur, and which, may lead to undesirable results.

I Situation:

- the collection tube was refrigerated for 30 min
- centrifugation made after more than 1 hour at 4600rpm
- CaCl2 amount: more than 4 drops in F1 fraction; more than 3 drops in F3 fraction

II Situation**:**

- blood haemolysis in blood collection tube occured
- centrifugation at 4600rpm
- CaCl2 amount: 3 drops in F1 fraction, 2 drops in F3 fraction

III Situation:

- centrifugation made after 30 minutes, at 4600rpm
- CaCl2 amount: 2 drops in F1 fraction, 1 drop in F3 fraction
- the activation took place in Petri vessel

IV Situation:

- centrifugation made after 2 hours 20 minutes, at 4600rpm
- CaCl2 amount: 3 drops in F1 fraction, 1 drop in F3 fraction

V Situation:

- centrifugation made at 2750rpm
- CaCl2 amount: 1 drop in F1 fraction, 1 drop in F3 fraction

VI Situation:

- centrifugation at 2750rpm
- CaCl2 amount: 2 drops in F1 fraction, 2 drops in F3 fraction
- the activation took place in cryovials

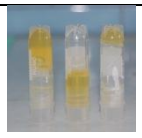
VII Situation:

- centrifugation at 2750rpm
- CaCl2 amount: 1 drop in F1 fraction, 1 drop in F3 fraction
- the activation took place in cryovials which were slightly warmed

RESULTS

After repeated attempts to obtain PRGF, facing several unexpected situations, consulting the producer related to the preparation of the product, the

results were becoming more promising. Following the formation of the fibrin membrane and PRGF, considering the different situation that occurred, the results were different; they are exposed in the following table:

I Situation	- no fibrin membrane obtained - no PRGF obtained	 
II Situation	- red fibrin membrane obtained - no PRGF obtained	
III Situation	- fibrin membrane obtained - PRGF obtained - the fractions were not fully activated so that the fibrin membrane and the PRGF were quantitatively smaller	 
IV Situation	- fibrin membrane obtained - PRGF obtained	 
V Situation	- fibrin membrane obtained - PRGF obtained - activation time around 40min	
VI Situation	- fibrin membrane obtained - PRGF obtained - activation time > 50min	
VII Situation	- fibrin membrane obtained - PRGF obtained - activation time around 15	

DISCUSSIONS

On market more than ten different autologous glues or biomaterials are available. Differentiation of these products is very difficult to appreciate and also very controversial, because all are based on platelet concentrates, and the differences, if there are any, are minimal. Controversial is also the PRGF preparation technique's ; is known that platelets are concentrated, after centrifugation, together with leukocytes into the 'buffy coat' (the layer above red blood cells). Considering PRGF preparation technique, the third fraction (obtained after centrifugation), situated immediately above the 'buffy coat' is considered to be rich in growth factors. More research must be done to clarify the properties of product's based on concentrated platelets.

CONCLUSIONS

After a period of time with more sa mpling, processing, consultation of releva nt literature, as well as consultations with some companies in the field, results began to appear, so that we could draw some conclusions on the causes of successes and failures in the preparation of PRGF.

We encountered situations that should be avoided in PRGF preparation technique, such as:

- refrigeration of harvested blood
- hemolysis of harvested blood
-centrifugation at
more than 1h since harvesting
- high speed centrifugation

A slight warming of blood collection tube or of separated fractions can facilitate obtaining PRGF.

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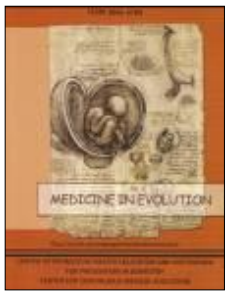
ASPECTS REGARDING ORAL HEALTH AND ORAL HYGIENE HABITS IN HEMODIALYSIS PATIENTS



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ABSTRACT

Aim and objectives: To assess oral health and the attitude and behavior regarding oral health in hemodialysis patients.

Materials and Methods: A cross-sectional study was conducted on 204 hemodialysis patients, in Bucharest. They answered questions regarding demographical data and their attitude and behavior regarding oral health. The data was analyzed using SPSS (16.0)

Results: The mean age of the group is 59.3 years. The mean value of the DMFT index is 17 and 14.2% are edentulous or have few teeth. Moderate or severe periodontal disease is found in 75% of the patients. Only 28.9% brush twice a day. The need of dental treatment is perceived by 64.7% of the subjects and 68.6% haven't visited a dentist in more than 2 years.

Conclusions: The prevalence of periodontal disease is high within the studied group. The attitude and behavior regarding oral health and visits to the dentist requires improvement.

Key-words: hemodialysis, oral health, oral hygiene, periodontal disease

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INTRODUCTION

During the last 15 years, an increasing number of studies have revealed the serious impact of malnutrition and inflammation on the outcome of patients with kidney failure undergoing chronic hemodialysis (HD) [1, 2]. In the last decade, several studies have assessed the oral health of chronic kidney disease (CKD) and hemodialysis (HD) patients. Those studies reported higher prevalence of poor oral health [3] and the impact that oral infections, especially periodontal disease, have on malnutrition and general inflammation [4-6]. Periodontal disease is more prevalent among CKD and HD patients and has been found as an important source of inflammation in these subjects. Moreover, the severity of the periodontal disease seems to be associated with malnutrition and inflammation [4, 7, 8].

Periodontal disease can be prevented through education regarding oral hygiene habits, healthy diet and no smoking. Its progression can be slowed or ceased through a correct dental treatment and regular follow-ups and by providing a good oral hygiene education to the patient [9].

AIM AND OBJECTIVES

We aimed to assess the oral health status, oral hygiene habits and attitude and behavior towards oral health and dentistry services of patients undergoing haemodialysis.

MATERIALS AND METHODS

A cross-sectional study on hemodialysis patients from two dialysis centers in Bucharest – Fresenius Nephrocare Dialysis Center and IHS sf. Pantelimon Dialysis Center – was performed in 2011 and 2012.

Data gathering was done by a single person, through an interview and a dental examination, during the dialysis session.

