ISSN 2065-376X

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ISSN 2065-376X

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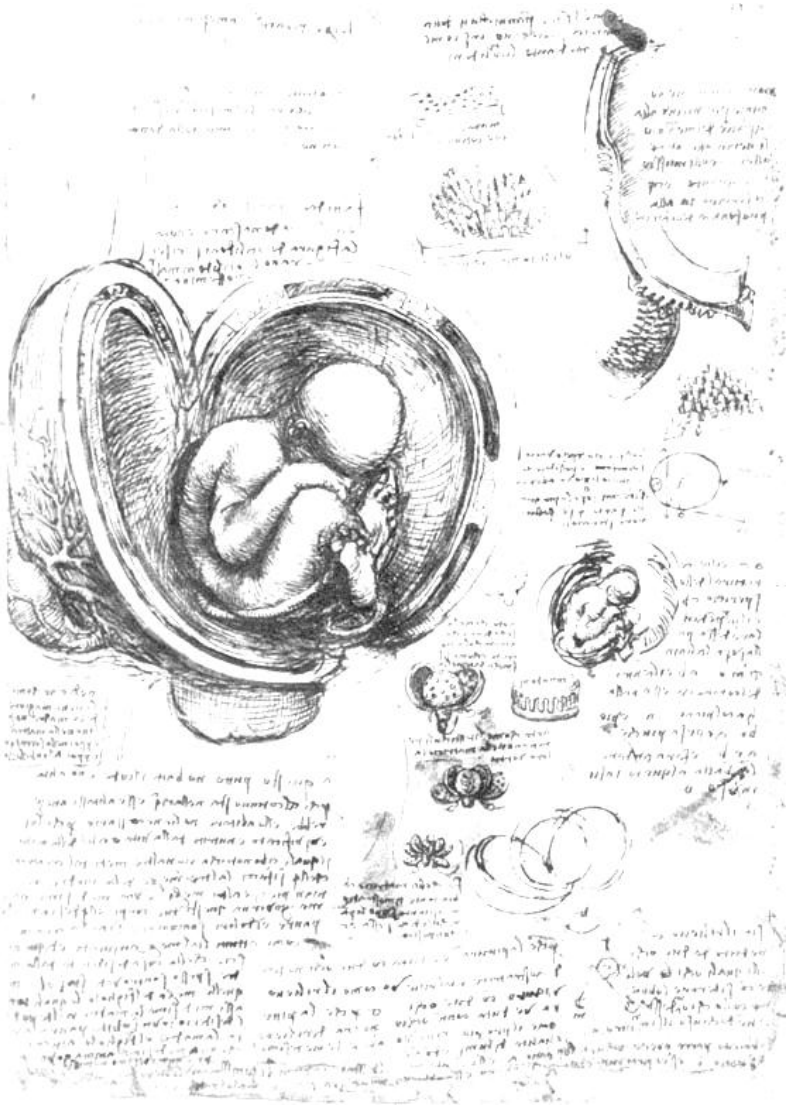

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Edited at: EUROSTAMPA, Timisoara
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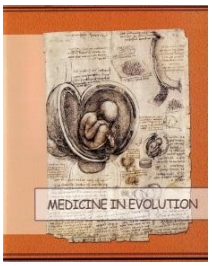
KERATOACANTHOMA - A DIAGNOSTIC CHALLENGE IN A PATIENT WITH MULTIPLE ACTINIC KERATOSES



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ABSTRACT

Keratoacanthoma (KA) is a cutaneous tumor located especially on the sun exposed skin. Sometimes, KA displays histopathological features similar to those found in squamous cell carcinoma (SCC). According to some authors KA is a variant of invasive SCC. We report the case of a patient with multiple actinic keratoses on her face, who presented to our clinic for the occurrence of a tumoral lesion located on the frontal region. In this context, several questions were raised regarding the differential diagnosis. Our case emphasizes the significant role of histopathological diagnosis.

Key words: *keratoacanthoma, squamous cell carcinoma, differential diagnosis*

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INTRODUCTION

KA was first described in 1889 by Jonathan Hutchinson as a"crateriform ulcer of the face". KA is a cutaneous tumor with a rapid growth occurring more frequently on the sun exposed skin areas, especially on the face and hands. It has its origin in the hair follicles. In rare cases it may develop on mucous membranes. It was reported mainly in men aged between 45-69 years (1). The main risk factors are the exposure to ultraviolet radiation, chemical carcinogens (e.g. pitch tar, coal and mechanic oil), smoking and trauma. Other conditions involved in the pathology of KA are genetic and immunologic factors. KA was encountered more frequently in immunosuppressed individuals (1,2).

Some authors consider KA as a type of invasive SCC. SCC is a malign tumor, which also occurs on the sun exposed skin having as a precursor an actinic keratosis (3). Unlike KA, SCC is a slow growing tumor, but it has an aggressive and invasive behavior and may metastasize. Nonetheless, cases of KA with an aggressive behavior, resulting in metastases were reported (4). Studies have emphasized that one third of KAs shows o malignant conversion progressing into SCC (5). Sometimes the differentiation between KA and SCC is difficult; dermoscopic and histopathological examinations are needed for establishing a diagnosis. Even if KA may regresses spontaneously, in most cases the treatment is based on surgery. To differentiate SCC from KA a complete excision is required (6,7).

CASE PRESENTATION

An 82-year-old patient, living in a rural area, was admitted to our clinic for the occurrence of a tumoral lesion on her forehead. The lesion had appeared three months earlier as a small nodule, which progressively increased. It was painless and nonpruritic. The patient's medical history included ischemic heart disease, arterial hypertension, osteoporosis (with favorable evolution under treatment with bisphosphonates) and hyperthyroidism. In addition the patient had a history of actinic keratoses. The first lesions had appeared 20 years before. Regarding the conditions of life and work, the patient stated that she had worked in a toxic environment, being exposed to arsenic for five years. In addition, she had been constantly exposed to sunlight. She was non-smoker.

Local examination revealed a tumoral lesion sharply defined with a central corneous plug and several peripheral teleangiectasias, located on the frontal region, on the left side. The lesion measured 1.5 cm (Figure 1). The patient also presented multiple yellow-brown rough plaques with an adherent scale, scattered on her face, with a diameter between 0.5-1 cm, more accesible to palpation than direct observation. Otherwise the physical examination was unremarkable.

The laboratory parameters were within normal range. The tumoral excision and a biopsy were performed by curettage followed by electrosurgery. The biopsy specimen displayed a cutaneous tumor with a central crater made of orto- and parakeratin (Figure 2). Epithelial cells had nuclear and cytoplasmic pleomorphism and atypical mitoses. In the dermis small tumoral islands composed of spinous cells with marked nuclear and cytoplasmic pleomorphism and as well as an inflammatory infiltrate were noticed (Figure 3). The histopathological diagnosis was keratoachantoma with areas of transformation into invasive

SCC. In respect to the other forementioned skin lesions, the clinical examination was compatible with the diagnosis of actinic keratoses, therefore no other investigation was considered to be necessary.

The patient underwent local radiotherapy after the healing of the surgical intervention site. Cryotherapy was used to treat the co-existing actinic keratoses. The evolution of the patient was favorable, with no re-occurrence of the lesions within a follow-up period of 6 months.

Figure 1. A large tumoral lesion sharply defined with a central corneous plug and several peripheral teleangiectasias, located on the frontal region

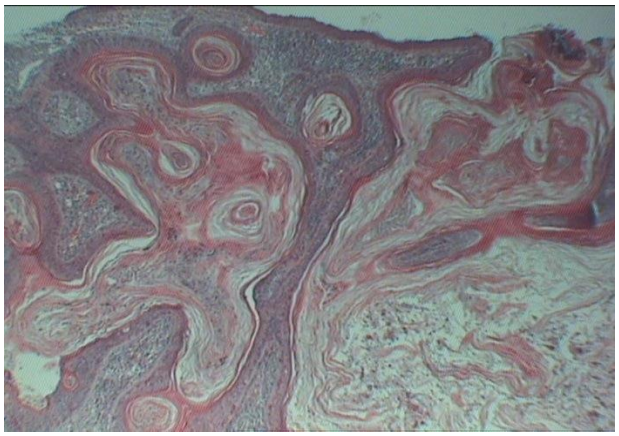

Figure 2. A cutaneous tumor with a central crater made of orto- and parakeratin

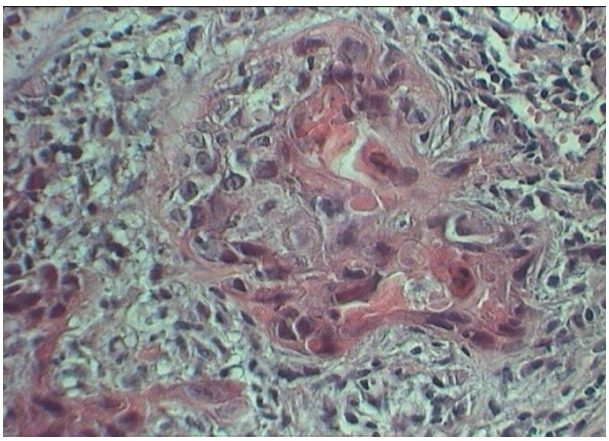

Figure 3. Small tumoral islands composed of squamous cells with marked nuclear and cytoplasmic pleomorphism and as well as an inflammatory infiltrate in the dermis

DISCUSSIONS

In most cases KA is a solitary tumor, which develops in three stages (proliferative, mature and resolving) (8). The tumor evolves within 6-8 weeks and regresses spontaneously over a 3-6 months period of time (9). The healing leaves a hypopigmented scar. Clinically, KA is a painless nodule with a diameter of 1-2 cm, presenting a central crater, with a plug of keratin. The differential diagnosis should include SCC, basal cell carcinoma (BCC), molluscum contagoisum and actinic keratoses. In some cases the

clinical appearance is not characteristic and the differential diagnosis may be difficult. Mainly we should rule out a SCC. KA differs from SCC by a rapid growth pattern and the presence of the central crater (1). However, cases of rapid growing SCC were reported, mimicking KA (3). In some cases KA may coexist with a SCC or progress to SCC (1). The malignant transformation may occur in any stage, including the involution stage. In our case, based on clinical features and growth pattern the presumptive diagnosis was keratoachantoma. Nonetheless, the history of actinic keratoses raised the suspicion of SCC. Our patient presented multiple actinic keratoses on her face. She was chronically exposed to ultraviolet radiation and this may explain the occurrence of multiple actinic keratoses. In Caucasian subjects actinic keratoses are frequently seen, in a proportion of 40-50% (10,11).

Studies have shown that the transformation risk of actinic keratoses into SCC varies between 0.15 – 80%. The risk is higher in the patients with more than five lesions (10). On the other hand, in our case we consider that peripheral teleangiectasias may represent an argument for BCC. Dermoscopy and histopathological examination are useful tools in establishing the diagnosis.

Dermoscopy is an easy and non-invasive procedure. Regarding the differential diagnosis between KA, SCC and BCC, the presence of coiled vessels represents a sign in favor of KA or SCC (6). Moreover, branched and dilated vessels are more frequently observed in KA (12). The central arrangement of keratin may be a strong criterion for the diagnosis of KA. In addition the white circles, that corresponds to acanthosis and hypergranulosis of infundibular epidermis characterize KA and SCC, differentiating them from other tumors (6).

The histopathological examination plays the most important role in the diagnosis. Histopathologically KA is an exo-endophytic nodule presenting a central crater filled with keratin. In the proliferative stage KA exhibits features resembling SCC such as infiltration and cellular atypia (9). These findings were more frequently noticed in elderly on the sun exposed skin. In our case the presence of cells with nuclear and cytoplasmic pleomorphism and atypical mitoses and the small tumoral islands composed of spinous cells with marked nuclear and cytoplasmic pleomorphism noticed in the dermis suggested the malignant transformation of KA. Recent studies have revealed that cells of KA possess mutations in the p53 gene or an activated ras-oncogene, such findings being also seen in the cells of SCC (5). It seems that laminine 322 is associated with an increased risk of progression to SCC. Laminine 322 is a component of basement membrane, being involved in the pathogenesis of SCC by contributing to the cell mobility, tumor progression and invasion (13). Numerous studies have tried to distinguish between KA and SCC according to histopathological criteria, chromosomal abnormalities or tumoral markers (e. g. p53, Ki67). But all of these criteria failed to distinguish clearly between KA and SCC (5).

There are data, suggesting that KA is an immunodependent tumor. A strong immune response against tumoral cells plays a role in malignant transformation. There is evidence that highlights the importance of immune response in malignant transformation. Inflammatory infiltrate stimulates the cell growth through the macrophages, which release growth factors and activators of angiogenesis. During tumoral development, the inflammatory infiltrate changes and KA regresses, the cells not achieving immortality (14).

Surgery is the treatment of choice in most cases of KA, given the resemblance to SCC. In the recurrent or

giant tumors, the Mohs surgery may represent an option. Other therapeutic options are cryotherapy, radiotherapy, intralesional administration of chemotherapeutic agents (e.g methotrexate) and local application of 5 fluorouracil (2, 15). Recent studies have demonstrated the effectiveness of topical imiquimod in the treatment of KA (16).

Our case is particular given the differential diagnosis challenge; the tumoral lesion did not have a striking clinical appearance. The medical history and the local examination suggested the diagnosis of KA, SCC or BCC. SCC remains the main diagnosis in a patient with a cutaneous tumor and multiple actinic keratoses, but other diagnosis should be considered. Histopathological findings play the most important role in establishing the diagnosis.

CONCLUSIONS

Based on clinical, demoscopic and histopathological criteria in most cases we may achieve the differentiation between KA and SCC. However, there are cases when differentiation is very difficult and in these cases the diagnosis becomes a challenge. Many researchers have attempted to define the relationship between SCC and KA; whether KA is a benign tumor or a variant of invasive SCC is still a topic under debate; further research in the field might yield to an adecquate set of clinical and histological criteria of differentiation.

Acknowledgement

This paper is partly supported by the Sectorial Operational Programme Human Resources Development (SOPHRD), financed by the European Social Fund and the Romanian Government under the contract number POSDRU 141531.

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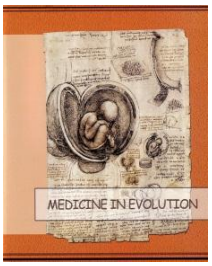
BULLOUS PEMPHIGOID INDUCED BY RADIOTHERAPY IN A MALE PATIENT WITH BREAST CANCER



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ABSTRACT

Radiotherapy can rarely be associated with cases of autoimmune blistering dermatoses such as bullous pemphigoid, pemphigus vulgaris and epidermolysis bullosa acquisita. We report the case of an 82-year-old male with a generalized pruritic eruption developed three months after the last radiotherapy session for breast cancer. The eruption was initially located on the irradiated area of the left thoracic wall and became generalized after a few days with involvement of the oral mucosa. The histopathological and immunological findings confirmed the diagnosis of bullous pemphigoid. This case underlines a very rare but severe side effect of radiotherapy that requires recognition both by dermatologists and oncologists but also radiotherapists in order to provide prompt and adequate treatment, reducing morbidity and mortality associated with bullous pemphigoid.

Key words: *bullous pemphigoid, radiotherapy, breast cancer*

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INTRODUCTION

Bullous pemphigoid, first described by Lever in 1953, is an autoimmune skin disease characterized by the development of subepidermal blisters that most often affects the elderly. The onset and course of bullous pemphigoid depends on the interaction between predisposing and inducing factors. The presence of trigger factors can be identified in less than 15% of patients (1). Various physical agents including UV radiation, thermal and electrical burns, trauma, surgical procedures and skin grafts have been reported to play a role in triggering bullous pemphigoid (1-3). Radiotherapy is rarely associated with autoimmune blistering skin diseases such as bullous pemphigoid, pemphigus vulgaris, lupus erythematosus or epidermolysis bullosa acquista (4).

CASE REPORT

An 82-year-old male patient was admitted in our department for a three-week history of a high pruritic blistering generalized eruption associated with oral ulcers. Three years before he had been diagnosed with breast cancer and underwent surgical resection (total left mastectomy and axillary lymph node dissection), adjuvant radiotherapy and hormonal therapy with anastrozolum. A year later, he developed local recurrence of breast cancer and underwent surgery, radiotherapy and started treatment with tamoxifen 20 mg per day.

Three months after completion of the second radiotherapy session, the patient developed multiple itchy blisters with denuded areas on the irradiated area of the left thoracic wall. The eruption became generalized after 10 days with involvement of the oral mucosa.

Clinical examination at the time of hospital admission revealed a generalized eruption with a predilection on the flexural areas of the skin consisting of urticarial plaques with an annular appearance, multiple, tense bullae that ranged in size from 0.5 cm to several centimeters, filled with a clear serum on an erythematous, normal or urticarial base, and multiple superficial erosions covered by hemorrhagic crusts (Figure 1). There was also mucosal involvement characterized by slightly painful ulcers.

The results of laboratory investigations, including hemogram, blood sugar levels, liver and renal function tests, chest radiography, were all within normal limits with the exception of leukocytosis (13100/μL) with neutrophilia (10600/μL) and hypochromic microcytic anemia. No manifestations of tumor activity were noticed during oncologic examination.

Skin biopsy from an intact lesion localized on felt arm revealed a sub-epidermal blister with dermal infiltration mainly of eosinophils and lymphocytes (Figure 2). Direct immunofluorescence showed linear deposits of Ig G and C3 at the level of the dermo-epidermal junction.

The clinical, histopathological and immunological findings confirmed the diagnosis of radiotherapy-induced pemphigoid bulous.

Treatment with systemic corticosteroids (prednisone 50 mg/day, hydrocortisone sodium succinate 100 mg i.m/day, 15 days), antihistamines, antiseptics and topical clobetasol propionate 0.05% was started, with significant healing of the lesions and without new lesions developed after 2 weeks of treatment. A month later the patient returned in our clinic with a relapse of the cutaneous eruption when oral prednisone dosage was decreased

to 40 mg daily. We decided to increase the oral prednisone back to 50 mg daily in association with hydrocortisone succinate, with a favorable course during hospitalization, without new lesions even after prednisone dose reduction. The oral corticosteroid dosage was gradually reduced in the next 3 months with complete resolution of the blisters.

Ten months later, he developed a single tense bullous lesion within the radiotherapy area, cleared with topical clobetasol ointment (Figure 3).

Figure 1. Clinical appearance at presentation

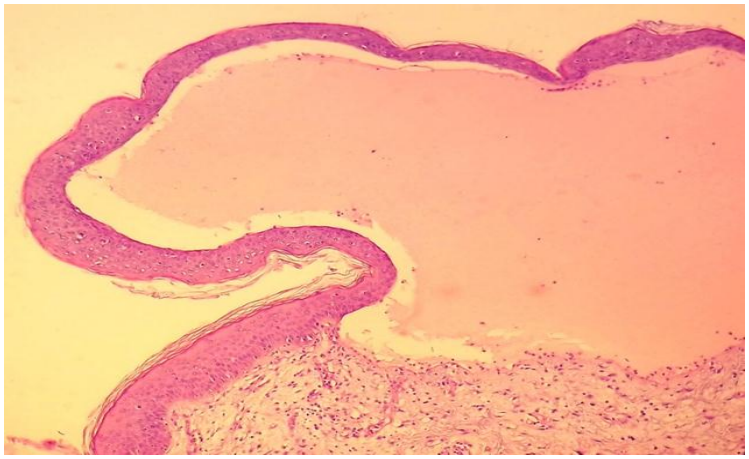

Figure 2. Histopathological appearance

Figure 3. Clinical appearance ten months later

DISCUSSIONS

Bullous pemphigoid is the most common form of autoimmune bullous dermatosis, characterized by the presence of autoantibodies directed against BP180 (collagen XVII, BPAG2, COL17) and BP230 (BPAG1-e) (5-7). The bullous pemphigoid autoantibodies predominantly belong to the IgG1 and IgG4 subclasses but, in addition, IgE against BP180 autoantibodies may be found in the majority of untreated patients.

The BP230 antigen is an intracellular hemidesmosomal plaque protein belonging to plakin family while BP 180 is a trans-membranar hemidesmosomal protein that belongs to the collagen family(8). Anti BP180 autoantibodies are detected in 79-93% of bullous pemphigoid patients and the serum levels are correlated with the activity of the disease. Anti BP230 autoantibodies are detected by ELISA in 57-63% of bullous pemphigoid cases and are not related to the disease activity but are correlated with localized forms of bullous pemphigoid (9).

The disease usually affects elderly persons and is rare in children and young people. Childhood bullous pemphigoid etiology is not clear; drug intake and vaccination have been incriminated in some cases (10).

Typically, bullous pemphigoid is clinically characterized by tense serous or hemorrhagic blisters, with variable size (usually varying between 1 and 3 cm) arising on normal, urticarial or erythematous skin. Blisters evolve to erosion and crusting, and usually heal without scarring formation. Sometimes, post-inflammatory hyperpigmentation or milia may occur (11, 12).

Sites of predilection include lower abdomen, proximal extremities, flexural aspects of the forearms and intertriginous areas, but any skin surface may be affected. Oral mucosa involvement is rare (10-30% of cases) (5).

Inducing factors and associated conditions

Bullous pemphigoid was associated with a variety of conditions and triggering factors identified in less than 15% of patients. Neurologic diseases have been often reported in association with bullous pemphigoid, 36 % of bullous pemphigoid patients presenting at least one neurologic disease. Bullous pemphigoid seems to

be associated with degenerative neurologic diseases that may involve autoimmune mechanisms such as Alzheimer and Parkinson disease. It has been associated with dementia (5.1%), stroke (6.7%) and in rare cases with amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, hemiplegia, miastenia gravis and Shy-Dragger syndrome (13).

Some autoimmune diseases and inflammatory dermatosis have been described in association with bullous pemphigoid. Autoimmune diseases reported in association with bullous pemphigoid include rheumatoid arthritis, Hashimoto thyroiditis, dermatomyositis, lupus erythematosus and autoimmune thrombocytopenia. The association between bullous pemphigoid and inflammatory dermatosis, particularly psoriasis and lichen planus, lacks statistical significance (5). It seems that the inflammatory process at the dermal-epidermal junction is responsible for antigen exposure to auto-reactive T-cells exposure leading to a secondary immune response (14). The majority of bullous pemphigoid cases reported in literature in association with psoriasis appeared after antipsoriatic treatment: PUVA, UVA and UVB light (10).

Many drugs (spironolactone, furosemide, beta blockers, nonsteroidal anti-inflammatory drugs, D-penicillamine, amoxicillin, ciprofloxacin, captopril, gold salts, simvastatin, metformin etc) have been proposed as possible bullous pemphigoid trigger factors, but the exact mechanism for the pathogenesis of drug-induced bullous pemphigoid is not fully understood. A theory sustains that these drugs may alter basal membrane immune response, in patients with genetic predisposition (10, 15).

There are some controversies regarding the association between bullous pemphigoid and malignant disease. Some studies have found an increased risk of different cancers (digestive tract, pulmonary, breast, urinary bladder) and lymphoproliferative disorders but the majority of large studies have not found significant associations between bullous pemphigoid and malignancies (16, 17).

Bullous pemphigoid was also reported after fototherapy, vaccination, viral infections, surgical procedures, burns and organ transplantation (15).

Possible pathogenic mechanisms

In rare cases, radiotherapy was associated with cutaneous afflictions such as bullous pemphigoid, pemphigus vulgaris, lupus erythematosus or epidermolysis bullosa acquisita.

The mechanism through which radiotherapy induces bullous pemphigoid is not clearly understood. It seems that radiotherapy changes the antigenic proprieties and induces antibody formation through direct alterations of the basement membrane zone and the exposure of antigens (18).

Another theory suggests the presence of circulating auto-antibodies in those patients before the initiation of radiotherapy and the disruption of the tissues through radiotherapy contributes to local accumulation of those antibodies by the increase of the vascular permeability (4, 19-21). Furthermore, it was proved that radiotherapy alters the levels of the vascular endothelial growth factor (VEGF). The role of VEGF in bullous cutaneous disorders is not well clarified but high levels of VEGF were found in the bulla fluid and the perilesional epidermis of patients with bullous pemphigoid, but also in the epidermis of patients with other bullous disorders such as dermatitis herpetiformis and erythema multiforme (4, 22).

Recently, a synergic interaction between the plasminogen/plasmin signaling pathway and MMP 9 (matrix metallopeptidase 9) during the initiation of antibody induced bullae

formation was reported (23). Furthermore, radiotherapy can induce local changes in the immune system. This immune imbalance may lead to inhibition of the suppressant activity of T lymphocytes, which could result in a high production of antibodies (24).

For those patients in which bullous pemphigoid occurs during radiotherapy the theory of radiotherapy-induced tissue injury with the increase of antibody deposits through alterations of vascular permeability is preferred. The theory of the existence of circulating antibodies prior to the initiation of radiotherapy could be valid in patients receiving medication known as trigger factors for bullous pemphigoid. Bullous pemphigoid with late onset after radiotherapy cessation and extensive or generalized forms occurring on the radiation area can be explained through an immunological phenomenon called epitope spreading by which a relatively limited immune response extends thus involving various regions of the same auto-antigen and other auto-antigens (18).

Paradoxically, a case of bullous pemphigoid healing after radiotherapy was reported, probably due to the remission of the underlying disorder (mycosis fungoides) (25) but also cases of bullous pemphigoid exacerbation after adjuvant radiotherapy for breast cancer (26).

Bullous pemphigoid and radiotherapy in the literature

In a review published in 2007, the association between bullous pemphigoid and radiotherapy was found in 27 patients, with a mean age of 75 years. Most of them only presented lesions on the radiation area (24/27, 89%) and had breast cancer (21/27, 78%). Other cases were described after radiotherapy for squamous cell carcinoma, cervical or vulvar cancer and thoracic cancers (lungs, esophagus). In most cases, bullous pemphigoid occurred after more than one year following radiotherapy; only 5 patients developed lesions several weeks later and 6 patients 1-6 months post-radiation. Of the reported cases, 5 used hormonal therapy (tamoxifen) (18).

In July 2014, 31 cases of radiotherapy associated bullous pemphigoid were reported. Of those, 23 cases occurred after breast cancer radiotherapy, 3 cases for gynecological cancers, the rest being associated with lung cancer, esophagus cancer and non-Hodgkin lymphoma (4, 25). Another article from 2014 describes a case of bullous pemphigoid located on the radiation area after an oral mucosa carcinoma (25).

Bullous pemphigoid treatment

The treatment of bullous pemphigoid depends on the multifactorial pathogenesis of this affliction. The imbalance between autoreactive T helper lymphocytes and T regulatory lymphocytes, the activation of toll-like receptors (TLR) and Th17/Il 17 pathway represent three possible autoimmune trigger factors fundamental for bullous pemphigoid (13).

The main pathogenic mechanism for bullous pemphigoid is based on an immune response against the structural components of desmosomes, BP 180 and BP 230. The binding of auto-antibodies to antigens leads to complement activation, with inflammatory cells recruitment and proteolytic enzymes release. This inflammatory cascade can also be triggered directly, through activation of LTh17, but without auto-antibody intervention. In fact, inflammation seems to be more important in bullae formation in bullous pemphigoid as compared to other autoimmune disorders such as pemphigus. Therefore, even though the first-line treatment is the immunosuppressant therapy, new therapeutic agents target the key pathogenic mechanisms above mentioned (13, 27).

Systemic corticotherapy proved its efficacy in bullous pemphigoid since 1950, the effect being rapid, with inflammation and bullae formation suppression after 1-4 weeks of treatment with prednisolone 0.75-1 mg/kg/day, after that the dose being slowly tapered. Intravenous methylprednisolone (1g/day) for three consecutive days followed by prednisone 30-40 mg/day also proved efficient, being associated with a faster therapeutic response but also with more frequent recurrences.

Some studies suggested that treatment with high potency topical corticosteroids such as clobetasol propionate cream 0.05%, applied twice a day, is as efficient and safer in localized and moderate forms of disease as compared to oral prednisolone (28, 29, 30, 31, 32). Nevertheless, their use in extensive forms can be limited by practical factors such as the ability or availability of the patient to apply the treatment and the fact that they can be associated with systemic absorption and the occurrence of adverse events. When possible, they should be considered as first-line treatments, especially in localized forms (32).

Topical tacrolimus (0.1% twice a day) could be a useful alternative to topical corticosteroids in the treatment of localized and limited forms, without having the disadvantage of causing skin atrophy (33, 34).

Immunosuppressants like azathioprine (100-150 mg/day) and mycophenolate mofetil (1 g twice a day), are used for the treatment of bullous pemphigoid either alone or in association with corticosteroids. Azathioprine seems to induce remissions slightly faster than mycophenolate mofetil. (32, 35, 36).

Methotrexate can be successfully used in monotherapy or in association with topical or systemic corticosteroids but should be prescribed with caution in elderly due to the bone marrow suppression and important liver toxicity it has been associated with (37).

Chlorambucil as an adjuvant therapy to systemic corticosteroids proved useful as an alternative to other immunosuppressants if those are not well tolerated or are contraindicated (32, 38).

Cyclophosphamide and cyclosporine proved their efficacy in the treatment of refractory bullous pemphigoid but they cannot be routinely recommended due to their high toxicity (32).

Intravenous immunoglobulins have been more frequently used in association with oral prednisolone and other agents (32, 39, 40). They should be considered as adjuvant treatment in patients with very severe disease, when a rapid control is necessary or when there is no response or other therapies are contraindicated.

The role of antibiotics like doxycycline, minocycline, limecycline and erythromycin as well as nicotinamide in the treatment of bullous pemphigoid is subject to controversy but they are well tolerated and can be useful for elderly patients with multiple comorbidities (32, 41).

There were reported cases of bullous pemphigoid only responding to high doses of prednisone which were treated with leflunomide (an isoxazole derivate which blocks pyrimidine synthesis by irreversibly inhibiting dihydrooroate dehydrogenase, also presenting anti-inflammatory effects), the patients remaining in clinical remission even after tapering the dosage (13, 42, 43).

The role of biological agents in bullous pemphigoid remains limited. Etanercept was successfully used for treating a patient with bullous pemphigoid and psoriasis (44) but in other cases, the long term treatment with etanercept for rheumatoid arthritis is presumed to be one of the trigger factors for bullous pemphigoid (45). Cases of bullous pemphigoid induced by adalimumab treatment

were also reported (46, 47). Rituximab and omalizumab on the other hand proved effective in some refractory cases of bullous pemphigoid, alone or in association with other immunosuppressants (48-51).

CONCLUSIONS

Bullous pemphigoid is a frequent bullous cutaneous affliction but was rarely reported after radiotherapy. It was especially described in women who underwent radiotherapy as a conservative or adjuvant treatment for breast cancer. Bullous pemphigoid can occur during, early or late after radiotherapy, presenting as localized forms on the radiation area or generalized forms initially located on the radiotherapy area, being considered an adverse effect of radiotherapy. The trigger mechanism remains unknown and the molecular cascade involved was not yet clarified.

The particularity of the case is the occurrence of bullous pemphigoid affecting the oral mucosa after radiotherapy for recurrent breast cancer in a male patient also undergoing hormonal adjuvant treatment with tamoxifen.

Acknowledgement

This paper is partly supported by the Sectorial Operational Programme Human Resources Development (SOPHRD), financed by the European Social Fund and the Romanian Government under contract number POSDRU 141531

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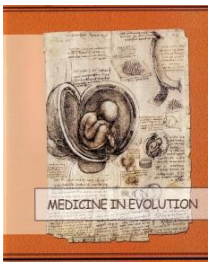

DIFFUSE LEFT VENTRICLE POST-SYSTOLIC CONTRACTION AFTER CARDIAC RESYNCHRONIZATION THERAPY OPTIMIZATION- A CASE REPORT

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ABSTRACT

A number of patients undergoing cardiac resynchronization therapy have a suboptimal response, thus being deprived of its important beneficial effects. Optimization of the device's intervals in order to increase the response to this therapy is still uncertain because of the heterogeneous results of the studies.

We report the case of a patient in which the high left ventricle afterload due to aortic wall calcification, prevented the achievement of a maximal beneficial effect after optimization of cardiac resynchronization therapy, identifying an important correctable problem in this field.

Key words: *cardiac resynchronization therapy; optimization; afterload; speckle tracking strain; post-systolic contraction*

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INTRODUCTION

Cardiac resynchronization therapy (CRT) is a new, revolutionary therapy that proved to reduce all-cause mortality by 28% and new heart failure hospitalizations by 37% in patients with dilated cardiomyopathy [1]. The lack of a beneficial response/ a suboptimal response to this therapy in some patients (30%) is one of the most important issues in this field [2]. The standard programming of the device may be unsuitable for some patients by lacking the achievement of synchrony. One of the attempts to increase the response rate is to optimize the device's parameters for each individual. For this purpose, echocardiography is the most frequently used to evaluate contraction synchrony and hemodynamics for different settings of the device's parameters [3,4,5]. The beneficial effects of optimization are still uncertain though, because of the heterogeneous results of the studies that need further research and explanations [6].

We report the case of a patient in which we identified an unexpected problem after optimization of cardiac resynchronization therapy. If left unsolved, this problem might have prevented the achievement of a maximal CRT beneficial effect.

CASE REPORT

A 66-year-old woman came to our clinic presenting fatigability, shortness of breath even with minimal physical activity and paroxysmal nocturnal dyspnea. Her history included an anterior myocardial infarction accompanied by acute pulmonary edema two years ago, three vessel coronary artery disease with stent placement within the left main, anterior descendent and circumflex arteries in the acute setting, a consequent ischemic dilated cardiomyopathy, diffuse calcified atheromatosis of the aorta and carotid arteries and stage 3 arterial hypertension (with constant values of 100-110/60-65mmHg after the myocardial infarction because of the low cardiac output). Her symptomatology occurred despite adequate medical therapy for heart failure.

The chest x-ray revealed a globally enlarged heart with bilateral central stasis and the electrocardiogram showed sinus rhythm and a left bundle branch block with QRS duration of 200ms. Transthoracic echocardiography revealed a severely dilated left ventricle with a severely reduced ejection fraction (~20%), interventricular and intraventricular dyssynchrony, apical akinesia, a grade III functional mitral regurgitation, mild aortic stenosis, and a grade II functional tricuspid regurgitation with mild secondary pulmonary hypertension.

The patient had a class IA indication for cardiac resynchronization therapy according to the 2013 European Society of Cardiology Guideline on cardiac pacing and cardiac resynchronization therapy [6] and therefore she underwent implantation of a triple chamber implantable cardioverter defibrillator (ICD) with the leads placed in the right atrium, right ventricle (RV) and a postero-lateral branch of the coronary sinus for the left ventricle (LV).

Mid-ventricular speckle-tracking radial strain with the standard programming of the device's intervals revealed a residual delayed post-systolic contraction especially of the inferior and posterior walls. The radial strain analysis (time to contraction) during RV versus LV pacing alone,

suggested these segments to be depolarized by the impulse coming from the interventricular septum (right ventricle lead and the impulse coming through the atrioventricular node) during biventricular pacing. Indeed, we obtained a synchronized contraction of all segments after prestimulating the RV by 15ms, but all these segments had a delayed post-systolic contraction (their maximal contraction occurred after aortic valve closure) (figure 1), problem confirmed also by speckle-tracking longitudinal strain examination, without any clear explanation. The patients' blood pressure at the moment of examination was 150/90mmHg. She was reexamined after her blood pressure was controlled with nitroglycerine (at a value of 110/70mmHg) when mid-ventricular speckle tracking radial strain revealed perfectly synchronized contraction without any delayed post-systolic contraction (figure 2).

The ejection fraction, calculated by echocardiography (Simpson method), increased first from 20% to 25-30%, and to 30-35% after administration of nitroglycerine. Mitral regurgitation reduced from grade III to grade I. Also, the"6 minute walk test"showed a significant improvement of exercise capacity- 120m with standard programming of the CRT device and 200m after complete optimization.

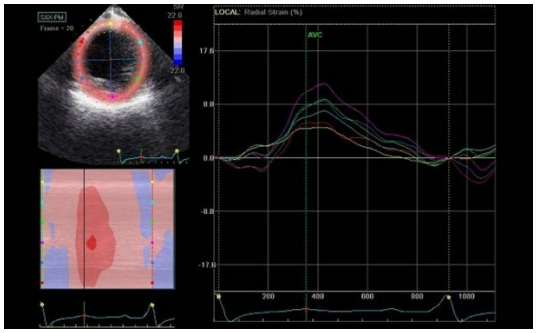

Figure 1. Diffuse synchronized post-systolic contraction of mid-left ventricular segments (maximal contraction of all segments occurs after aortic valve closure)- speckle-tracking mid-ventricular radial strain (AVC- aortic valve closure)

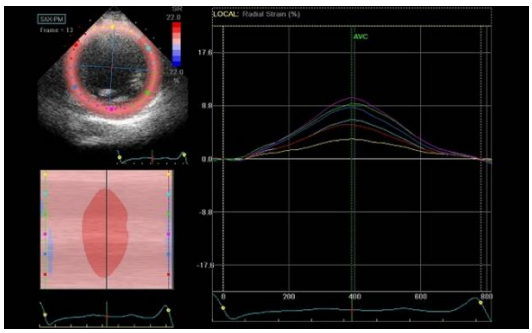

Figure 2. Normal synchronized contraction of mid-left ventricular segments- speckle-tracking mid-ventricular radial strain

DISCUSSIONS

The mechanism of action of cardiac resynchronization therapy consists in pacing certain segments of the heart (from the right atrium, right ventricle and left ventricle) in order to determine a synchrone atrio-biventricular contraction, to prevent the delayed postsystolic contraction of certain segments of the left ventricle (especialy the postero-lateral segment), and consequently to increase the systolic volume and pressure. On a molecular basis, the resynchronized contraction seems to increase the myofilament calcium ions sensitivity, thus further increasing contractility [7].

A reasonable explanation for the totally unusual and unexpected diffuse postsystolic contraction of the left ventricle's segments in our case could be the calcified atheromatous aortic wall.

The aortic pulse pressure wave is formed by the left ventricle systolic pressure and the waves reflected from the periphery (arterial bifurcations, muscular arteries), which return to the aortic valves [8,9,10]. With an increased left ventricle systolic pressure (by cardiac resynchronization therapy), thus with a stronger incident wave in a calcified athermatous noncompliant aorta, the reflected waves may return

earlier and exert a stronger pressure on the aortic valve compared to the contraction of the myopathic left ventricle (even if this contraction is synchronized), increasing the blood pressure and determining the valve to close while the left ventricle's segments are still contracting. Besides aortic wall stiffness, muscular arteries, by altering their tonus, can modify the speed and timing of the reflected waves. Vasodilators, especially nitric oxide donors, proved to reduce the tonus of these arteries, thus reducing the pressure of the reflected waves and delaying the time they arrive back to the heart in systole [10]. This explains why the problem solved and we obtained an optimal synchronized contraction before aortic valve closure after administration of nitroglycerin.

This aspect highlights the importance of a high afterload in a suboptimal response to cardiac resynchronization therapy, and that optimization of the device's intervals is not always enough to obtain the best response. This may be an important correctable issue for CRT optimization in some of the patients.

Conflict of interest: none to declare.

Acknowledgements. This work was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007- 2013, project no. POSDRU/159/1.5/S/136893.

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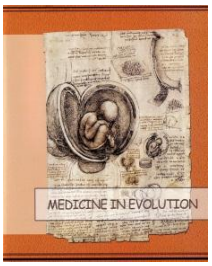
CLINICAL AND MORPHOLOGICAL ASPECTS IN RECTAL CANCER



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ABSTRACT

Introduction. Rectal cancer is a major healthcare issue around the world. We aim to analyze a population of patients with rectal cancer with no adjuvant treatment from two major hospitals situated both in the western part of Romania.

Methods. The present cohort prospective study enrolled 31 patients from two hospitals – Arad and Timisoara County Hospitals, who underwent different types of procedures which included a resection for rectal cancer. We analyzed our study population regarding sex, age, tumor localization (superior or inferior rectum), staging (tumor grading, tumor local spreading, distant spreading – especially lymph nodes), the histopathology of the tumor.

Results. There were more males 58%. The histological grading were 87.1% were G2, and 12.9% G3. A percentage of 45.1% did not have any lymph node metastasis, being classified as N0. N1 stage was more frequently present in the tumors situated in the superior rectum, N2 had a similar distribution among the superior and inferior rectal localization of the tumors. Anemia was present in half of the patients enrolled in our study in all T stages. Leukocytosis was an important parameter in order to relate it to local spreading.

Conclusions. Our study had a heterogeneous and representative population. Male gender represented a risk factor for developing rectal cancer. Leukocytosis was associated with more advanced local stages of rectal cancer.

Key words: *Rectal cancer, staging, cancer spreading, leukocytosis*

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INTRODUCTION

Rectal cancer is a major healthcare issue around the world. The number of cases is rising, despite awareness and screening campaigns. One of the drawbacks is that in many studies rectal cancer cases are evaluated side by side with colon cancer cases, this fact makes it difficult to interpret statistics and epidemiological data, thus creating research issues. Rectal cancer incidence throughout the European Union is about 35% of all the colo-rectal cancers, reaching 15-25/100.000 every year. Mortality is between 4 and 10 per 100.000 subjects every year; lower rates are reported for women.

We aim to analyze a population of patients with rectal cancer with no adjuvant treatment from two major hospitals situated both in the western part of Romania in order to see if there are any demographics differences between this area and other studies in the literature.

METHODS

The present cohort prospective study enrolled 31 patients from two hospitals – Arad and Timisoara County Hospitals, who underwent different types of procedures which included a resection for rectal cancer. Patients were evaluated in the gastrenterology department prior to surgery. All the patients refused neoadjuvant chemoradiation, this being a criteria for inclusion in our study, as we tried to find out the natural evolution of rectal cancer.

In this paper we studied the epidemiological data of our patients. We analyzed our study population regarding sex, age, tumor localization (superior or inferior rectum), staging (tumor grading, tumor local spreading, distant spreading – especially lymph nodes), the histopathology of the tumor.

RESULTS

The age of the patients varied between 40 and 90 years. We used different age intervals to stratify them. Most of our patients were in their 6th and 7th decade of their life. The smallest numbers of cases were forming the first and the last decade, before 50 and after 80.

Figure 1 Percentage distribution of patients age

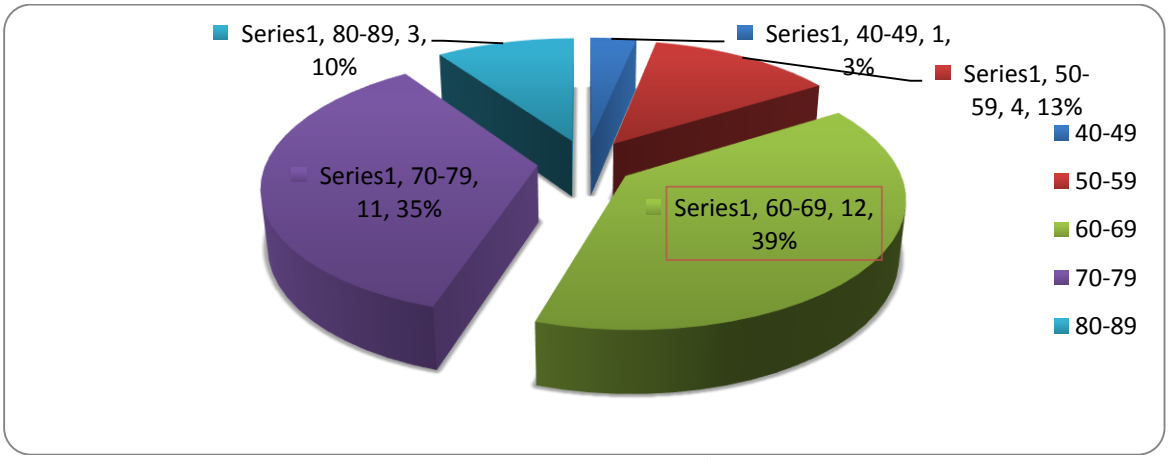


Figure 1. Percentage distribution of patients age

There were more men included in our study than females, 58% of the patients being males. Most males with rectal cancer were in the 6th decade of their life. In females the incidence peak was ten years later, in the 7th decade.

Looking over the analysis of the tumor localization in the superior and inferior rectum we found an even distribution of the total case number, with a slightly increased incidence for the superior rectum with 54.8%. For females the incidence of the cancer of the superior rectum was double than the one for inferior rectum.

The histological grading of the tumors included in our study revealed only two grades, 87.1% were G2, and the remaining tumors were G3. There were no G1 or G4 tumors.

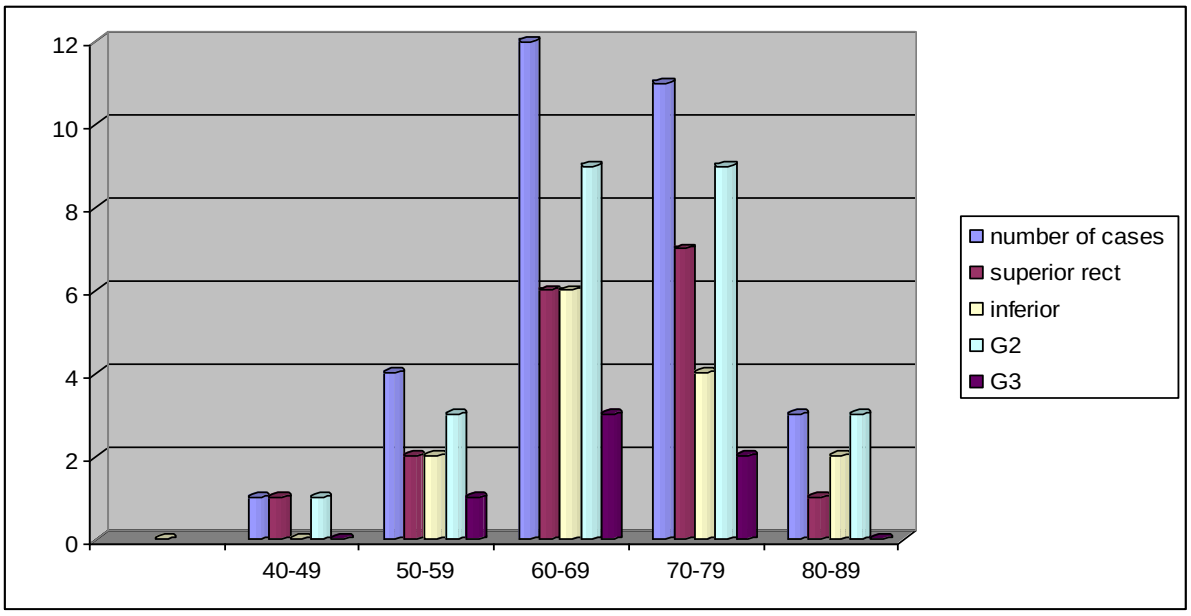
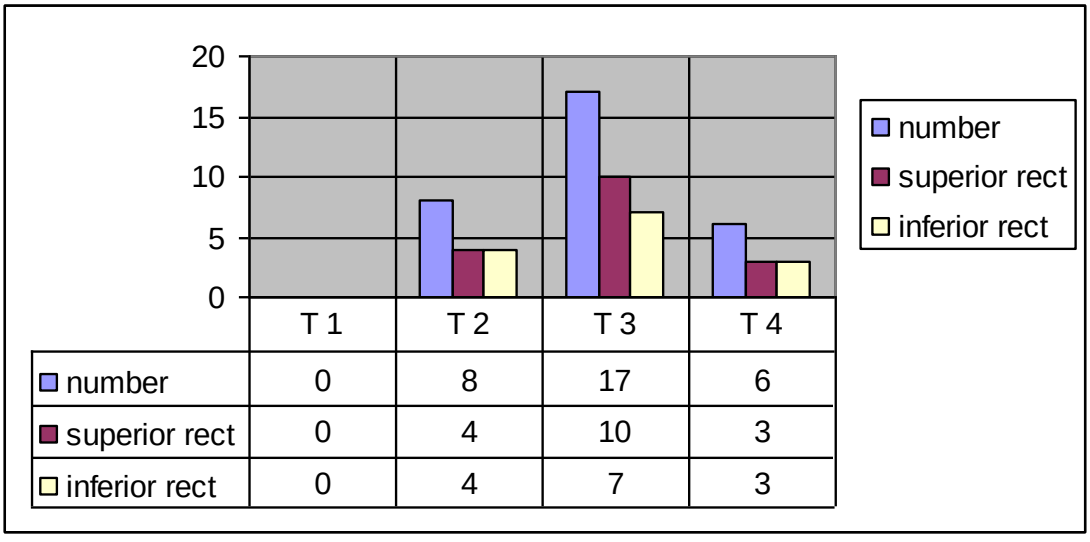


Figure 2. Grading and localization of the rectal tumors

Regarding the size of the tumor, there was an increased incidence of large tumors situated in the superior rectum; the symptoms of these tumors were mainly given by the grade of the obstruction. The rest of the tumors were distributed between small tumors and those who were invading surrounding structures.



	T 1	T 2	T 3	T 4
number	0	8	17	6
superior rect	0	4	10	3
inferior rect	0	4	7	3

Figure 3. T stage and localization of the rectal tumors

A percentage of 45.1% did not have any lymph node metastasis, being classified as N0. The N0 stage was evenly distributed between superior and inferior rectal tumors. N1 stage was more frequently present in the tumors situated in the superior rectum, N2 had a similar distribution among the superior and inferior rectal localization of the tumors.

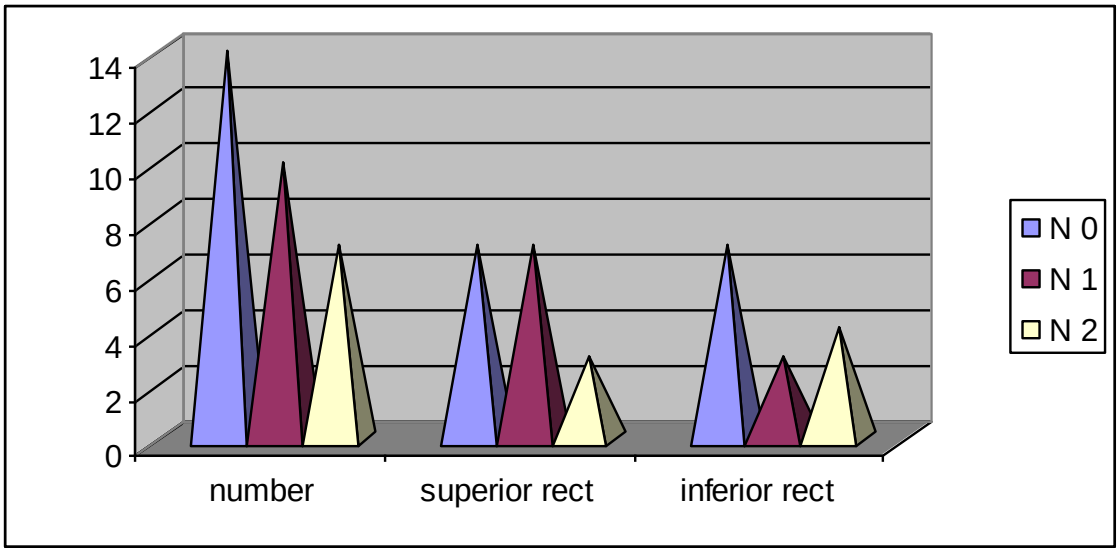


Figure 4. Limphnodes metastasis and localization of the rectal tumors

Blood tests were performed for all the patients and anemia and leukocytosis were selected as parameters in order to evaluate cancer evolution and the relation between the size of the tumor, anemia and systemic inflammatory response.

Anemia was present in half of the patients enrolled in our study in all T stages.

Leukocytosis was an important parameter in order to relate it to local spreading and to further study the microvessel density.

DISCUSSIONS

There are reports that rectal cancer risk increases with age, most of the cases being diagnosed around the age of seventy, with a mild increase in most of the European Union countries (1), most likely due to general population increase in life expectancy. A recent study from USA on a large number of cases that included 393.241 of patients diagnosed with colo-rectal cancer in the period between 1975 to 2010, reported a decrease in incidence until 1998, than a small increase (2).

In our study one third of the patients were 70 to 79 years old, a result of the increased life expectancy in our country during last 25 years.

This percentage is the same as those published in literature. As a particular feature of our study we noticed an incresead number of patients (38,7%) with the age between 60 and 69 years enrolled in our study. Literature data certified that in the future less people will suffer from rectal cancer, mostly because of the diet and lifestyle changes.

The median age of rectal cancer appearance is lower in the western part of Romania than that reported in developed countries. One of the possible factors associated with this difference might be the high number of undiagnosed patients with Diabetes mellitus type 2, but also the excessive use of alcohol and tobacco in the area.

Compared to other studies who reported that 15% of the rectal cancer

cases had a genetic component (3), in our study we didn't get any information in this direction.

Also, we evaluated patients according to sex distribution. Literature data separated the incidence of rectal cancer mainly according to male and female sex (4). We noticed a larger number of male population with rectal cancer, the number of cases being three times higher than in the female population. This difference was not statistically significant when the patients life style was taken in consideration (5). According to age, the incidence of rectal cancer was higher for males of age between 60 and 79, and a slightly higher incidence was observed for females of age between 50 and 59, when compared to the male population. Most cases were reported for females of age between 70 and 79. However, when analyzing the age group 50-79, the incidence of rectal cancer was similar in the male and female population. Literature data showed significant differences in rectal cancer incidence when reported to race for both genders (6,7).

Identifying proximal or distal localization of rectal cancer has significant importance because of the differences reported regarding surgical curative operations and local recurrence (8). This is the reason why we considered important to divide the cases of rectal cancer according to patients gender and tumor localization (superior rectum vs. inferior rectum). When reported to the total number of cases, our data revealed an approximately equal number of cases for superior and inferior localization of rectal cancer cases. When dividing cases according to sex, significant differences were noted regarding the inferior localization of rectal cancer in the female gender compared to the male gender; inferior rectal cancer was three times less frequent in females than in males. For superior rectal cancer the number was approximately equal. Our results were different from the literature data, which reports a higher incidence for inferior rectal cancer in both male and female populations, while maintaining the higher prevalence for male gender (9).

Tumor staging has been evaluated in our study in relation to TNM classification, but tumor localization has also been taken into consideration, therefore we divided the population in two groups according to cancer localization (superior part of the rectum, inferior part of the rectum). If literature data reported up to 44% percent of the rectal tumors being T1 (9), in our study there were no tumors in this stage. We had this discrepancy most likely due to lack of screening programs for the general population and especially for the population at high risk for developing rectal cancer. The rather poor medical education of the general population prevents us, the healthcare professionals, to diagnose people in early stages of this disease. The main reason for addressing a doctor is the occurrence of a complication. This matches the fact that most of the cases we enrolled were T3 and T4, literature data showing a decrease of the number of cases with T3 and T4 stages. As a particular feature of our study, we separated the rectum in two halves, superior and inferior, and consecutively analyzed the tumors according to their origin in the superior or inferior region. For both T2 and T4 tumor cases there was an equal distribution among superior and inferior rectum, as for T3 there was a greater number in the superior rectum. A single study divided the patients using the T criteria and the terms proximal and distal rectum (10). According to this study most of the cases were T2 and T3, the larger proportion of the patients presenting T3 tumors, just like in our study. Also the T3 had the same incidence between proximal and distal localization in the rectum, not like in our study, where the proximal tumors had an advanced T staging. The impact of anemia upon

rectal cancer evolution was not researched properly when low levels of hemoglobin where associated with neoadjuvant therapy. Lee et al. showed that a preoperative anemia with a level of hemoglobin lower than 9g/dL tends to associate with a worse outcome of the post-operative chemo-radiation therapy (11), this being the reason why correction of the anemia was recommended before starting the postoperative adjuvant therapy. We did not find any reports about the association between anemia and T staging. In our study patients presented anemia regardless their T stage, but the maximum incidence was found for T3 stage cases.

Leukocytosis had been studied more often than clinical data or anemia when the mechanisms of rectal cancer progression were researched and it looked like leukocytosis had a bigger impact in prognosis of the response to chemoradiation therapy. There were reports emphasizing that pre-therapy leukocytosis lead to a worse prognosis, shorter disease-free interval, shorter long term survival comparing to their normal value of leucocytes counterparts. The pre-operative association of anemia and leukocytosis had a worse outcome than anemia or lekocytosis occurring alone (12). Hannisdal and Thorsen demonstrated that leukocytosis with a raised ESR correlated significant with Dukes staging and were a sign of poor prognosis, together they defined a group of patients with shorter survival. Our data was concordant with those published in the literature, as leukocytosis was related to local advanced cancers, most of the patients presenting with leukocytosis having T3 stage (13).

CONCLUSIONS

Our study had a heterogeneous and representative population. Male gender represented a risk factor for developing rectal cancer, but the number of male patients included in our study was not so large compared with female patients as in other papers. A higher incidence of rectal cancer in the younger population was observed in our study. Leukocytosis was associated with more advanced local stages of rectal cancer.

Aknowlegde

All authors have contributed equally in preparing this manuscript.

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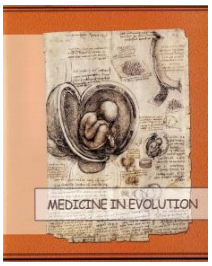
UNUSUAL CAUSE OF A CHRONIC DIARRHEIC SYNDROME IN A YOUNG PATIENT



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ABSTRACT

Cryptosporidium parvum (Phylum Apicomplexa) is a protozoan pathogen responsible for a diarrheal illness called cryptosporidiosis. In immunocompetent hosts it produces a short, self-limiting diarrhea but in immunocompromised patients can lead to chronic, trennant, watery diarrhea associated with malabsorbtion. The histopathological findings can be similar to those seen in celiac disease. The detection of the parasite oocytes attached to the epithelial cells and the lack of the antibodies associated with celiac diseaseis is the key of the correct diagnosis. The best management of these patients is to improve the immune status which will cause the diarrhea to resolve itself. We report a case of a 25-years-old female with chronic diarrheic syndrome and malabsorbtion, first diagnosed in another unit with celiac disease based on the histological aspect, whose symptomatology didn`t improve after gluten-free diet initiation, but resoluted after therapeutical test with trimethoprim-sulfamethoxazole.

Key words: *Chronic diarrhea, HIV infection, Cryptosporidiosis, Celiac disease*

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CLINICAL PRESENTATION

We report on a case of a 25-years-old female with a history of Herpes Zoster in childhood, firstly presented to a medical unit complaining for chronic diarrhea (8-10 watery stools /24 h), significant weight loss (15 kg in the last 2 months) and diffuse abdominal pain. She also associated oligomenorrhea.

The physical examination revealed an underweight patient (BMI= 15,6), multiple hyperchromic macules on legs, arms and abdomen (Figure 1 and 2) and aphthous ulcers of the oral cavity (Figure 3). Laboratory data returned within normal limits, except for a mild hepatocytolisis syndrome (ALT=67, AST=70) with negative viral markers for hepatitis B and C, negative antibodies for Celiac Disease and normal thyroid hormones and TSH.

Figure 1. Hyperchromic macules on legs

Figure 2. Hyperchromic macules on the abdomen

Stool analyisis showed the absence of parasites/bacteria and the test for *Clostridium difficile* toxin A/B in stool was also negative. Both gastroscopy and ileocolonoscopy were normal. Anyway, for excluding celiac disease or microscopic colites, multiple biopsies from the jejunum and colon were performed.

Before the histopathological results were communicated we initiated a gluten-free diet treatment without any clinical improvement for aproximatley 3 weeks of compliance.

Figure 3. Aphtous ulcers of the oral cavity

The decision was to associate Trimethoprim-sulfamethoxazole (suspicion of Whipple Disease). The results were spectacular and clinical improvement was observed within 48 hours (2 consistent stools/day).

The histpathological findings were as follows: villous atrophy with Lieberkuhn crypts hyperplasia associated with increased number of intra-epithelial lymphocytes, images suggestive for Coeliac Disease (Marsh III). The colonic mucosa was normal.

Given this diagnosis, we stopped the treatment with Trimethoprim-sulfamethoxazole and started again the Gluten-free diet combined with Budesonid 9 mg/day. Two weeks later, the symptoms reappeared (diarrhea – 10 watery stools /24 h and abdominal pain) .

This is the moment when the patient, seeking for a second opinion, was addressed to our clinic. The histopathological slides were reviewed and revealed the presence of *Cryptosporidium parvum* in the duodenum (Figures 4 and 5).

The patient was then tested for HIV-antibodies and found to be HIV positive (Anti HIV Ab-251, 8 ELISA). She was transffered to a Infectious Diseases Hospital, where the HIV infection was confirmed and, with the proper treatment initiated, the symptomatology improved.

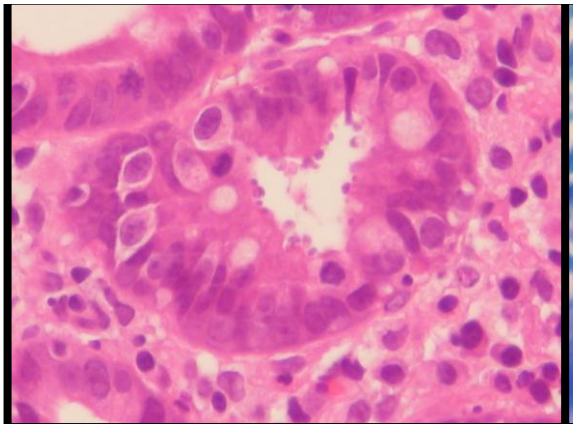

Figure 4. Spherical basophilic bodies in a duodenal crypt with alterative and reactive changes, HE stain, 600x

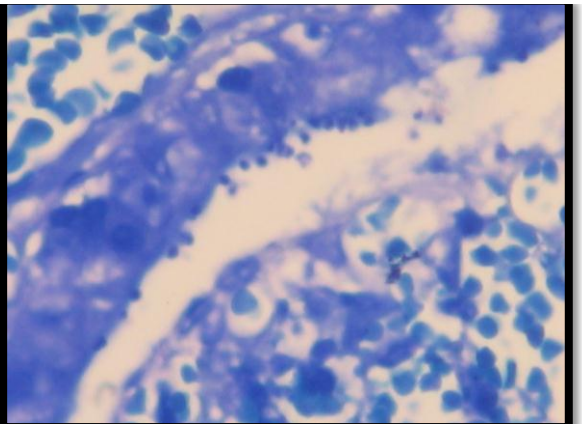

Figure 5. Cryptosporidium on the surface epithelium of enterocytes, Giemsa stain, 600x

DISCUSSIONS

Cryptosporidium was first identified as a cause of gastrointestinal disease in humans in 1976 [1, 10]. It is an intracellular protozoan parasite that along with *Giardia*, it is among the most common enteric parasitic pathogens in humans.

Althought the organisms infect and reproduce in the epithelial cells of the gastrointestinal or respiratory tracts [11], the infection is predominantly associated with diarrhea and biliary tract disease [2].

Transmission can be made by spread from an infected person, or from an environmental source (food/water) that is fecally contaminated. *Cryptosporidium* produces a sporadic, self-limited diarrhea in immunocompetent patients but chronic, life-threatening illness in patients with immunocompromised status (particularly HIV infection)[3]. It is associated with a secretory diarrhea and malabsorption [4].

The diagnosis can be made using the following methods [2,4]:

- Microscopic identification of the oocysts in stool / tissue (biopsy from GI tract), respiratory or bile secretions, bile aspirates.
- Enzyme immunoassay (ELISA) (epidemiologic tool).

In the evaluation of a chronic diarrheic syndrome in a HIV-infected patient, we must consider that it can be caused by infectious agents (most frequently), but it may also be due to infiltrative diseases, such as lymphoma or Kaposi`s sarcoma.

Patients with CD4 cell counts <100 cells/microL [5,6] are at risk for opportunistic infections, such as [7]: *Cryptosporidium*: severe watery diarrhea and weight loss; *Mycobacterium avium, tuberculosis; Histoplasmosis*; CMV*; HSV; Isospora* sp.; *Microsporidium sp*.

As mentioned before, the proper treatment for these patients means to improve the immune system, with specific HIV therapy (HAART), so that the diarrhea will resolve itself without needing specific antibiotics.

In the presented case, the first diagnosis was of Coeliac disease because of the deceitful hystopathological aspect. We remind here the diagnosis citeria for this disease [8,9]: serological screening compatible with Coeliac disease: antigliadin antibody (AGA), antiendomysium antibody (AEA), tissue transglutaminase (tTG) antibody, (all negative in our case); histological findings compatible with Coeliac disease: villous atrophy + crypts hyperplasia + chronic inflamation in lamina propria (lymphocyes, plasmocytes)- all features were positive in the presented case; obvious clinical and serological response to a gluten free diet (GFD) – negative in the presented case.

Rule out other conditions mimicking Coeliac disease such as Coeliac-like lesions (villous atrophy): acquired or ereditar immunodeficiency syndromes (subtotal atrophy, no plasmocytes, Antigliadin antibodies absent); Tropical sprue (epidemiological findings); Intestinal lymphoma; Parasites such as *Giardia sp., Strongyloides sp.,Cryptosporidium sp.*[12].

Association of malabsorbtion but different histological findings we must consider also: Whipple disease (*Trophyrema Whippeli*)-(1/1000000, PAS + spumous macrophages); Crohn Disease (transmural inflammation); chronic pancreatitis; eosinophilic gastroenteritis; Zollinger–Ellison syndrome and intestinal bacterial overgrowth.

CONCLUSIONS

Cryptosporidiosis is rarely the cause of a chronic diarrheic syndrome, so each time we diagnose it, we should immediately test the patient for HIV infection given the strong association between these two conditions. Also, HIV infection should be suspected in any young patient who presents with chronic diarrhea with negative tests for the most frequently associated infectious ethiological causes.

Conflict of interests: none declared

Acknowledgement and Financial support

This paper is partly supported by the Sectorial Operational Programme Human Resources Development (SOPHRD), financed by the European Social Fund and the Romanian Government under contract number POSDRU 141531

We undersign, certificate that the procedures and the experiments we have done respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000 (5), as well as the national law.

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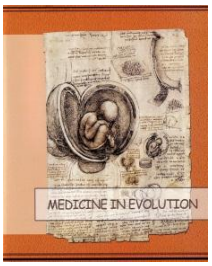
THE ROLE OF HPV INFECTION IN CERVICAL UTERINE CANCER



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ABSTRACT

Human papillomavirus (HPV) is the main cause of cervical cancer; it annually affects more than 490.000 women and causes over 270.000 deaths around the world. In Timis County, there are around 150 women diagnosed with cervical cancer every year. HPV test detects the presence of HPV DNA in a sample taken from the cervix. It detects the presence of HPV infection, as well as the types of virus involved in the infection. This study is a prospective study which presents a statistical analysis of a group of 982 female patients who underwent HPV vaccination as primary prevention against cervical uterine cancer (CUC). At the moment, HPV vaccine is a reality. Vaccination against cervical uterine cancer, Cervarix, was administrated in 3 doses, over a period of 6 months.

Key words: *Human papillomavirus, cervical uterine, cancer prevention, Vaccines availab HPV types, 16 and 18*

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INTRODUCTION

Knowing these methods of prevention and intervention in early detection of CUC has not only theoretical significance, but also a practical one, as it is known that Romania recorded the highest incidence of cervical uterine cancer in Europe. In Europe, every 18 minutes a woman dies of cervical uterine cancer. HPV is the cause of more than 90% of all cases of cervical uterine cancer, the second leading cause of cancer death in women. However, only 1%-5% of HPV infected women will develop cancer. Information on HPV prevalence are contradictory, because there is no standard program on detecting and treating this infection (1, 2, 3, 4).

Cancer prevention is a too broad and important action to be considered a doctors-exclusive activity. Many economic and socio-cultural factors are employed to contribute in increasing the efficiency of cancer disease prevention (5). Therefore, it results into some peculiarities of anticancer fight, which refers to environmental factors and a few harmful habits of the individual – all constituting means of primary prevention, so that those referring to the ill and to the early detection and diagnosis of the disease, to compile elements of secondary prevention.

Primary prevention remains a major challenge with huge medical and economic value, still hard to assess; only multidisciplinary epidemiological studies can make progress in detecting and neutralizing the aggressive factors in the life and work environment, as well as detecting high-risk population groups, with conditions endogenously predisposed, possibly hereditary determined. The opinion by which 80%-90% of human cancers are directly or indirectly caused by environmental factors, must not necessarily lead to the assumption that in the near future a just as high percentage of illnesses from cancer could be prevented (6, 7). However, applying effective measures against smoking, for instance, could prevent various localization neoplasias, in a significant proportion.

MATERIAL AND METHODS

This study is a prospective study which presents a statistical analysis of a group of 982 female patients who underwent HPV vaccination as primary prevention against cervical uterine cancer (CUC). CUC does not occur in the absence of HPV infection; therefore, the vaccines that prevent it will also protect against the development of this form of cancer.

Cervarix is a vaccine recommended for the prevention of precancerous cervical lesions and cervical uterine cancer, determined by human papillomaviruses (HPV = human papillomavirus), types 16 and 18 (8, 9, 10, 11, 12, 13).

Thus, our group which has undergone vaccination is presented in Table 1 and Chart 1, 2 and 3:

Table 1. *Anti - HPV vaccination*

Age Group	Dose 1 (D1)	Dose 2 (D2)	Dose 3 (D3)	Total number of doses
12-14 yo	207	148	125	480
15-19 yo	12	2	9	23
20-24 yo	637	407	142	1186
>24 yo	126	112	29	267
Total	982	669	305	1956

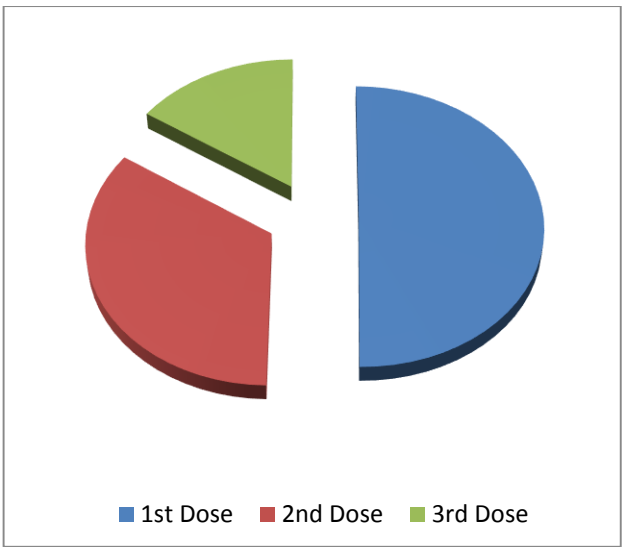

Chart 1. Representation of number of vaccine doses used in our study, for all age groups

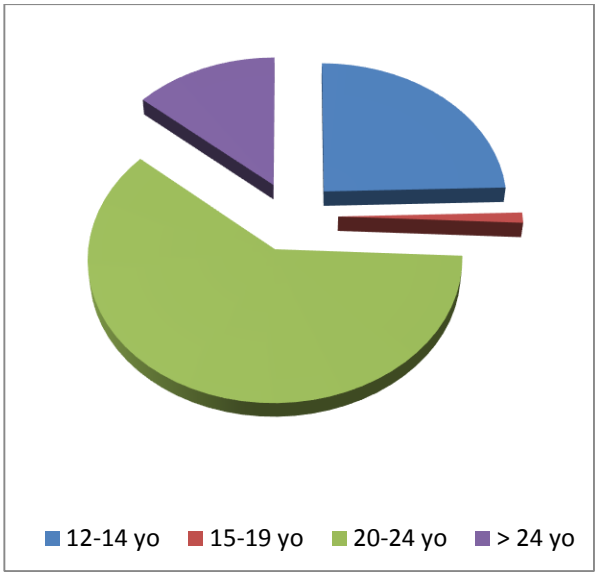

Chart 2. Representation of the number of vaccine doses used in our study, depending on age groups

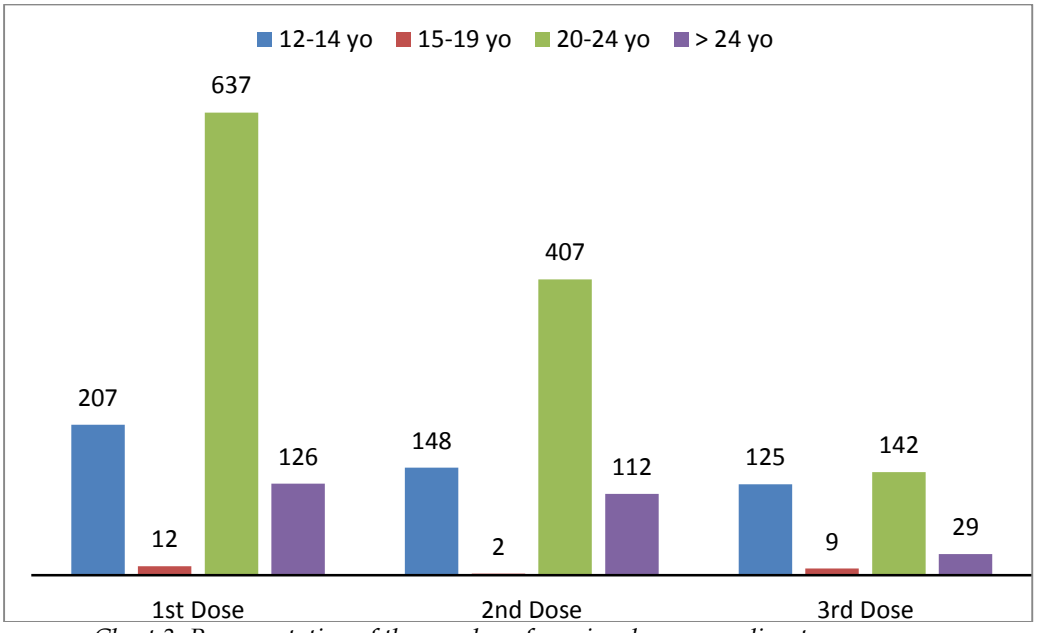

Chart 3. Representation of the number of vaccine doses according to age groups

RESULTS AND DISCUSSIONS

Vaccination against cervical uterine cancer, Cervarix, was administrated in 3 doses, over a period of 6 months. In general, vaccination was well tolerated, but there were some side effects such as redness or mild pain at the injection side, but fleeting.

According to the charts, one can notice a decrease in the vaccinations number on all age groups, in terms of 2nd and 3rd doses, decrease caused by the patients' non-addressability regarding the HPV vaccine continuance.

According to the charts, there is a higher addressability in patients with ages between 20 and 24: 1186 doses, which represents 60.6% of the total of 1956 doses used (out of a total of 2946 doses which should have been applied to our group). Unfortunately, health education and self interest are poor. There have not been ran any specific studies regarding this vaccine in pregnant women. During a clinical development program, before approval, a total of 1737 pregnancies were reported, of which 870 received Cervarix. Overall, the proportion of pregnant women who had specific outcomes (eg. normal infant, abnormal infants, including congenital anomalies, premature birth and spontaneous abortion) was similar among the groups receiving this treatment (14).

There was no case of pregnancy in our group.

Animal studies do not indicate direct or indirect harmful effects on fertility, pregnancy, embryonic/fetal development, birth or postnatal development. The data is insufficient to recommend use of Cervarix during pregnancy. Therefore, vaccination should be postponed until after completion of pregnancy (15, 16, 17, 18, 19).

CONCLUSIONS

- Vaccines are now available to protect against the two of the most common oncogenic HPV types, 16 and 18, which together determine about 2 out of 3 cases of CUC and many abnormalities shown by cytological testing.
- One can observe a decrease in the number of vaccinations at all age groups, in terms of 2nd and 3rd doses, decrease due to patient's non-addressability regarding the HPV vaccine continuance.
- Adolescents' vaccination against HPV type 16 and 18, along with a cytological screening for cervical uterine cancer, every 3 years, may reduce the incidence of cervical uterine cancer by 94%, compared to the lack of any intervention (20). Moreover, it will significantly reduce the number of anomalies detected by screening test, abnormalities which, in time, require medical attention. The efficacy of the screening, regarding the cervical uterine cancer, increases simultaneously with its frequency. Aggressive forms and premalignant lesions are less likely to go undetected when the testing interval is shorter. However, the additional benefits that come with every supplementary testing progressively decrease with increasing frequency.
- In addition, we showed that the vaccine ensures cross-protection to 40.6% of the women vaccinated against any cytological disturbances, caused by other oncogenic types of virus. The seroprotective level of anti-HPV 16 and anti-HPV 18 antibodies (igG

determined by serologic testing) has been reached in a percent of over 100% vaccinated women and has been kept for more than 82 months after the administration of the last vaccine dose. The maximum immune response was detected immediately after completing vaccination cure. Antibodies were stored for 7.3 years (at present) of continued surveillance, starting with the administration of the first dose. Moreover, the neutralizing ability of the developed antibodies has been demonstrated. In women who initially were HPV-16 and/or HPV-18 seropositive, the vaccine caused the development of an antibody level similar with the one in women who initially tested seronegative, their titer being considerably higher (minimum 11 times higher) than the one produced by the naturally endured infection. The data show the futility of HPV testing before vaccination.

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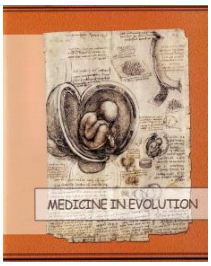

ANALYSIS OF MORTALITY AND MORBIDITY IN PATIENTS WITH ACUTE STROKE ADDMITTED IN THE INTENSIVE CARE UNIT. A RETROSPECTIVE STUDY

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ABSTRACT

Statistics show that stroke is the third leading cause of death worldwide after cardiovascular diseases and neoplasms [1].

Our study compared the number of days of ICU staying, the duration of mechanical ventilation and mortality of patients with stroke. From all the patients addmitted with stroke, 20% were diagnosed with ischemic stroke (Group I) and 80% had hemorrhagic stroke (Group II). Group I had a mortality rate of 69% and Group II had a mortality rate of 55%. A detailed analysis of data showed differences between the two groups regarding age, sex, duration of mechanical ventilation, lenght of ICU stay and comorbidities.

Duration of hospitalisation and mechanical ventilation time is influencing the management in the Intensive Care Unit. Stroke is still a challenge for the intensivists and neurologists.

Key words: *Stroke, Ischemic, Hemorrhagic, Mortality*

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INTRODUCTION

The acute manifestation of cerebrovascular disease, stroke, can cause considerable morbidity and mortality. In the same time, stroke can be followed by persistent residual disability and socio-economic consequences.

Statistics show that stroke is the third leading cause of death worldwide after cardiovascular diseases and neoplasms [1]. This entails high costs for hospitalization and recovery.