Demographical data, educational level, oral hygiene and smoking habits and visits to the dental office data were obtained through an interview of the patient. The clinical data revealed by dental examination regarded: the DMFT index, the plaque index according to Silness and Loe, the total number of functional teeth, the clinical attachment level (CAL), the periodontal pocket depth (PD), the type of existing prosthetic treatment and the need of prosthetic treatment. Periodontal measurements were done using the probe recommended by the World Health Organisation (WHO).

Statistical analysis was performed using the 16.0 version of the SPSS.

RESULTS

The total number of examined patients is 204 of which 55,9% (114) are males. The mean age of the group is 59.3 (SD 12.5). Among the subjects, 81.4% (166) don't smoke, 22.1% (45) have higher education and 48% (98) have secondary education and 81.9% (167) live in an urban environment.

The mean of the Silness-Loe Plaque Index (PI) is 1.4 (SD 0.6) for the entire group, with a higher mean value for men 1.5 (SD 0.7). The PI values are higher than 1.91, which is indicative for an unsatisfactory level of oral hygiene, for 20.1% (41) of the patients.

The DMFT index has a mean value of 17 (SD 8.3), with a mean value of 1.6 (SD 2.2) for the decayed teeth, 3.3 (SD 4.4) for the filled teeth and 12 (SD 8.8) for the missing teeth.

In regard to the mean of the number of functional teeth inside the group is 16.6 (SD 9.5), with a higher value for males – 17.3 (DS 9.6)- than for females. (Fig. 1)

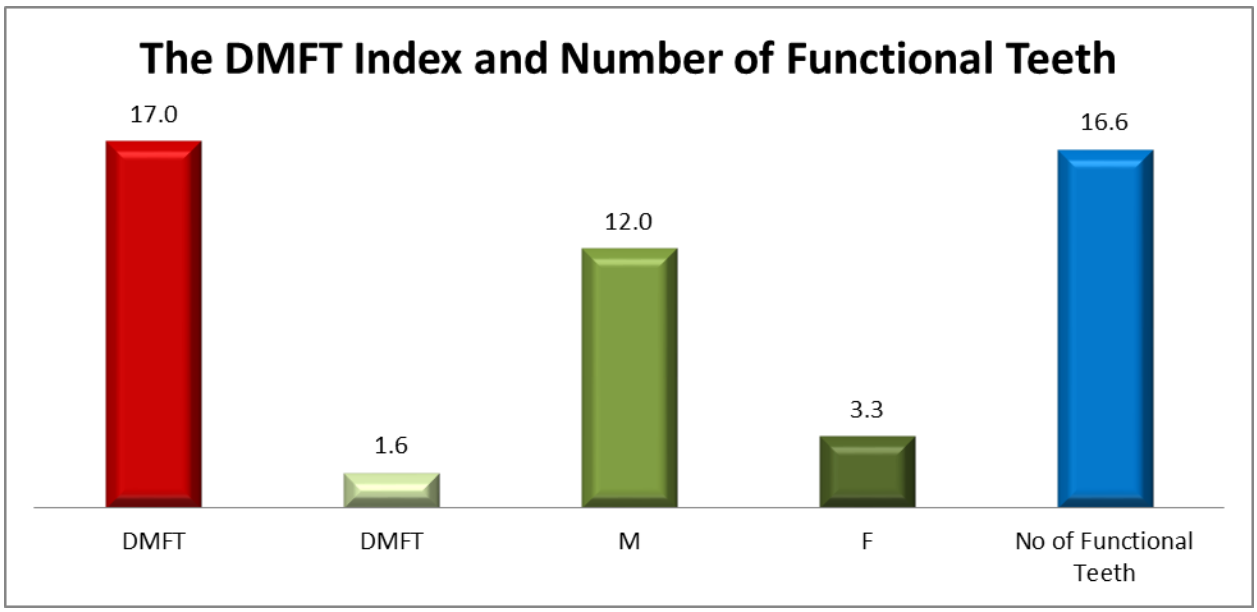

Figure 1 Mean values for the DMFT index and number of remaining Functional Teeth

The percentage of subjects, who are either edentulous or have few remaining teeth is 14.2% (29). We evaluated the periodontal status for 175 patients and we discovered that 28.9% (59) have a severe form of periodontal disease and 46.1% (94) a moderate one (Fig. 2). The evaluation was made using both CAL and PD values, taking into consideration the suggestion of the Center for Disease Control and Prevention (CDC) and of the American Academy of Periodontology from 2003 [7]. In this evaluation system the diagnose for severe periodontitis is given by the finding of 2 or more proximal areas with CAL ≥ 6 mm and one or more proximal areas with PD ≥ 5 mm, on different teeth; moderate periodontitis means a CAL ≥ 4 mm or two or more proximal areas with PD ≥ 5 mm, on different teeth.