The common term for cerebrovascular disease is `` stroke``. Immediate and long-term prognosis of stroke is different depending on the type (embolic, thrombotic, hemorrhagic) and it can be regressive or fatal [1].

Stroke is defined as an acute focal neurological deficit caused by cardiovascular disease, which lasts longer than 24 hours or is causing death before 24 hours [2]. The neurological dysfunction can be focal and is typical for vascular occlusion, or global, when vascular rupture occurs followed by hemorrhage, sometimes with mass effect.

Brain tissue infarction (ischemic stroke) occurs as a result of inadequate perfusion derived from occlusion of cerebral blood vessels in combination with inadequate collateral circulation [2]. A hemorrhagic stroke is defined as an acute, spontaneous, nontraumatic extravasation of blood into the brain parenchyma wich may extend to the ventricular system or subarrachnoid space [3].

Strokes are considered major emergencies because they can cause severe profesional disability for the patient and may present serious complications such as: pulmonary embolism, aspiration pneumonia, myocardial infarction, cardiorespiratory arrest, vascular coma, peripheral venous thrombosis. We already know that reducing major risk factors could prevent a big number of deaths and disabilities resulting from brain vascular disease, but the management of the acute onset of stroke has a high relevance.

AIM AND OBJECTIVES

This study presents a retrospective analysis of data from Intensive Care Unit of the Emergency County Hospital Timisoara. Data usage and correlation was approved by the Ethic Committee from our hospital: informed consent was waived because of the retrospective nature of the study.

Patients and inclusion criteria

In this single center retrospective study were analyzed data obtained from patients admitted in Intesive Care Unit between January 2014 and December 2014. Patients with the following diagnosys were selected for further detailed analysis: documented clinical and imaging (computed tomography or magnetic resonance investigations) diagnosis of stroke (ischaemic or hemorrhagic); men and women aged 25 to 80 years old. Exclusion criteria were: traumatic brain injuries, patients younger than 25 or older than 80 years; patients with uncertain diagnosis, suspected brain tumor, encephalitis or meningoencephalitis with acute onset, syncope, epilepsy, drug intoxication, poliomyelitis. A complex statistical analysis was performed regarding age, sex, length of ICU stay, discharge status (died or improved), duration of endotracheal intubation and patient comorbidities.

Statistical analysis

Results are reported as mean ± standard deviation. For comparison of the variables t test was used. Statistical

significance is defined as: where $p<0.001$ the difference is significant for the group were the numerical value is greater, and where $p<0.05$ there is no statistical significance. Analysis was performed with Microsoft Office Excel for Mac 2011 v.14.4.7. (Microsoft Corporation) and Prism 6 for Mac OS X v.6.0. (GraphPad Software, Inc.).

RESULTS

Between January 2014 and December 2014, 1292 patients were admitted in the Intensive Care Unit of the Emergency Clincal County Hospital, Timisoara. 15% of them (194 patients) were diagnosed with stroke and they all benefited of the management of stroke in the Intensive Care Unit (mechanical ventilation, medication for patients comorbidities and complications developed during the hospitalisation in the Intensive Care Unit, monitoring of vital signs, imaging and laboratory tests). From all the patients with stroke 80% (155 patients) were with haemorrhagic stroke (classified into intracerebral haemorrhage-12% (18 patients) and subarrachnoid haemorrhage-88% (137 patients)) and 20% (39 patients) had ischaemic stroke (divided into: left 69% (27 patients) and right 31% (12 patients)).

Demographic and clinical characteristics of the study population is showed in Table 1.

Table 1. Demographic and clinical characteristics of the study population

Characteristics	Ischaemic Stroke (n = 39)	Haemorrhagic Stroke (n =155)	
Sex (% M)	58% (23)	48%(75)	
Lenght of ICU stay			
0-7 days	21%(24)	79%(91)	
8-15 days	16%(6)	84%(32)	
16-23 days	20%(3)	80%(12)	
24-30 days	18%(2)	82%(9)	
30-60 days	42%(5)	58%(7)	
>60 days	0	100%(4)	
Discharge status			p value
Died		55%(86)	p < 0.01
Improved	69%(27)	45%(69)	p < 0.01
	31%(12)		
Duration of endotracheal intubation			
0-23 h	26%(10)	36%(56)	
24-95 h	36%(14)	13%(20)	
>96 h	38%(15)	51%(79)	
Comorbidities			
Hypertension	74%(29)	58%(90)	
Heart disease	56%(22)	14%(22)	
Lung disease	44%(17)	5%(8)	
Kidney disease	15%(6)	6%(10)	
Liver disease	5%(2)	6%(9)	
Diabetes	36%(44)	10%(15)	
Obesity	8%(3)	8%(12)	
No comorbidities	10%(4)	30%(47)	

The mean age of the patients was 59 years old (with limits between 28 and 80 years old), being 65 years old for the ischemic stroke (64 for the patients with ischemic stroke in the left cerebral hemisphere and 68 for the patients with ischemic stroke in the right hemisphere) and 55 years old for the haemorrhagic stroke (mean age of 60 years old for the patients with intracerebral haemorrhage and 57 for the patients with subarrachnoid haemorrhage).

Mortality rate of all the patients admitted with stroke regardless of etiology was 58% (113 patients), compared with the global mortality rate in the Intensive Care Unit of 45% (583 patients).

Depending of the type of stroke the study group was divided into 2 categories: Group I (ischaemic stroke) and Group II (haemorrhagic stroke).

Group I: 39 patients had ischemic stroke at addmission (20% from all the patients addmitted with stroke in the Intensive Care Unit). Patients from this group had a global mortality rate of 69% (27 patients). We emphasize ischemic stroke in the left hemisphere with a mortality rate of 74% (20 patients) and ischemic stroke in the right hemisphere of 58% (7 patients). (Table 2).

Table 2. Discharge status

Discharge status	Group I (n = 39)	Group II (n = 155)	**p value**
Died	69%(27)	55%(86)	p <0.01
Improved	31%(12)	45%(69)	p <0.01

From the total of patients with ischemic stroke in the left hemisphere, 71% died (20 patients) and 65% of them (13 patients) were men. A lower mortality rate was seen in patients with ischemic stroke in the right hemisphere, 58% (7 patients), and 28% of them were men (2 patients).

Group II:155 patients had haemorrhagic stroke at addmission (80% from all the patients addmitted with stroke in the Intensive Care Unit). This group had a global mortality rate of 55% (86 patients). Differentiated by diagnostic, mortality rate was 77% (14 patients) for the patients with intracerebral haemorrhage and 52% (72 patients) for the patients with subarrachnoid haemorrhage, caused by aneurisms and arteriovenous malformations.(Table 2).

From the patients with intracerebral haemorrhage, 77% died (14 patients) and 64% of them were men (9 patients). Patients with subarrachnoid haemorrhage had a lower mortality rate of 52% (72 patients) and 54% of them were men (39 patients)(Figure 1).

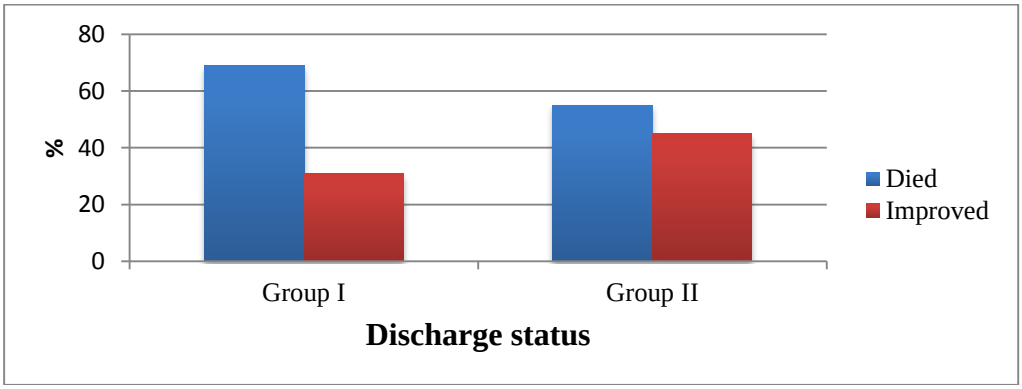

Figure 1. Discharge status: From both groups the mortality was higher than the survival rate, but in Group I the mortality rate was more than twice higher compared with the survivals

The lenght of hospitalization varried between one day and 76 days, with an average of 11 days. Analysing the duration of Intensive Care Unit stay, we differenttiated the hospitalization time for the deceased patients from the lenght of Intensive Care Unit stay for the patients who survived. Thus, patients who's cause of death was the ischemic stroke had a mean hospitalization time of 13 days. The patients who survived from the ischemic stroke group had a mean hospitalization time of 8 days. From the haemorrhagic group, the patients who deceased had a mean lenght of ICU stay of 15 days. Appart of them, the survivors with haemorrhagic stroke had a mean hospitalization time of 7 days.

Depending of the lenght of stay in ICU, Group II had a longer hospitalization time compared with Group I (Table 3), and there were no patients from Group I with more than 60 days of intensive care hospitalisation.

A high statistical significance had the hours of mechanical ventilation in the ICU (Table 4).

Table 3. Depending of the lenght of stay in ICU, Group II had a longer hospitalization time compared with Group I

Lenght of ICU stay	**Group I (n = 39)**	**Group II (n = 155)**	**p value**
0-7 days	21%(24)	79%(91)	0.9282
8-15 days	16%(6)	84%(32)	p <0.01
16-23 days	20%(3)	80%(12)	p <0.01
24-30 days	18%(2)	82%(9)	p <0.01
30-60 days	42%(5)	58%(7)	p <0.01
>60 days	0 (0)	100%(4)	-

Table 4. Depending on the duration of the orotracheal intubation,, the mortality was higher in Group II if the ventilation time was less than 23 hours or more than 96 hours. Between 24-95 hours the higher mortality rate occured in Group I

Duration of endotracheal intubation	Group I (n = 39)	Group II (n = 155)	**p value**
0 – 23 h	26%(10)	36%(56)	p <0.01
24 – 95 h	36%(14)	13%(20)	p <0.01
> 96 h	38%(15)	51%(79)	p <0.01

The analisys of the necesity of mechanical ventilation revealed the followings:

a) For Group I, 26% of the patients needed less than 24 hours of mechanical ventilation. From the patients with ischemic stroke in the left hemisphere 22% from the group, and for the patients with ischemic stroke in the right hemisphere, 33% from the group. Another 36% needed between 24 and 95 hours of ventilatory support, and from those, 33% were from the subgroup with ischemic stroke in the left hemisphere and 41% from the subgroup with ischemic stroke in the right hemisphere. The rest of 38% from Group I needed more than 96 hours of mechanical ventilation, with a percentage of 44% for the patients with ischemic stroke in the left hemisphere and 25% for the patients with ischemic stroke in the right hemisphere.

b) For Group II, 36% of the patients needed less than 24 hours of ventilatory support; 27% from the patients with intracerebral haemorrhage and 37% from the patients with subarrachnoid haemorrhage. Between 24 and 95 hours of mechanical ventilation they were only 13% from the patients with haemorrhagic stroke; 11% were from the patients with intracerebral haemorrhage and 13% from the patients with subarrachnoid haemorrhage. 51% from Group II needed more than 96 hours of

mechanical ventilation; 61% from the patients with intracerebral haemorrhage and 49% from the patients with subarrachnoid haemorrhage.

Regarding the comorbidities, the higher risk factor for Group I was hypertension (74%), followed by heart disease (56%), lung disease (44%), diabetes mellitus (36%) and kidney disease (15%). 10% from the group had no comorbidities at the admission time. In Group II, the higher risk factor was hypertension (58%), followed by heart disease (14%), diabetes mellitus (10%) and obesity (8%). 30% from the group had no comorbidities at the admission time (Table 1).

In hypertensive patients, no specific agent has been proven to be clearly superior to all others for stroke protection [4].

DISCUSSIONS

In our study the haemorrhagic stroke had a four time higher incidence than ischemic stroke.

The global mortality rate in patients addmitted in the Intensive Care Unit with stroke is still much higher than the total addmissions (58% versus 45%). The higher incidence regarding the etiology was the subarrachnoid haemorrhage 88%, followed by the ischemic stroke in the left hemisphere 69%. The mean age for the ischemic group is higher in our analisys, 65 years old versus 55 years old for the haemorrhagic group.

Our study revealed that male sex had a higher mortality rate in both ischemic and haemorrhagic strokes.

A report from the American Heart Association showed that women have lower age-adjusted stroke incidence than men; however, sex differences in stroke risk may be modified by age [5,6]. Male sex was another independent predictor of prolonged hospital stay. Cohort studies indicate that women and men have differences in risk factors profiles, acute stroke presentation, and stroke etiology [7] but clinical outcome as well as the number of patients with a favorable clinical outcome did not differ significantly between women and men [8]. Male sex was also a predictive factor of prolonged hospitalization in the study of Chang et al. [9]. It is not clear whether the influence of sex on length of hospital stay reflects the impact of culture difference or is due to other factors.

We compared the lenght of stay in ICU for Group I and Group II. The statistical analysis showed a significant difference between Group I and Group II, with a higher number of patients in Group II. Analysing for each group separately, the hospitalisation time for the first 30 days is almost constant for both groups (16-21% from patients in Group I compared with 79-84% for patients in Group II). Between 30 and 60 days of intensive care and management, the lenght of stay in ICU was higher in Group I (42%) compared with the first 30 days, but in Group II was lower (58%). There were no patients with a lenght of ICU stay more than 60 days for the ischemic stroke patients.(Figure 2).

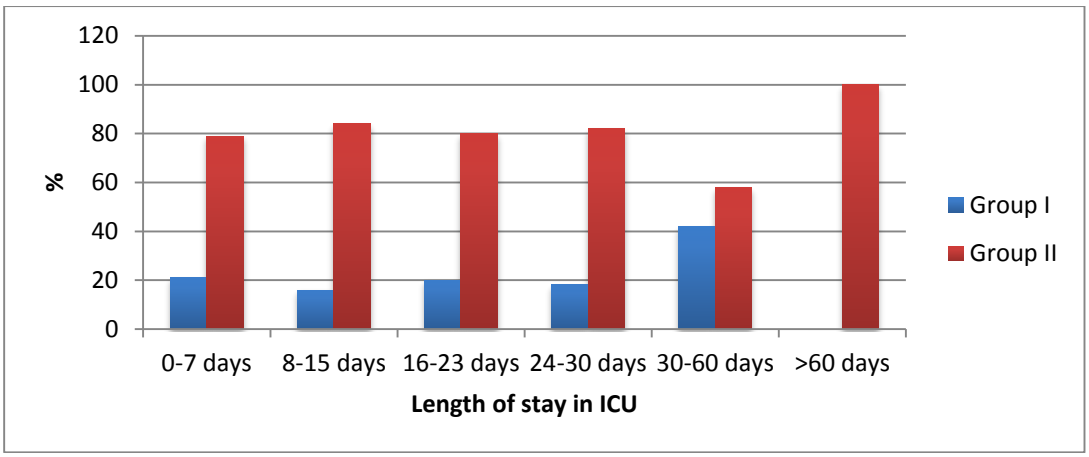

Figure 2. Lenght of stay in ICU: Group II had a longer hospitalization time compared with Group I

A high statistical significance had the hours of mechanical ventilation in the ICU (Table 4). Taking in consideration the duration of orotracheal intubation, the mortality rate is significantly higher for Group II for ventilation time less than 23 hours (36%) or more than 96 hours (51%), while in Group I the ventilation time less than 23 hours was 26% and for mechanical support more than 96 hours was 38%. Between 24-95 hours of ventilation, the mortality was higher in Group I (36%) in comparation with Group II (13%) (Figure 3).

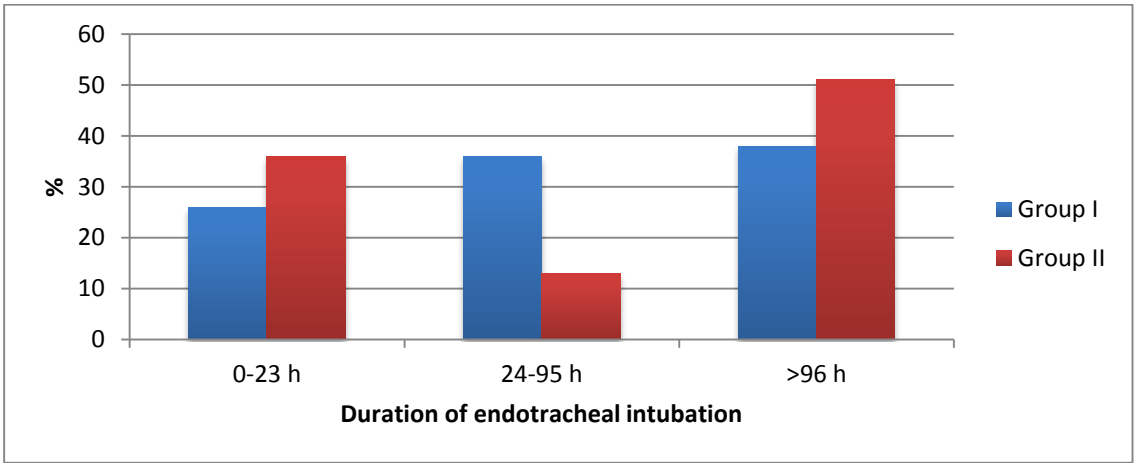

Figure 3. Depending on the duration of orotracheal intubation, the mortality was higher in Group II if the ventilation time was less than 23 hours or more than 96 hours. Between 24-95 hours the higher mortality rate occured in Group I

Unfortunately, statistical analysis of the influence of specific treatments was not feasible in this study because treatments differed considerably in patients with ischemic and hemorrhagic stroke. Symptomatic management of stroke will be more important in the next future as new therapies are being developed. Mechanical ventilation should be considered as an additional measure for the control of intracranial hypertension in patients with severe stroke. Our study showed that most ventilated patients with stroke still die within the first few weeks after admission.

The present study showed that mortality in stroke depends on several factors, others that the preexisting risk factors.

For a long time, early death has been shown to be higher in patients with hemorrhagic stroke than in those with ischemic stroke [10,11]. In this study, the mortality rate was higher in patients with ischemic stroke. Several other studies including general

population-based studies of stroke [12,13] have also found older patients with stroke to have a poorer prognosis, not only those mechanically ventilated [10].

During ICU admission, clinical and biological investigations were done on a regular basis.

Potentially modifiable risk factors are those that can be modifiated or ameliorated with appropriate behavioral, pharmaceutical or other medical interventions. Modifiable risk factors account for the majority of strokes [3,5]. This paper focuses on seven comorbidities (hypertension, other cardiac disease, lung disease, kidney disease, liver disease,diabetes mellitus, obesity). Hypertension looked to be the first on the etiology factors, but in the haemorrhagic group there were more patients with no comorbidities (30%) compared with the ischemic group (10%).

CONCLUSIONS

Accurate information about hospital resource utilization is necessary for management of health care services. The analysis of prolonged length of hospital stay can provide valuable data for planning and policy in the health care system.

Stroke is a major cause of death and disability. While acute interventions in acute management of stroke have advanced in recent years, prevention remains key in reducing the high number of patients with stroke.There are important known risk factors for stroke. Non-modifiable risk factors include age, sex, race and family history. While not amenable to intervention, these later factors warrant attention, as they may help identify populations and individuals who are at increased risk and who may benefit from screening and from intensive treatment or prevention of modifiable risk factors.

The management of acute stroke, ischemic or hemorrhagic, still remains a big challenge for the intensivists and neurologists. Not only the verry known risk factors represent a danger for the evolution of the patients with stroke, but the duration of hospitalisation and the mechanical ventilation time are good predictors of survival. The key remains the prevention of stroke from both health and financial point of views.

Treatment with antihypertensive therapy was associated with significant reductions of between one fifth and one quarter in stroke, nonfatal stroke, MI, and combined vascular events [14].

Finally, we concluded the followings:

1. Mortality rate of all the patients admitted with stroke regardless of etiology was higher compared with the global mortality rate in the Intensive Care Unit.

2. Admission rate was higher for the haemorrhagic stroke group compared with the ischemic stroke group, but mortality remained higher in the ischemic stroke group. Analysing separatelly for each group, mortality was higher in patients with ischemic stroke in the left hemisphere from Group I, and for patients with intracerebral haemorrhage from Group II.

3. Mean hospitalisation time did not vary to much for the deceased patients compared with the survivors for each group, but Group II had a longer Intensive Care Unit stay compared with Group I.

4. After we analysed the mechanical ventilation hours, we concluded that the mortality rate was higher in the haemorrhagic stroke group for ventilatory support less than 23 hours or more than 96 hours. Between 24 and 95 hours, mortality rate was higher in the ischemic stroke group.

5. Comorbidities like hypertension, other cardiac disease,

lung disease, kidney disease, liver disease, diabetes mellitus, obesity, are still considered high risk factors in the mortality of stroke.

Compliance with Ethical Requirements

Lavinia Anca Mozos, Ovidiu Horea Bedreag and Liviu Matcau declare that they have no conflict of interest. For the study we have the approved form to use the clinical and demographic data of the patient from the Ethical Committee of Emergency County Hospital Timisoara.

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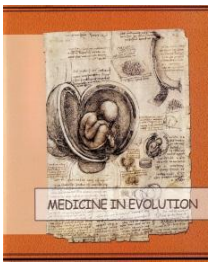
IMPLEMENTING EURAHS–QOL SCORE TO EVALUATE THE SURGICAL OUTCOMES FOR VENTRAL HERNIA REPAIR



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ABSTRACT

Introduction. *EuraHS-QoL score can be applied pre- and postoperatively thus the impact of surgical treatment on the patients' quality of life can be investigated.*

Materials and methods. *The EuraHS-QoL questionnaire was applied pre- and 30 days postoperative to all 70 consecutive patients from July 2012 until December 2013 (18 months) at Surgery Clinics II – Emergency County Hospital Timisoara. Data were collected and analyzed separately for each answer designated by letters for statistical reasons. Mean values and standard deviation have been calculated for each question and 95% confidence interval, with p value = 0.05 have been used.*

Results. *Statistic significance was valid for all answers, the biggest difference was for the pain at the site of hernia/hernia repair (2.71±1.70; p=0.23). One answer (out of nine) described a different behavior pre- and postoperative. We have interpreted it as being more subjective than other questions about the pain, being related to a general feeling over a period of time. Ratio between figures for every question indicated that pain increased more postoperative, (1.92-2.39) than the cosmetic discomfort (1.23-1.32).*

Conclusions. *Questionnaire is objective, the patient followed a similar approach pre- and postoperative. The pain was perceived as being more important than the cosmetic appearance*

Key words: *EuraHS–QoL score, surgical outcomes, ventral hernia repair*

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INTRODUCTION

Data about relationship between risk factors, postoperative complications, recurrence rates and satisfaction before and after ventral hernia repair for incisional hernia with implantation of a surgical mesh are collected for randomized clinical trials [1]. National Scandinavian registries, like the Swedish Hernia Database and the Danish Hernia Database on hernia surgery were designated to allow the detection of poor and good results, if they appear more frequently than expected [1-5]. Veterans Affairs Medical Centers database and the National Surgical Quality Improvement Program database have been also able to detect outcome results in hernia surgery [1, 6-8]. Several quality-of-life (QoL) scores have been used after surgery. The SF-36 has become the most widely used measure of general health in clinical studies throughout the world. [9] Short Form 36 (SF 36) is a validated QoL assessment tool that is used for surgery in general. For evaluation of patients after hernia repair and specifically after mesh implantation, a QOL score has been developed by Heniford et al. at the Carolina Hernia Centre in Charlotte, NC, USA [10]. This Quality-of-Life scale is commonly referred to as the Carolina Comfort Scale (CCS). In order to develop a registry for operations on abdominal wall hernias the European Hernia Society board launched the project named EuraHS (European Registry for Abdominal Wall HerniaS) [1]. The EuraHS working group proposed a"EuraHS-QoL"score for evaluation of QOL before and after ventral hernia repair. The EuraHS-QoL adds in particular, assessment made pre- and postoperatively and by including a cosmetic dimension which is an important but understudied element in ventral hernia repair.

Aim and objectives

The primary objective of our study was to implement in our Clinics the EuraHS-QoL score for the measurement of surgical outcomes of hernia repair and to practically use it to evaluate the quality of life for patients that underwent that surgical procedure in a period of 18 months.

MATERIAL AND METHODS

Electronic medical records of all 70 consecutive patients who had a ventral hernia repair from July 2012 until December 2013 (18 months) at our Surgery Clinics II from Emergency County Hospital Timisoara were retrospectively reviwed. We have used the"EuraHS-QoL"score for evaluation of QOL before and after ventral hernia repair that is based on a Numerical Rating Scale for three dimensions: pain at the site of the hernia or the hernia repair, restriction of activities and cosmetic discomfort (fig. 1) [1]. The questionnaire was applied pre- and 30 days postoperative to all 70 consecutive patients. All of them agreed to answer the questionnaire. We have discussed all the issues in advance in order to be sure that the questions were completely understood. For statistical reasons we have noted each question with a letter in alphabetical order and as they appear in the questionnaire, irrespectively"a, b, c, d, e, f, g, i, h". The results were analyzed according to the three big domains of questions (three columns) and the corresponding sub-columns designated by letters accordingly: the first three letters refer to the pain at the site of the hernia, the next four letters to restrictions of activities because of the pain or discomfort and the last two

letters to cosmetic discomfort. Mean values and standard deviation have been calculated for each question and 95% confidence interval, with *p value =0.05* have been used.

EuraHS-QoL Preoperative

Pain at the site of the hernia												
	0 = no pain										10 = worst pain imaginable	
In rest (lying down)	0	1	2	3	4	5	6	7	8	9	10	
During activities (walking, biking, sports)	0	1	2	3	4	5	6	7	8	9	10	
Worst pain felt during the last week	0	1	2	3	4	5	6	7	8	9	10	
Restrictions of activities because of pain or discomfort at the site of the hernia												
	0 = no restriction										10 = completely restricted	
Daily activities (inside the house)	0	1	2	3	4	5	6	7	8	9	10	X
Outside the house (walking, biking,driving)	0	1	2	3	4	5	6	7	8	9	10	X
During sports	0	1	2	3	4	5	6	7	8	9	10	X
During heavy labour	0	1	2	3	4	5	6	7	8	9	10	X
											X = If you do not perform this activity	
Cosmetic discomfort												
	0 = very beautiful										10 = extremely ugly	
The shape of your abdomen	0	1	2	3	4	5	6	7	8	9	10	
The site of the hernia	0	1	2	3	4	5	6	7	8	9	10	

EuraHS-QoL Postoperative

Pain at the site of the hernia repair												
	0 = no pain										10 = worst pain imaginable	
In rest (lying down)	0	1	2	3	4	5	6	7	8	9	10	
During activities (walking, biking, sports)	0	1	2	3	4	5	6	7	8	9	10	
Worst pain felt during the last week	0	1	2	3	4	5	6	7	8	9	10	
Restrictions of activities because of pain or discomfort at the site of the hernia repair												
	0 = no restriction										10 = completely restricted	
Daily activities (inside the house)	0	1	2	3	4	5	6	7	8	9	10	X
Outside the house (walking, biking,driving)	0	1	2	3	4	5	6	7	8	9	10	X
During sports	0	1	2	3	4	5	6	7	8	9	10	X
During heavy labour	0	1	2	3	4	5	6	7	8	9	10	X
											X = If you do not perform this activity	
Cosmetic discomfort												
	0 = very beautiful										10 = extremely ugly	
The shape of your abdomen	0	1	2	3	4	5	6	7	8	9	10	
The site of the hernia and the scars	0	1	2	3	4	5	6	7	8	9	10	

Figure 1. EuraHS-QoL Preoperative and EuraHS-QoL Postperative Questionnaires (1)

RESULTS

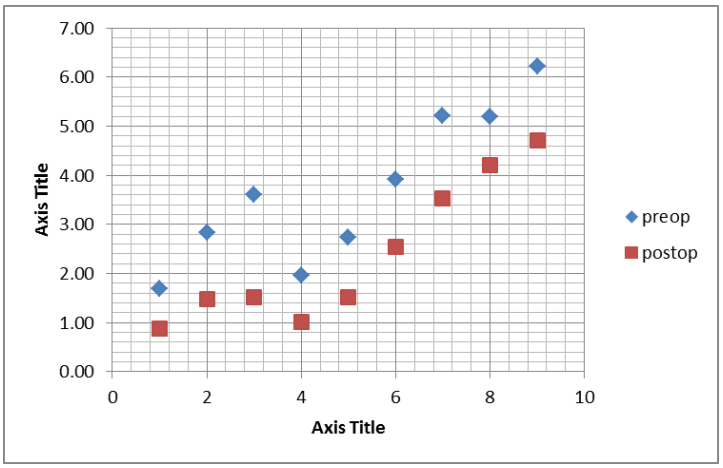
We have colected all the questionnaires and the consecutive raw data for all the questions. The mean value and standard deviation (SD) have been calculated for each answer, pre- and postoperative. In tabel no.1 we have compared the three big domains of questions in terms of mean values ± SD and the *p value*.

Table 1. Comparison between mean values± SD and *p value* pre-and postoperative

Status of the pacient	*Pain at the site of hernia/hernia repair*		*Restrictions of activities at the site of hernia/hernia repair*		*Cosmetic discomfort*	
	media± SD	*p value*	media± SD	*p value*	media± SD	*p value*
preoperator	2.71±1.70	0.23	3.46±1.96	0.23	5.70±2.04	0.34
postoperator	1.29±0.97	0.13	2.15±1.50	0.17	4.45±1.89	0.31

Considering the three big domains of questions we could notice the biggest difference for the pain at the site of hernia/hernia repair, with statistical significance (2.71±1.70; *p=0.23*). For the other two domains of questions the answers had also statistical significance.

Analyzind the behavior pattern of each question we have plotted the graphic no.1. The same patern of distribution have been obvious for restrictions of activities and for cosmetic discomfort.



Graphic 1. Behavior pattern of the answers to the questionnaire

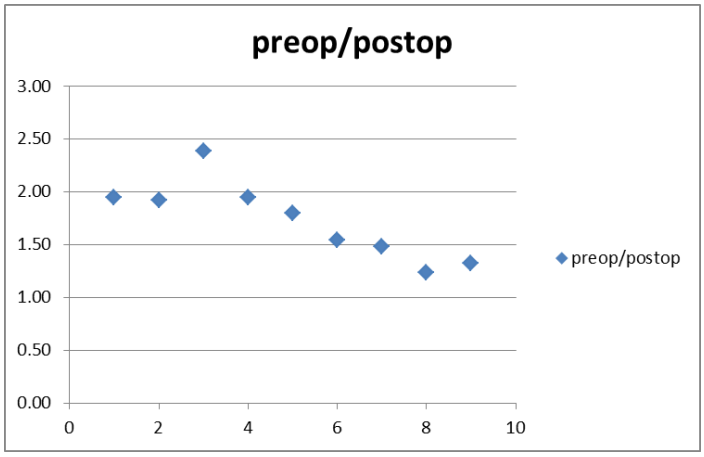
Out of the total number of nine questions, the question noted as"c", meaning"worst pain felt during the last week"described a different behavior pre- and postoperative. We have interpreted it as being more subjective than the other questions reffering to the pain, because is related to a general feeling over a period of time.

The correlation coeficients calculated pre-and postoperative and the mean values differences as well as the ratio between the figures obtained for every question is shown in table 2.

Table 2. Pre-and postoperative correlation coeficients

Parameter	**Pain at the site of hernia/hernia repair**			**Restrictions of activities at the site of hernia/hernia repair**				**Cosmetic discomfort**	
	a	b	c	d	e	f	g	h	i
Correlation coeficient pre/postoperative	0.70	0.68	0.19	0.73	0.78	0.77	0.75	0.86	0.86
Mean values differences pre/postoperative	0.83	1.36	2.10	0.96	1.21	1.37	1.69	0.99	1.51
Ratio pre/postoperative	1.95	1.92	2.39	1.94	1.80	1.54	1.48	1.23	1.32

The ratio between the figures obtained for every question is indicating pain as an indicator of quality of life declines more post operative, (1.92-2.39) than the cosmetic discomfort with just around 23% (graphic no. 2).



Graphic 2. Ratio between the figures obtained for every question.
Legend: preop/postop= pre and postoperative

DISCUSSIONS

Assessing the quality of life (QoL) in patients undergoing a ventral hernia repair is extremely useful to evaluate and measure the surgical outcomes in terms of reasonable functional and cosmetic outcomes, patient satisfaction, and acceptable complication rates [11].

The concern for estimating the QOL after this type of surgery has been constant in the last decades. Recently, in June 2012 EuraHS working group has launched an international online platform for registration and outcome measurement of hernia operations [1]. They provided a questionnaire to evaluate the quality of life, EuraHS-QoL, for 3 dimensions: pain, restriction of activities and the cosmetic outcome by using a numerical rating scale. Carolinas Comfort Scale (CCS) questionnaire was used to assess the outcomes in different reported studies. For instance Zaborszky et all [12] compared two hernia patient groups and found that the type of mesh used in their surgical repair made only a minor difference to their QoL outcomes. They found that responses on the CCS questionnaire clustered into two subscales: 'mesh sensation' and 'pain+movement limitations' were able to discriminate between those who had a recurrence compared with those who had not ($p<0.003$) [12]. In the meanwhile the CCS total score did not reveal which issues (sensing mesh versus experiencing pain and movement limitations) were more important to these patients [12]. Snyder et all, have performed a cohort study included patients from sixteen Veteran's Affairs Medical Centers across the United States who underwent elective incisional hernia repair between 1997 and 2002 [7]. Patient satisfaction, chronic pain (McGill pain scale and visual analogue scale), and health-related quality of life (Short Form 36) were evaluated with a mailed survey at a median of five years after repair[7]. Poelman et all [13] has investigated the long-term health-related quality of life (HRQL) of 101 patients who were treated for incisional hernias using an onlay technique over a period of 10 years (1997–2007). The Short Form 36 (SF-36©) and the Karnofsky Performance Status Scale (KPS) and a semi-structured interview were used to measure HRQL. The results showed that HRQL was the same in patients treated for an incisional hernia compared to the matched controls. [13]. Beltrán et all [14] conducted a study to validate a standard measurement instrument in the form of a questionnaire to report the evolutionary outcomes of a specific open technique to repair incisional hernias. The follow-up consisted in an interview and physical examination 30 days after surgery, at 6 months, 1 year, 2 years, 3 years, and 4 years. Primary outcome measure was reporting the outcomes of hernia repair according to a standardized scale obtained from a questionnaire [14].

Because the EuraHS-QoL score can be applied pre- and postoperatively, the impact of surgical treatment on the patients' quality of life can be investigated. In our study we have used this questionnaire for 70 consecutive patients who had a ventral hernia repair from July 2012 until December 2013 (18 months) at our Surgery Clinics II – Emergency County Hospital Timisoara with the purpose to introduce it as an instrument for further studies on this topic. The results were analyzed comparatively according to the three big domains of questions: pain at the site of the hernia, restrictions of activities because of the pain or discomfort and cosmetic discomfort. We could notice that all the answers were statistically significant (p value=0.05) and the biggest difference was for the pain at the site of hernia/hernia repair pre-and postoperative (2.71±1.70; $p=0.23$). The

ratio between the figures obtained for every answer is indicating that pain declines more postoperative (1.92-2.39) than the cosmetic discomfort (1.23-1.32). The existence of correlation between answers pre-and postoperative is suggesting that the patient had the same reasoning of treating the questions.

CONCLUSIONS

The questionnaire is objective, the patient has followed a similar approach pre- and postoperative. The pain was perceived as being more important than the cosmetic appearance. EuraHs QoL is a valuable tool to assess the surgical outcomes and a practical research instrument for surgical hernia repair.

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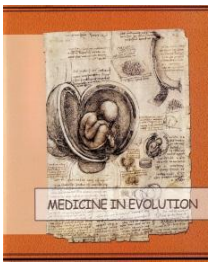
BENIGN LARYNGEAL LESIONS – A CLINICOPATHOLOGICAL STUDY



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ABSTRACT

Introduction: *Benign vocal cord mass lesions are commonly encountered causes of dysphonia which are offen surgically correctable.*

Objectives: *The aim of the study was to evaluation of the age, incidence, sex ratio, occupational factors and symptomatology in correlation with clinical, microlaryngoscopic and histopathological features in benign lesions of the larynx.*

Material and method: *This is a retrospective cohort study carried out over a period of two years from January 2013 to December 2014. The patients were selected on the basic of the following inclusion criteria: hoarseness in voice, foreign body sensation in the throat, throat pain and fatigue of voice. All the patients included in the study were questioned about age, gender, occupation and area of residence. The treatment underwent was microlaryngeal surgery with cold instruments or CO2 laser with biopsy. The results were correlated with white light endoscopy and histopathological findings .*

Results*: The highest incidence was among the age group between 41 and 70 years old (125 cases). The youngest patient was 5 year old and the oldest being 70 years old. The common complaints recorded were hoarseness (100%), followed by vocal fatigue in nearly 59 % and 22% of them complained of foreign body sensation in throat. Most of the cases in the series were observed to have vocal cord polyp (44.97%), followed by papilloma (30.17), leukoplakia and keratosis (5.9%), Reike's edema (5.32%), granuloma and cyst (3.55% each), vocal nodules (2.95%) and TB laryngitis (2.36%).*

Conclusion: *The benign laryngeal lesion produces symptoms which can vary from mild hoarseness to life-threatening stridor. Early diagnosis of the lesions can lead to effective management and good recovery. Surgical removal with microsurgical instruments remains the mainstay of the therapy for laryngeal polyps, cysts and recalcitrant nodules.*

Key words: *benign laryngeal lesions, microlaryngeal surgery, study*

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INTRODUCTION

Benign lesions of the larynx, an uncommon condition, are classified into rare neoplastic and relatively common non-neoplastic tumor-like conditions [1].

The significance of benign lesions of the larynx lies in the importance of its function in speaking and the contribution of the voice to one's identity [2].

Benign vocal cord mass lesions are commonly encountered causes of dysphonia which are often surgically correctable. They include lesions like vocal polyps, vocal cysts, vocal nodules and Reinke's edema. Since 1854 when Manuel Garcia first observed the movement of his own vocal cords using a laryngeal mirror and sunlight as the light source, a number of techniques for demonstrating laryngeal anatomy have evolved [3,4].

Hoarseness of voice is a common complaint in today's high –stressed life and describes terms such as dysphonia, aphonia, voice break and adynophonia and most commonly vocal fatigue. Bartha et al. observed the largest number of cases of hoarseness (70%) in the age group of 20-50 years with a male: female (M: F) ratio of 2.6:1. He also suggested fiberoptic laryngoscopy to be an invaluable tool in the diagnosis of functional voice disorders primarily affecting young adults, frequently non-vocal, non-professional resulting from vocal abuse. Failure of voice therapy to improve or alleviate vocal symptoms is the most common indication for the surgical removal of the lesions. Surgical removal with microsurgical instruments remains the mainstay of the therapy for laryngeal polyps, cysts and recalcitrant nodules [5].

Restoration of normal vocal function in patients with benign glottic lesions is of fundamental importance[6]. A generally accepted principle of vocal fold surgery is that any intervention should be as conservative as possible. Preservation of both healthy mucosa and intermediate and deep layers of lamina propria is important for voice restitution [7].

Aim and Objectives

- Categorization of specific lesions, mucosal disease of the glottis and benign neoplasms and other tumors of the larynx
- Correlation of clinical, microlaryngoscopic and histopathological features
- The role of microlaryngeal surgery in laryngeal lesion
- Age, prevalence, gender and occupational factors evaluation

MATERIAL AND METHODS

A retrospective cohort study was performed on 169 specific lesions, mucosal disease of the glottis and benign neoplasms and other tumors of the larynx over a period of 2 years from January 2013 to December 2014.

Laryngeal specific lesions included Reinke's edema, hypertrophic laryngitis, laryngeal tuberculosis, mucosal disease of the glottis (vocal nodules and polyps), benign neoplasms and other tumours of the larynx (recurrent respiratory papillomatosis and keratinised papilloma), NonEpithelial tumors were vascular and cartilage and bone tumors and pseudotumours include granuloma, amyloid and laryngeal cysts.

All the patients included in the study were questioned about age, gender, occupation and area of residence. A detailed history was obtained, focused on presenting

symptoms and their duration, preexistent or persistent smoking habit, the presence of gastro-esophageal reflux and voice abuse.

The patients were selected on the basic of the following inclusion and exclusion criteria.

Inclusion criteria: hoarseness; foreign body sensation in the throat; throat pain and fatigue of voice; difficulty in swallowing and breathing. Exclusion criteria: patients with clinical diagnosis of malignancy of larynx; all cases with inflammatory lesions; patients with speech defect due to central nervous system (CNS) lesions.

Routine investigations were carried out in each patient. Each patient was evaluated endoscopically in HDTV using 70 degree rigid hypopharyngoscope (Karl Storz, Germany) followed by microlaryngoscopic excision with cold instruments or CO2 laser with biopsy of the benign vocal cord lesion under General Anesthesia with Orotracheal Intubation. Results were compared to histopathological findings. Postoperative management included complete voice rest for 3 weeks followed by gradual resumption of voice in order to resume the normal function of the vocal cords. Along with this regime, the patients were also advised to avoid extremely hot and cold foods, foods with strong seasonings, exposure to air pollutants, smoking, tobacco, alcohol, coughing and clearing the throat. All the patients were subjected to objective measurements at interval of 4 weeks; 12 weeks; and 6 months.

RESULTS

Demographic profile:

The youngest patient in our study was 5 year old; the oldest being 70 years old. The highest incidence was among the age group between 41 and 70 years (125 cases). The mean age in years was 36.75. The average age in males was 41 years old, while in females was 32.5 years old. In our study males/females ratio was 2:1 (i.e. 113 males to 56 females). The distribution of patients according with specific lesions, mucosal disease of the glottis and benign neoplasms and other tumors of the larynx are shown in Table 1.

Occupation and symptoms:

Labor class constituted single largest group of patients (40.12%), followed by housewives (18.32%), teachers (10.5%), students (8.28%) and professional voice singers (1.18%).

The common complaints recorded were hoarseness or change in voice (100%), followed by vocal fatigue in nearly 59% of the patients, foreign body sensation in nearly 22%, throat pain in 13% of the patients and 6% of them complained of difficulty in swallowing and breathing. The duration of symptoms ranged from 1 month to 24 months; the mean duration of illness in months was 4.50+/- 2.32.

Specific lesions, mucosal disease of the glottis, benign neoplasms and other tumors of the larynx and their site of origin:

Most of the cases in the series were observed to have vocal cord polyp (44.97%), followed by papilloma (30.17%), leukoplakia and keratosis (5.9%), Reike's edema (5.32%), granuloma and cyst (3.55% each), vocal nodules (2.95%) and TB laryngitis (2.36%). We found also one man of 47 years old with laryngeal chondroma and one woman of 56 years old with laryngeal amyloidosis.

The commonest site of origin of the tumor was vocal cord with 45.56% on the left vocal cord, 38.46% on the right vocal cord, and for 5.33 %, the site of origin was bilateral. Arytenoids and epiglottis were the next common sites (10.65%). Two cases of papilloma in

adults evolved into malignant tumor as squamous cell carcinoma. In our series we encountered 4 cases of laryngeal tuberculosis; all were secondary to pulmonary affect; only two patients being smokers. Microsurgical interventions were performed according to a conservative technique, as promulgated by Remacle et al. [6] and based on the principle of improving voice by respecting and maintaining the functional structure of the larynx. All the clinically diagnosed cases were later confirmed by histopathology reports. All the patients were totally symptom free with complete recovery. No tracheotomy was necessary.

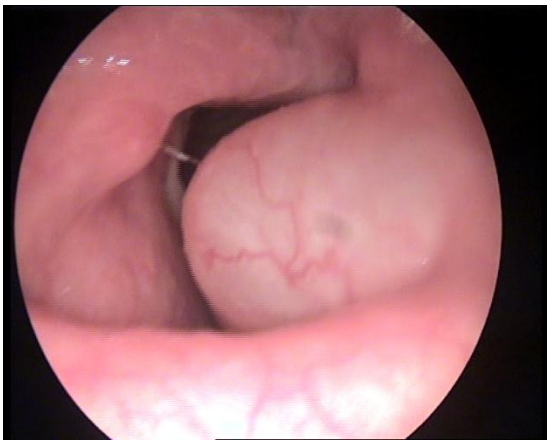

Figure 1. Laryngeal cyst

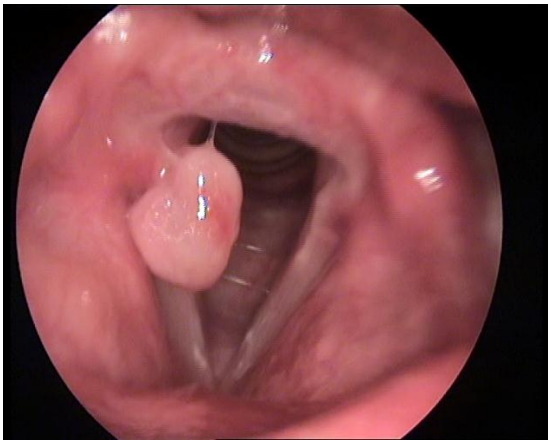

Figure 2. Laryngeal granulloma

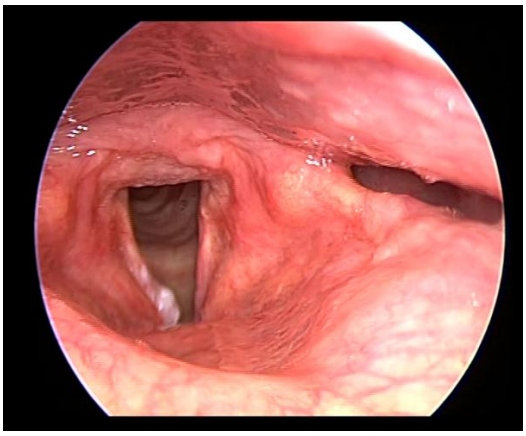

Figure 3. Laryngeal papilloma

Figure 4. Reinke's oedema

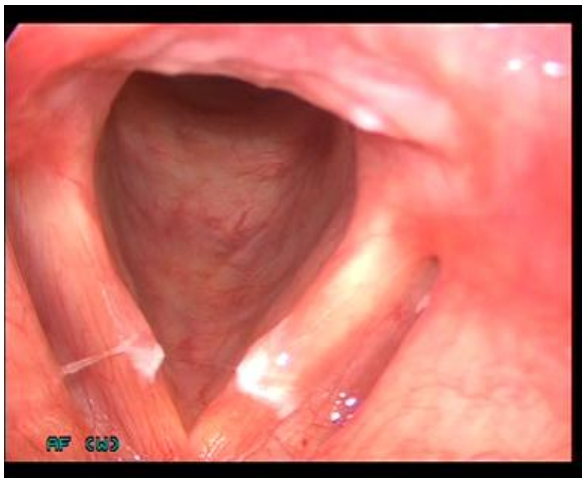

Figure 5. Vocal cord nodules

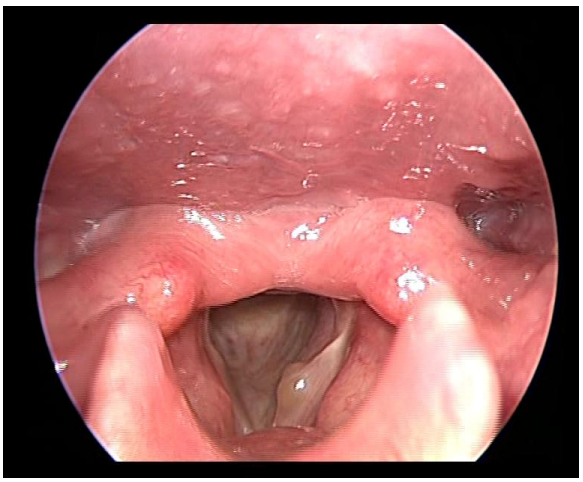

Figure 6. Vocal cord polyp

Table 1. Patient distribution under different types of benign tumor of the larynx according to age

Type of tumors	Males (age in years)			Females (age in years)			Total
	5-20	21-40	41-70	5-20	21-40	41-70	
Vocal Cord Polyps	0	15	34	0	9	18	76
Laryngeal Papilloma	2	4	36	1	4	4	51
Vocal Cord Nodules	0	0	0	2	3	0	5
Reinke 's oedema	0	0	2	0	6	6	9
Granullomas	0	1	2	1	1	1	6
TB Laryngitis	0	0	3	0	0	1	4
Cyst	0	0	3	0	0	3	6
Laryngeal Chondroma	0	0	1	0	0	0	1
Laryngeal Amyloydosis	0	0	0	0	0	1	1
Leukoplakia , keratosis	0	0	10	0	0	0	10
Total	2	20	91	4	18	34	169

DISCUSSIONS

The results in our series were in concurrence with most of the reviewed studies. Our data reported a higher incidence of benign tumor of the larynx among males (66.86%) as compared with females (33.13%), similar to Kleinsasser's (76%) and Bastian's (1990) study [8]. Males predominated in every category of benign laryngeal lesions except in cases of vocal nodules. This is in accordance with a study conducted in Chicago in 1197 patients by Paul H Holinger and KC Johnston(1951) [9] in which 70% of cases were males and 30% females. On the other hand female far exceeded the males in cases of vocal nodules and Reinke's edema.

Shaw et al. [10] in their series of 1505 cases surprisingly had no single case of vocal nodules. We had one case of laryngeal amyloidosis and one case of laryngeal chondroma.

Various studies have reported a higher incidence of benign tumors of the larynx in the age groups between 20 and 60 years.

In our study most of the patients (73.96%) were between 41-70 years.

Vocal cord polyp (44.97%) was the commonest mucosal disease of the glottis encountered in our study as also reported by Kleinsasser (1982) and Snow (1984) [11]. Bilateral lesions were observed in 5.33% of cases in the present series as opposed to 10% in Kleinsasser's study. However Chopra et al. [12] had an incidence of 16% only. In their series the incidence of vocal nodules was 33.33%.

Vocal cord polyps (44.97%) were followed by papillomas (30.17%), leukoplakia and keratosis (5.9%), Reinke's edema (5.32%), granuloma and cyst (3.55% each), vocal nodules (2.95%) and TB laryngitis (2.36%). These findings indicate preponderance of non-neoplastic tumors over neoplastic tumors, confirmed by histopathological examination and have supported by the elaborate classification given by Myerson and revised by Friedmann [13]. However Stewart[14] reported the incidence of

neoplastic tumors to be 24.03%; Shaw[10] reported 14%; Lowenthal[15] reported 51.9% and Oliver[16] reported 31.1%, and in our series we observed 36.07% cases of neoplastic tumors (papilloma).

Less number of cases in neoplastic group in our series can be ascribed to less number of cases (169 cases) studied over 2 years as compared to Shaw et al.[10] who studied larger number of cases (1505 cases) over a longer period of time.

Laryngeal papilloma constituted 30.17% of cases in the present study. Considering to low cost involved and no life threatening complication as opposed to cold instruments application, microsurgical removal with laser CO2 (McCabe et al, 1983) [17], offered an efficient method to treat laryngeal papilloma.

Tuberculosis of the larynx is the commonest granulomatous disease of larynx. In our series we had 4 cases of laryngeal tuberculosis and all were secondary to pulmonary tuberculosis. This is in accordance with Chopra et al. [12] who had 3 cases of tuberculosis of larynx, of which 2 were secondary to pulmonary tuberculosis.

With regards to the site of origin of the benign tumors, true vocal cords were found to be the commonest site for the origin of all neoplastic and non-neoplastic tumors. These findings are in accordance with the findings reported by Hegde et al. and Baitha et al. [18].

Nearly 89.35% of lesions were from true vocal cords. Vocal polyps and vocal nodules which together constituted 47.92% of cases were found at the junction of anterior 1/3rd and posterior 2/3rd of the true vocal cords. This is because the mechanical force of vibration is most intense at this site.

Hoarseness of voice can occur due to local as well as systemic disorders.

This symptom was found to be the most prominent and presenting feature of these tumors in the current series. All patients (100%) had this symptom.

Surgical treatment was the treatment of voice in all of the cases studied. Of the different modes of management mentioned in the literature: medical, physical, immunological and surgical, the last one remains the standard treatment of choice in all types of tumors and in all age group. However, it has been advised that surgical treatment of benign tumors of the larynx must invariably be followed by post-operative voice correction therapy, otherwise recurrence are liable to occur. Following this regime in the present study showed encouraging results as 93,6% of patients were normal without any recurrence after single operation.

The success of the treatment depends upon the team approach involving otolaryngologist, anesthesiologist, voice therapist and the complaint of the patient.

CONCLUSIONS

The specific lesions, mucosal disease of the glottis, benign neoplasms and other tumors of the larynx produces symptoms which can vary from mild hoarseness to life-threatening stridor. Laryngeal amyloidosis was an unexpected finding observed in our series. Early diagnosis of the lesions can lead to effective management and good recovery. As such, the standard treatment of choice in all types of benign tumors of the larynx should consist of a triad of approach by microlaryngeal surgery (either microscopic or endoscopic, with or without use of CO2 laser surgery), voice rest and vocal rehabilitation.

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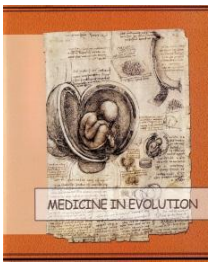
NODULAR LYMPHOID HYPERPLASIA IN THE LUNG - A RARE FINDING – CASE REPORT



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ABSTRACT

Nodular lymphoid hyperplasia of the lung is a rare pathological entity with tumoral appearance which microscopically corresponds to localized reactive lymphoid infiltrates in the lung with predominant follicular disposition. It is a controversial entity due to its rare incidence toward lymphomas originating in bronchus-associated lymphoid tissue to which it has to be distinguished. Therefore, immunohistochemical evaluation is required to demonstrate reactive phenotype of polyclonal proliferating lymphocytes to settle the diagnosis. Sometimes molecular studies for Ig H rearrangement are needed. We report on a a case of pulmonary nodular lymphoid hyperplasia in a 77-year-old patient and discuss the difficulties of this rare diagnosis and current knowledge in literature considering this rare entity.

Key words: *lymphoid hyperplasia, lymphoid infiltrate, lung pathology*

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INTRODUCTION

Nodular lymphoid hyperplasia (NLH) of the lung is a rare pulmonary disease with tumoral appearance characterized by pulmonary reactive lymphoid hyperplasia of unknown cause. In one recent study it was linked to the family of IgG4-related sclerosing disease [1]. NLH has its place in the spectrum of reactive pulmonary lesions that ranges from follicular hyperplasia to diffuse hyperplasia of the bronchus-associated lymphoid tissue (BALT) [2]. It is also a controversial entity because with current immunohistochemical techniques, authors in this field concluded that most of these lesions can prove to belong to B-cell lymphomas of BALT [3,4].

In3rd edition of Histology of Lung and Pleura Tumors published by World Health Organization, diagnosis of NLH was restricted to rare localized reactive lesion, whereas new criteria for primary pulmonary non-Hodgkin's lymphoma emerged based on accurate histochemical investigations [5].

Histologically, NLH consists of numerous intact reactive follicles with germinal centers and interfollicular polyclonal plasma cells in a circumscribed distribution. The infiltration into the adjacent lung parenchyma is limited. Distinction from the atypical lymphoid infiltrate and infiltrative growth of pulmonary BALT lymphoma is usually possible by histology and immunohistochemistry[6].The immunohistochemical evaluation of NLH shows polyclonality of plasma cells and an admixture of B-cells and T-cells in lymphocytes population [7].Although in cases of BALT lymphoma lacking plasmacytic differentiation plasma cells polyclonality may be preserved, NLH clonality may be confirmed by flow cytometry or by polymerase chain reaction (PCR) for IgH gene rearrangement [6].

CASE PRESENTATION

C.D, a 77-year-old male patient, presented at our Pulmonology Department acussing fatigability lasting for several months and twinge pain localized in the left hemithorax. He has a medical history of stage II arterial hypertension, chronic ischemic heart disease, hiatal hernia and benign prostate hypertrophy.He is nonsmoker and has no toxic habits.

Thoraco-pulmonary X-ray revealed an inhomogeneous opacity with irregular contour which obliterates the left costophrenic angle (Fig. 1).Laboratory findings were between normal limits.

Surgical intervention was performed with atypical resection within anterior basal and lateral basal segments of the left inferior pulmonary lobe corresponding to the mass detected on chest radiography.Intraoperatory microscopical examination of frozen section raised the suspicion of a lymphoid tissue proliferation.The lesion was completely excised and subsequently was fixed in 10% neutral buffered formalin for 24 hours.

Gross examination showed a nodular mass of 3 cm in the maximum diameter, with greyish-white appearance and elastic consistency, with clear and irregular demarcation from adjacent lung parenchyma. The specimen was sectioned in alternative slices of 0.5 cm. Five sections including center and margins of the lesion were submitted for routine processing for histologic examination by paraffin embedding, sectioning, and hematoxylin-eosin (H&E) staining.

Microscopical examination of the slides showed a well-demarcated lymphoid proliferation with follicular disposition and interfollicular sheets of lymphocytes and plasma cells (Fig 2). The lymphoid follicles have unequal germinal centers rimmed by well-defined mantle zone, resembling aspect of hyperplastic reactive follicles. Van Gieson stain emphasized nodular appearance with follicles being separated by variable amount of collagen fibers (Fig 3). Within lymphoid proliferation,small vessels with thickened walls and rare small airway conducts were observed. In our case there was no clinical suspicion of IgG4-related sclerosing disease and IgG4 investigation was not performed.

Immunohistochemical evaluation showed CD 20 positive B-cells distributed mainly in follicles (Fig 4) and interfollicular admixture of plasma cells and T-cells. Plasma cell population showed positivity for both κ siλ immunoglobulin light chains (Fig 5 a,b).CD 10 immunohistochemical evaluation was also performed with sparsely positivity in germinal centers.

In this context, both H&E stain examination and immunohistochemical phenotype of the proliferative lymphocytes sustained the diagnosis of pulmonary nodular lymphoid hyperplasia.

18 months after the surgical intervention, the evolution of the patient was uneventful, with a good clinical course and subjective improvement of respiratory function.

Figure 1. X-ray image showedanopacity with irregular contour localized in left costophrenic angle

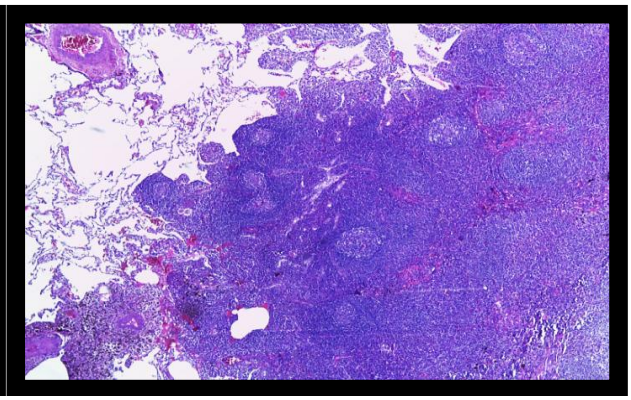


Figure 2. Lymphoid proliferation with apparent reactive follicles with unequal germinal centers,clearly demarcated from adjacent lung parenchyma. H&E stain, ob 4x

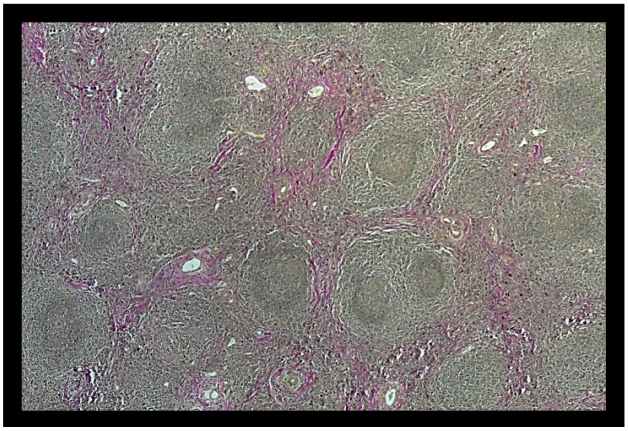


Figure 3. Van Gieson stain shows nodular appearance with follicles separated by variableamount of collagen fibers.Ob. 10x

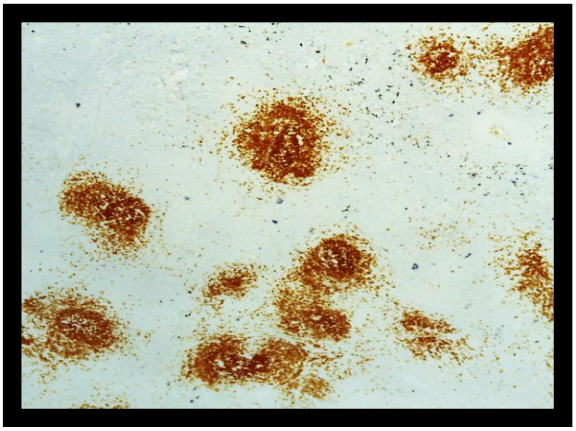
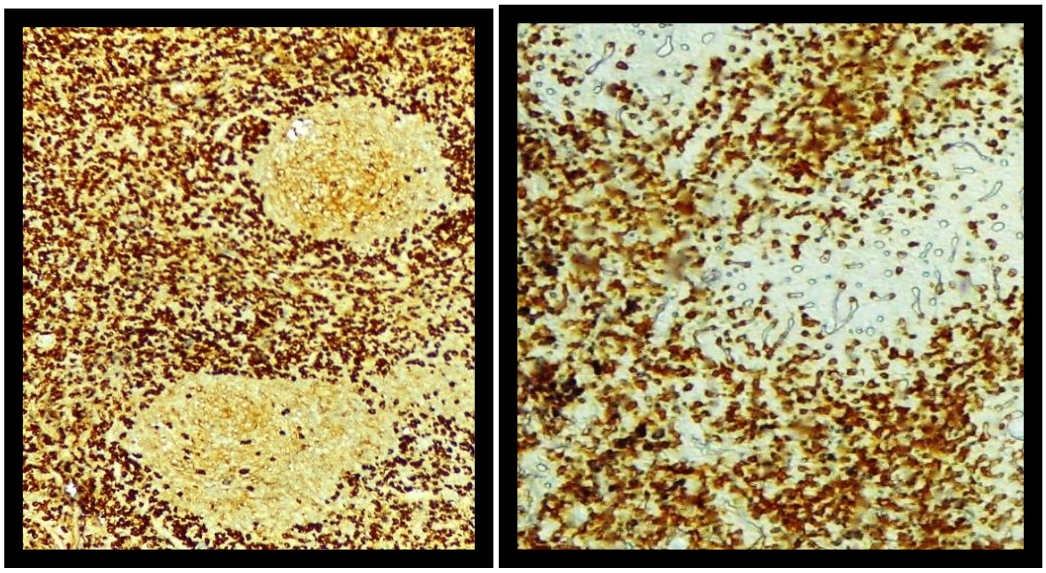


Figure 4. CD 20 brightly positive in reactive follicles. Ob 10x



A B

Figure 5. Both immunoglobulin light chains are represented in plasma cell population:(A) kappa light chains; (B) lambda light chains.Ob. 20x

DISCUSSIONS

Nodular lymphoid hyperplasia was first used by Kradin and Mark in 1983 to describe reactive lymphoid tissue in the lung which appear as nodular or localized lymphoid infiltrates [8]. Transiently, the term pseudolymphoma was used as synonym [9].

The current opinion about the concept of reactive lymphoid hyperplasia in the lung continues to be controversial because nearly all lymphoid masses in lung are neoplastic, even when they are microscopically low-grade, they contain an admixture of lymphocytes, plasma cells, occasionally granuloma and numerous germinal centers,[4,10,11]substantially resembling aspects of reactive lymphoid tissue.

This rare entity underlines the uncertain demarcation between low grade neoplasia and reactive hyperplasia when clonality of lymphocytes population cannot be precisely assessed due to a possible reactive component within neoplastic tissue or inconclusive immunohistochemical and molecular tests.In addition, the good prognosis of low-grade lymphomas in the lung may clinically overlap with the uncomplicated evolution of lymphoid reactive hyperplasia.

Histologically, the NHL lesions are localized and well-demarcated [6,10]. Constant feature of NHL are numerous reactive germinal centers with well-preserved mantle zones and the interfollicular sheets of mature plasma cells. Also, variable degrees of interfollicular fibrosis is frequently seen[10,11]. On routine stain, clues for reactive lesions include polarization of lymphocyte distribution within germinal centers, with a darker zone containing more proliferative cells and tingible body macrophages and a paler zone containing a heterogeneous population of cells, including larger and smaller lymphoid cells.

Differential diagnosis with pulmonary lymphomas relies on histological and immunohistochemical examination, and sometimes molecular studies are needed [7,11]. Other authors consider that both immunohistochemical and molecular studies should be performed in any case in which the diagnosis of nodular

lymphoid hyperplasia is considered [10].

Immunohistochemical stains demonstrate a reactive pattern of B and T cells. In particular, the germinal centers shows intense staining for the B-cell marker CD20, while interfollicular lymphocytes are immunoreactive for CD3, CD43, and CD5. When lymphoepithelial lesions are present, coexpression of CD20 and CD43 is not identified in lymphocyte population, in contrast with low-grade B-cell lymphoma [2]. Reactive germinal centers can also be highlighted by a very high Ki-67 proliferative rate and lack of staining of bcl-2. Absence of staining for bcl-2 does not exclude the presence of follicular lymphoma but should lead to evaluation for other features that can assist in distinction of neoplastic follicles, such as identification of abnormal staining for CD10 or bcl-6 outside of germinal centers, discordance in the pattern of staining for CD10 and bcl-6, or the presence of an unusually low Ki-67 proliferation rate [11].

Immunohistochemical staining for IgG4 and total IgG can be performed if there are morphologic features that suggest the possibility of IgG4 disease, such as marked plasmacytosis, fibrosis, obliterative vascular changes, and eosinophilia [1,12]

In NLH immunoglobulin light chain reactivity is polyclonal. Molecular genetic analysis shows no rearrangement of the immunoglobulin light or heavy chain gene [2]

NLH was found to be associated with Common Variable Immunodeficiency (CVID) [13], Sjogren syndrome [14], familial autoimmune disease [15], and recently it was linked to IgG4–related sclerosing disease [1].

Reactive lymphoid hyperplasia (RLH) usually with nodular appearance and reactive germinal centers, termed pseudolymphoma, is also described in other anatomical location, as skin, lung, orbit, gastrointestinal tract, breast [16], liver and pancreas [17] with similar histological pattern of pulmonary NLH.

It was suggested that nodular reactive lymphoid lesions can potentially be precursors to lymphoma [6] but the absence of recurrence in one series of cases [2] ameliorates this concern. In some pulmonary disease publications, NLH is classified as a borderline entity within rare primary pulmonary tumors [18].

CONCLUSIONS

NLH in the lung is a reactive lymphoid infiltrate with incomplete knowledge of pathogenesis. It is a non-neoplastic lymphoid lesion which has to be demonstrated by immunohistochemical evaluation of the lymphocytes. In some cases NLH still may be indiscernible from low-grade lymphomas and this is due to limits of current knowledge and available investigations concerning hallmarks of lymphoid malignant transformation versus lymphoid hyperplasia.

We undersign and certificate that the procedures and the experiments we have done respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000 (5), as well as the national law.

Conflict of interests: none to declare.

Acknowledgments

This work received financial support through the project entitled"CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760,
cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007-2013 for A.M.

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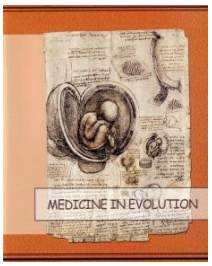
OUTLOOK OF THORACIC SURGERY ON LUNG CANCER IN BANAT REGION



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ABSTRACT

According to American Cancer Society (ACS), the incidence of bronchopulmonary cancer has critically increased, being the second most often newly diagnosed cancer in both men and women. Incidence of this type of neoplasia in Romania has significantly increased, reaching the first place in men and the third in women (after breast and uterine corpus cancers).

The retrospective study on oncologic thoracic pathology between 2008 and 2012 in Banat Euroregion, aiming at analysing the following parameters: age, sex, environment (urban/rural), pulmonary localisation, histopathological types and thoracic surgery type.

Out of the 926 patients diagnosed with bronchopulmonary cancer, 279 were selected (diagnosed by thoracotomy), with the mean age of 58.24 ± 9.26 years, 2.53:1 male/female ratio. Patients from urban areas accounted for 62.36% of the studied population. The most common localisation of bronchopulmonary cancer is in the upper lobes. Among these patients, 15.41% underwent curative oncologic surgery. NSCLC (93.85%) accounted for the highest number of cases, while 48.37% of these were adenocarcinomas.

If early detected, bronchopulmonary cancer is a disease that may benefit of radical oncologic surgery. As this pathology is asymptomatic for a long time, the number of patients benefiting of radical surgery is limited. Histopathologically, the most frequent lung cancer type is the adenocarcinoma.

Key words: *thoracic surgery, lung cancer, incidence, Banat, histopathological type*

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INTRODUCTION

Bronchopulmonary cancer is a silent disease as it manifests clinically late, many cases developing asymptomatically for a long period of time. Bronchopulmonary cancer is a frequent malignant tumour, accounting for over 90% of lung tumours; it is defined as the development of malignant cells in the lungs starting from the bronchial and lung structures. According to American Cancer Society (ACS), the incidence of bronchopulmonary cancer has critically increased, being the second most often newly diagnosed cancer in both men and women. Incidence of this type of neoplasia in Romania has significantly increased, reaching the first place in men and the third in women (after breast and uterine corpus cancers).[1,2,3]

Only 15% of the cases are surgically treatable at the time of diagnosis. Most of them are diagnosed in inoperable stages. According to data in the literature, at the time of diagnosis, a very high number of patients (approximately 55%) have advanced disease, with metastases, 30% have locoregionally advanced disease and only 15% are in an early localised stage. Smoking is an important risk factor, often incriminated in the development of this pathology. The 5-year survival rate of patients diagnosed with bronchopulmonary cancer is low, ranging from 17% - 18% for those diagnosed in advanced stages to 0% – 3% for those diagnosed with metastases. Mortality from bronchopulmonary cancer is frequently due to secondary tumours complications. Patients diagnosed in early stages have long survival rates, of approximately 70% - 90%.[4,5]

MATERIAL AND METHODS

A retrospective study has been conducted during 1 January 2008-31 December 2012, in the Thoracic Surgery Clinic, City Clinical Emergency Hospital Timisoara. The study group included 926 patients diagnosed with bronchopulmonary neoplasms, only 279 of them by thoracotomy. The exclusion criterion was diagnosis made by other methods: fibrobronchoscopy with bronchial biopsy, thoracoscopy with pleural biopsy, lymph node biopsy, mediastinoscopy with biopsy, cytological examination of pleural aspirate, CT-guided thoracic puncture. This study was approved by the Ethics Committee of City Clinical Emergency Hospital Timisoara. All the patients consented to have their personal data processed and used for scientific purposes. The main analysed parameters were: age, sex, environment (urban/rural), pulmonary localisation, histopathological types and thoracic surgery type.

We have used Microsoft Office Excel 2007 software for statistical analysis and graphical representation of data. Statistical parameters were interpreted using a normal value interval > 95%. The presented data were expressed as standard deviation and standard error. A p value ≤ 0.05 was considered statistically significant.

RESULTS

Patients were divided into two groups according to gender: group A, comprising 200 male patients (71.68%) and group B, consisting of 79 female patients (28.31%). Bronchopulmonary cancer was definitely more frequent in

male patients, their number being practically 2.53 greater than that of female patients. No statistically significant differences regarding the average diagnosis age (58.87±8.63 years vs. 56.78 ± 10.58) were recorded between the two groups (p = 0.10).

Patients mainly came from urban areas (62.36% vs. 37.63%), with mean age of 58.24 ± 9.26 years. As regards bronchopulmonary cancer localisation, it is of note that the most frequent was in the upper lobes: 31.54% left upper lobe (LUL) (n= 88 pts.); 30.82% right upper lobe (RUL) (n = 86 pts.); 17.20% right lower lobe (RLL) (n= 48 pts.); 15.05% left lower lobe (LLL) (n = 42 pts.); 3,22 % middle lobe (ML) (n= 9 pts.); 2.15% bilaterally (n = 6 pts.). (Table 1).

Atypical pulmonary resection was performed in 48.02% of cases, 36.55% needed lung tumour biopsy and only 15.41% of cases underwent radical surgery (Table 2).

Table 1. Tumour localisation in the whole studied group

Localisation		Number of cases - pts. (%)	Males (%)	Females (%)
Right lung	Upper lobe	86 (30.82%)	73 (26.16%)	13 (4.65%)
	Middle lobe	9 (3.22%)	5 (1.79%)	4 (1.43%)
	Lower lobe	48 (17.20%)	33 (11.82%)	15 (5.37%)
Left lung	Upper lobe	88 (31.54%)	62 (22.22%)	26 (9.31%)
	Lower lobe	42 (15.05%)	26 (9.31%)	16 (5.73%)
Bilateral		6 (2.15%)	1 (0.35%)	5 (1.79%)

Table 2. Type of surgery performed

Intervention type		Number of cases - pts.		%	
Radical	Right upper lobectomy	43	13	15.41	4.65
	Right lower lobectomy		10		3.58
	Middle lobe lobectomy		2		0.71
	Left upper lobectomy		7		2.50
	Left lower lobectomy		3		1.07
	Right pneumonectomy		2		0.71
	Left pneumonectomy		4		1.43
	Middle lower bilobectomy		1		0.35
	Culmenectomy		1		0.35
Palliative - diagnostic	Atypical resection	236	134	84.58	48.02
	Lung biopsy		102		36.55

Radical excision of the mediastinal lymph nodes is the most important prognostic factor for survival.

Histopathologically, 135 patients were diagnosed with adenocarcinoma (48.37%), 77 (27.59%) with squamous-cell carcinoma, 17 (6.09%) with small cell lung cancer, and 9 patients (3.22%) with typical carcinoids. By analysing the presence of metastases we have seen that most of them (17 cases, 6.09%) were adenocarcinoma metastases. The remaining four cases had other histological types of metastasis (pulmonary sarcoma, leiomyosarcoma, malignant melanoma). It can be seen that adenocarcinoma and squamous-cell carcinoma were the main histopathological types in both males and females. Ninety-six (24.40%) adenocarcinoma cases and 62 (22.22%) squamous-cell carcinoma cases were recorded among men, and 39 (13.97%) adenocarcinoma cases and 15 (5.37%) squamous-cell carcinoma cases were seen in women (Table 3).

Table 3. Histopathological types of lung cancer

Histopathological type	Nr. of cases - pts.(%)	Males (%)	Females (%)
Adenocarcinoma	135(48.37%)	96(24.40%)	39(13.97%)
Squamous cell carcinoma	77(27.59%)	62(22.22%)	15(5.37%)
Neuroendocrine carcinoma	3(1.07%)	1(0.35%)	2(0.71%)
Small cell carcinoma (SCLC)	17(6.09%)	12(4.30%)	5(1.79%)
Bronchogenic carcinoma	2(0.71%)	2(0.71%)	0
Undifferentiated non small cell lung carcinoma (NSCLC)	15(5.37%)	11(3.94%)	4(1.43%)
Typical carcinoid	9(3.22%)	4(1.43%)	5(1.79%)
Lung metastases	21(7.52%)	12(4.30%)	9(3.22%)

A slight increase over the studied 5-year period has been seen when analysing the annual distribution of cases, the most important growth being recorded in 2009 (66 cases), as compared with the previous year (Figure 1).

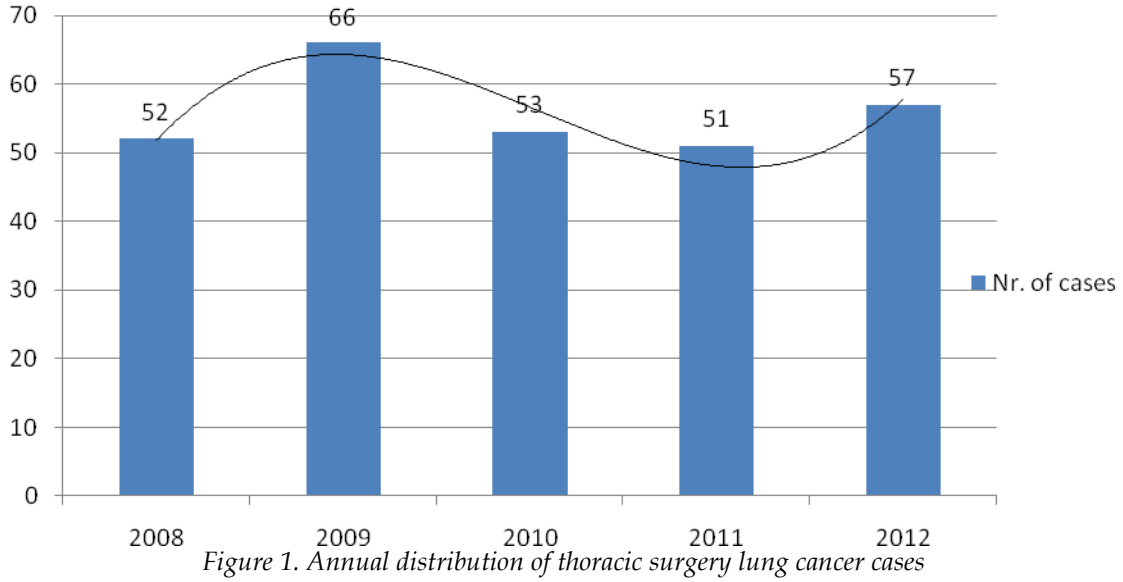

Figure 1. Annual distribution of thoracic surgery lung cancer cases

DISCUSSIONS

The most recent report released by the International Agency for Research on Cancer (GLOBOCAN 2012) specifies that 78,800 new cases of cancer arise in Romania annually, an increase by almost 4,000 cases towards the previous report in 2008.[6,7] Over the past 5 years, the total number of patients was 178,400 cases, bronchopulmonary cancer being the most frequent type of cancer in our country.

The Banat Euroregion is located in Western Romania and comprises three counties (Timis, Arad, Caras-Severin, Hunedoara), with a total number of 1,828,313 inhabitants.[8] Bronchopulmonary cancer incidence in the various geographic regions of the country is currently not known. This study has been performed within the largest thoracic surgery centre in the Banat Euroregion, although thoracic surgery compartments also exist in the other counties. The bronchopulmonary neoplasm was more often diagnosed in male patients, but it continues to grow in women also. The data acquired by us are consistent with those in the literature[9,10,11].