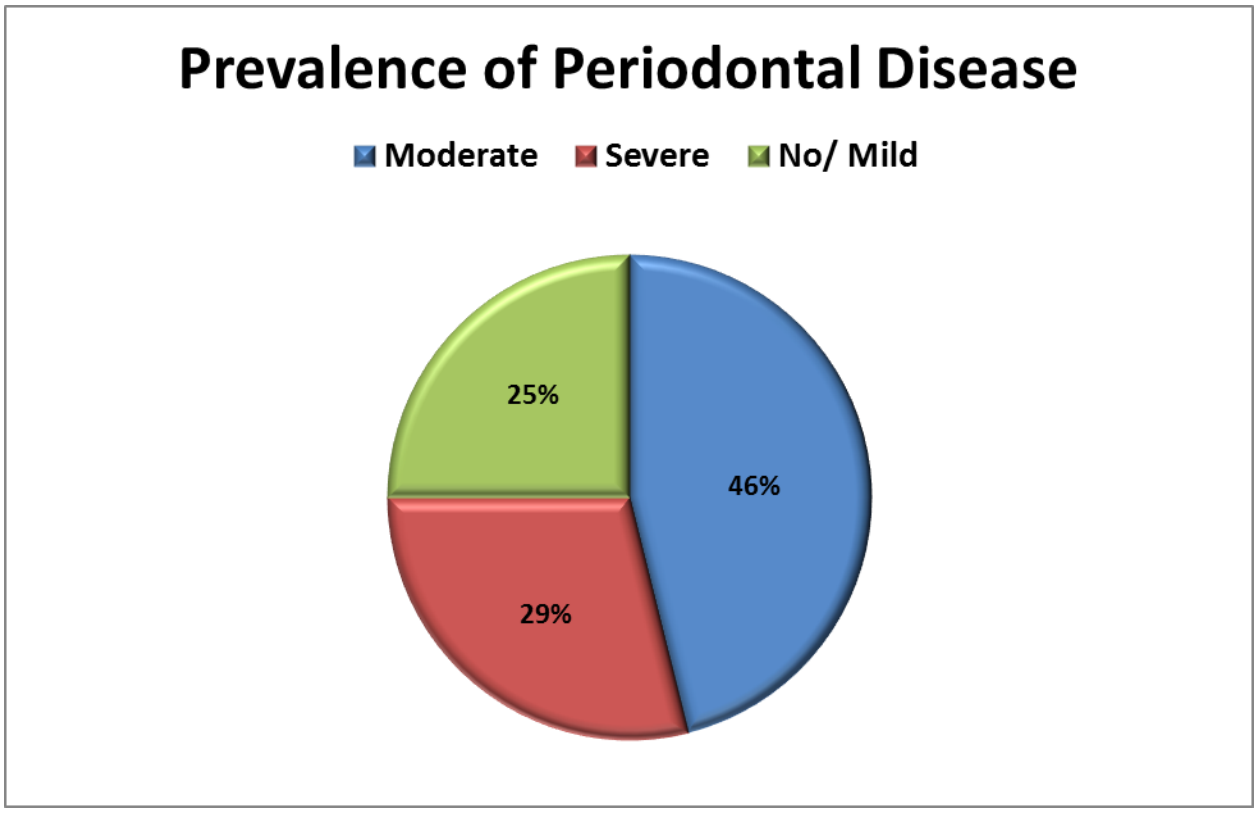

Figure 2 Prevalence of periodontal disease among the hemodialysis patients in the group

Mean values of CAL index and PD were, respectively 3.7 (SD 1.6) and 2.8 (SD 0.9), for the study group.

Regarding the prosthetic treatment need, 49.5% (101) of the patients needed fixed prosthodontic treatment or a combination between fixed and removable dentures and 15.7 % (32) needed total removable dentures.

When asked about dental hygiene habits, 28.9% (59) answered that they brush their teeth twice ore more every day and 52.9 % (108) just once per day (Fig. 3). The others are performing oral hygiene occasionally or have never brushed their teeth. Mouth wash is used only by 22.5% (46) of subjects.

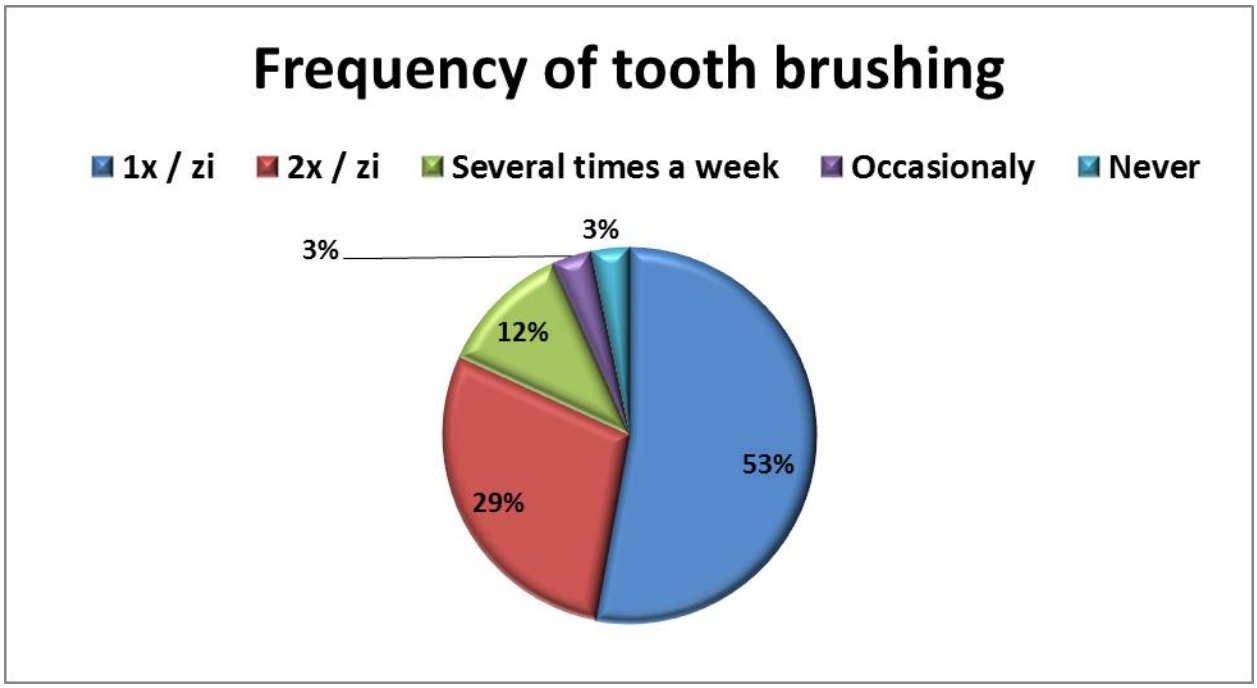

Figure 3 Frequency of tooth brushing within the hemodialysis group.

Interviewed regarding their perception of their own oral health, 34.8 % (71) from the subjects considered that it is bad, 29.9% (61) satisfactory, 30.4% (62) good and 4.9% (10) very good.

A percentage of 64.7% (132) subjects considered they needed dental treatment at the moment of the interview.

Nevertheless, 68.6% (140) haven't been to the dentist for over two years and only 6.9 % (14) have visited a dental practice in the last 6 month (Fig. 4).

Asked about the moment they seek dental treatment, 73.5 % (150) answered that they go only if the dental problem doesn't go away on its own or following drug therapy and only 12.3 % (25) attend dental services on a regular basis.