We have asked ourselves why bronchopulmonary cancer is much more frequent in urban areas as compared with the rural ones. A possible answer would be that access to health services is much easier in urban than in rural areas. Patients included in the studied group were adults aged between 24 and 79 years, mainly originating in urban areas (62.36%). There are studies confirming the high incidence of this pathology in urban areas.[12] Another cause could be the presence of several known risk factors (such as air pollution), which, according to the International Agency for Research on Cancer (IARC), prevails in urban areas.[7] It is estimated that Iasi city is the most polluted Romanian urban area, with 54 $\mu g/m^3$ (microgram per cubic metre of air), followed by Timisoara with 51 $\mu g/m^3$ (microgram per cubic metre of air).[13] The European Union set an annual average limit value of 25 micrograms per cubic metre of air, while the World Health Organisation (WHO) recommends up to 10 micrograms per cubic metre of air yearly. Taking into account the significant differences in our study, a future research theme could be related to the study of risk factors involved in this pathology onset and evolution.

We have also noticed a slight preponderance of right lung involvement as compared with the left one (51.24% and 46.59%, respectively). Considering the volumes of the two lungs (55% - 45%), we can consider there was no localisation of bronchopulmonary cancer for a certain lung.

Possible explanations for the fact that bronchopulmonary cancer is more frequently localised in the upper lobes were suggested, as follows: right bronchus is much shorter and is greater in diameter than the left one, or the much larger volume of right lung compared to the left one, or the PO_2 amount is much higher in the upper lobes, as there are many anatomical, physiological and functional differences between upper and lower lobes[14].

In Romania there is no screening programme for bronchopulmonary cancer detection in general population, even though this disease is a major public health issue.

If early detected, bronchopulmonary cancer is a disease that may benefit of radical oncologic surgery. As this pathology is asymptomatic for a long time, those benefiting of radical surgery (lobectomy, pneumonectomy) are representing only 15.41%, while the remaining patients undergo palliative

surgery (atypical pulmonary resection, tumour biopsy)[15,16].

Surgeries were performed by thoracotomy, most cases were discovered in advanced stages of the disease, 48.02% of patients had atypical pulmonary resection, 36.55% tumour biopsy, and only 15.41% of cases underwent curative oncologic treatment (lobectomy, pneumonectomy). Most cases were identified as adenocarcinomas, representing 48.37%.[17,18,19] The histopathological type is correlated with aggressiveness and thus with presence of metastases.[20,21,22,23,24] The large majority of metastases were of adenocarcinoma origin, accounting for 6.09%.

Pulmonary cancer is more frequent in men (M:W: 2.53: 1), but it also raises in women, with a mean age of 58.24 ± 9.26 years, the youngest patient aging only 24 years. It is more frequent in the patients from urban areas, being mostly localised in the upper lobes and involving in about the same proportions both LUL and RUL (31.54% vs. 30.82%). Surgeries were performed using the open approach, most cases being detected in advanced stages; therefore, the curative oncologic treatment was only possible in 15.41% of the cases. Most neoplasms were NSCLC (93.85%), and the adenocarcinoma prevailed (135 cases).

Acknowledgements

The authors would like to thank Prof. Dr. Mihalas Gheorghe Ioan for excellent support with the statistical analysis and to Carmen Pitic for linguistic review.

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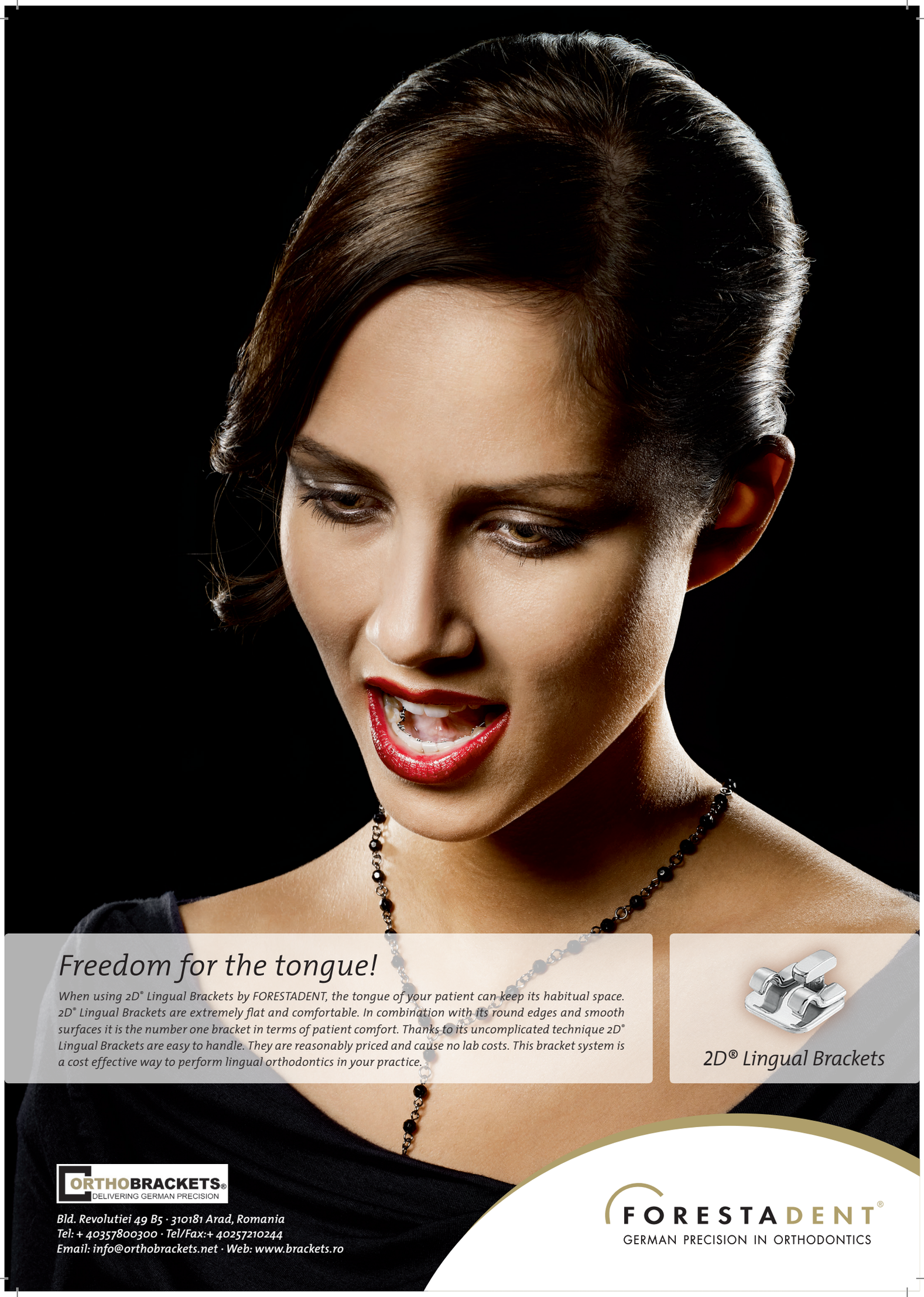
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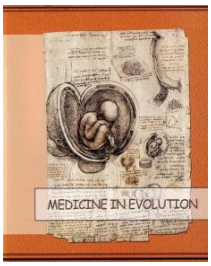

ANTIOXIDATIVE EFFECTS OF THE GRAPE SEED EXTRACT BURGUND MARE DURING POSTINITIATION OF ORAL CARCINOGENESIS IN A RAT MODEL

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ABSTRACT

Cancer treatment is often problematic because conventional drugs are cytotoxic to cancer cells as well as normal cells. Since oxidative stress mediates the complex events that occur during carcinogenesis and phytochemicals, lacking toxicity, exert antioxidative benefits, we aimed to evaluate the effects of a grape seed extract Burgund Mare (BM) on serum sulfhydryl groups (–SH) (as indicators of oxidative stress) during 4NQO induced oral carcinogenesis in rats. BM administered during postinitiation of carcinogenesis adjusted serum –SH that were significantly increased by 4NQO at levels comparable with controls. We concluded that BM exerted antioxidant effects during oral carcinogenesis and deserves being studied as a possible antioxidative chemotherapeutic agent.

Key words: *oral cancer, oxidative stress, sulfhydryl groups, grape seed extract Burgund Mare*

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INTRODUCTION

Oral cancer, like any other cancer, develops through three stages: initiation (the carcinogen initiates the cells to divide more rapidly), promotion (cells fail to fix the damage and aberrant growth is promoted) and progression (angiogenesis and metastasis). The complex events that occur during these steps are mediated by endogenous and exogenous stimuli and among them an important one is oxidative stress (Choudhari SK et al., 2014).

Cancer treatment is often problematic since conventional chemotherapeutical drugs are cytotoxic to cancer cells as well as normal cells and can result in damaging the organism.Chemoprevention by natural safe, non-toxic products have arousen the interest of scientists. Several antioxidant phytopharmaceuticals like resveratrol, curcumin, gren tea extract, oligomeric proanthocyanidins have been studied and proved to exert beneficial effectsin oral cancer (Bagchi D et al., 2014).

Aim and Objectives

In this study we aimed to evaluate the effects of a grape seed extract on oxidative stress oral carcinogenesis.

MATERIAL AND METHODS

Chemicals

4-nitro-quinolin-1-oxid (Sigma-Aldrich®) was dissolved 0,5% wt/vol in polyethylene glycol (Sigma-Aldrich®) (4NQO).

The hydroethanolic extract from grape seeds (Vitis vinifera L), variety Burgund Mare (Romania) (BM) was prepared from 1:10 w/v mixture of finely powdered dried seeds and water/ethanol 50/50 (v/v). The total polyphenolic content of BM extract was measured spectrophotometrically by Folin-Ciocalteu method. The HPLC revealed the presence of procyanidin B, catechin hydrate, epigallocatechin, epicatechin and gallic acid in BM extract (Postescu ID et al., 2007).

Animals

30 male Wistar albino rats (8 weeks old and weighting 220±20 g) were purchased from „Iuliu Haţieganu"University of Medicine and Pharmacy Bioabase and housed at the Department of Physiology Biobase:5 per cage; at a temperature of 21±2°; humidity of 70±4%; 12h dark/ 12h light cycle; fed a standard pellet laboratory diet; receiving water ad libitum; acclimatized one week before any procedure.

The rats were randomly divided into three groups. To initiate oral carcinogenesis one group was treated with 4NQO for 12 weeks while the control group received the vehicle only (polyethylene glycol). To evaluate the chemopreventive effect of BM during postinitiation of carcinogenesis, rats in the treatment group received 4NQO for 12 weeks and then BM for 4 weeks (fig. 1). The rats were restrained from drinking water two hours after each administration. After 16 weeks animals were anaesthetized and blood was collected from the retroorbital sinus.

Assay of oxidative stress parameters

Blood samples (5ml) were centrifuged for 5 minutes at 3500 rpm. After serum was obtained sulfhydryl groups (–SH)were assayed as indicators of oxidative stress. The results were expressed in µmol/ml(Hu ML, 1994).

All procedures in this study were approved by The Ethical Committee on Animal Welfare of „Iuliu Haţieganu"University of Medicine and

Pharmacy in accordance with the Romanian Ministry of Health.

Statistical analysis

Data were expressed as mean ± SD. Kolmogorov-Smirnov test was used followed by ANOVA test and a p value ≤ 0.05 was considered significant. Statistical analysis was done using SPSS 13.0 and Microsoft Excel.

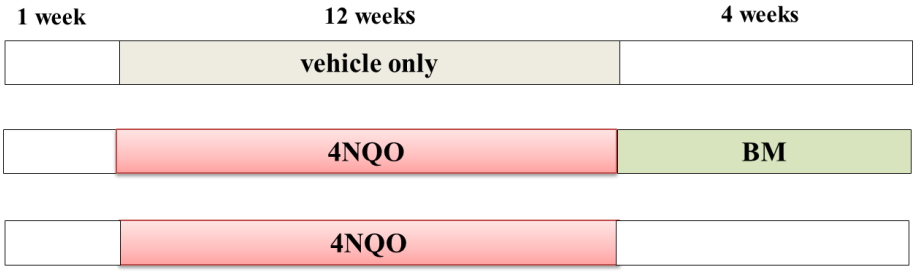

Figure 1. Experimental design. 30 Wistar rats were randomly divided intothree groups (n=10): group 1 received vehicle only (polyethylene glycol) topically applied on oral mucosa thrice a week for 12 weeks; group 2 received 25µl 4NQO topically applied on oral mucosa thrice a week for 12 weeks and thenBM by oral gavage 50mg/kg wt/day thrice a week for 4 weeks; group 3 received 4NQO the same way as group 2. At the end of the experiment blood was sampled from the retroorbital sinus of rats and oxidative stress was evaluated in serum

RESULTS

4NQO administration for 12 weeks resulted in increased serum –SH. Median levels of –SH were significantly elevated in the carcinogen group compared with the median values in the control group ($p<0.05$). BM administered during postinitiation of carcinogenesis significantly reduced serum median values of –SH($p<0.05$) (fig. 2).

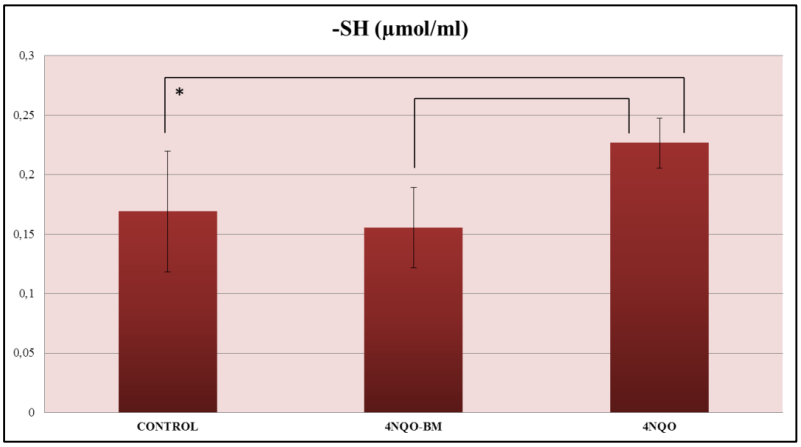

*Figure 2. Serum –SH after BM treatment during postinitiation of 4NQO induced oral carcinogenesis in Wistar rats. 30 rats were randomly and equally divided in three groups: group1 – the negative control group received vehicle alone for 12 weeks; group 2 – the treated group received 4NQO for 12 weeks and BM for 4 weeks after 4NQO cessation; group 3 – the carcinogenetic group received 4NQO for 12 weeks (see"Material and methods"). At the end of the experiment blood was sampled from all rats and serum –SH was assayed. BM administration lowered –SH levels increased by 4NQO at values comparable with the ones in the control group. Data are depicted as mean ± SD. Statistical analysis was done by a one-way ANOVA test (*p<0.05).*

DISCUSSIONS

In our study BM adjusted serum –SH levels that were increased by 4NQO when administered during postinitiation of oral carcinogenesis in rats.

–SH, also called thiols, arethe most chemically reactive group in the biological systems. Thiol mechanisms control the redox systems biology and are implicated in oxidative stress related diseases includingcancer (Go Y-Met al., 2013). In our study increased levels of serum –SH in 4NQO induced oral carcinogenesis could be an expression of increased oxidative stress. Our results are in concordance with one of the few studies in which high thiol levels were found to promote rather than suppress oxidative stress in plasma and that regardless of their metabolic origin increased thiols were associated with increased plasma oxidative stress (Wronska-Nofer T et al., 2007).

Researches demonstrated grape seed extracts to have pharmacological and therapeutic effects against oxidative stress and several degenerative diseasesby: scavenging free radicals even better than other antioxidants like vitamin C, E and β-caroteneand prevent biomolecules damage by oxidative stress; protecting tissues against chemical-induced toxicity; inducing selective cytotoxicity toward cancer cells with no damaging effects on normal cells (Bagchi D et al., 2014). Our results support this theory as they show that BM administered during postinitiation of carcinogenesis reduced oxidative stress in terms of adjusting –SH to values comparable with the ones in controls.

CONCLUSIONS

We can conclude that BM, administered during postinitiation of carcinogenesis, decreased serum –SH levels that were increased during 4NQO induced oral carcinogenesis. Therefore, BM deserves to be studied as a promising antioxidative therapeutic agent in oral cancer.

Acknowledgements

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

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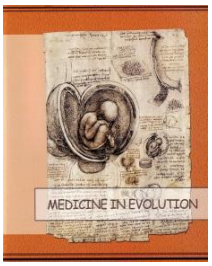
CBCT EXAMINATION – WHERE IS MOST USEFULL IN MAXILLO-FACIAL PATHOLOGY?



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ABSTRACT

The purpose of this article is to analyze the cases and type of pathology where CBCT examination is the most helpful. The study did not include the importance of CBCT examination in dental implantology, considering this field as particular. After a review of cases where CBCT was used, we can make the following classification of the examination utility: 1. Supranumerary teeth 2. Foreign body 3. Odontogenic and nonodontogenic tumours 4. Impacted teeth 5. Benign bone tumours and cysts. Other areas of interest, less explored are: clefts, orthognathic surgery, ATM disorders.

Key words: *CBCT examination, pathology*

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INTRODUCTION

Cone beam CT (CBCT) produces three-dimensional information on the facial skeleton and teeth and is increasingly used in many of the dental specialties, including orthodontics, orthognathic surgery, trauma and implantology [1,3,4]. The method is known by several names: dental volumetric tomography, cone-beam volumetric tomography, dental computed tomography, cone-beam imaging. CBCT uses a cone-shaped source of ionizing radiation and a two-dimensional area detector fixed on a rotating gantry to acquire multiple sequential projection images in one complete scan around the area of interest. The method was first developed in 1982 for angiography, and thereafter applied to maxillofacial surgery. After 1990, the method grew by producing lower volume CBCT equipments at a acceptable price cost.[1,2]

Main features of the method

- CBCT equipment has a small volume compared with conventional and the investigation costs are diminished in comparison with the CT costs value.[1]
- For the most CBCT equipments the scanning time is short, less than 30 seconds, due to a single rotation to capture the necessary data.[1]
- CBCT provides convenient low radiation dose. The majority of CBCT equipments have a radiation dose varying between 52 and 1025 microsieverts, equivalent to 4 to 77 digital panoramic radiographs. In comparison with conventional CT, the irradiation dose is lower from 2 to 50 times.[1,6]
- Is based by analyzing the anatomic region in a personal computer. The possibility to generate a 3D analysis and reconstruction give the method a high value.[1]

Limitations of the technique

The main limitations of the technique, unsolved until now, remains the image noise and the poor soft tissue contrast, caused mainly by the cone beam projection geometry, detector sensitivity and contrast resolution.[1]

MATERIAL AND METHODS

The study was retrospective, made on the patients who received CBCT for maxillo-facial pathology.

Pathology	Number of cases
Supranumerary teeth	5
Foreign body	3
Odontogenic and nonodontogenic tumours	4
Impacted teeth	4
Benign bone tumours and cysts	5

Pathology	CBCT utility
Supranumerary teeth	Very useful
Foreign body	Very useful
Odontogenic and nonodontogenic tumours	Useful
Impacted teeth	Useful
Benign bone tumours and cysts	Useful

DISCUSSIONS

We present below representative cases for each type of pathology where the preoperatory CBCT examination was very helpful.

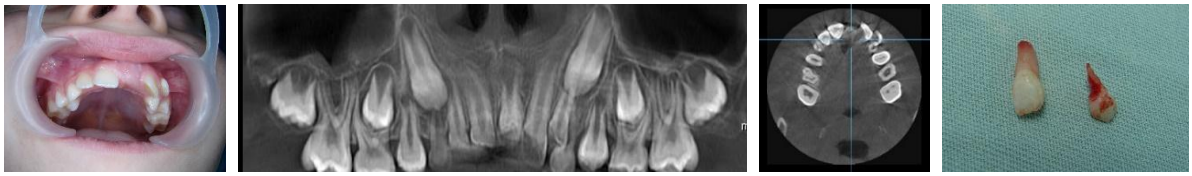
Supranumerary teeth

A 7 year old girl with dental eruption disturbance was examined in the Maxillofacial clinic. She presented an edentulous space corresponding to the 21 tooth site, an erupted tooth with an appearance of a central incisor corresponding to 22 space, an ectopic vestibular placed tooth with the appearance of an incisor in 23 space and an oral erupted tooth with temporarily appearance. Distally, the first and second left maxillary bicuspid teeth were present.

The panoramic radiography showed in the edentulous space an impacted tooth with a radiological appearance of a central incisor. Supplementary, we observe the existence of a supernumerary tooth in the left jaw region and an impacted tooth corresponding to 23 space, without other clarifications of the situation. After clinical and radiographic examination, performed both by the surgeon and the orthodontist, we were not able to identify the teeth and to know which tooth to extract.

Only after the patient performs the CBCT, we could clarify the situation: in the 11 space a supranumerary impacted tooth was present, followed distaly by 21 tooth (erupted), 22 tooth (vestibular), 63 (palatal), 23 tooth included, first and second left maxillary premolar teeth. We perform the odontectomy of supranumerary included tooth and 63 tooth extraction. After surgical treatment the patient continued with the orthodonthic treatment.

It was one of the cases in which without the CBCT examination, we could not make a correct diagnosis.

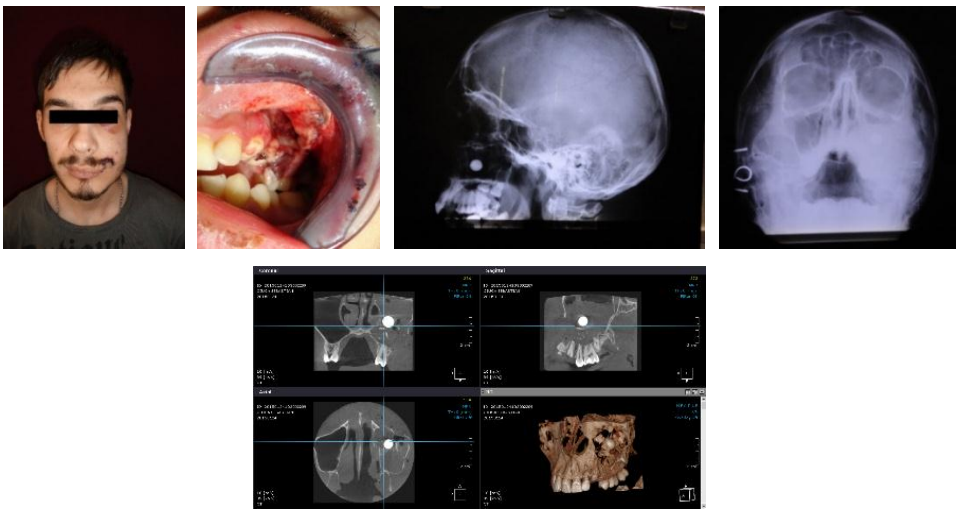


Foreign bodies

A 23 years old male patient presents in the emergency room with a left labio-vestibular gunshot rubber bullet wound. We suspected that the bullet was still present in the facial soft or bony tissue depth. Skull profile and facial sinuses radiographs were performed, but without being able to specify the bullet exact location. The emergency treatment consisted in suturing the facial open wound.

Subsequently, CBCT examination confirmed the foreign body and its position in the upper pole of the left maxillary sinus. We performed the surgical cleansing of the left maxillary sinus and removal of the radioopaque mass, find easily in the upper pole of the left maxillary sinus.

It was one of the cases in which the CBCT examination was very helpful to determine the exact position of the foreign body and contributed to an easier and more accurate surgery. The surgeons know very well how difficult, unpredictable, and sometimes unsuccessful is the finding of the foreign bodies in deep tissue, without a preoperatory precise localization.



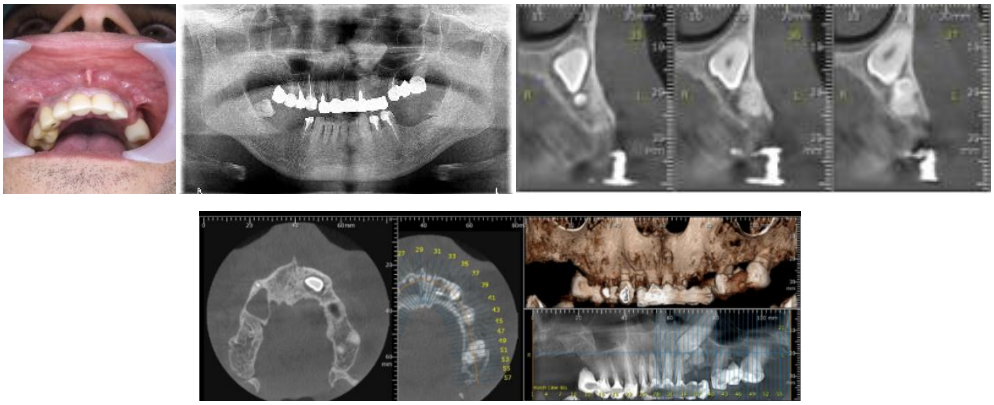
Odontogenic and nonodontogenic tumours

A 32 year old man presents in the Maxillo-facial clinic, sent by the dentist to clarify the clinical and radiographic aspects of an edentolous area corresponding to the 23 and 24 teeth. In the left maxillary zone, no vestibular or oral clinical signs were present, except the missing 23 and 24 teeth.

Panoramic radiography showed in the edentulous space an opaque inhomogeneous zone and the existence of the impacted 23 tooth, without further clarify the situation. After clinical and radiographic examination, we suspected an odontoma placed lower from the impacted teeth 23.

Only after the patient performs the CBCT, we can clarify the situation: in the edentolous space it was confirmed the radioopaque mass made from two soldered fragments, without contact with surrounding teeth, and the 23 impacted tooth placed higher. We perform the enucleation of the radioopaque mass and the odontectomy of the impacted tooth. The histological diagnosis of the radioopaque mass was compound odontoma.

It was one of the cases in which the CBCT examination clarified the diagnosis, localized the two pathological entities, and contributed to a more accurate surgery.



Impacted teeth

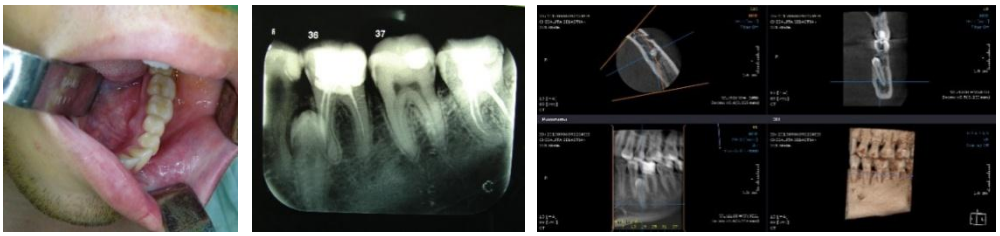
A 24 year old man presents in maxillo-facial clinic, send by the dentist, with pain in the left mandibular zone. At the clinical examination, in the left oral mandibular zone, a small bulge is observed. All left mandibular teeth were presents, without cavities or periodontal pathology.

Retroalveolar radiography showed an opaque zone with a dental contour, placed near the inferior edge of the mandible. After clinical and radiographic examination, we

suspected an impacted supranumerary premolar tooth.

After the patient performs the CBCT we received additional information that confirmed the supranumerary impacted tooth, placed orally, between second premolar and first molar. The impacted tooth had no relation with the vasculo-nervous mandibular bundle, but a close relation with the mesial root of the first molar. We perform the odontectomy of the impacted tooth and the apicectomy of the mesial root of the first molar, without any accidents.

It was one of the cases in which the CBCT examination clarified the diagnosis, and contributed to a more accurate surgery.



Benign bone tumours and cysts

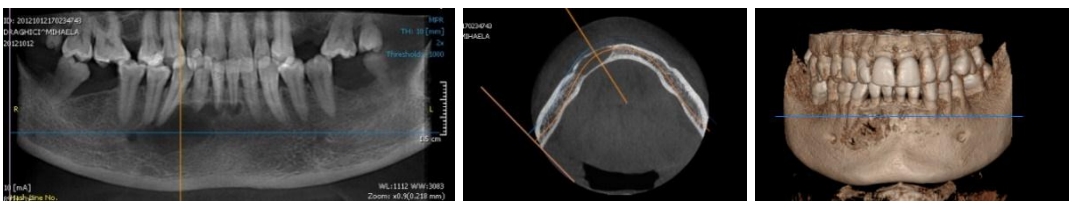
A 33 year old woman presents in Maxillo-facial clinic, sent by the dentist to clarify the clinical and radiographic aspects of the area corresponding to the 31-44 teeth. The clinical examination showed a light congestion of the vestibular mucosa near 42 and 43 teeth, without oral or vestibular bone enlargement.

Panoramic radiography showed in the mentioned space an inhomogeneous radiotransparent zone, without further clarification of the situation. After clinical and radiographic examination, we suspected a mandibular cyst, without defining the extent, volume and relationship with the surrounding teeth.

In evaluating cysts or benign tumours, intraoral or panoramic radiographs show only the two dimensions of the lesion. Because of superimposition of large tissue volume, extraoral plain film radiographs often cannot provide reliable information on the internal structure of a lesion. Information on the spatial relationship of the lesion with other anatomic landmarks on such images is limited, and often difficult to analyse.[5]

In contrast, CBCT record all three dimensions (axial, coronal and sagittal planes) and provide important information on the presence and extent of bone resorption, sclerosis of neighbouring bone, cortical expansion and internal or external calcifications, and proximity to other anatomic structures.[7]

In our clinical case, the CBCT examination contributed to diagnosis and also to establish the real volume of the tumor and the relation with the surrounding teeth. All these lead to a more accurate surgery.



CONCLUSIONS

The close contacts between the local anatomical structures and the teeth, tumors, foreign bodies, etc. are risk factors in dentoalveolar surgery.

However, the local anatomical structures may not be reliably interpreted in a bidimensional image. Panoramic or periapical radiographs are often inadequate to locate impacted teeth, tumors, foreign bodies. This is the main reason that a CBCT examination is necessary.[5]

Although relative recently introduced in practice, the CBCT method is likely to become one of the main methods of investigation in oral and maxillofacial surgery. Using the CBCT, we are now able to get more data to characterize the patient. Increased diagnostic capacity will propel this technology. [5,7]

Basically, we can not speak of a contraindication to CBCT, apart from the cost and radiation it produces (greater than conventional radiography). If the medical information obtained by conventional radiography (panoramic, maxillary, mandibular incidence) is enough, sure we will not use the CBCT, which has a higher radiation dose and a higher cost price.[6,7]

Situations where we identified the method as having a maximum value (conventional radiography was insufficient) were in descending order: supranumerary teeth, foreign body, odontogenic and nonodontogenictumours, impacted teeth, benign bone tumours and cysts. We can say in these cases that preoperative information obtained was maximum and surgery without CBCT would have been poor or subject to unacceptable errors.

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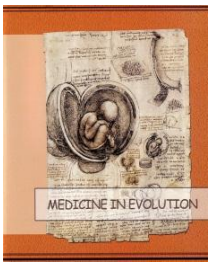

ORAL HEALTH PROFILE OF SCHOOLCHILDREN INCLUDED IN NATIONAL PROGRAMME „SMILE ROMANIA"IN BUCHAREST

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ABSTRACT

This current study is part of the national educational program „Smile Romania", which is included in an European program – „Platform for a Better Oral Health", developed by a cooperation between the Association for Dental Education in Europe (ADEE), the Council of European Chief Dental Officers (CECDO), the European Association of Dental Public Health (EADPH) and the International Dental Health Foundation (IDHF).

Objectives: the study was developed to assess oral health status, oral hygiene level, preventive dental sealants and the orthodontic treatment need among 6-11 years old school children in Bucharest. Material and method: there were included in the study 2599 children, 49.9% girls an 50.1% boys, 6-11 years old, from 12 state elementary schools in Bucharest. Oral examinations were performed in the classroom, using disposable dental examination tools, based on WHO 1997 examination criteria and dental chart. Results: for temporary dentition that caries prevalence is very high (71.1%), we found a medium to high value of def-s = 5.44 and def-t = 3.18, both based mostly on the ds value. For permanent dentition, the prevalence was smaller, of 31.8%, the mean value of DMF-S was 0.92 and DMF-T is 0.82. We found that sealing were applied on only 8.5% of examined molars, there are only 25% of subjects that keep the tooth surfaces plaque-free and in equal proportions children have a low (34%), medium (32%) and high (33%) need of orthodontic treatment need. Conclusions: therefore, efforts should be made to continously decrease the prevalence of caries both in temporary and permanent dentition and intensify the prevention actions.

Key words: *oral health, caries prevalence, national educational programme*

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INTRODUCTION

According to World Health Organization reports published during the last decade, oral diseases are still widely spread in populations (1). Despite the efforts of international organizations that activate in the health field, aimed to raise the educational level and awareness among communities regarding prevention of oral diseases, gouvernamental efforts are still at unsatisfactory level in many countries. Globally, there is offered support for all events that focused on reducing that is know as the „10/90 gap"which characterizes the fact that only 10% of the funding used in health research are reserved for diseases that affect 90% of the population (2). Dental cavities are an ongoing major public health issue. Among children, there has been observed an improve in the oral health status and an increase of the number of caries-free indivuduals, due to the changes in the lifestyle and life conditions, improved oral hygiene performance, decreased quantities of sugar consumption, increase in fluoridated tooth paste, topical applications of fluoride and increased number of oral health educational programs in schools (3). Nevertheless, dental caries affect between 60% and 90% of the children in industrialised countries (4). Advanced caries affect children quality of life by producing pain, modifying facial aspect, leading to acute or chronical infections, developing masticatory or sleeping disorders (5). Also, late diagnostic of the decayed teeth increases the costs of dental treatment and decrease learning abilities (6,7).

Recent studies developed in Romania regarding the assessment of the oral health status showed medium to severe forms of dental diseases. A study conducted in Bucharest in 2008 on a sample of 510 schoolchidren with an avarage age of 7 years, showed a high prevalence for dental cavities, only 28.4% of subjects being cavities-free. For temporary teeth, cavities experience indices had values between 0 and 57. For 6 years old children, defs value was 7.23, at the age of 7 the avarage value found was 8.4, for 8 years old was 6.89. When it comes to permanent teeth, the DMFS values were 0.32 at children at age of 6, 0.57 at the age of 7 and 0.53 at the age of 8. The avarage value for entire sample was 0.47. Regarding the gingival status, prevalence of gingival bleeding was 6.7%. The avarage value for the O'Leary Plaque Index was 57.31% representing a medium oral hygiene level. The orthodontic treatment necessities found were medium for most of the children – 37.4%, low for 35.8% and high for 5.6% od the examined subjects (8).

Another study published in 2013 included 1595 subjects of an age between 10 and 17 years and aimed not only to assess the oral health status but also reveal the impact of the socio-economic status on the oral health status. Results showed a high prevalence of cavities affected children – 75%, of which 64% had untreated cavities. The mean value of DMFT was 2.8 and the major part of it was constituted of the decayed component – mean DT=2. There were statistically significant differences in prevalence of dental caries between children from graduate education families and children whose parents only graduated highschool. Moreover, children studying in schools with dental office had a statistically significant better oral health that the other children (9).

This current study is part of the national educational program „Smile Romania", which is included in an European program – „Platform for a Better Oral Health", developed by a cooperation between the Association for Dental Education in Europe (ADEE), the Council of European Chief Dental Officers (CECDO), the European Association of Dental Public

Health (EADPH) and the International Dental Health Foundation (IDHF). „Smile Romania"is a health education program developed to raise awareness about oral health, to control the initiation and evolution of caries and other oral diseases through their early diagnosis, to present children oral health status to their parents (10) and to improve children oral hygiene behavior.

Aim and objectives

In the current study we look into considerations the objectives of WHO for 2020 regarding the necessity to significantly reduce the impact of oral diseases on general health, using modern prediction methods, also the disparities of oral health status among population, addressing to communities with high risk for caries and offering access to oral health care programs. Thus, the study was developed to assess oral health status, oral hygiene level, preventive treatment with dental sealants and the orthodontic treatment need among 6-11 years old school children in Bucharest.

MATERIAL AND METHODS

In order to reach our goals, we designed a cross-sectional study which was applied in Bucharest between April 1st and May 16th 2013. The study was a part of a national oral health educational program „Smile România", which was developed as a partenership between Faculty of Dentistry („Carol Davila"University of Medicine and Pharmacy) and Glaxo-Smith-Kline S.R.L. Romania. There were included in the study 2599 children, 6-11 years old, enrolled in 0 to 4 grade, from 12 state elementary schools in Bucharest. Oral examinations were performed in the classroom, using disposable dental examination tools, by 15 dentists, and for the filling of the dental chart there were involved 5th and 6th year dental students. There was used the WHO dental charts which consist of 4 sections: personal data, dental status, gingival status and orthdontic treatment need. In addition, the presence and level of dental plaque was assessed. Thus the indices used were def (decay-extraction-filling) for surfaces and teeth for temporary dentition, DMF (Decay-Missing-Filling) for surface and teeth for permanent dentition, Gingival Index (GI), Orthodontic Index for Treatment Needs (OITN) and Simplified Debris Index (DI-S). After the examinations, the children were offered information and demonstrations about the proper oral hygiene technique and healthy diet. Also, they received oral hygiene products and notes for parents in order to be informed about their child's oral status and dental treatment need. The data were analyzed using statistical program SPSS vers. 13.0.

RESULTS

From the total number of 2599 examined subjects, 2395 were brought to be analyzed, and after introducing the data in the database, only 2288 were considered valid, the rest were excluded due to the unproper filling. The subjects were 49.9% girls an 50.1% boys, with an age between a minimum of 5 years and a maximum of 11 years: 27% at 7 years old, 37% at 8 years old an 22% at 9 years old. There were found that 36% of children were in the 1st grade, 42% in the 2nd grade, 16% in the 3rd grade, only 5.3% in the 4th grade and 1.5% in the preparatory grade.

Caries experience for temporary dentition shows that caries prevalence is very high (71.1%), thus only 28.9% of

temporary teeth are healthy, cavity-free (Table 1).

Table 1. Severity zones distribution for temporary teeth

Severity zones distribution for temporary teeth

		Frequency	Percent	Valid Percent	Cumulative Percent
	Zone 1	646	28.2	28.9	28.9
	Zone 2	543	23.7	24.3	53.2
	Zone 3	584	25.5	26.1	79.3
	Zone 4	461	20.1	20.6	99.9
					100.0
	Total	2238	97.8	100.0	
Missing	System	50	2.2		
	Total	2288	100.0		

Regarding the location of the cavities found in temporary dentition for this group of children, we found small diferences between percentages for occlusal area (zone 2) – 24.3%, proximal surfaces (zone 3) – 26.1% and buccal/oral surfaces and frontal teeth (zone 4) – 20.6%. Only 29% of the total number of the surfaces examined were cavities – free (zone 1) (Table 1).

Regarding the def-s index, we found a medium value of 5.44 (SD=6.606) of tooth surfaces affected by carious lessions (cavities, extracted teeth due to the severe cavities and fillings), of which most of the surfaces had untreated cavities (ds = 4.78±6.244 surfaces) (Table 2**).**

Table 2. Defs, deft indices and their components for entire sample

Defs, deft indices and their components for entire sample

N	ds	es	fs	defs	dt	et	ft	deft
Valid 2235	4.78± 6.244	.23± 1.654	.45± 1.527	5.44±6.606	2.84± 2.855	.07± .404	.29± .896	3.18± 2.93
Missing 53								

When it comes to def-t index, the medium value was 3.18 (SD=2.93), based mostly on the ds value, 2.84 (SD=2.85) teeth presenting decays, and only 0.29 (SD=.896) with fillings (15% of the total number of examined teeth) and 0.07(SD=.404) teeth (3.5% from total number) extracted due to the advanced phases of cavities (Table 3). When we analyzed the def index per surfaces and per tooth, taking the grade as the independent variable, we found the following results for def-s, def-t indexes and their components (ds, es, fs, dt, et, ft) (Table3).

Table 3. Mean defs, deft indices and their components in primary dentition for children grade 0-4

Mean defs, deft indices and their components in primary dentition for children grade 0-4

Grade	N	ds	es	fs	defs	dt	et	ft	deft
0	34	8.44± 12.54	.00	.44±1.211	8.88± 12.438	3.35± 3.347	.00	.41± 1.019	3.76± 3.403
1	767	5.50±7.031	.19±1.303	.53±1.736	6.22±7.305	3.27±3.138	.06±.328	.33±.948	3.64±3.168
2	890	4.39±5.549	.19±1.316	.36± 1.368	4.94 ±5.903	2.79 ±2.790	.05±.331	.23 ±.760	3.07 ±2.860
3	320	3.87± 5.284	0.3±0.305	0.7± 1.721	4.61±5.448	2.01±2.155	.05±.318	.461±.200	2.50±2.417
4	86	3.81±5.420	.813±.331	.12±.788	4.746±.327	1.941±.972	.16±.666	.05±.262	2.15±2.044

For permanent dentition, the prevalence found for this group of children was 31.8%, thus 68.2% of permanent teeth examined were cavities-free (Table 4).

Table 4. Severity zones in permanent dentition

Severity zones in permanent dentition

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	Zone 1	1553	67.9	68.2	68.2
	Zone 2	559	24.4	24.5	92.8
	Zone 3	53	2.3	2.3	95.1
	Zone 4	112	4.9	4.9	100.0
	Total	2278	99.6	100.0	
Missing	System	10	.4		
Total		2288	100.0		

Most of the affected surfaces were caries-free (zone 1) - 68%, followed by the occlusal surfaces (zone 2) – 24.5%, for very small percent, proximal surfaces (zone 3) – 2.3% and buccal/oral surfaces and frontal teeth (zone 4) - 4.9% of the examined surfaces (Table 4).

The mean value of DMF-S found was 0.92 (SD= 1.778) and most of the examined surfaces were decayed – 0.88 (SD= 1.735) (Table 5). The average DMF-T is 0.82 (SD=1.693), examined teeth being in equal proportions decayed (31%), treated (32%) and even extracted (37%) (Table 5).

Table 5. Mean DMFS, DEFT indices and their components for permanent dentition

Mean DMFS, DEFT indices and their components for permanent dentition

N	DS	MS	FS	DMFS	DT	MT	FT	DMFT
Valid 2278	.88±1.735	.01±.252	.05±.355	.92±1.778	.77±1.551	.01±.147	.04±.338	.82±1.693
Missing 10								

Regarding the Orthodontic Index for Treatment Need, we found that in equal proportions children have a low (34%), medium (32%) and high (33%) need of this type of treatment (Table 6).

Table 6. Orthodontic treatment need, expressed by IOTN

Orthodontic treatment need, expressed by IOTN

IOTN		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	1	714	31.2	34.5	34.7
	2 and 3	666	29.1	32.2	67.0
	4	683	29.9	33.0	100.0
	Total	2067	90.3	100.0	
Missing	System	221	9.7		
Total		2288	100.0		

We found that sealing were applied on only 8.5% of examined molars, mostly on two molars (2,8%), folllowed by one molar (2.7%), very few children have all four molars sealed (1.8%).

Regarding the quality of oral hygiene performances, we found dental plaque covering one third of surface in 35% of case, two thirds – 28% and even all the surface in 11% of cases. There are only 25% of subjects

that keep the tooth surfaces plaque-free (Table 7). The average number of areas screened for gingival inflammation was 0.87.

Table 7. Plaque Index

Plaque Index

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	=0	509	22,2	25,5	25,5
	=0.1-0.6	708	30,9	35,4	60,9
	=0.7-1.8	564	24,7	28,2	89,1
	=1.9-3	219	9,6	11,0	100,0
	Total	2000	87,4	100,0	
Missing	System	288	12,6		
Total		2288	100,0		

CONCLUSIONS

The present study reveals a high prevalence of dental caries – two thirds of both children are affected, not only at temporary dentition, but also at permanent dentition. For deciduous teeth, indices for carious experience are high, mostly because of the presence of the untreated cavities. Regarding the location of the caries, all three types of surfaces were equally affected. Results showed that the most affected children are those in grade 0 and the opposite – less affected were those in the 3rd grade. Permanent dentition was less affected – there was a mean 1 tooth and 1 surface per child affected by caries. There were found equally untreated, treated with filling or the tooth was extracted because of the advanced lesion. The location of the cavities were eather occlusal or proximal but no child was found with the buccal or vestibular surface affected.

When it comes to othodontic treatment needs, children examined are equally distributed in the 3 levels – high, medium, low. Only 1 of 11 children were applied sealants for preventing the occlusal cavities. Regarding the oral hygiene performance, in most cases this was insufficient. Only one quarter of the subjects had the examined surfaces plaque-free. Most children had plaque covering one third or two thirds of the surfaces examined and even one in 10 children had these surfaces completelly covered by plaque. Gingival bleeding was observed at one in ten examined teeth.

Compared to results of the studies developed in the past in Bucharest, children in the present study had a lower prevalence and a lower number of teeth or surfaces affected. But when it comes to permanent dentition, even though the prevalence of caries decreased, the number of teeth or surfaces per child affected by caries increased.

Therefore, efforts should be made to continously decrease the prevalence of caries both in temporary and permanent dentition, intensify the prevention actions including the increase of applications of sealants, encourage and support the children to inhance their oral hygine techniques, using also auxilliary products to clean the prone surfaces and also to promote the need and the role of the orthodontic treatment. Regarding the high number of permanent molars extracted by this age, it is necessary to raise awareness among parents of the role played by these molars and thus to insist on maintaining their integrity, early treatment, avoiding the extraction or considering an interceptive tratement immediately after the extraction.

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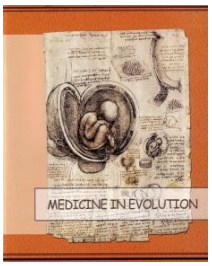
THE IMPLICATION OF HERPESVIRUSES IN PERIODONTAL DISEASES- CLINICAL REPORT



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ABSTRACT

Findings revealed that specific periodontal injury may be induced by viral agents, especially herpes viruses that are present in millions of genomic replicas on this level.

The aim of this study is to emphasize on the basis of one clinical study, a supposed involvement of the herpetic viruses in the development of the periodontal disease. The occurrence of the viral DNA in the periodontal tissue has been performed by the genetic amplification, following an"in house"method (High pure PCR template preparation kit -Roche). The obtained results showed the occurence of the EBV DNA in the collected tissue. The CMV DNA did not occured.

The data obtained reveals that a herpesviruses (EBV and CMV) involvement is a possibility to be accepted regarding the evolution of the periodontal disease, fact that it should be further researched.

Key words: *CMV, EBV, Periodontal diseases, PCR*

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INTRODUCTION

Findings reveal the frequent associations of the periodontal disease with major systemic disorder and inflammatory process caused by viral agents (herpesviruses).

The complexity of the periodontal disease is generated by the interplay between the supposed pathogen agents and the host organism's defence response.

There are insufficient informations regarding the fact that, at hosts with the same level of risk factors, many of the periodontal infections lead to the loss of the periodontal support and of the bone structure, meanwhile other infectious cases remain limited to an inflammatory process of the gums with clinical unobtrusive consequences.

Findings revealed that specific periodontal injury may be induced by viral agents, especially herpes viruses, but also by papilloma and hepatic viruses, that are present in millions of genomic replicas on this level (1, 2).

The aim of this study is to evaluate on the basis of one clinical study, the hypothesis that herpetic viruses play a role in the development of the periodontal disease.

The genome of the Epstein –Barr (EBV) and cytomegalovirus (CMV) were identified in the periodontal injuries in frequencies varying from a few percents to 80%.

Studies conducted so far display a 65% incidence of the EVB in aggressive periodontal pathology cases and only 49% in chronic injuries, meanwhile CMV was revealed in 44% of the active and chronic cases (3, 4, 5, 6, 7).

In this study we will present a clinical case with chronic periodontitis, followed by the revealing of the viral DNA in the periodontal diseased tissue.

CASE REPORT

The 49 years old female patient came to the dental clinic with periodontal issues. The patient did not suffer any systemic disease. The clinical examination revealed tartar, gum retractions to a maximum of 5 mm, and probing depth up to 6 mm.

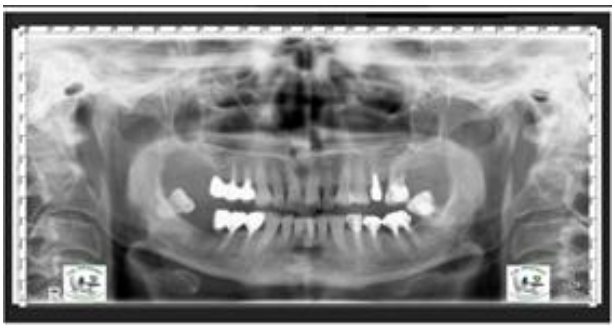

Figure 1. Initial panoramic radiography

Figure 2. Initial clinical situation in the frontal mandible

Figure 3. Initial situation in the frontal dental-maxillary

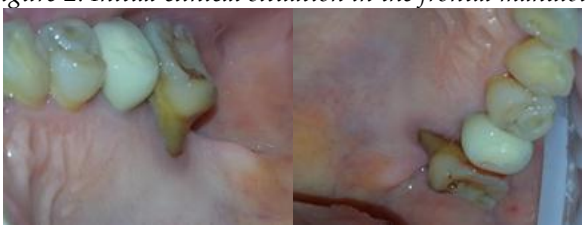

Figure 4. Initial situation in the maxillary side group

After the patient gave her full consent, injured tissue was collected from the periodontal pochets. The tissue was transported in EPPENDORF recipients to the Ştefan S.Nicolau Virusology Institute Bucharest, where the sample was analysed.

The occurrence of the viral DNA in the periodontal tissue has been performed by the genetic amplification, following an"in house"method (High pure PCR template preparation kit - Roche), as prior described (8). The target DNA's integrity was verified by amplification of a 110 bp β-globin gene fragment using PC 03/04 primers synthetized by Invitrogen).(9)

The concentration and purity of each DNA sample was evaluated with NanoDrop spectrophotometer.

The HCMV primers' sequences:

Fw: 5'-GAGCGCGTCCACAAAGTCTA-3'

R: 5'-GTGATCCGACTGGGCGAAAA-3'

The EBV primers' sequences:

Fw: 5'-AGGGATGCCTGGACACAAGA-3'

R: 5'-GCCTCGGTTGTGACAGAG-3'

PCR conditions for HCMV and EBV are shown in the following table:

Table 1. PCR conditions

Initial distorsion	94°C	5 minutes	1 cycle
Distortion	94°C	30 seconds	30 de cycles
Primers' alligment	59°C	30 seconds	
Elongation	72°C	30 seconds	
Final elongation	72°C	5 minutes	1 cycle

The obtained results: The occurence of the EBV DNA (figure 3-sample 2) in the collected tissue. The CMV DNA did not occured.

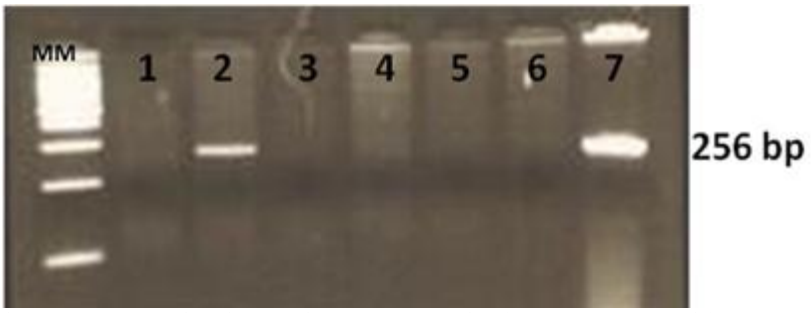

Figure 5. Legend EBV MM= molecular marker; 1-5= samples (2= positive sample); 6= negative control'; 7= positive control

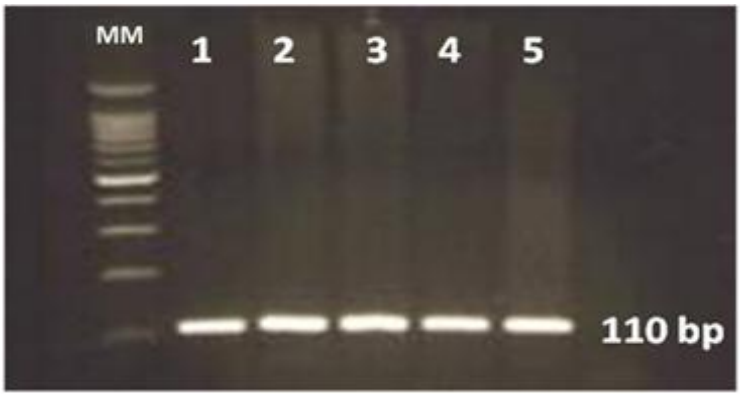

Figure 6. Legend β globin MM= molecular marker; 1, 2 3, 4 = samples; 5= positive control

Figure 7. Dental arches after cleaning

DISCUSSIONS

The abundance of the herpesviruses in the periodontal injuries is expressed through a big viral load at the level of the entire periodontal system, in patients with severe and extended periodontal pathology.

Research revealed that herpesviruses (capable to produce systemic infections following the activation of the latent infections under the state of immune suppression or under trauma and inflammatory diseases) were isolated in the saliva and the gum tissue of the patients suffering from periodontal pathology (3,10).

In the study case presented, the viral DNA occurrence may explain the aggressive evolution of the disease. This shows the importance of complementary testing, whose results might help us understand the evolution and the aggressiveness of the injury.

CONCLUSIONS

The data obtained reveals that a herpesviruses (EBV and CMV) involvement is a possibility to be accepted regarding the evolution of the periodontal disease, fact that it should be further researched.

Understanding the significance of herpesviruses in human periodontitis may allow for improved diagnosis and, ultimately, disease prevention.

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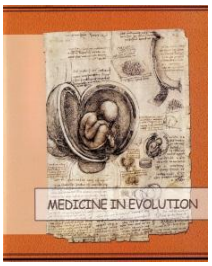
ORAL HYGIENE BEHAVIOR AMONG CHILDREN OF 9-10 AGE OLD. CASE STUDY OF THREE SCHOOLS FROM TIMISOARA, ROMANIA



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ABSTRACT

Literature on dental health behavior is consistently developed and mainly based on data from adults. There are also researches realized on children`s dental health behavior, but there are less frequent and realized just in small number of country. No data to the actual oral hygiene behavior on children from the primary schools exist in Romania. Therefore our aim is to identify the specific behavior in terms of keeping oral hygiene between children from primary school. In order to achieve our aim we designed our research using quantitative approach, conducting specialized social survey. The research sample was constructed from 9-10 years old children coming from different but not representative schools from Timisoara area. The sample was done by 418 selected children representing the entire population of children from the 3 schools, respecting age selection criteria. The study presents the oral hygiene behavior of 9-10 years children associated with gender specificity. In order to increase the children' good practice in oral hygiene, our research indicate the opportunity of using schools as new channels for teaching children on oral health behavior

Key words: *Oral hygiene behavior, children, Timisoara, Romania*

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INTRODUCTION

Scholars in oral health have agreed that assessments of oral hygiene practice have a great importance in developing oral health care and prevention. The literature shows that oral self-care practice, individual belief and attitudes are considered to have an important role in oral health care. The relation between psychosocial dimension and oral health behavior has been analyzed by several different studies. As example, one of the theories used to predict patients' tooth brushing and dental flossing, it was the theory of reasoned action. (Ajzen and Fishbein, Understanding attitudes and predicting social behavior. 1980). The results of this study show that attitude and subjective norms explain the variance in intention to brush and to floss. In additional, the study realized by Freeman and Linden in 81 college students among 214 participants (Freeman and Linden 1995), indicate that an adequate oral hygiene behavior has been associate with individual's attitude toward oral health and with the perceived influence of the others persons that are part of the respondent's social capital.

However, even consider the large number of studies realized, we, as the others authors (Buunk-Werkhoven, Dijkstra and van der Schans 2011) consider that so called"state-of-art"with regard of oral health behavior is still not satisfactory. Continuing oral hygiene behavior index, as recommended in the previous studies, is characterized by the lack of the complete measure of this behavior. For this reason this study aims to develop a new and more elaborate index for oral hygiene behavior based on process analyze. The first and the most important aspect is that our study is exclusively on realized actions not on intention to perform behavior. We consider oral behavior not just a matter of"just tooth brushing and flossing"(Buunk-Werkhoven, Dijkstra and van der Schans 2011), but also as a complex and multidimensional process that include instruction, motivation, a matter of doing and specifics effects. In this way, our study was oriented by the next research question: Who are the persons that instruct children on oral hygiene behavior? Which are the motivations of keeping teethe clean? When, how frequent, how long and with what effects oral self-care practices are realized?

Aims

Oral hygienic behavior is an important predictor of oral health care and moreover for the quality of life. Literature is consistently developed and mainly based on data from adults. There are also researches realized on children`s oral hygiene behavior, but there are less frequent and realized just in small number of countries. No data to the actual oral health behavior on children from the primary schools exist in Romania. Therefore, our aim was to identify the oral hygiene behavior of children at the end of primary school.

MATERIAL AND METHODS

In order to achieve our aim we designed our research using quantitative approach, conducting specialized social survey. The research sample was constructed from 9-10 years old children coming from different but not representative schools from Timisoara area. The 418 selected children represented the entire population of children from the 3 schools, respecting age selection criteria. The first school was from an appreciated rich neighborhood closed to Timisoara (n=118 children). The

second one was from downtown Timisoara (n=184 children) and the third one was from a middle class suburb Timisoara (n=116 children). From the initial sample size of 443 interviewed children, 25 cases were drop outs because of the declared age of 8 years old. The research instrument was a questionnaire with 37 closed and opened questions related to specific oral hygiene behavior. The instrument was firstly tested on a pilot sample of 20 children and afterwards optimized and adapted for 9-10 years old children. The University „Victor Babes"ethics committee approved the study and the school informed consent was obtained for each subject of our research.

RESULTS AND DISCUSSIONS

Our first research interest was regarding the instruction in oral hygiene behavior. The results show that 379 (91.6%) children declared that at least one person talk to them about brushing teeth.

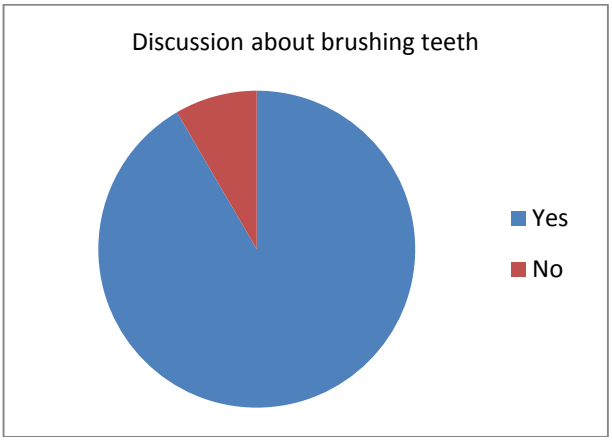

Figure 1. Discussion about brushing teeth

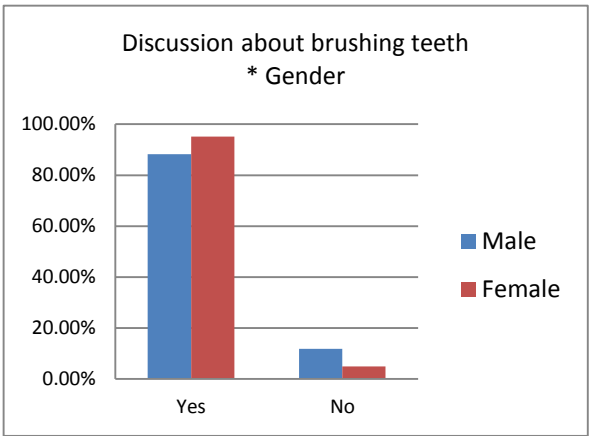

*Figure 2. Discussion about brushing teeth * Gender*

In order to have a better understanding of the process we conduct a comparative analyze in order to identify the variance across gender. The results reveal an existing statistical difference between female 200 (94.8%) and male 173 (88.3%). Even if this variance is not a strong one, as a preliminary discussion we can assume that girls pays more attention or receive more information regarding oral hygiene practice.

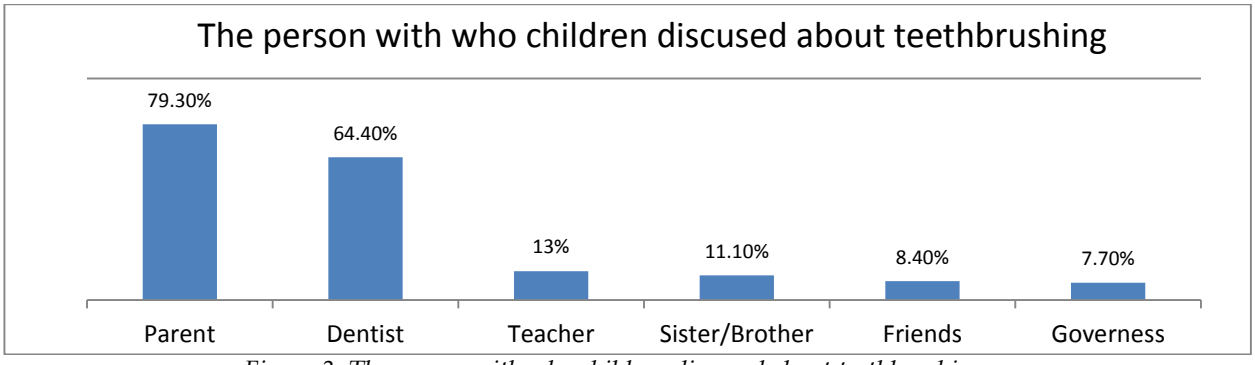

Figure 3. The person with who children discused about teethbrushing

The person who took care of that was one of the parents 330 (78.9%), the dentist 268 (64.1%) and, only for 32 (7.7%) cases the governess or the teacher 54 (12.9%). Based on this results we can say that informing about oral hygiene practice is made by two important agents: the family and the

professional in oral dentistry. An interesting aspect that can be observed analyzing this graph is the importance of teacher in this process of information. Even if the teacher is most closed person of the children in this period, its contribution could be considered as being very limited. For this reason, considering the proximity between the children and the teacher at this age, the teacher could be considered as a resource person and the school as an adapted place for informing children in oral hygiene behavior.

As follow, another point of interest for our research was the perception of children regarding their knowledge on the good practice of oral hygiene. Our result show that an average of 207 (49.5%) children declared that they knew very well to brush their teeth. The gender comparative analyze indicated that 85 (42.9%) male and 118 (55.4%) female appreciate that they new very well to brush their teeth. Considering this result we can assume that the difference between genders was statistically significant not only in term of informing, but also in terms of perception.

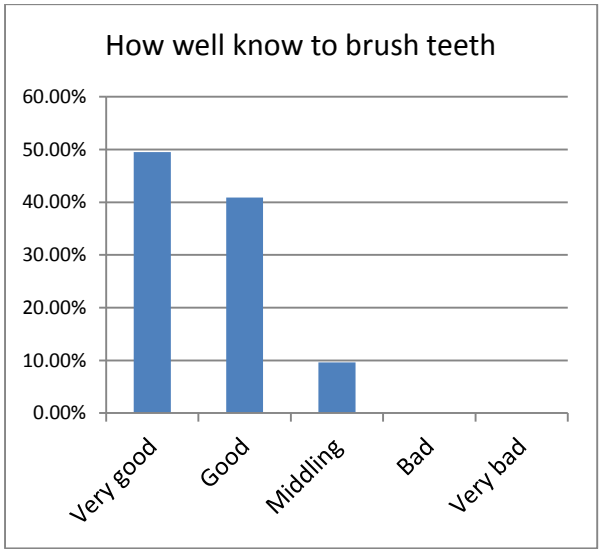

Figure 4. How well know to brush teeth

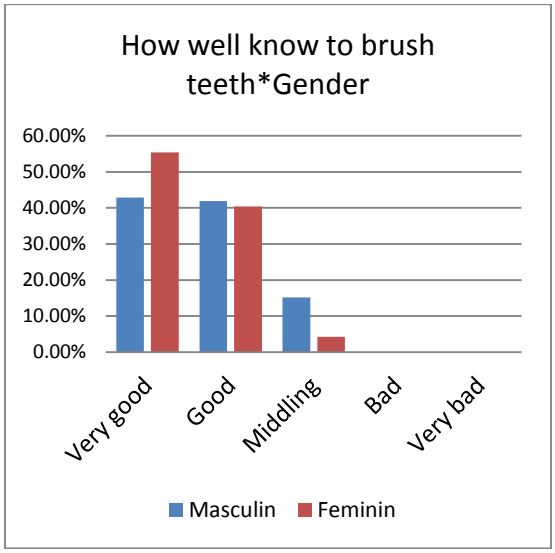

*Figure 5. How well know to brush teeth*Gender*

The daily behavior in terms oral hygiene was probably the most important indicator of our study. Considering brushing after each meal as the most adequate practice in term of oral hygiene, we conduct a comparative analyze between girls and boy on this issues. Once again our results show a significant variance across gender 37 (18.7%) male compared with 58 (27.2%) female declared that they used to brush their teeth after each meal.

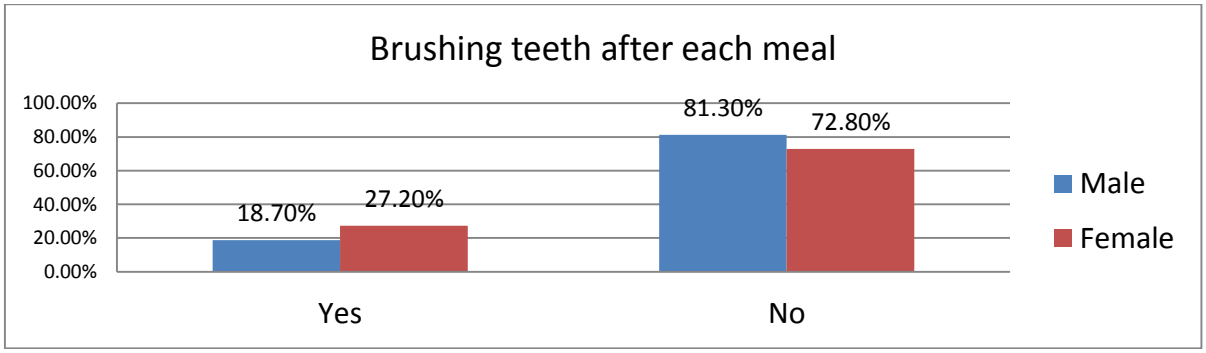

Figure 6. Brushing teeth after each meal

CONCLUSIONS

The study presents the oral behavior of 9-10 years children associated with gender specificity. Considering the significant difference between male and female behavior, we could propose that boys more than girls need more attention in doing prevention and promoting good practice of oral hygiene behavior. In the same time, based on our research results we suggest and indicate the opportunity of using the teachers as agents and the schools as new places for teaching children on oral health behavior.

Acknowledgment

This work was realized with the support of the project:"Parteneriat strategic pentru cresterea calitatii cercetarii stiintifice din universitatile medicale prin acordarea de burse doctorale si postdoctorale – DocMed.Net_2.0"project no. POSDRU/159/1.5/S/136893.

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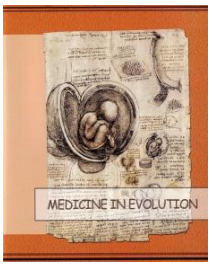
THE MARKERS OF SYSTEMIC INFLAMMATION IN PERIODONTAL DISEASE ASSOCIATED WITH PREMATURITY



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ABSTRACT

Objectives. *During pregnancy periodontal pathology can know exacerbation closely linked to specific hormonal changes occurred. The existence of inflammatory periodontal disease may have negative influences on pregnancy. The aim of the research was to analyze possible correlations between periodontal disease during pregnancy and premature birth.*

Material and method. *To achieve its purpose 109 pregnant women were studied in the second trimester of pregnancy. They have been monitored for the values of C-reactive protein, fibrinogen and a dental exam was conducted. The pregnancy has been monitored and it ended in a premature birth.*

Results and discussion. *In the control group, the PCR value was below the cut-off limit, being 1.73 mg / dL ± 0.65 SD. Mean values of CRP were higher in pregnant women with periodontal disease thus: in women with gingivitis, the average PCR was 6.16 mg / dL ± 0.78 SD, in women with slight PMC mean PCR were 6.20 mg / Mr ± 0.49 SD, and in pregnant women with moderate to severe PMC, mean PCR were 10.88 mg / dl ± 3.07 SD.*

Fibrinogen values were highly statistically significantly different between the 4 groups, ANOVA p <0.001, loads with gingivitis or mild PMC with lower values, 6.29 mg / l respectively ± 0.76 SD 6.18 mg / L ± 0.86 SD, unless the lot with medium and severe PMC, 7.15 mg / l ± 0.48 SD, and high significantly higher than the control group.

Conclusions. *The existence of periodontal disease at pregnant women who gave birth prematurely, associated with high inflammatory markers (CRP, fibrinogen), constitutes a relationship between the pathogenic mechanisms of periodontal disease and preterm birth criminalized.*

Key words: *periodontal disease, CRP, preterm birth*

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INTRODUCTION

Pregnancy is a period during which periodontal pathology can know exacerbations due to hormonal changes, but also the periodontal inflammatory processes can have negative effects on some developments in the state of pregnancy. Prematurity accounts for approximately 75% of perinatal mortality, and half of long term morbidity (4). The association of periodontal disease with complications of pregnancy is a highly debated issue. A number of studies have shown that periodontal pathogens can enter the systemic circulation, such as maternal periodontal diseases can be associated with premature birth by mechanisms involving inflammatory molecules or direct invasion of the amniotic membranes of bacteria (5). It has been demonstrated that women with periodontal disease are two to four times more likely than healthy women to give birth prematurely or have a low birth weight baby (9).

MATERIAL AND METHODS

For this study a total of 109 pregnant patients were selected, being in the second trimester of pregnancy, from the ones existing in the files of the Obstetrics and Gynecology Clinic of Filantropia Hospital from Craiova. Patients participating in the study were divided into three groups as follows:

1. a sample of 56 pregnant patients with gingivitis

2. a group of 26 pregnant patients with periodontitis including:

- 16 cases with mild chronic periodontitis = easy PMC

- 8 cases with average chronic periodontitis = moderate PMC

- 2 cases with severe chronic periodontitis = severe PMC

3. a group of 27 healthy pregnant patients as a control group

Each patient has been achieved dosages of C-reactive protein (CRP) and fibrinogen and was examined in the oral cavity. Data were recorded in a personal form of periodontal control of the pregnant woman, adapted from the model proposed by professor Dumitriu (3).

For all patients the study was carried until they gave birth or if there have been premature births. The increase of the PCR may be caused by the existence of periodontal disease (chronic bacterial infection that starts at the gums).

The aim of this study was to examine the relationship between periodontal disease and CRP levels in plasma fibrinogen, serum markers of inflammatory status among pregnant women.

RESULTS AND DISCUSSION

I especially chose as the cut-off for CRP the value of 2.5 mg / dl, given by statistical analysis performed. The statistical analysis of this parameter has shown a strong correlation between periodontal disease and pregnancy complications.

Table 1. Statistical analysis of the mean values of CRP levels in the studied groups

Group	*Number of cases*	*Media*	*Standard deviation*
Control	26	1.73	0.65
Gingivitis	58	6.16	0.78
Slight PMC	15	6.20	0.49
Moderate + severe PMC	10	10.88	3.07
p ANOVA	**0.000**	**HS**	

In the case of the control group, the PCR value was below the established cut-off limit, being 1.73 mg / dL ± 0.65 standard deviation. Average values of CRP were higher in pregnant women with periodontal disease. In women with gingivitis, the average PCR was 6.16 mg / dL ± 0.78 standard deviation. In women with slight PMC, average PCR have almost the same value as in women with gingivitis, 6.20 mg / dL ± 0.49 standard deviation.

For pregnant women with moderate to severe PMC, the average PCR were much higher than in other subgroups, 10.88 mg / dl ± 3.07 standard deviation.

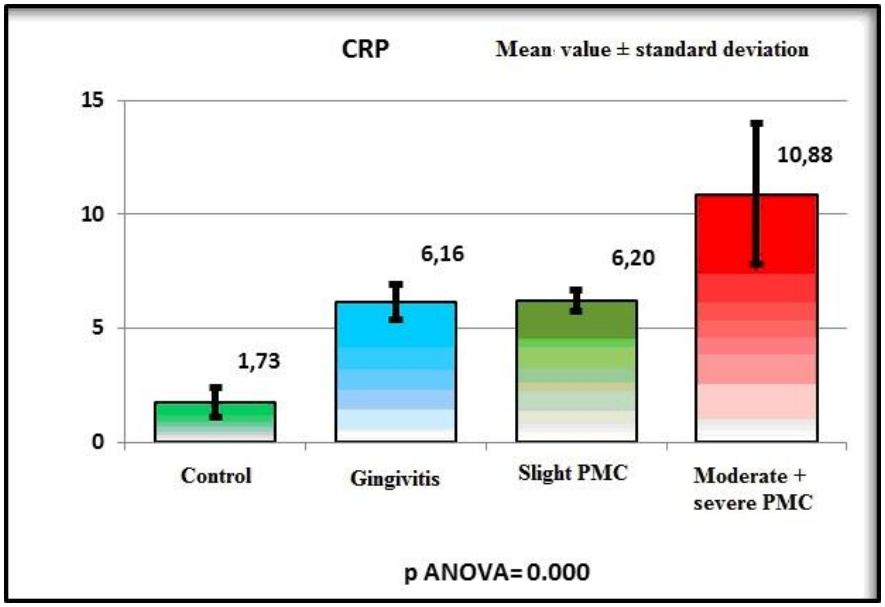

Chart 1. Statistical analysis of the mean values of plasma PCR in the studied groups

Making the pANOVA test to compare the values of C reactive protein, we found statistically significant differences between groups ($p < 0.001$). Continuing the analysis with the Fisher LSD post-hoc test we found that lots of gingivitis and mild PMC had significantly higher values than the control group, but significantly lower than the group with moderate to severe PMC.

The association between CRP and periodontitis in pregnant women may or may not be a causal one. High levels of C-reactive protein may be caused by periodontal infection or inflammation. In this case, C-reactive protein may enhance the inflammatory response by complement activation, tissue damage, inflammatory cytokines, the induction of monocytes, and thus may mediate the relationship between periodontal disease and adverse effects during pregnancy (8).

CRP involvement in producing premature birth was statistically analyzed to find if elevated CRP may be predictive of periodontal disease to premature birth.

Table 2. Statistical analysis of plasma CRP from the premature birth

	Premature bitrh		*Term birth*		*Total*	
CRP>2.5	32	37.21%	54	62.79%	86	100.00%
CRP<2.5	4	17.39%	19	82.61%	23	100.00%
Total	36	33.03%	73	66.97%	109	100.00%
p Chi square	**0.07265**	**NS**				

Chi square test result exceeds the threshold of significance (p> 0.05), so there's a useful relationship between the increased value of CRP and the possibility of premature births, although we found values> 2.5 mg / dl in most cases that gave birth prematurely.

OR	*2.815*	
I.trust 95%	0.879	9.011

RR	*2.140*	
I.trust95%	0.668	6.849

The fact that the neutral value, 1, is found in the range of 95% of OR and RR leads us to consider that the OR and RR results are not statistically significant, so we can not conclude that the identification of CRP may help detect future premature births.

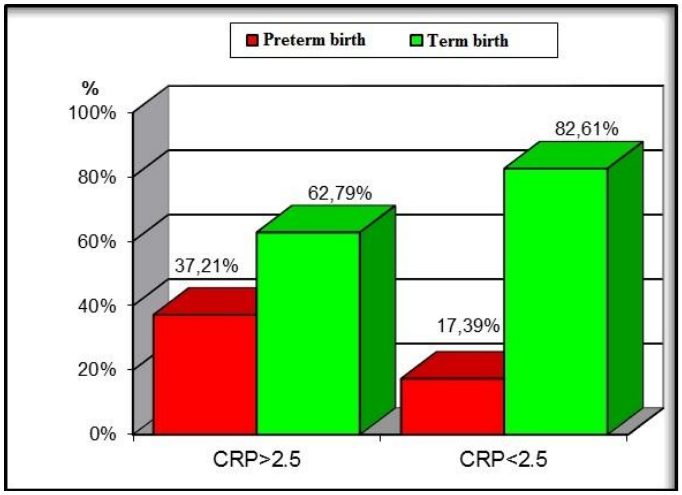

Chart 2. Statistical analysis of plasma PCR from the premature birth

Statistical parameter	*%*
Accuracy	***46.79%***
Sensitivity	***88.89%***
Specificity	***26.03%***
Positive predictive value	***37.21%***
Negative predictive value	***82.61%***

The low accuracy of this test of 46.79%, as the specificity 26.03%, makes this test not to have precision just by itself in determining the likelihood of premature births. Only the negative predictive value may be practical because it shows that the values below 2.5 mg / dl, only 17.39% of pregnant women will give birth prematurely.

The association between CRP and premature birth has not been clearly linked to the pathogenesis of the premature birth. Studies so far have produced conflicting results and not yet developed that can determine exactly this correlation (6,1).

Fibrinogen is a soluble plasma glycoprotein synthesized by the liver, converted by thrombin to fibrin during coagulation. Fibrinogen, along with its role in clotting, can cause inflammation development.

It seems that people with periodontal disease are more likely to have a rare form of the gene responsible for the expression of fibrinogen than people without

periodontal disease (5). In this context we analyzed this parameter, fibrinogen, in pregnant women with periodontal disease, statistically establishing a cut-off of 5.4 g / l.

Table 3. Statistical analysis of the average values of plasma fibrinogen

Group	*Number of cases*	*Media*	*Standard deviation*
Control	26	4.04	0.76
Gingivitis	58	6.29	0.86
Slight PMC	15	6.18	0.87
Moderate + severe PMC	10	7.15	0.48
p ANOVA	**0.000**	**HS**	

Fibrinogen values were highly statistically significantly different between the 4 groups, ANOVA p <0.001, the groups with gingivitis or mild PMC having lower values, 6.29 mg / l respectively ± 0.76 SD 6.18 mg / L ± 0.86 SD, only groups with moderate to severe PMC, 7.15 mg / l ± 0.48 SD, and significantly higher than the control group.