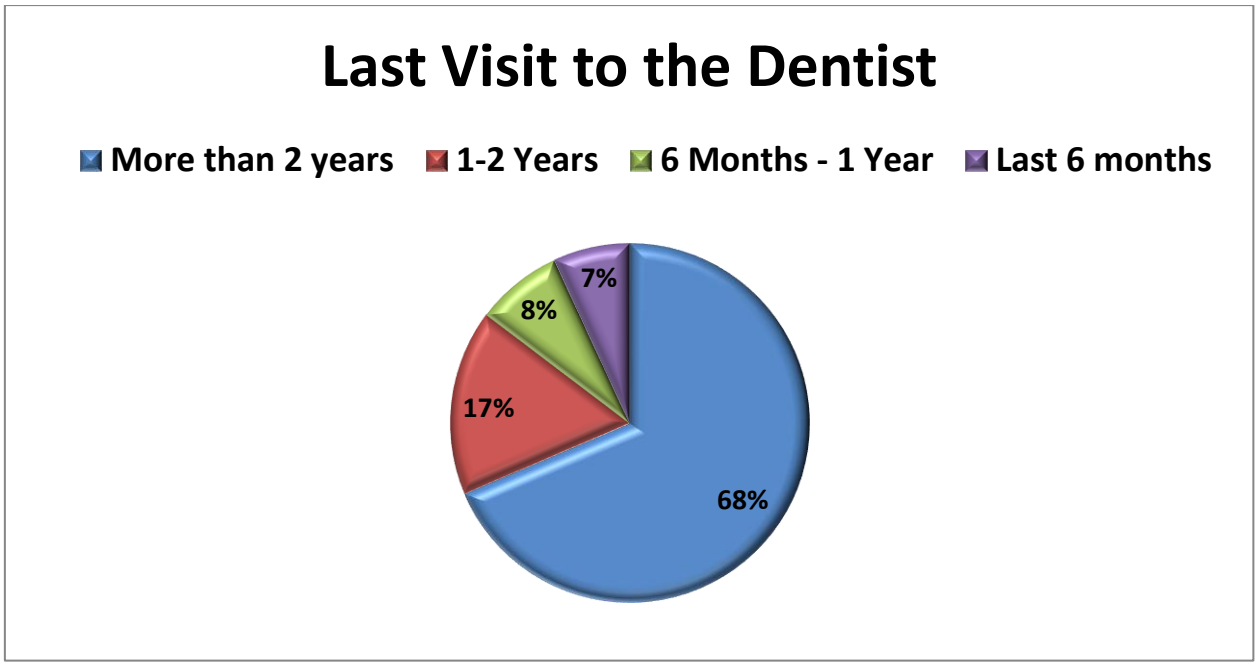

Figure 4 Attendance of dental services: time since the last visit to the dentist

Among the dialysis patients, 11.8% (24) went to dental offices, but they were refused because of their pathology and the hemodialysis treatment.

DISCUSSIONS

Our present research revealed a mean value for the DMFT of 17 (SD 8.3) which is greater than the 11.3 (SD 8.4) obtained by Chamani et al [10] on a Persian population. It is also greater than the DMFT mean reported by Bayraktan et al [8] of 11.8 (SD 7.6) on a Turkish population.

The mean value for the decayed teeth is similar to the one obtained in the Bayrakthan et al study [8], but the mean values for the missing and filled teeth are smaller, 9 (SD 7.2) and 1 (SD 2), respectively. This may be evidence of a lesser prevalence of oral diseases that can lead to tooth loss, but also of a decreased use of dental services among those populations.

The percentage of subjects in our study, who are either edentulous or have few remaining teeth, 14.2%, is similar to the one reported by Gurkan et al [11], but smaller than the 18% revealed by Borawsky et al [7] among Polish dialysis patients.

In the present study, moderate or severe periodontal disease can be found 75% of the subjects. The percentage of 28.9% patients with severe periodontal disease is higher than the one reported by WHO in April 2012 [12] of 15-20% worldwide. It is also higher than the one obtained by Chen et al [4], of 20.2%, in Taiwan.

Mean values of CAL and PD revealed in our study are higher than the ones observed in the Chamani et al study [11] – 3.2±2.2 and 2.2±1, respectively.

Oral hygiene knowledge and behavior are insufficient as only 28.9% of the patients brush their teeth or dentures twice a day and 18.1% of them brush occasionally or have never brushed their teeth.

Oral hygiene habits have also been evaluated by interview in the Gurkan et al [11] study and have revealed the following: 14.3% subjects brushed twice daily, 29.3% once daily, 39.7% occasionally and 16.7 % have never brushed their teeth. It seems that, at least according to what patients declared, the present study population has better oral hygiene habits than the one in the above mentioned study.

Attendance of dental services is also low, as almost 70% of our studied population hasn't been in a dental practice for over two years and 22.1% have never been to the dentist. The lack of correct attitude towards oral health is even more serious as it doesn't correlate with the lack of knowledge regarding dental treatment need perceived by the patients. The majority of patients, 64.7%, acknowledge their need of dental treatment and this is in accordance with the observed clinical data.

Incorrect attitude towards oral health and prevention of oral diseases may be partly due to their complicated comorbidities and their busy dialysis schedule (three times a week), that backgrounds all other chronic illnesses. Another probable cause, which is characteristic of the entire Romanian population, is the lack of general public education regarding hygiene habits and prevention of diseases.

Interviewed regarding their visits to the dentist, almost 12% reported being refused dental treatment or referred by some dentists because of their general disease and dialysis treatment. This may also contribute to their low attendance of dental services. Similarly, none of the interviewed patients have been referred for dental examination and treatment, by the nephrologists, upon the diagnosing of the CKD or the beginning of the dialysis sessions.

A lack of knowledge regarding dental treatment of CKD and dialysis patients seems to become evident among dentists. This can be a drawback for these patients and their oral health, especially as dental treatment for this group of patients can be safely performed by a dentist, in a regular dental practice, provided some simple precautions are taken and that there is a good interdisciplinary communication between the latter and the nephrologist.

Also, the nephrologist, being aware of the disturbances caused by chronic renal failure through the entire body, oral cavity included, should refer their patients to a dentist for oral prophylaxis, dental treatment and oral hygiene education.