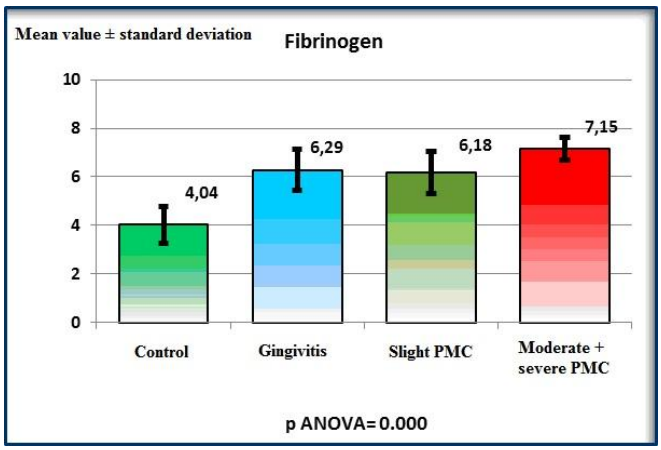

Chart 3. Statistical analyses of the average values of plasma fibrinogen

Fibrinogen involvement in premature birth was analyzed in order to see if increased fibrinogen values from the periodontal disease may be predictive for preterm birth.

Table 4. Statistical analysis of plasma fibrinogen in premature birth

	Premature birth	*Premature birth*	*Term birth*	*Term birth*	*Total*	*Total*
Fibrinogen>5.4	28	40.58%	41	59.42%	69	100.00%
Fibrinogen<5.4	8	20.00%	32	80.00%	40	100.00%
Total	36	33.03%	73	66.97%	109	100.00%
p Chi square	**0.02767**	**S**				

Chi square test result is statistically significant, p <0.05, so there is the possibility that high fibrinogen found value to be useful in the detection of future premature births.

Statistical parameter	*%*
Accuracy	*55.05%*
Sensitivity	*77.78%*
Specificity	*43.84%*
Positive predictive value	*40.58%*
Negative predictive value	*80.00%*

The amount of fibrinogen> 5.4 seems to have no practical utility in detecting premature births, according to the statistical parameters followed.

Early increase in fibrinogen values was associated with a reduced risk of premature birth in some studies (7).

CONCLUSIONS

The determination of inflammatory markers can be used as a diagnostic tool for identifying clinical infection, in monitoring of the effects of treatment and early detection of recurrent disease and, therefore, can be useful to determine the progression of the disease.

Our study showed a high statistical significance between periodontal disease and systemic inflammatory markers, CRP and fibrinogen, but they can not be used as predictive markers for the evolution of pregnancy complications if determined in the second trimester.

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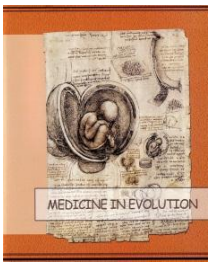
ORAL HEALTH EDUCATION CLASS IN PRESCHOOL CHILDREN COMMUNITIES



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ABSTRACT

Health education is considered a basic and mandatory activity of health services. It must be regarded as an element of continuous education on a background previously prepared by the public instruction system. Regarding health education of preschool children, there is a series of objectives and specific problems according to morpho-functional and neuro-psychological peculiarities of children.

Aim: The main objectives of health education in kindergartens are: to elaborate hygiene habits and practices necessary for human health and to increase the resistance to diseases, to acquire harmonious physical and mental development; to provide children with elementary notions on age-specific individual and collective hygiene and to develop hygienic behaviours and habits allowing children to act for the benefit of their own health and also for the health of the collective they belong to.

Material and method: In the group of small, 3-4 years old children, education will focus on acquiring personal hygiene for body and clothing. For this purpose, emphasis will fall on shaping habits and practices of personal hygiene; in 4-5 years olds, activities will continue with improvement of habits and practices initiated during small ages and with additional practices according to age specific hygiene and sanitation requirements. Of great importance at this age is the early removal of harmful habits. In 5-6 years old children, emphasis is mainly set on intensification of educational influences.

Conclusions: Health education of preschool children has a particularly important role in their healthy development and growth, in the acquisition of healthy hygiene practices. These practices are vital for the formation of future healthy adult organisms, free of nutrition or hygiene deficiencies.

Key words: *education, oral health, hygiene, nutrition*

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INTRODUCTION

Health culture is formed by the general instruction of the population throughout successive generations. Health education is considered a basic and mandatory activity of health services. It must be regarded as an element of continuous education on a background previously prepared by the public instruction system.

Health education is the main trigger for both health and education services in order to reach good results in improving population health. It expands its influence onto the entire population with different objectives, content and methods depending on peculiarities of target groups.

Regarding health education of preschool children, there is a series of objectives and specific problems according to morpho-functional and neuro-psychological peculiarities of children. In the group of small, 3-4 years old children, education will address learning personal hygiene, both bodily and clothing aspects. For this purpose, emphasis will be put on the formation of personal hygiene habits. In the group of 4-5 years old children, the improvement of habits already initiated in small age groups is continued and further habits are added according to age specific hygiene and sanitation requirements. In the group of 5-6 years old children, emphasis is mainly put on the intensification of educational influences for a harmonious development of the preschool child and for the strengthening of the defence capacity of the organism, as well as for maintaining the health status. For these purposes, the accommodation of the child with natural strengthening factors such as air, water and sun is required.

PURPOSE OF EDUCATION

The main objectives of health education in kindergartens are: to elaborate hygiene habits and practices necessary for human health and to increase the resistance to diseases, to acquire harmonious physical and mental development; to provide children with elementary notions on age-specific individual and collective hygiene and to develop hygienic behaviours and habits allowing children to act for the benefit of their own health and also for the health of the collective they belong to.

The instructive-educational process in kindergartens is a formative process. Health education in preschool institutions expands its action onto adults who, depending on their health education level and by fulfilling occupationally related activities, may positively or negatively influence children's health and health education.

The various categories of care personnel i.e. administrative, parents and teaching staff, together with institution management must be, in their turn, instructed and educated regarding hygiene and sanitation requirements in preschool institutions.

Thus, for the good organisation and development of health education activities in preschool institutions, two distinct categories are needed: healthcare and education personnel (educators).

The main tasks of the medical staff are coordination and guidance of health education performed by educators with preschool children, health education of the members of the education, administration and management departments and health education of parents, conducted in collaboration with the teaching staff.

The members of the teaching staff (educators) who have the task of integrating children's health education in the educational process, also take part in the health education of care staff members and parents together with a physician or another competent healthcare staff member employed by the kindergarten.

MATERIAL AND METHODS

Regarding the content of health education, this must be adapted to each age group according to physical and psychological development.

The volume of transmitted knowledge and influencing actions exerted on children is adjusted to the level of hygiene habits and practices required and achievable in different age groups.

In the group of small, 3-4 years old children, education will focus on acquiring personal hygiene for body and clothing. For this purpose, emphasis will fall on shaping habits and practices of personal hygiene; in 4-5 years olds, activities will continue with improvement of habits and practices initiated during small ages and with additional practices according to age specific hygiene and sanitation requirements. Of great importance at this age is the early removal of harmful habits.

In 5-6 years old children, emphasis is mainly set on intensification of educational influences.

Preschool age adapted health education types are varied. Transmitting knowledge for shaping healthy habits and practices is done by current classic work methods with preschool children, adapted for health education and age specific peculiarities: group activity as a mean of health education, free discussions with children, occasional or chance story telling, observational visits, creative, motility games used as health education means, etc.

For long lasting results, parents have to be also educated. Both in preschool institutions and in families, direct influences are the most effective: discussions and advice offered to care takers (parents, grandparents, other care takers); private conversations at home, whenever appropriate; dissemination of leaflets, brochures, and health education literature among parents; control of fulfilment of offered recommendations and advice; conversations organised during meetings with parents; informing parents on the results of children's health check-ups; involving the parents in various health education actions and activities, etc.

Education lessons were organised in some kindergartens in Timisoara and included 3 successive sessions.

The first session was entitled”Teeth and healthy food”and was included into the weekly topic:”Health from everything”. This was a lesson aiming to acquire knowledge and the purpose was to shape and develop knowledge on the importance of oral hygiene in order to have happy teeth. The session lasted for 20-25 minutes.

Framework objectives: to inform children on the diseases that may occur if they do not brush their teeth at least twice a day and on the danger to oral health, using a simple language; to describe the benefits of healthy feeding, daily physical activities and relaxation in order to have a healthy body;

Operational objectives: to identify friends of teeth: tooth brush, tooth paste, dental floss, mouth rinse; to acquire practices for correct tooth washing; to follow the personal hygiene rules; to follow a healthy lifestyle in order to maintain physical and mental health.

Teaching strategy:

- methods and procedures: conversation, explanation, exercise,
- organisation forms: frontal, in pairs, individual
- teaching means: working chart, letter, tooth brush and paste, dental model
- evaluation: verbal assessment, exercising brushing on the dental model.

The lesson started with an animation presenting teeth-related information: dentition and types of teeth in each dentition, role of each type of teeth, dental structure. Discussions on foods and their role in shaping and maintaining a healthy dentition followed. Using the dental model, I explained correct brushing for the entire group; the next step was for each child to exercise the correct dental brushing under my supervision. We discussed the importance of visits to the dental practice, starting with simple and attractive explanations intended to remove children's anxiety or fear of the dentist.

The second session was entitled"A happy smile". This was a knowledge consolidation type of lesson. The aim of the lesson: to develop and consolidate knowledge on the importance of oral hygiene in achieving a caries-free smile. The duration was between 25-30 minutes.

Framework objectives: to identify diseases and the threat they represent for oral health, using a simple language; to describe the benefits of healthy feeding, physical activity and relaxation for a healthy body;

Operational objectives: to identify positive behaviours leading to maintaining health, to identify negative behaviours leading to loss of health; to follow personal hygiene rules; to adhere to a healthy lifestyle in order to keep physical and mental health.

Teaching strategy:

- methods and procedures: conversation, explanation, exercise,

- organisation forms: frontal, in pairs, individual

- teaching means: independent work charts, work charts, letters, tooth brush and paste.

- evaluation: verbal assessment.

Our aims with this health education lesson were the assessment and consolidation of knowledge acquired 2 months earlier, as well as learning and acquiring new knowledge on: teeth, tooth brushing, dental brushes, tooth paste, dental floss, formation of caries, aspect of healthy teeth, aspect of carious teeth, symptoms of caries, importance of visits into the dental practice, loss of temporary teeth, emergence of permanent teeth.

The third session was dedicated to the verification of accumulated knowledge and consisted of interactive discussions with each child on the notions acquired following previous presentations; exercises of correct dental brushing on models.

RESULTS

On the second lesson presented on the 9th of April 2014, we observed the acquired notions and the visible clinical results as a consequence of the first health education lesson held on the 20th of March 2013. The results of the evaluation are presented below:

- 90% of the children correctly performed the technique of dental brushing
- The intake of healthy food increased while the consumption of cariogenic aliments decreased. Thus, 70% of the children in the group preferred fruits for desert instead of sweets
- The number of visits to the dentist remained constant for the percent of children who had been used to attend these sessions for treatment or dental health check-ups; Children who had never visited a dental practice before our first health education lesson, attended their first dental check-up during this period.
- The number of treated caries increased due to repeated dental practice visits at 6 months interval

- The number of new dental caries decreased due to correct dental brushing
- 95% of children learned body and oral hygiene practices: hand washing before and after eating, teeth washing twice a day (in the morning, after breakfast and in the evening, before going to bed)
- 85% of children learned correct food hygiene rules: washing raw fruits and vegetables before consumption
- Feeding practices for healthy teeth, i.e. foods rich in calcium, iron, proteins (milk, cheese, meat, eggs), were acquired by 80% of children.

CONCLUSIONS

After presenting the first lesson, children were interested in the correct nutrition for the purpose of keeping healthy teeth, a very high percent starting to consume foods rich in calcium, vitamins, proteins and minerals. Children acquired the correct technique for dental brushing.

After presenting the second health education lesson on the 9th of April 2014, I noticed an increased interest in both children and parents for:

- Correct brushing techniques
- Use of age appropriate tooth brushes and pastes
- The importance of a constant use of the dental floss
- The effects of fluoride upon children's development and the methods by which an increased fluoride intake can be achieved
- Loss of temporary teeth and the emergence of permanent dentition
- The importance of extracting temporary teeth in a dental practice by the dentist
- Identification of carious teeth by examination in the mirror
- Dental practice visits every 6 months for the detection of novel dental problems, if any
- Treating new caries

Health education of preschool children has a particularly important role in their healthy development and growth, in the acquisition of healthy hygiene practices. These practices are vital for the formation of future healthy adult organisms, free of nutrition or hygiene deficiencies.

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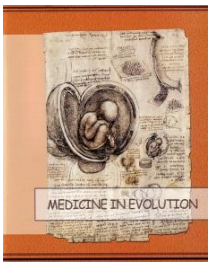
EPIDEMIOLOGICAL FEATURES OF PERIODONTAL DISEASE



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ABSTRACT

Periodontal disease, a chronic inflammatory disease of the supporting tooth tissues, has been extensively investigated. However, actual epidemiologic data is difficult to be interpreted, due to lack of homogeneity among studies. Differences in methodology, as well as in case definitions, conducted to limitations in comparisons and conclusions. The present paper aims to discuss the main epidemiological features of periodontal disease in light of recent studies.

Key words: *Periodontitis; assessment tools; epidemiological diagnosis*

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INTRODUCTION

Periodontal disease is a chronic inflammatory disease of the supporting tooth tissues, contributing significantly to the global burden of oral disease (Petersen and Ogawa, 2005).

PREVALENCE

Globally, the signs of gingivitis predominate among children, while among adults, the initial stages of periodontal diseases are prevalent. Severe stages of periodontitis are found in 5-15% of most populations. Aggressive periodontitis, a severe periodontal condition, affecting individuals during puberty and leading to premature tooth loss, counts for 2% of youth (Petersen, 2003). Still, giving the fact that chronic periodontitis is a multifactorial disorder (Albandar, 2002), the rates of severity, prevalence and extent of periodontitis may differ from population to population. Any prevalence information should be interpreted in light of the population studied and the periodontitis case definition applied (Burt, 2005).

ETIOLOGY – RISK FACTORS

Tonetti & Claffey described the risk factors as being established, such as specific plaque bacteria, smoking or poorly controlled diabetes, and putative, among which age, gene polymorphisms, socio-economic status, gender, osteoporosis and obesity are included (Tonetti and Claffey, 2005). It has been demonstrated an existing association between putative periodontal pathogens and clinical periodontitis (Beck et al., 1992, Socransky et al., 1991). Suppression of specific bacteria following treatment has been previously associated with disease resolution. Treatment studies of patients with diabetes mellitus have shown an association between inadequate metabolic control and unsatisfactory treatment outcome (Silva-Boghossian et al., 2014, Tonetti and Claffey, 2005). Current smoking may influence negatively the therapeutic outcome, as it has been previously demonstrated (Ardais et al., 2014, Nociti et al., 2015). Regarding age as a risk factor, opposite to old assumptions that periodontitis is a disease of aging, actual opinions consider that greater periodontal destruction in the elderly is reflecting a lifetime disease accumulation, rather than an age-specific condition (Burt, 2005). When discussing gender as a putative risk factor, it has been observed that clinical attachment level of different severity is usually more prevalent among males than females. Generally, males' oral hygiene is poorer than females', no matter the way it's measured (Abdellatif and Burt, 1987, Burt, 2005). Instead, women with osteoporosis are prone to developing periodontitis (Lin et al., 2015). While gingivitis and poor oral hygiene are clearly related to lower socio-economic status, the relationship between periodontitis and this condition is less direct (Burt, 2005). Patients with obesity exhibit lower reduction in probing depth (PD) than patients without obesity, after scaling and root planning (Goncalves et al., 2015). With respect to gene polymorphisms, it has been suggested that individuals with positive IL-1 genotype show increased susceptibility to periodontitis and disease progression (Cullinan et al., 2001).

EPIDEMIOLOGICAL DIAGNOSIS AND CLASSIFICATION

Diagnosis of the disease is based on severity and extent of clinical attachment loss and PD and generally categorized as mild, moderate, or severe disease (Page et al., 1997). It mostly depends on methodologies of data collection and case definitions adopted in periodontal research. However, diagnostic criteria proposed in dental literature use different thresholds to define the number of affected teeth, clinical levels of gingival inflammation, and clinical attachment level. Moreover, lack of universally accepted case definitions for periodontitis led to challenges for surveillance of periodontitis, being a major limiting factor in determining and comparing prevalence estimates of periodontitis across surveys (Eke et al., 2012, Eke et al., 2010). Costa et al. emphasized that case definition of periodontitis is a fundamental requirement in epidemiologic research, as it may affect the validity, estimates of odds ratio and relative risk, as well as the conclusions (Costa et al., 2009). It has also an impact on the prevalence and extent rates of periodontitis.

Case definitions for surveillance of periodontitis proposed by Eke et al., following a collaboration between the Centers for Disease Control and Prevention (CDC) and American Academy of Periodontology (AAP), use measurements for interproximal sites (Eke, 2012). Severe and moderate periodontitis are defined in terms of PD and attachment loss. The number of affected sites, as well as the thresholds of PD and attachment loss, are important in order to define the cases. In Europe, Tonetti & Claffey defined two types of cases, that is, sensitive cases (including incipient cases) and cases with substantial extent and severity (Tonetti and Claffey, 2005). The measurements are performed on six sites, and the attachment loss is the primary outcome variable. In addition, bleeding on probing and/or PD should be also assessed.

RADIOGRAPHIC EXAMINATIONS

Radiographs are not frequently available in epidemiological studies of periodontal disease mostly because of ethical reasons, limited information due to two-dimensional view, and lack of clear case definition of the threshold of bone loss (Leroy et al., 2010).

MOUTH RECORDINGS

Full-mouth versus partial-mouth recordings are still under debate. Full-mouth periodontal assessment provides an optimal examination of periodontal conditions, increases the probability of detecting disease prevalence, although it is time consuming (Savage et al., 2009). Partial-mouth periodontal assessment either underestimates the prevalence of periodontal disease in populations less susceptible, or overestimates the prevalence when the selected index teeth are, for example, first molars and mandibular incisors (Beck and Loe, 1993, Carlos et al., 1986). However, this type of recording is quick and minimizes patient's discomfort (Savage, 2009).

CONCLUSIONS

Actual epidemiologic data on periodontal diseases is difficult to be interpreted, due to lack of homogeneity. However, the prevalence of periodontitis seems to increase, mostly because of decline in edentulism in older age groups. Use of full-mouth examination protocol in future population-based surveys may reveal fair trends in periodontal status and disease progression over time.

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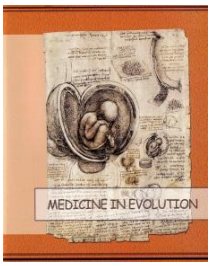
EFFECTS OF NON-SURGICAL MECHANICAL THERAPY ON PERIODONTAL PATHOGENS



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ABSTRACT

Aim and objectives: The present study develops an analysis of the levels of periodontal pathogens before and after non-surgical mechanical therapy.

Material and methods: Two groups of patients, the first group diagnosed with aggressive periodontitis comprised of 17 patients and a group of 20 patients with chronic periodontis were included in this study. Clinical and microbiologic examination were performed at baseline and three months after non-surgical mechanical therapy (SRP).

Results: Significant reductions were shown in clinical parameters in both groups as well as a significant decrease of the level of bacterial species.

Conclusions: Non-surgical mechanical therapy is effective on the level of periodontal pathogens except for Aggregatibacter actynomicetemcomitans.

Key words: *periodontitis, SRP, Aggregatibacter actynomicetemcomitans*

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INTRODUCTION

Periodontal disease results from the inflammatory response to the bacterial challenge in the gingival crevicular area. Aggregatibacter actynomicetemcomitans (Aa), Porphyromonas gingivalis (Pg), Treponema denticola (Td) and Tanerella forsythia (Tf) are considered major periodontal pathogens. [1]. Recently, the polymerase chain reaction (PCR) has demonstrated its usefulness in detecting periodontal pathogens in clinical crevicular fluid samples taken out of periodontal pockets. Microbiological monitoring may be a method of evaluating treatment efficacy.[2,3]

A number of studies have shown the effect of scaling and root planing (SRP) on periodontal disease, namely mechanical debridement could alter the composition of the subgingival biofilm..[4,5,6,7] .Levels of subgingival periodontal pathogens are important for the establishment and progression of periodontal destruction in both chronic and aggressive periodontitis. [8,9] Therefore, given the microbial etiology of periodontal disease it would be useful to be able to asses and predict treatment efficacy based on microbial markers.

PURPOSE OF STUDY

This study aims to asses the combination and levels of Aggregatibacter actynomicetemcomitans (Aa), Porphyromonas gingivalis (Pg), Treponema denticola (Td) and Tanerella forsythia (Tf) before and after non-surgical treatment of aggressive and chronic periodontitis by evaluating the relationships to probing depth (PD) reduction, bleeding on probing (BOP) and attachement loss (CAL).

MATERIAL AND METHODS

The study population was comprised of 17 patients with untreated aggressive periodontitis (mean age: 34.41± 7.55, 58.8% males and 47.1% smokers) and 20 patients with untreated chronic periodontitis (mean age: 46.35±5.88, 50% males and 65% smokers) who sought treatment in the Department of Periodontology, Faculty of Dental Medicine, University of Medicine and Pharmacy"Victor Babes", Timisoara.In order to participate in this study all patients were informed about the nature of this study and a signed consent form was obtained from each individual. Patients had ≥20 teeth and were diagnosed according to criteria described by the American Academy of Periodontology.[10,11]

Clinical measurements were performed at six sites per tooth excluding the third molar (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) at baseline and 3 months post-therapy. They included probing depth (PD), measurement of clinical attachement loss (CAL), bleeding on probing (BOP) determined with a conventional periodontal probe by two calibrated examiners. Crevicular fluid samples were collected from the 4 deepest sites (sites with BOP,PD>4, CAL≥3) per patient, one week after clinical examination. Sites to be sampled were isolated with cotton rolls and dried gently with an air syringe. Spragingival plaque was removed before sampling. Thirty-second gingival crevicular fluid samples were collected using sterile paper points. Levels of Aa, Pg, Td and Tf were detected by PCR at baseline and 3 months after non-surgical treatment from the same sites.

After baseline examinations, patients received non-surgical

periodontal treatment which consisted of full mouth scaling and root planing (SRP) under local anesthesia with theextensive use of chlorhexidine solution 0.2% (Glucochex) and chlorhexidine gel 0.2% (Glucosite) performed by one periodontist with manual and ultrasonic instruments. Oral hygiene instruction were also given.[12]

Reevaluations were performed 3 months after completion of non-surgical treatments.[13]

Statistical analysis was performed by using a software program (SPSS version 20). Clinical parameters were computed for each subject and then averaged across subjects in each group. Microbial data were expressed as mean counts for bacterial species (Aa, Pg, Td, Tf) computed for the two groups (aggressive periodontitis and chronic periodontitis). Mean values (BOP, PD, CAL) were calculated for each individual and averaged across patients in each group at baseline and 3 months after periodontal treatment. The significance of changes in clinical data with time was determined using the Wilcoxon rank sum test for each clinical group and clinical parameter separately. Levels of periodontal pathogens were averaged across patients for each clinical group. The significance of changes with time in microbiologic parameters was also determined using the Wilcoxon rank sum test for both groups.

RESULTS

Mean clinical and demographic parameters for both groups are presented in Table 1. There were statistically significant reductions in clinical parameters in both groups 3 months after non-surgical mechanical therapy.

Figure 1 presents the mean counts for each of the 4 bacterial species examined before and after therapy in patients with chronic periodontitis. Significant reductions were observed 3 months after SRP for levels of Porphyromonas gingivalis (Pg), Treponema denticola (Td) and a low reduction of Tanerella forsythia (Tf). Aa was not detected in any of the samples in the group with chronic periodontitis. Figure 2 presents the mean counts for Aa, Pg, Td and Tf before and after periodontal treatment in patients with aggressive periodontitis. Before treatment, Aa was detected only in two of the 17 patients diagnosed with aggressive periodontitis. Three months after SRP, the level of Aa was not significantly reduced. Significant reductions were especially observed for levels of Tf, Pg and Td. Data suggested that the microbiologic impact of therapy was similar in both groups for levels of Pg, and Td.

Table 1. Mean clinical and demographic parameters

PARAMETER	**AGGRESSIVE PERIODONTITIS (n=17)**		**CHRONIC PERIODONTITIS (n=20)**	
Age (years)	34 ±7		46±8	
Males%	58.8		50	
Smokers%	47.1		65	
	Baseline	**3 months**	**Baseline**	**3 months**
PD (mm; mean ±SD)	4.91±1.03	4.05±0.74	4.81±0.87	3.30±0.75
CAL (mm; mean ±SD)	3.66±1.66	3.08±1.20	3.37±1.47	2.56±1.47
BOP (%; mean ±SD)	*76.17±15.95*	*24.35±8.49*	54.5±13.09	21.92±11.34

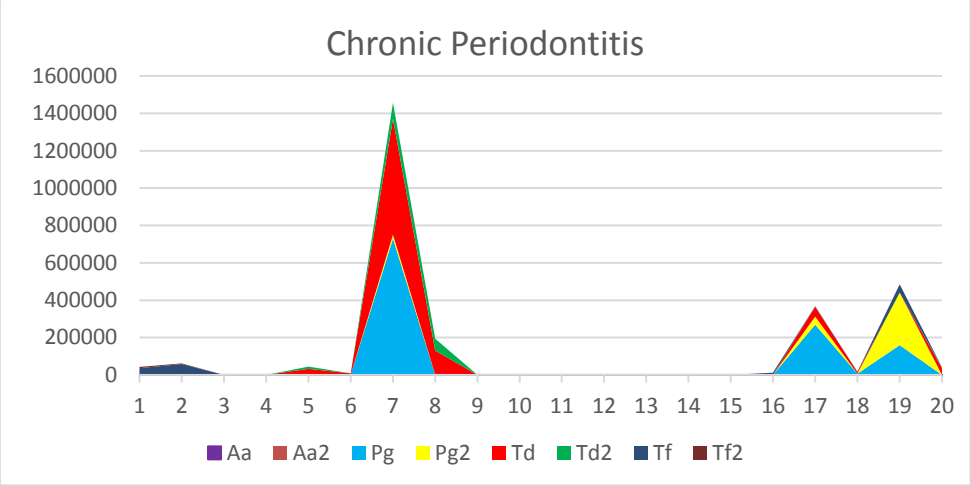

Figure 1. Bacterial species in patients with chronic periodontis at baseline and 3 months after periodontal treatment

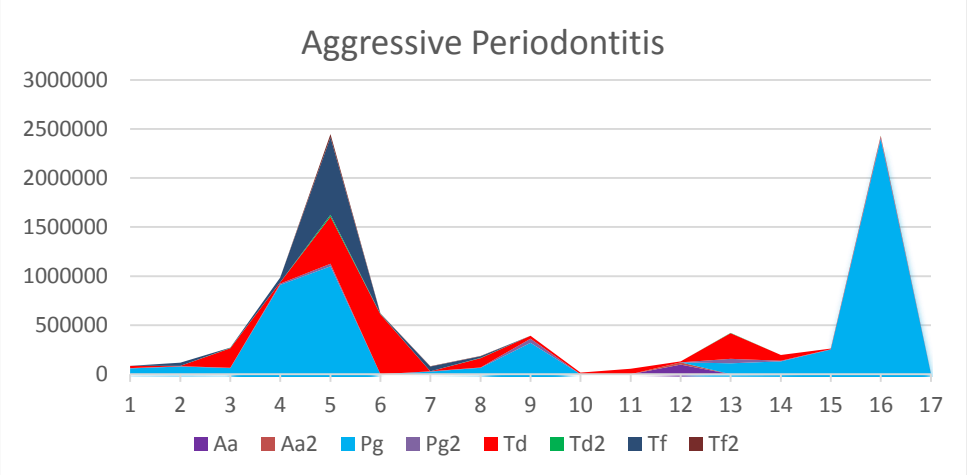

Figure 2. Bacterial species in patients with chronic periodontitis at baseline and 3 months after periodontal treatment

DISCUSSIONS

Because of the relatively small size of this study, it was not possible to state that the two forms of periodontitis responded equally to non-surgical treatment, but within the limits of the study, some significant differences were shown. Clinical parameters (PD, CAL, BOP) show a relatively higher improvement in the group with chronic periodontitis. Microbiologic data also reveal some significant differences. Aggregatibacter actynomicetemcomitans is present only in the patients with aggressive periodontitis but SRP seems not to have a major impact on the level of this bacterial species. Also the level of Tanerella forsythia is higher in patients with aggressive periodontis. However, periodontal treatment majorly reduces the level the of members of the red complex (Pg, Td, Tf) in both study groups.

CONCLUSIONS

Non-surgical mechanical therapy seems to have a major effect on periodontal pathogens reducing their level and activity. [14] .Clinical parameters can be improved significantly both in patients with aggressive and chronic periodontitis. However, the level of Aggregatibacter

actynomicetemcomitans seem not to be influenced by mechanical debridement in patients with aggressive periodontis.

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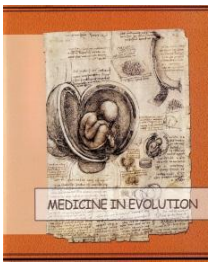
THE STATUS ASSESSMENT OF THE ORAL HEALTH DURING MIXED DENTITION (PART 1)



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ABSTRACT

The aim of the study *was to establish some aspects of the current situation in our country regarding the oral health status of children, by assessing the orodental status during mixed dentition.*

Material and methods*: The study was conducted in children aged 6-8 years old, from Galati and Iasi, having clinical examinations performed in school offices, using the consultation kit. The assessment of the dental status was achieved by using the ICDAS assessment system (International Caries Detection and Assessment System), and orthodontic status through the IOTN index (Index of Treatment Need).*

The results *show that under the conditions of this study, the average value of CAO.S is very high, 9.47 surfaces affected by caries and their complications, predominantly the decay component - cs (decayed surfaces) with an average value of 8.13 compared to filling component of only 0.62. In these teeth, the caries prevalence reported on surfaces was of 83.19%. For permanent dentition, the caries prevalence compared to the total examined area was of 81.4%, which is high for this age group (6- 8 years old).*

Conclusions: *From the assessment of the orthodontic status, it results that most children with abnormalities are from 1st grade (78.8%), only a small percentage of abnormalities in the 2nd (15.4%) and in the 3rd grade (2.2%).*

Key words: *dental status; mixed dentition; oral health; maxillary dental anomalies*

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INTRODUCTION

Maxillary dental anomalies continue to be a major public health problem, although oral health status of the population in many parts of the world experienced a clear improvement. Fundamental elements of oral health are established in childhood and the child's health in this regard is an important health indicator of the community.

Therefore, the existence of a state of optimal oral health in children creates the prerequisites for its perpetuation from adolescence and then to adulthood. At the same time, improving, as we age, the sanogene knowledge and attitudes will secure a state of optimal health in adult and then elderly times, with positive effects on quality of life in terms of oral health.

Although oral health status of the population in many parts of the world experienced a clear improvement, oral diseases, however, continue to be a major public health problem.

The data collected so far show a downward trend in the prevalence and severity of oral diseases, a characteristic tendency for the western European countries and the countries of the North American continent. This trend is especially evident in children, by reducing dental caries index, which is mainly due to improving dietary habits (eating balanced products with high content of carbohydrates, especially highly refined), oral hygiene attitudes and general usage of general fluoridation (water fluoridation) and local (topical applications, rinses with fluoride solutions, toothpaste with fluoride).

Many of these issues are the result of educational campaigns to improve the level of knowledge on oral health and the implementation of primary prevention programs in schools.

In the countries of central and southeast Europe, due to political and economic changes occurred in the last 15 years, health systems are in transition. The oral health at the population level in these countries is below that of the Western and Northern Europe; this is mainly due to the socio-economic differences.

Based on these facts, we wanted to establish some aspects of the current situation regarding oral health status of children.

The aim of the study was to evaluate the oral-dental status of a group of schoolchildren, between 6-8 years old, from Galati and Iasi.

MATERIAL AND METHODS

The study was conducted in children aged between 6-8 years old, from Galati and Iasi, having clinical examinations performed in school offices, using the consultation kit. The assessment of the dental status was achieved by using the *ICDAS* assessment system (International Caries Detection and Assessment System), and orthodontic status through the *IOTN* index (Index of Treatment Need).

RESULTS

The study group consisted of 137 children, aged between 6 and 8 years old, pupils in the first grade, from Galati and Iasi. The average age of the study group was 7.26 ± 0.5 years (Table 1).

Table 1. The number and age of individuals included in the study group

Number and age		
Total no	Validated	137
	Excluded	0
Average age		7,266
Standard deviation		0,5117
%		8,700

Gender distribution analysis indicates a balanced distribution of the batch, 51% of pupils are female and 49% male. (Table 2) Most subjects (93.4%) come from urban areas (Table 3).

48.9% of caregivers of children have a high educational level, the remainder being approximately equal distribution between caregivers with medium and low level of education (Table 4).

Supervision of pupils after school is provided by grandparents in a percentage of 43.8% and the parents in proportion of 19.7%. In 19% of the cases, children are alone, unattended (Table 5).

Table 2. Distribution of the study group in relation to gender

Gender		No	%	% valid	% cumulative
Valid	Female	70	51,1	51,1	51,1
	Male	67	48,9	48,9	48,9
	Total	137	100,0	100,0	100,0

Table 3. Distribution of the study group compared with residence

Residence	No	%	% valid	% cumulative
Rural	9	6,6	6,6	6,6
Urban	128	93,4	93,4	93,4
Total	137	100,0	100,0	100,0

Table 4. Distribution of the study group compared to the level of education of caregivers of children

Level of education of caregivers of children		No	%	% valid	% cumulative
Valid	Not specified	2	1,5	6,6	1,5
	High Education level	67	48,9	48,9	50,4
	Average Education level	35	25,5	25,5	75,9
	Low Education level	33	24,1	24,1	100,0
	Total	137	100,0	100,0	100,0

Table 5. Distribution of the study group compared to how supervision of students after school

The supervision method after school		**No**	**%**	**% valid**	**% cumulative**
Valid	After-school	8	5,8	5,8	5,8
	Nanny	5	3,6	3,6	9,5
	Grandparents	60	43,8	43,8	53,3
	Brothers	11	8,0	8,0	61,3
	Parents	27	19,7	19,7	81,0
	Alone (male)	19	13,9	13,9	94,9
	Alone (female)	7	5,1	5,1	100,0
	Total	137	100,0	100,0	100,0

Assessment of Oral Status

Evaluation of dental status indicates a significant carious experience for this age group, the index *cao.d* (for temporary teeth) being 4.55. This value consists of component *cd*

(decayed teeth) value of 4.09, *ad* (absent teeth) and *od* 0.15 (obturated teeth) 0.31. It can be observed the significant value of the decayed teeth component, in the detrimental of obturated teeth structure (Table 6).

The Odontal status assessment based on the number of surfaces indicates the seriousness of oral diseases. The recorded *cao.s* value was 9.47, *cs* component (decayed surfaces) having a value of 8.13 (Table 7).

The assessment for permanent teeth dental status indicates a value of 0.85 CAO.D, and the CD component (decayed teeth) has the highest value: 0.80 (Table 8).

Table 6. Assessment of dental status by index cao.d

		cd	ad	od	cao.d
Total no.	Validated	137	137	137	137
	Excluded	0	0	0	0
Average value		4,09	0,15	0,31	4,55
Standard deviation		2,970	0,483	0,763	3,053
- Minimum		0	0	0	0
- Maximum		11	4	5	11
Total		561	21	42	624
%		11,00	4,00	5,00	11,00

Table 7. Assessment of dental status by cao.s index

		cs	as	os	cao.s
Total no.	Validated	137	137	137	137
	Excluded	0	0	0	0
Average value			0,72	0,62	9,47
Standard deviation			2,319	1,362	8,585
- Minimum			0	0	0
- Maximum			20	7	36
Total			99	85	1298
%		36,00	20,00	7,00	36,00

Table 8. Assessment of dental status by index CAO.D

		CD	*AD*	*OD*	*CAO.D*
Total no.	Validated	137	137	137	137
	Excluded	0	0	0	0
Average value			0,00	0,05	0,85
Standard deviation			0,000	0,280	1,179
- Minimum			0	0	0
- Maximum			0	2	4
Total			0	7	117
%		4,00	0,00	2,00	4,00

The assessment dental status for teeth permanently affected, CAO.S has the value of 0.94, of which 0.88 represents the component CS - decayed surfaces (Table 9).

Of the total number of teeth examined, 1460 were permanent teeth, teeth at the front side 878, and 582 sidewise (Table 10). Of the 1721 examined temporary teeth, 1028 were located sidewise (Table 11). The total number of examined dental surfaces was of 6422, for permanent teeth (Table 12) and the 7912 temporary teeth (Table 13).

Table 9. Assessment of dental status by index CAO.S

		CS	*AS*	*OS*	*CAO.S*
Total no.	Validated	137	137	137	137
	Excluded	0	0	0	0
Average value			0,00	0,07	,94

	CS	AS	OS	CAO.S
Standard deviation		0,000	0,325	1,322
- Minimum		0	0	0
- Maximum		0	2	5
Total		0	9	129
%	5,00	0,00	2,00	5,00

Table 10. Distribution of the study group compared with the total permanent examined teeth

		No.dt.ex.P	No.dt.fr.ex.P	No.dt.lat.ex.P
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value		10,66	6,41	4,25
Standard deviation		2,542	2,067	,821
- Minimum		4	0	2
- Maximum		20	12	8
Total		1460	878	582
%		20,00	12,00	8,00

Table 11. Distribution of the study group compared with total temporary examined teeth

		No. dt.ex.tmp	No.dt.fr.tmp.ex	No.dt.lat.tmp.ex
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value			5,06	7,50
Standard deviation			2,054	1,065
- Minimum			0	2
- Maximum			12	8
Total			693	1028
%		20,00	12,00	8,00

Table 12. Distribution of the study group compared with total dental surfaces in permanent examined teeth

		S.fr.ex.total.P	S.lat.ex.total.P	S.ex.Total.P
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value			21,24	46,88
Standard deviation			4,103	10,748
- Minimum			10	18
- Maximum			40	88
Total			2910	6422
%		48,00	40,00	88,00

Table 13. Distribution of the study group compared with total dental surfaces examined in deciduous teeth

		S.fr.ex.total.tmp	S.lat.ex.total.tmp	S.ex.Total.tmp
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value			37,52	57,75
Standard deviation			5,325	11,463
- Minimum			10	15
- Maximum			40	88
Total			5422	7912
%		48,00	40,00	88,00

The assessment of Odontal status reveals that only 1198 of the permanent teeth examined were free of cavities, most of them located in the front area: 875 teeth (Table 14). Among deciduous examined teeth, only 1095 teeth were free of cavities, most in the front area: 645 teeth (Table 15). In total, there were several areas free of decay for temporary teeth: 6962 surfaces, versus

the permanent ones: 5330 surfaces (Table 16).

Regarding the distribution of caries lesions in ICDAS system, permanent teeth had fewer surfaces with superficial cavities in enamel (code 03) compared with temporary teeth, most of which are located on the occlusal surface: 79 surfaces (Table 17).

In the case of temporary teeth, of the 119 carious lesions in enamel surfaces (code 03), most were located on the occlusal front: 82 surfaces, followed by the buccal and lingual surfaces: 25 surfaces (Table 18).