CONCLUSIONS

The prevalence of moderate and severe periodontal disease is high among the studied hemodialysis group.

The results of this study suggest the need to further improve oral hygiene habits, attitude and knowledge regarding oral health maintenance and attendance of dental services among dialysis patients.

There is a need to better the knowledge and attitude of dentists regarding dental treatment of CKD and dialysis patients.

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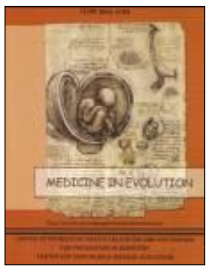
ORTHODONTIC TREATMENT OF ANTERIOR CROWDING WITH A COMPLETELY CUSTOMIZED LINGUAL APPLIANCE



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ABSTRACT

There is a big demand for invisable treatment appliances in orthodontic treatment all over world. Most adults prefer treatment with invisible or nearly invisible appliances because they want to achive a treatment objective without aesthetic impairement. Ceramic brackets are an alternative to metal brackets, but they are still visable.

Aligners need to be worn 24 hours by our patients and are not invisible because they cover the labial surface of teeth. Lingual braces are completely invisible from the outside. There are different lingual brackets on the market and in contrast to labial brackets, lingual brackets always require a lab side positioning procedure. Bonding is usually indirect with an individual tray. Due to the heterogenic morphology of the lingual tooth surfaces an individual bracket base is required in order to achieve a prefect fit of the bracket.

As a solution individual brackets are the best option because they are custom made for every patient and tooth. The same applies to the arch wires. Individual arch wires compensate the different teeth thicknesses with first order bends. As a result the combination of customized brackets and individual arch wires leads to a flat appliance with high patient comfort. It seems that the Incognito lingual appliance(3M – Bad Essen / Germany) meets this requirements.

This article presents two adult patients with anterior crowding treated with the Incognito lingual appliance(3M – Bad Essen / Germany).

Key words : Incognito lingual appliance, anterior crowding, invisible orthodontic treatment.

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INTRODUCTION

There is a big demand for invisable treatment appliances in orthodontic treatment all over world.

The Incognito lingual appliance is a completely customized system , that can be used for young and adult pacients. Most non-extraction patients have some degree of crowding in one or both arches, so a key component of non-extraction treatment is resolving crowding. As a solution for crowding we have interproximal reduction, expansion ar a combination of the two.

Most non-extraction patients can be treated with fewer wires using the Incognito System.

MATERIAL AND METHOD

Two cases with one with minor anterior crowding and the other with severe crowding shall demonstrate treatment steps in order to align the teeth. The second case shows a situation where one tooth could not be bonded initially due to severe crowding. After space opening the bracket could be bonded in a direct procedure. The impressions were send to 3M Bad Essen / Germany where the Incognito appliance was produced.

Fig. 1 Patient 1 Pre-Treatment *Patient 2 Pre-Treatment*

RESULTS

After bonding and arch wire insertion the patients were checked every 4-5 weeks in the practice. In the first case all brackets could be bonded initially.

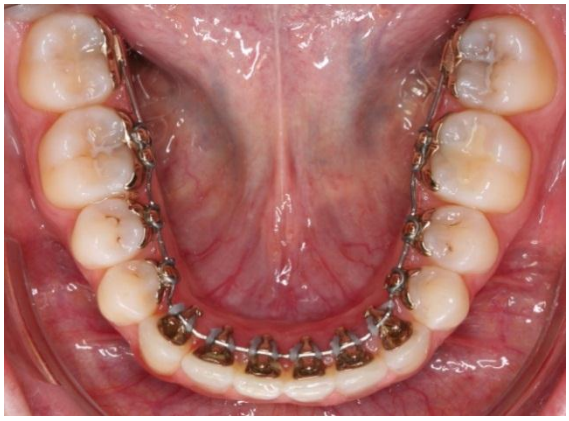
The arch wire could be inserted in the self retaining slot from 3-3 before the rectangular wire was inserted with elastics and ligatures in the vertical slot (3-3) and horizontal in the lateral.

In the second case the lower right canine could only be bonded after space opening with compressed archwire in the front.

A crimpable stop was placed mesial to the left lower canine in order to gain additional arch wire length in the front. After placing the bracket on the lower right canine leveling and aligning was easy.

Both cases were finished after 9 month of treatment time. Retainers were placed from 3-3 after debonding.

A B



C

Fig. 2 Patient 1 A: The first archwire was inserted into the self-ligating clips. B. The 016x0.22NiTiarch archwire . C. 0.0182x0.0182 Beta III Titanium finishing archwire.

A B

C **D**

Fig. 3 Patient 2 A. Compressed archwire. B.Space opening. C. Bonding 43. D. 0.0182x0.0182 Beta III Titanium archwire.

DISCUSSION

The Incognito lingual appliance is a fully customized orthodontic device. The brackets and wires are all produced individually.

On the bases of the orthodontic treatment plan an individual set up is produced for every patient which is the fundament for the lab side production of the individual brackets and wires. The archwires are produced in different dimensions and quality. The quality are superelastic NiTi, stainless steel and beta III Titanium.

All arch wires are only programmed with first order bends. The slot dimensions of the brackets is 0.018 x 0.025 inch. The archwires are ribbonwise. The insertion of the wires is vertical in the front from canine to canine and horizontal in the lateral. Different individual features can be ordered by the doctor such as tubes with hooks and half of an occlusal pad. To produce such an individual and precise appliance makes it necessary to take impressions with silicon. An indirect procedure in order to position brackets is essential.

CONCLUSIONS

Lingual treatment is a key orthodontic technic that provides invisible high end treatment.

Due to the different teeth morphologies customized appliances with individual archwires support the orthodontist to achieve the desired result. Bonding , rebonding and debonding are comparable to labial treatment.

Aligning frontal crowdings even in cases when one bracket could not be bonded initially is easy and fast .

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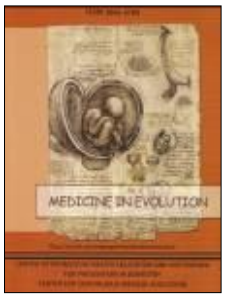
RESEARCHES UPON INDIGENOUS HERBAL PRODUCTS FOR THERAPEUTIC VALORIFICATION IN METABOLIC DISEASES NOTE II. POLYPHENOLS' ANALYSIS OF BETULAE FOLIUM



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ABSTRACT

Aim and objectives: *verification of the herbal product's identity (Betula pendula Roth. leaves) and determination of optimum time for harvesting the leaves, in order to obtain pharmacological active extracts with the highest content of polyphenols (flavonoids, tannins, proanthocyanidins, phenolcarboxylic acids = PCA).*

Material and methods: *silver birch leaves harvested in: May (batch M_1), June (batch M_2), July (batch M_3), end of August (batch M_4), scanning electron microscopy (SEM), qualitative (chemical analysis, thin layer chromatography=CSS) and quantitative (high liquid chromatography = HPLC, spectrophotometric) methods.*

Results: *morphological and anatomical characteristics correspond to those mentioned in literature. The highest content of: total polyphenols (8.1791±0.2249g% tannic acid), flavonoids (2.8006±0.0488g% hyperoside), PCA (2.9369±0.0905g% chlorogenic acid), proanthocyanidins (0.5814±0.0325g% cyanidin chloride), tannins (2.8176±0.3433g% tannic acid) was found for batch M_1. Using CSS, hyperoside and quercitrin were identified in all batches; their content (0,8%; 0,24%) was determined (HPLC) for batch M_1.*

Conclusion: *young leaves are rich in polyphenols and will be used for future valorification.*

Keywords*: Betulae folium, HPLC, polyphenols*

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INTRODUCTION

Silver birch (*Betula pendula* Roth.) leaves are used as a folk medicine to treat urinary diseases, rheumatic complaints and gout.

According to scientific literature birch leaves contain: flavonoids (hyperoside = 0.8%, quercitrin = 0.14%, avicularin = 0.3-0.6%, rutin = 0.24%, myricetin-3-O-galactoside); phenolcarboxylic acids (chlorogenic, caffeic, ferulic, *p*-coumaric = PCA); gallotannins = 1.07-9%; triterpenic saponosides = 3%; proanthocyanidins; macroelements (calcium, potassium); microelements (selenium vanadium, manganese, zinc), voltatile oil = 0.11% [1]. European Pharmacopoeia 7th edition (Eph 7) foresees a concentration of minimum 1.5% flavonoids, expressed as hyperoside [2].

Birch leaves are not mentioned by scientific literature for hypoglycaemic and hypolipidaemic properties and the benefits in gout's treatment aren't supported by scientific data.

The herbal product might be used as an adjuvant for diabetes, dyslipidaemia and hyperurichaemia treatment, since flavonoids, tannins and proanthocyanidins are known for insulin-like effects, antioxidant properties and aldose reductase inhibition. Caffeic, *p*-coumaric and chlorogenic acids are HMG-CoA reductase inhibitors and myricetin is an inhibitor of xanthinoxidase's activity [1].

AIM AND OBJECTIVES

The objectives of the study were the verification of the herbal product's identity and the determination of the optimum time for harvesting the leaves, in order to obtain extracts with the highest content of polyphenols (flavonoids, PCA, tannins, proanthocyanidins), that might be used as adjuvants in some metabolic disorders' treatment (diabetes, dyslipidaemia, hyperuricaemia).

MATERIAL AND METHODS

Birch leaves were harvested in 2011, from Morăreşti, Argeş District, Romania, in different growing stages: May (**batch M_1**), June (**batch M_2**), July (**batch M_3**), end of August (**batch M_4**).

The herbal products were naturally dried, in shade, and stored in laboratory conditions.

The identity was checked by macroscopic and microscopic (scanning electron microscopy - SEM) exam. For SEM investigations, small leaf pieces were fixed in FEA (formol : 70% ethanol : acetic acid = 5 : 90 : 5 v/v/v) for 48 hours, washed with distilled water and stored in 70% ethanol. After dehydration in a graded ethanol series (80%, 90%,100%) and acetone, the material was critical point dried with CO_2 (EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (EMS 550X Sputter Coater) and examined by scanning electron microscopy - Tescan Vega II SBH (Tescan, USA) at an acceleration voltage of 29,64 kV [3].

For polyphenols' analysis, qualitative (specific chemical reactions on etheric, methanolic and aqueous solutions; thin layer chromatography = TLC) and quantitative (spectrophotometric and high

liquid chromatography = HPLC) methods were applied.

For TLC, the following parameters were used: stationary phase - ready made silicagel GF_{254}, 20 x 20 plates (Merck, Germany) kept 1 hour at 105°C before use; mobile phase - ethyl acetate : methylethylketone : formic acid : water = 50 : 30 : 10: 10 (v/v/v/v); test samples - methanolic solutions; standards - mixture of 1 mg caffeic acid (Fluka, Switzerland), 2.5 mg hyperoside, 1 mg chlorogenic acid , 2.5 mg rutin (Carl Roth, Germany) dissolved in 10 mL methanol and methanolic solution 1mg/mL quercitrin (Carl Roth, Germany); application - bands (10µL), distance path - 10 cm, detection reagent - mixture of methanolic solutions 10g/L of diphenylboryloxyethylendiamine and 50g/L of macrogol 400 (Fluka, Switzerland) = DFBOA/PEG [2]. After spraying with DFBOA/PEG reagent, the plate was examined in UV light (λ = 365 nm), using a Camag Reprostar Lamp with Epson Photo PC 850 (Camag, Germany).

Preparation of samples for TLC: 1 g of powdered herbal product (all batches) was heated under a reflux condenser for 5 minutes with 10 mL methanol. After cooling and filtration (plug of absorbent cotton) the extract was completed up to 10 mL in a volumetric flask (**SM_1, SM_2, SM_3, SM_4**)[2].

Spectrophotometric determinations of total polyphenols, flavonoids, AFC and tannins were carried out using Jasco V-530 spectophotometer (Jasco, Japan).

Determination of total phenolic and tannins' content was performed using the modified Folin Ciocalteu method [4]. Tannins were determined as the difference between total phenolic compounds and non-tannins phenols, based on their property to precipitate with hide powder.

Preparation of samples for analysis of total phenolic compounds: 0,5 g powdered herbal product (all batches) were twice heated with 50 mL of 50% ethanol, under a reflux condenser, for 30 minutes. The extracts were filtered (quantitative paper white band, Carl Roth, Germany) and made up to 100 mL with the same solvent, in a volumetric flask (solution A). 10 mL solution A were diluted with 50% ethanol in a 50 mL volumetric flask (solution B). 0.2-0.6 mL solution B were diluted to 1mL with distilled water, then 1mL Folin Ciocalteu reagent was added and the mixture was completed up to 10 mL (volumetric flask) with Na_2CO_3 200g/L. Blanks were prepared with all reagents and solvents, excluding the extracts. The flasks were left in dark, for 40 minutes and the absorbance was measured at λ = 763 nm. A standard curve was calculated using tannic acid (Riedel de Häein, Germany) concentrations ranging 2.04-9.18 µg/mL ($y = 0.1044x + 0.0257$, $R^2 = 0.9994$, n =8). Results were expressed as g% tannic acid.

Preparation of samples for determination of non-tannins phenols: 5 mL solution B were shaken, for 60 minutes with 0.1000 g hide powder (Sigma-Aldrich, Germania) using a magnetic stirer (Velp Scientifica, Italy). The solution was filterd through qualitative paper (Carl Roth, Germany) and 0.4-0.9 mL were analysed following the same procedure, descriebed above.

Determination of proanthocyanidins'content was performed using buthanol-clorhidric acid assay, based on their conversion to red coloured cyanidins [5].

Preparation of samples: 1g of powdered herbal drug (all batches) were twice heated with 50 mL 70% ethanol under a reflux condense, for 30 minutes. The extracts were filtered through quantitative filter paper and completed up to 100 mL, with the same solvent, in a volumetric flask (solution A). 2-3 mL solution A were treated with 0.2 mL ferrous ammonium sulphate dodecahydrate (Carl Roth, Germany) and 15 mL of buthanol : HCl mixture (95 : 5 v/v) in a 25 mL volumetric flask. Samples were heated at 95°C, for 60 minutes using a water bath (BAD2 Raypa, Spain) and after cooling were completed up to 25 mL

with the same mixture. Absorbance was measured at λ = 550 nm against blank samples, prepared in the same manner, but without heating. A standard curve was calculated using cyanidin chloride (Carl Roth, Germany) concentrations ranging 1.63-9.79 µg/mL ($y = 0.0972x - 0.0154$, $R^2 = 0.9995$, n =11). Results were expressed as g% cyanidin chloride.

Phenolcarboxylic acids' (g% chlorogenic acid) and flavonoids' (g% hyperoside) content was determined according to EPh 7 (*Ash leaf* and *Birch leaf* monographs)[2].

All spectrophotometric determinations were calculated on a dry basis and are presented as means ± standard deviation of nine determinations (Microsoft Excel, 2003).

Flavonoids (the main polyphenols from birch leaves) were also analysed using HPLC. Determinations were carried out using a Varian Star (Varian Inc., SUA) HPLC with UV detector and a silicagel C_{18} 150 x 4.6 nm, 5 µm particle column. The mobile phase was a binary gradient prepared from trifluoracetic acid solution 0.1% (A) and acetonitril; the elution program was: 0 min (80% A , 20% B), 3 min (95% A, 5% B), 7 minutes (50% A, 505 B), 25 minutes (20% A , 80% B). The flow rate was 1 mL/min, the injection volume 20 µL and the working temperature 25°C. The UV detector was set at 270 nm. The following standards were used: hyperoside 0.1 mg/mL , quercitrin 1 mg/mL, quercetol 0.5 mg/mL (methanolic solutions) (LGC Standards, UK). Calibration curves in the 0.1-0.3 mg/mL (hyperoside), 1-3 mg/mL (quercitrin), 0.5-1.5 mg/mL (quercetol) range had good linearity ($R^2 > 0.99$, n = 5).

Sample preparation for HPLC: 5 g of powdered herbal product (batch M_1) was heated with 25 mL methanol under a reflux condenser for 30 min. After cooling, the extract was filtered and completed to 25 mL in a volumetric flask (solution A). 5 mL solution A were diluted with methanol in 25 mL volumetric flask, filtered through a 0.45 µm filter (LGC, UK). (**SMM**)

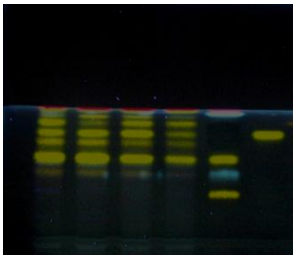
RESULTS

The herbal product's morphological and anatomical characteristics correspond to those mentioned by scientific literature[2]. The microscopic examination (figure1) showed the presence of: glandular and non-glandular trichomes and anomocytic stomata. Using specific chemical reactions, flavonoids, tannins, proanthocyanidins and AFC were identified in all batches. TLC technique revealed the presence of hyperoside ($R_f = 0.67$) and quercitrin ($R_f = 0.93$) in all analysed solutions. Oppsosite to scientific literature, rutin ($R_f = 0.42$) was not identified [2]; one spot, with blue fluorescence, similar to caffeic acid ($R_f = 0.97$) was observed only in SM_1. Chlorogenic

acid ($R_f = 0.67$) was not identified, although a spot, with similar R_f , but with yellow fluorescence, was seen in all samples. By analysing the TLC chromatogram, one can note the presence of other spots, corresponding to flavonoid (yellow fluorescence) or AFC (blue fluorescence) compounds, not identified due to lack of standards.

A **B** **C**

Figure 1. *SEM examination of silver birch leaves: A , B - glandular and non-glandular trichomes, C - anomocytic stomata*



1 2 3 4 5 6

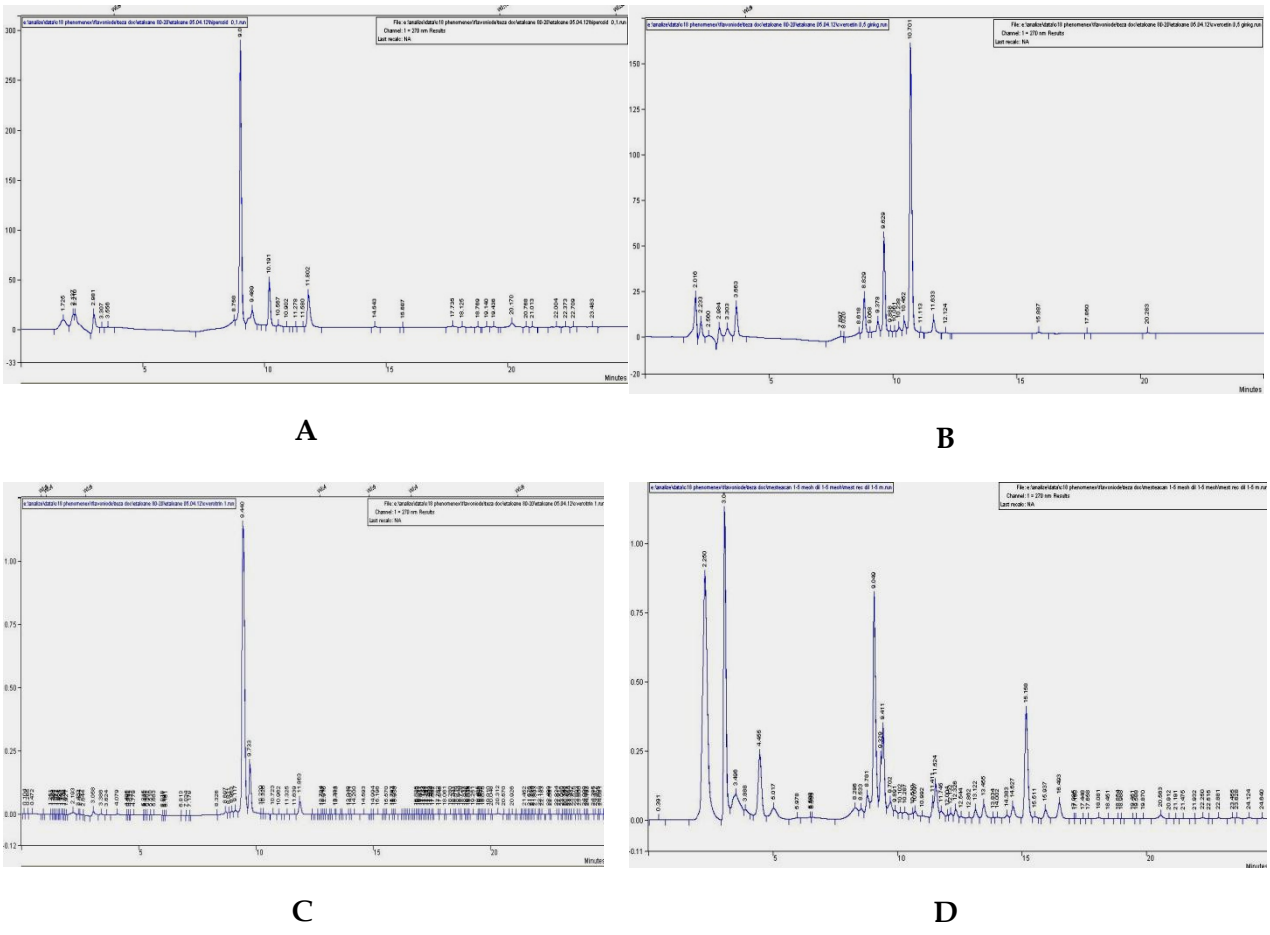
Figure 2. TLC separation and identification of flavonoids and PCA after detection with DFBOA/PEG reagent; UV light (λ = 365 nm)
Legend:
1 – SM_1, 2 - SM_2, 3 – SM_3, 4 –SM_4,
5 – rutin, chlorogenic acid, hyperoside, caffeic acid (from down topwards),
6 - quercitrin

Spectrophotometric determinations showed that **batch M_1** has the highest content of polyphenols (table I), so HPLC analysis upon flavonoids continued on young birch leaves. Hyperoside, quercitrin and quercetol were identified in SMM, by comparison with retention time (minutes) of standards (hyperoside = 9.002, quercitrin = 9.440, quercetol = 10.700 – figure.3); the content (0.86%, 0.24%, 0.11%) was also determined. The HPLC chromatogram for SMM (figure3) revealed the presence of other peaks, that were not identified due to lack of standards.

Table I

Results of spectrophotometric determination

Batch	Total polyphenols (g% tannic acid)	Tannins (g% tannic acid)	PCA (g% chlorogenic acid)	Flavonoids (g% hyperoside)	Proanthocyanidins (g% cyanidin chloride)
SM_1	8.1791 ±0.2249	2.8176±0.3433	2.9369±0.0905	2.8006±0.0488	0.5814±0.0325
SM_2	6.3016 ±0.3013	2.6782±0.3952	2.5934±0.0293	2.0965±0.0199	0.3662±0.0454
SM_3	5.6063 ±0.1876	2.4482±0.4182	2.0694±0.0978	1.9009±0.07822	0.3244±0.0342
SM_4	5.6665 ±0.2398	2.2171±0.2840	2.1402±0.3073	2.0840±0.0230	0.2647±0.0255



A **B**

C **D**

Figure 3. HPLC chromatograms for: A = hyperoside, B = quercetol, C = quercitrin, D = SMM

DISCUSSIONS

Total polphenols, PCA and tannins content decreased with leaf development. The flavonoids' and tannins' content (all batches) was similar to the one reported by scientific literature; for batch M_1, quercitrin content (0.24%) was higher[1,2]. Flavonoids showed the same pattern as for *Betula pubescens* Ehrh. leaves; a slight increase being observed with leaf development (batch M4), whereas proanthocyanidins' content continuosly decreased with leaf growth[6]. We consider that the present study contributes to the knowledge of *Betula pendula* leaves, no data regarding polyphenols' accumulation during leaf development being reported in literature. Future research is needed for HPLC anaysis of other phenolic compounds.

CONCLUSION

The highest content of AFC, flavonoids, tannins and proanthocyanidins was found in young birch leaves (batch M1) and these will be further used for obtaing a polyphenol standardized extract.

ACKNOWLEDGEMENT

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