Table 14. Distribution of the study group compared with the number of permanent teeth free of cavities

		Dt.P.00	Dt.fr.P.00	Dt.lat.P.00
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value			6,39	2,36
Standard deviation			2,059	1,513
- Minimum			0	0
- Maximum			12	7
Total		1198	875	323
%		18,00	12,00	7,00

Table 15. Distribution of the study group compared with the number of temporary teeth free of cavities

		Dt.tmp.00	Dt.fr.tmp.00	Dt.lat.tmp.00
Total no.	Validated	137	137	137
	Excluded	0	0	0
Average value			4,71	3,28
Standard deviation			2,266	2,797
- Minimum			0	0
- Maximum			12	8
Total			645	450
%		19,00	12,00	8,00

Table 16. Distribution of the study group compared with total number of Dental caries-free surfaces

		S.00.P	S.00.tmp
Total no.	Validated	137	137
	Excluded	0	0
Average value			48,61
Standard deviation			14,474
- Minimum			10
- Maximum			88
Total			6962
%		83,00	88,00

Table 17. Distribution of the study group in relation to the assessment in ICDAS system: superficial enamel caries (code 03) in permanent teeth

		S.Total.03	S.perm.03	S.perm.MD.03	S.perm.Ocl.03	S.perm.VO.03
Total no.	Validated	137	137	137	137	137
	Excluded	0	0	0	0	0
Average value			0,72	0,00	0,58	0,15
Standard deviation			1,041	0,000	0,889	0,375
- Minimum			0	0	0	0
- Maximum			4	0	4	2
Total			99	20	79	1
%		9,00	4,00	0,00	4,00	2,00

Table 18. Distribution of the study group in relation to the assessment in ICDAS system: superficial enamel caries (code 03) in deciduous teeth

		S.temp.03	S.temp.MD.03	S.temp.Ocl.03	S.temp.VO.03
Total no.	Validated	137	137	137	137
	Excluded	0	0	0	0
Average value			0,09	0,60	0,18
Standard deviation			0,332	1,003	0,488
- Minimum			0	0	0
- Maximum			2	4	2
Total			12	82	25
%		7,00	2,00	4,00	2,00

CONCLUSIONS

The assessment of dental status for permanent teeth indicates a value of 0.85 CAO.D, the CD (decayed teeth) component having the highest value: 0.80. Regarding permanent tooth surfaces affected CAO.S has the value 0.94, of which 0.88 is the CS component - decayed surfaces**.**

(Further analysis will be presented in the second part of the article).

Acknowledgment

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOPHRD) Financed from the European Social Fund by the Romanian Government under the agreement number HRD/159/1.5/S/137390/.

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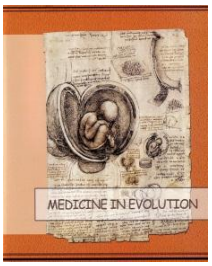
FROM PERIODONTITIS TO CVD. IS CRP THE `MISSING LINK`?



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ABSTRACT

Systemic inflammation determined by PD seems to influence the course and severity of CVD. Especially more severe forms of PD exhibit certain serological markers that confirm the inflammatory burden in the cardiovascular system and can even predict major CVD events. CRP is one of the most important of these biomarkers, easily detectable and displaying a high and independent predictive value.It is closely related to the presence of inflammation and swiftly drops its values as inflammation subsides.

Some studies have shown its values to decrease after periodontal treatment, although others proved a merely moderate association between CRP and PD treatment outcomes.

Nevertheless CRP is a determinable and to some extent controlable risk factor in the relation between PD and CVD, which cannot lightly be dismissed in the management of periodontitis patients with CVD.

Key words: *periodontal disease, cardiovascular disease, C-reactive protein, inflammation, biomarkers*

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INTRODUCTION

Periodontitis is a low –grade local inflammation of the supporting tissues of the teeth, caused by groups of specific microorganisms, which untreated leeds to the distruction of the periodontium and tooth loss.The response to this microbial invasion is a permeation of the periodontal tissues for inflammatory cells. This inflammatory progression also determines a systemic response, characterized by the exhibition of acute-phase reactants, such as IL1-β, TNF- α, fibrinogen and CRP.

There is now increasing evidence that these same markers of systemic inflammation are associated with an increased risk of cardiovascular disease. Many biomarkers have been shown to be raised years in advance of the first ever major cardiovascular event.

Atherosclerosis, which is the initial lesion in ischemic cardiovascular disease, progresses from microscopic endothelial events to the pathological formation of plaques in the arterial walls, that eventually grow and rupture. It can occlude large to medium sized arteries and lead to ischemic lesions in the heart, brain or peripherial areas.

Atherosclerosis seems to be initiated and sustained by periodontal pathogens and their bacterial byproducts. Estalished periodontal disease entertains a state of moderate systemic inflammation, due to circulating endotoxins and inflammatory cytokines.(1)

CRP seems to be the strongest and most indepent predictive factor for major cardio-vascular events, such as myocardial infarction and stroke. Furthermore, the relative risc of a first MI or ischemic stroke increases significantly with each quartile of baseline concentrations of CRP.

PD. FROM PLAQUE TO HOST RESPONSE

PD is the most common dental condition in the adult population and also one of the most common inflammatory diseases reaching all throughout gender and age groups around the world.

It was initially considered an age-related afliction, to be expected in the elderly population, and directly connected to the presence of dental plaque. Extensive evidence of the last decades proves the earlier conception wrong and although it does not deny the importance of plaque in the onset of PD, it links its progression and clinical form to host –related conditions. The host response to the bacterial challenge was proven to be the most important factor in the development and establishment of the disease and more importantly in disease severity. Of course bacteria can directly damage periodontal tissue, but the importance of the distruction by endotoxines, cytokines and enzymes resulted from the inflammatory reaction is far greater and determines disease course and the extend of periodontal damage.(2)

Bacteria colonize the surface of the teeth and grow into evermore organized biotops. So the biofilm evolves to dental plaque which enriches with new bacterial species as the colonisation and therefore the lesion progresses. These microbes also attach themselves through fimbriae to the surface of periodontal cells and so the invasion takes a physical shape in addition to the mediator-related agression.

As a response to this aggresion, the jonctional epithelium proliferates and produces tissue –damaging proteinases. Pattern recognition molecules, such as TLRs and GPCRs

recognize Pamps (LPS, PGN, fimbriae, LTAs etc) and induce the secretion of proinflammatory cytokines (TNF α, IL 1β, IL 6, IL8) in epithelial cells.

Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are able to invade the epithelium and due to its increased permeability in this situation, gain acces to the subepithelial connective tissue. Here microbial products and pro inflammatory mediators produced by these tissues attract inflammatory cells. Neutrophils are typical for the early stages of inflammation, followed by fibroblasts, macrophages, dendritic cells and limfocytes. This migration is part of the immune response in the periodontium and further leads to the release of inflammatory mediators from the migrated cells on site.In this stage phagocytosis and distruction of bacteria by PMNs strive to protect tissues from the invasion. It is also the stage of clinically recognisable gingivitis. (3)

Further, the host response modulates the progression of the disease. In patients who do not have a susceptibility to periodontitis the tissue will either recover, if the initiating factors (bacteria) are removed, or progress to cronic gingivitis in the presence of bacteria; whereas in patients susceptible to periodontitis the process of inflammation will extend and involve deeper connective tissue and bone.

Monocytes and macrophges migrated to this site further produce inflammatory mediators: PGEs, interleukins, TNF and MMPs who break down colagen fibres and subsequently stimulate osteoclastic bone resorbtion, creating and deepening periodontal pockets. This happens in an enviroment with disrupted homeostasis, were pro-inflammatory and protective (anti-inflamatory) factors are unbalanced.

As mentioned above, at this point an excessive inflammatory response becomes far more damaging than the initial bacterial aggresion.

CRP

CRP is a pentameric plasmatic protein syntetized in the liver, as part of the systemic response to inflammation. It was discovered in 1930 by Tillet and Francis in the serum of patients infected with streptococcus pneumoniae. (4)

CRP can bind phosphoethanolamine and phosphocholine of bacterial and host cell membranes, as well as laminin, chromatin and fibronectin, in the presence of calcium. Bound to these constituents it activates the complement and determines an increased inflammatory response to infections and trauma. It is also found on inflammatory cells such as macrophages, monocytes and neutrophils and thus reveals its role in phagocytosis.(5)

It`s secretion is regulated by interleukins, such as IL6, IL1 β and TNF-α, which also determine the synthesis and release of acute phase-proteins from the liver, and activate the complement cascade.

In case of an acute injury or inflammation its secretion increases abruptly and peaks at about 24-48 h from the onset of inflammation. It is an extremely sensitive pattern recognition molecule and a non-specific marker for acute inflammation. When inflammation is resolved CRP levels return to normal.

Low levels of CRP and other acute phase proteins appear in plasma even in the absence of inflammation. CRP level varies between 1.0 – 3.0 mg/l in different populations. It`s determination can not diagnose a

disease as it is merely a paraclinic adjuvant which must be correlated with other laboratory and clinical parameters.

Usually CRP is measured as:

-routine CRP, above 3mg/l

-high-sensitivity CRP (hs-CRP) measuring up to 3mg/l

CRP determination is usually used in screening programms for certain organic disease; it can also reflect the intensity and evolution of any infection, inflammation or malignancy, and is also a useful indicator in the management of recurrent infections.(6)

ROLE OF CRP IN THE MANAGEMENT OF PD

Since PD is an inflammatory disease, levels of acute phase proteins and CRP have been reported in some studies to appear elevated in the serum of patients suffering from this disease.

Especially patients with severe forms of PD seem to exhibit elevated levels of serum CRP, but since these raised values could be related to other factors like HTA, age, acohol and tobacco abuse, coffee consumption, inactive lifestyle,insulin resistence etc, PD could not be undoubtedly proven to be the main reason for CRP elevation.

On the other hand, cronical low grade inflammation such as PD was reported in many recent studies to determine the release of pro-inflammatory mediators (IL-6, IL-1, TNF-α) and stimulate the liver to produce CRP.

Different categories of cardiovascular risk were proposed in a joint consensus conference of the American Heart Association and the Center for Diseases Control, by Pearson et al., based on serum CRP levels:

-Low risk: serum CRP concentration <1 mg/l

-Medium risk: serum CRP concentration 1-3 mg/l

-High risk: serum CRP concentration > 3mg/l (7)

Koppolu et al., evaluated the levels of serum CRP and TNF α in periodontitis patients and found that they rose concomitently with the severity of the disease. The same study also reports a decrease of these markers in the same pacients after periodontal treatment.(8)

Paraskevas, in"A Systematic Review and Meta-Analysis of CRP in Relation to Periodontitis"found robust evidence that CRP is elevated in the serum of patients with PD. However, no significant improvement was reported in the levels of this biomarker after periodontal treatment. (9)

Gani reported in his study on the effect of PD on inflammmatory markers, higher levels of CRP in both localized and generalized forms of PD compared to the control group.(10)

Raised inflammatory markers, especially CRP might increase activity in aterosclerotic plaques and thereby cardiovascular risk.

Furthermore, studies have shown CRP and IL 6, to be elevated in patients with PD. All these elevated pro-inflammatory factors may increase atherosclerotic activity and the risk for major cardiovascular events.(11)

Determination of CRP in PD patienst seems to be an important and independent predictor of CV risk. Especially hs-CRP was proven to be linked to both PD and CV events.

CONCLUSIONS

More and more studies relate periodontal disease to CVD.

One of the most important and easily determinable serological markers in this relation seems to be

CRP. Its values have been shown to rise, especially in aggressive and severe periodontitis, as do other inflammtory mediators, and determine a state of long–term systemic inflammation. This may be the cause of an increased activity in atherosclerotic lesions and may determine the onset or progression of atherosclerosis-related CVD.

CRP levels are easily determinable and definetly strong and independent predictors of CV risk.

In light of these recent findings, should CRP not become a recomandable even compulsory determination in patients with severe PD? Even in dental clinics, cardiovascular risk in patients with PD could be assesed and their management extended through cooperation with general health practitioners.

Further findings link PD to a series of other systemic conditions and make its treatment and prevention compulsory not only from an oral but also from a general practitioner`s point of view.

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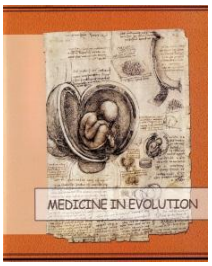
ESTHETIC SOFT TISSUE PARAMETERS IN ORTHODONTICS: A 3D APPROACH



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ABSTRACT

Recent treatment philosophies place great importance on the soft tissue of the face. The orthodontic treatment is starting to be planned from the external profile with regard to the patient soft tissue adaptation ability and contours. This approach has the potential to adapt the orthodontic treatment to the patient and obtain better function, stability and esthetics. The aim of our study was to evaluate the applicability of 3D photography with Planmeca ProFace in evaluating soft tissue parameters from a group of Romanian patients.

Key words: *facial profile, digital analysis, 3D, ProFace*

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INTRODUCTION

A proper facial analysis is essential for diagnosis and treatment planning for patients who will benefit from orthodontic treatment, especially in cases were orthognatic surgery is a possibility.

Facial anthropometry plays a more important role in the diagnosis dysmorphic syndromes so physicians are forced to seek and apply the best diagnostic pre and post-operative planning. With constant modernization and informatical growth, diagnostic methods have greatly diversified. These efforts were primarily aimed at reducing the time spent on examinations and improving measurement accuracy.

In order to achieve facial harmony, the interrelationships of the facial features must be in balance between themselves and also with the skeletal and dental components. Many attempts have been made to investigate the differences of the human face among various ethnic groups including African-Americans, Africans, Chinese, Japanese, Koreans and Indians [1,2][3][4].

The aim of our study was to evaluate, by 3D methods, the esthetic soft tissue parameters in a group of orthodontic patients. Physical appearance is an important characteristic of the face. It has long been established that self-esteem is strongly influenced by facial appearance. The perception of an attractive face is largely subjective, with ethnicity, age, gender, culture, and personality influencing average facial traits. The interrelationships of the facial features must be in balance in order to achieve facial harmony.

Various methods have been used to evaluate soft tissue facial characteristics. The need for three-dimensional (3D), noninvasive methods and diagnostic tools that can be used in addition to, or as an alternative to, radiographic methods has stimulated a growing interest in facial anthropometry. The analysis based on photogrammetry has been extensively described. Different research groups have defined various soft tissue parameters and landmarks of soft tissue facial analysis.

The aim of the present study was to evaluate the average variables that define the soft tissue facial profile of a sample group from Timisoara, Romania by means of angular and linear measurements conducted on a 3D photograph. These would serve as a guide for aesthetic treatment goals leading to more predictable results.

MATERIAL AND METHODS

The imaging system which was used for this study is the ProMax Planmeca ProFace™ that provides a three dimensional picture realistic face and is complementing traditional maxillofacial digital radiography.

One hundred and thirty five adult patients aged 18-35 years of age (83 female and 52 male). The study group consisted of orthodontic patients from Western part of Romania.

The 3D photographs were recorded using the ProFace™ option from Planmeca ProMax 3D. Planmeca ProFace™. The upgraded unit produces a realistic 3D face photo in addition to maxillofacial radiography. One imaging session was required for patients who also needed a CBCT volume to be recorded. Other patients required solely a 3D photo, in which case no radiation was emitted.

Recorded linear soft tissue parameters were: head width, middle face length, upper face width and upper/lower lip distance to profile line. Three angular parameters were

also recorded: naso labial angle, labio mental angle and inter labial angle.

The 3D photographs were measured using Planmeca Romexis® software, that facilitated accurate and detailed operations. The results were statistically analysed .

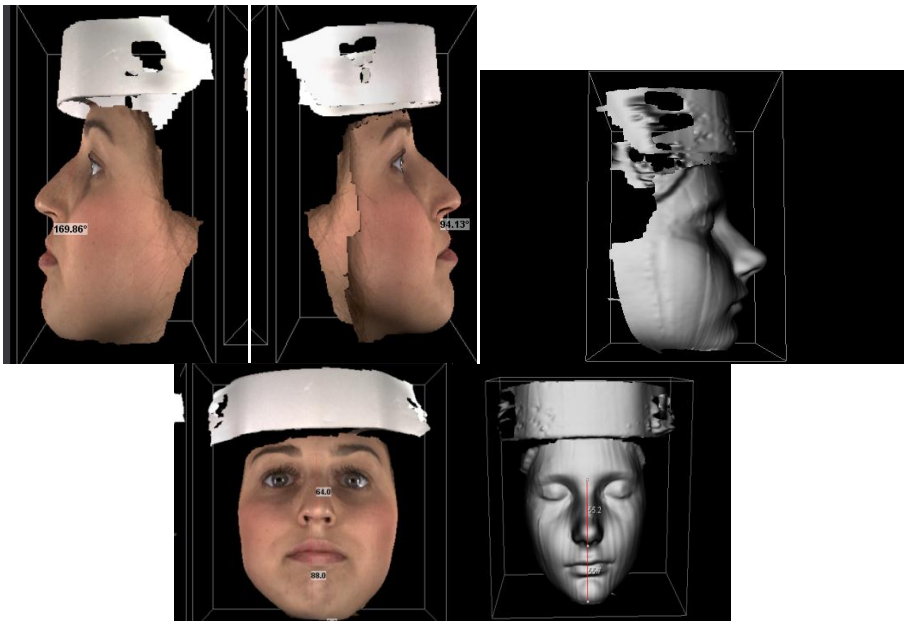

Figure 1. The ProFace system by Planmeca

RESULTS

The 3D photographs were measured using Planmeca Romexis® software, that facilitated accurate and detailed operations. The results were statistically analysed.

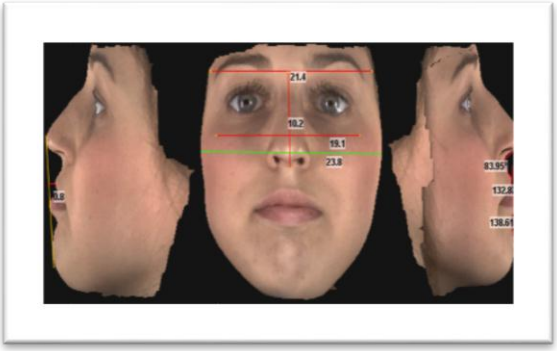

Figure 2. Some of the soft tissue parameters analysed with Planmeca ProFace

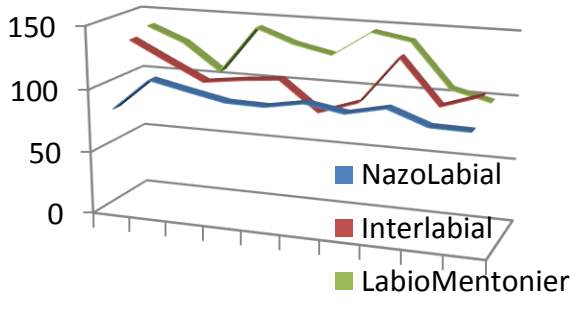

Figure 3. The variation of profile angles – nasolabial, interlabial and labiomentonier angles

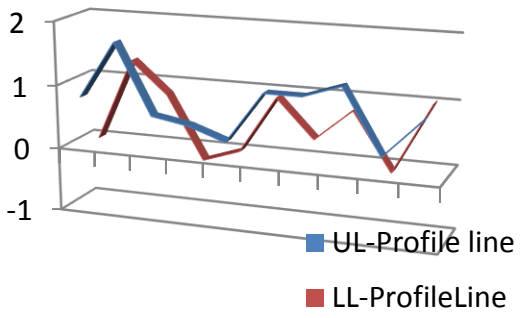

Figure 4. The variation of profile lines in the studied groups

The higher values for the females in this study could be explained by the fact that in general the facial contours of female subjects were softer than those of males, especially in the area of the nose, lips, and chin.

CONCLUSIONS

The 3D facial scanning allowed a more accurate representation of facial morphology and soft tissue landmarks. Thus, in the present tendency towards globalization and the increased occurence of mixed-raced individuals, more research is needed on theses variations in order to achieve complete esthetics along with the orthodontic treatment.

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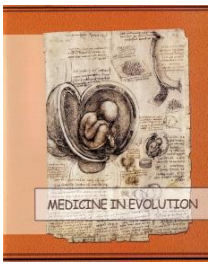
INDICATIONS OF VACUUM FORMED DEVICES IN ORTHODONTICS



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ABSTRACT

This article points out the indications of vacuum formed devices as an active or passive appliance in orthodontics. These devices are often used in an orthodontist's office to correct mild malocclusions (as an active appliance) or to prevent relapse (as passive retainers). Orthodontic-prosthodontic interdisciplinarity is another field in which vacuum formed devices are of great use: as space maintainers prior to implant placement. By studying different reviews on this topic and a few clinical cases we came to the conclusion that manufacturing these devices and using them in the daily practice has several advantages: they are more comfortable than fixed or wrap-around retainers, more esthetic, they produce less gingival recession, they are capable of maintaining space, they can correct mild malocclusion in adults patients or guide skeletal growth in children. These appliances are useful in several tooth movements, such as intrusion, overbite reduction, diastema closure, rotations, etc. By adding attachments or other accessories and inter-maxilary elastics they can reduce sagital discrepancies in adult's orthodontics.

Key words: *vacuum formed devices, retainers, orthodontics*

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INTRODUCTION

Vacuum formed devices as we know them have a vast history. The idea of using such an appliance belongs to Pontiz, when he discovered the use of thermoplastic materials in orthodontics. Nowadays, the major indication of these devices is maintaining orthodontic stability after achieving Andrew's six keys of ideal occlusion. In the last years, due to Clear Aligner's increasing popularity(12), vacuum formed devices became an important step in orthodontic treatment as active appliances.(2,5) Vacuum formed devices are easy to make, effective, esthetic and provide good results.

Study Objectives

Our study objective was to emphasize the role of vacuum formed orthodontic devices and their multiple indications by presenting some clinical cases.(10)

MATERIAL AND METHODS

We evaluated a few clinical cases after consulting orthodontic literature and came to the conclusion that vacuum formed devices have several indications: they replace missing teeth before full growth is achieved and implants can be placed,(9) they correct mild malocclusions, maintain stability after active treatment, proper oral functions and periodontal health. (7,18)These appliances, if used as a passive device, are more comfortable than wrap-around or fixed retainers and produce less gingival problems (plaque deposits, gingival recession)(3,4,16) When dealing with more severe malocclusions, several vacuum formed aligners can be used to achieve the desired result(ClearCorrect, Invisalign, Clear Aligner).

To support our findings we selected some of the cases we analised.

CASE 1

The first case presented in this study is a post-orthodontic patient with a missing 4.6. The space is maintained for implant insertion with a hard vacuum formed retainer.

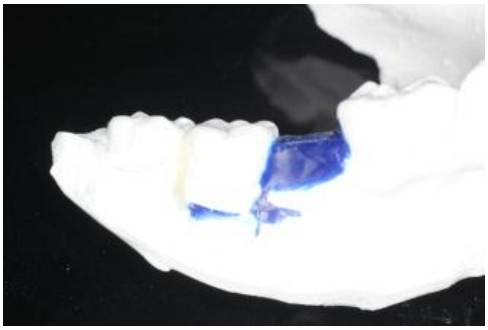

Figure 1. Dental cast: before the manufacturing of the appliance

Figure 2. The vacuum formed device: used to maintain space

CASE 2

The second case is a 8 year old boy with bruxism. A soft vacuum formed splint was indicated in this case. The appliance has also the role of a space mantainer, due to early loss of decidous molars.

Figure 3. A vacuum formed device used as a space maintaner

Figure 4. The soft splint on the stone model

CASE 3

A 33 year old patient was treated with pre-prosthetic orthodontics.

The completion of the treatment: a vacuum formed retainer was used. In order to mantain the space, acrylic lateral incisors were included in this device.

Figure 5a. Patient during orthodontic treatment

Figure 5b. Patient after orthodontic treatment

Figure 6. The orthodontic retainer in place

In this class 3 patient, an acrylic bite plane was adapted to the vacuum formed device. Its role was to guide the mandible of the 5 year old child in a retruded position.

Figure 7. Young patient with class 3 malocculsion

Figure 8. An active appliance was used to guide skeletal growth

RESULTS AND DISCUSSIONS

Orthodontic relapse is corelated with occlusal factors, periodontal and muscular balance. Therefore, the vacuum formed devices, as retainers, must satisfy these conditions.(8,15) If used as an active device, the thermoplastic appliance must not induce great tensions and heavy forces on the dental arches. It is best to increase the number of aligners when dealing with severe malocclusions in order to obtain the desired tooth movements. Orthodontists should consider the use of attachments to minimalise side effects or anchorage problems.(1,17) If used wisely, the

appliance will lead to good orthodontic results and a produce a physiologyc effect regarding tooth movement and periodontal tissues.(13,14)

CONCLUSIONS

Such appliances will replace brackets in the near future, especially in adults orthodontics, but recently among younger patients as well. They are comfortable, easy to manufacture and preffered by most patients because of their esthetic advantages.(6,11)

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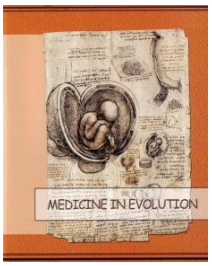
TOXICITY EVALUATION STUDY OF CHLORHEXIDINE USED IN DENTAL TREATMENT



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ABSTRACT

The aim of this study is the biological evaluation of chlorhexidine used in periodontal disease and disinfection of root canals. This compound is a broad spectrum antibacterial agent and is currently used as a local antiseptic. At low concentrations it has bacteriostatic effect and at high concentrations it has bactericidal effect. At low concentrations cell death occurred through apoptosis, and at higher concentrations, chlorhexidine induced necrosis.

Key words: *chlorhexidine, topical application, cytotoxic effect; non-invasive skin measurements*

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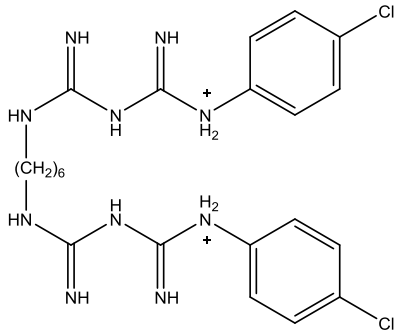
INTRODUCTION

Chlorhexidine is a cationic synthetic molecule that contains in his structure 2 cycles of 4-chloro- and 2 biguanide groups united by a central chain of hexamethylene (Figure 1). This compound is known as a broad spectrum antibacterial agent and currently is used as a local antiseptic [1,2]. At low concentrations has bacteriostatic effect and bactericidal at high concentrations [3].



Clorhexidina

Figure 1. Chemical structure of chlorhexidine

As a therapeutic recommendation, chlorhexidine is used to reduce the formation of dental plaque, gingivitis and disinfection of root canals. After administration, chlorhexidine is absorbed at oral mucosa and gastrointestinal level. It is metabolized in the liver and it's eliminated in the urine [4].

Regarding the toxicity of this compound in vitro and in vivo exist a considerable number of studies that shows an increased interest of researchers in this subject, but the toxic mechanism of action is incompletely understood. In a study carried out on a fibroblasts cell line isolated from mouse (L929), they were exposed to different concentrations of chlorhexidine (from 0.000025% to 0.016% by weight) for 24 h. At low concentrations (up to 0.002%) occurred cell death through apoptosis, and at higher concentrations, chlorhexidine induced fibroblasts necrosis. The results of this study indicated that the mechanism of action may involve the endoplasmic reticulum [5].

Giannelli M and his collaborators proposed checking the effects of different concentrations of chlorhexidine digluconate (form that is found in most dental products) on the viability of cell lines osteoblasts, fibroblasts and endothelial cells and mechanism of action involved. The results have indicated proapoptotic effect of dozo- and tempo-dependent solution especially if osteoblast effect associated with disruption of mitochondrial function, increasing intracellular calcium ions and oxidative stress [3].

Chlorhexidine had showed a cytotoxic effect on cells isolated from Chinese hamster ovary. At low concentrations, chlorhexidine took proapototic effect and necrotic effect at high concentrations [1].

Salim and his co-authors have demonstrated its efficacy as an antifungal agent against Candida spp species [6].

The potential toxic effect of chlorhexidine has been studied on different types of normal cell lines, which are found in oral cavity, evaluating the toxicity of this compound in vivo after 8 days by oral gavage / 1 time / day of 3 ml solution chlorhexidine digluconate 0.12% on Wistar rats. The results indicated an

increase of defects at DNA level of leukocyte and kidney cells, they represent potential targets of toxic action of chlorhexidine digluconate [4].

So far no attention was paid to the effects of chlorhexidine solution in the skin, where the solution comes into contact with skin. The objective of this study was to verify the effects of chlorhexidine gluconate solution (2%) on physiological parameters in mice skin after topical application SKH 1.

MATERIAL AND METHODS

SKH1 mice were obtained from Charles River Germany, female, 12 weeks. SKH1 mice were divided in 5 groups (4 mice/group): for each group mice were topically exposed to active agent, 1 application/day during 5 days. The solutions of chlorhexidine digluconat 2% from PPH CERKAMED.

All experimental procedures performed in this experiment were performed according to the Directive 2010/63/EU regarding the animal protection.

Non-invasive skin measurements

Determination of melanin and erythema values, markers with an important role in evaluating lesions in the skin were obtained with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of erythema were obtained by means of the MPA5 Mexameter® MX 18 probe, as quantitative results regarding erythema (haemoglobin) subjected to toxicological evolution. The units for erythema and melanin were determined by a spectrophotometer evaluation.

The haemoglobin values for erytema were measured using 2 wavelengths: 560nm (green) and 660 nm (red) and for melanin also at two wavelengths: 660 nm (red) and 880 nm (infrared) [7-10].

Statistical analysis

Data were analyzed using paired Student's *t* tests or One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between experimental and control groups; *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS AND DISCUSSIONS

This research was conducted on SKH1 mice in order to evaluate the effects of chlorhexidine digluconate 2%. The compound was topically applied on the dorsal side of the mice and were measured physiological skin parameters with the help of a non-invasive techniques.

The first evaluation, macroscopic evaluation indicated the presence of both redness and skin dryness in the mice treated with the solution.

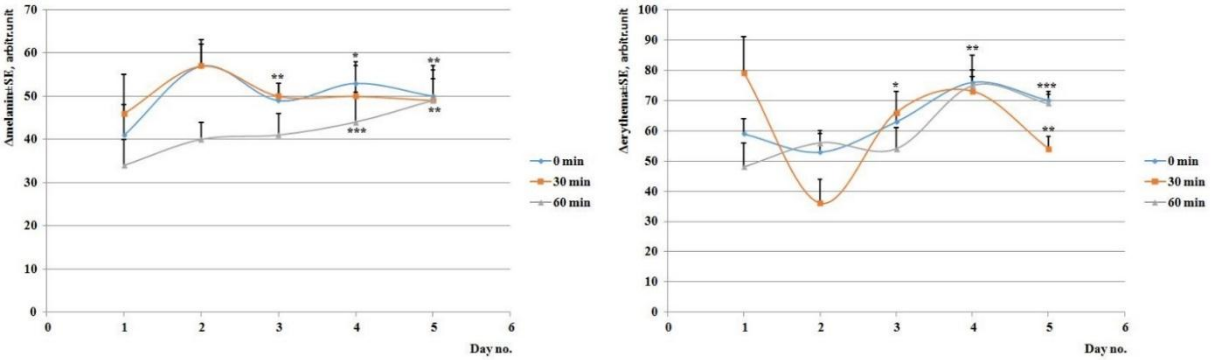


Figure 2. Melanin and erythema measurements prior to application, 30 minutes after application, 60 minutes respectively after application over the course of 5 days

Melanin and erythema (hemoglobin) indices are the indicators that quantify the intensity of pigmentation. The results showed a higher variation of melanin after immediate application of chlorhexidine digluconate as compared to the control group as can be observed in the figure 2. The same conclusion can be drawn regarding the assessment of erythema, higher values at 30 minutes after application, which persists until the last day of the experiment.

CONCLUSIONS

At these concentration chlorhexidine digluconate lead to minor changes in the skin which persist for a long time. Future study, involving use in various concentrations of chlorhexidine digluconate able to determine the value of which can exert harmful effects, need to be made.

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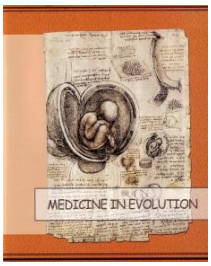
THE TOXICOLOGICAL ACTIVITY OF CITRIC ACID: A PRELIMINARY EXPERIMENTAL STUDY



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ABSTRACT

The aim of this study was the biological evaluation of citric acid used in dentistry. This compound was buccal applied in order to evaluate the values of pH and topically application on the dorsal side of the mice and measuring physiological skin parameters by means of a non-invasive techniques, mexametry in order to evaluate the values of erythema.

Key words: *citric acid, biological evaluation, cytotoxic effect; non-invasive techniques, erythema*

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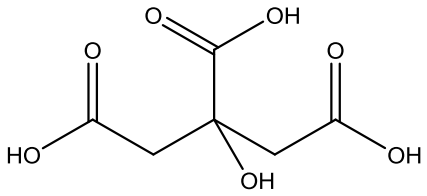
INTRODUCTION

The Citric acid (Figure 1) is a natural compound present in every living, in tissues, in body fluids and in many fruits and vegetables, taking part in the metabolism of the cells [1]. It was discovered in the ninth century by the Arab alchemist Jabir ibn by Hayyan (Geber, 721-815) and was first isolated in 1784 by the Swedish chemist Carl Wilhelm Scheele, who managed to crystallize from the lemon juice.



Acid citric

Figure 1. Chemical structure of citric acid

In dental medicine citric acid is used in a concentration of 30-40% and has the following properties:

- Helps to remove the sticky or slippery layer of the channel wall, exposing dentina's root canal;
- Allows precise cleaning of the channels from debris pulp;
- Ensure precise penetration of the root filling material;
- Improves the mechanical cleaning of the root canal
- Removes dentinal sawdust of the root canal walls making possible the discovery of dentinal tubule holes.

Smear layer adheres to the tooth surface, obturating the dentinal tubules. Studies on the efficacy of phosphoric acid in the removal of the smear layer in comparison with citric acid and EDTA have been made by various methods. One of these involves the use of scanning electron microscopy (SEM). Prado et al. have evaluated using SEM efficiency of 37% phosphoric acid solution compared to a 17% EDTA solution and a 10% citric acid. The result of studies show that none of these substances at the concentration used is not effective for removing smear in 30 seconds, but after three minutes all potential exercise [2].

The bacteria and their byproducts play a key role in establishing and discovery of pulp and periapical diseases. These include anaerobic organisms Enterococcus faecalis influencing endodontic treatment and contributes to enhancing infections. It is able to survive and persist as pathogen inside dentinal canals and tubules. Arias-Moliz et al. have researched in vitro studies Enterococcus faecalis bactericidal activity against irrigable substances phosphoric acid, citric acid and EDTA, using concentrations of 2.5% and 5% for phosphoric acid, 10% and 25% for citric acid and and 17% for EDTA. The experiments have revealed that the two concentrations of phosphoric acid exerts an intense activity, compared with citric acid and EDTA solution who does not present any kind of activity [3].
Consuming acidic food and drinks cause tooth enamel erosion, which is increasingly common nowadays, so study in dental materials has experienced a real upswing in recent years. In vitro performed studies have tested the hypothesis that addition of polymers to the solution of citric acid leads to a decrease in the erosion of tooth enamel and / or they form a protective layer formed on the surface

of the enamel [4-6]. Beyer et al. tested esterified pectin polymers, propylene glycol alginate and arabic gum, exposing the samples to different time periods, both solutions containing polymers with citric acid and citric acid solutions. Beneficial effects have been observed for all the the three polymers used [7].

Native dental film plays an important role in dental erosion and tooth decay occurrence. Its selective permeability reduces ion transportation in dental tissues and plays a mechanical barrier by reducing contact between the tooth surface and acidic environment. Because of the different composition dentina is considered responsible for this difference of its protein composition. Delecrode et al. identified a number of proteins resistant to acids, especially mucin, which may be related to their protective effect on the teeth homeostasis, but their mechanism has not been elucidated. An elucidation of this mechanism would be a real success for treatment and prevention of tooth decay and dental erosion [8]. Wegehaupt et al. have studied the possibility of sealing the surface coat with resin base, reaching the conclusion that it is able to reduce significantly the erosive demineralisation of tooth enamel caused by citric acid [9].

TOXICOLOGICAL DATA

Hans Krebs, proposed in 1937 citric acid cycle (tricarboxylic acid cycle) which revolutionized the metabolic chemistry. This cycle is composed of eight reactions that occur in the mitochondrial matrix, the oxidized acetyl group from acetyl-CoA to form two molecules of carbon dioxide by means of a process which allows recovery of the free energy by the synthesis of ATP. The main function of the Krebs cycle is to produce energy. As such an important compound in metabolic processes it cannot be considered a potential toxic compound. Citric acid poisoning can occur through inhalation or ingestion. Chronic effects on humans are just the destruction taking place at dental surface.

Considering several studies conducted in animals and humans conclude that citric acid is low acute toxicity. The value of repeated dose toxicity NOAEL for rats is 1200 mg / kg, at reproduction 2500 mg / kg and major toxic effects are reversible limited to changes in blood chemistry and absorption / excretion of metals. The major danger of toxicological exercised citric acid is the irritation, especially eyes, respiratory system and skin [10]. Oral exercises a low acute toxicity in rats (lethal dose 3000-12000 mg / kg) and mice (lethal dose 5400 mg / kg). General effects include physiological disturbances (acidosis and calcium deficiency), whereas high doses may cause central nervous system effects and serious damage to stomach lining. The subcutaneous lethal dose application is 5500 mg/kg to rats and 2700 mg / kg to mice. In various injection ways of citric acid in rats, mice and rabbits cause effects on the nervous system, lungs, spleen and liver partially attributable acidosis and calcium deficiency. A clinical case of ingestion can be recalled at a dose of 25 g of citric acid by a woman who has resulted vomiting and abdominal pain. Volunteers who have received a dose of about 4.7 g of citric acid in the form of potassium citrate or magnesium did not exhibit gastrointestinal effects. It hase no carcinogenic effects [10].

The aims of the present study were: (1) to observe the changes in the pH values after oral administration of citric acid solution and (2) to evaluate the evolution of the main physiological skin parameters after citric acid solution exposure in a SKH1 mouse model.

MATERIALS AND METHODS

The solutions of citric acid was purchased from PPH CERKAMED. SKH1 mice were obtained from Charles River Germany, female, 8 weeks. SKH1 mice were divided in 5 groups (4 mice/group): for each group mice were topically exposed to active compound, 1 application/day during 5 days.

Non-invasive skin measurements

All the non-invasive measurements on mice skin were carried out with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of erythema were obtained by means of the MPA5 Mexameter® MX 18 probe, as quantitative results regarding erythema (haemoglobin) subjected to toxicological evolution. It was used a spectrophotometer evaluation for obtain the units for erythema.

The haemoglobin values for erytema were measured using 2 wavelengths: 560 and 660 nm [11].

For the pH values was used a manual Mettler Toledo ph meter.

RESULTS AND DISCUSSIONS

At the beginning of the experiment on day before oral application of 40% citric acid solution, the experience animal present a pH value of 8. At 30 seconds after administration the pH drops suddenly to 1, and after 5 minutes from the application returns to a value close to the initial one, namely 7.8, and after one hour is 8.4 (Figure 2). In the next four days pH variations are similar: first animal has a pH of 8.4-9, 30 seconds after administration its value is about 1.4, but returns in a short time (about 5 minutes) to values between 7-7.6, one hour later is located between 8.3-8.8.

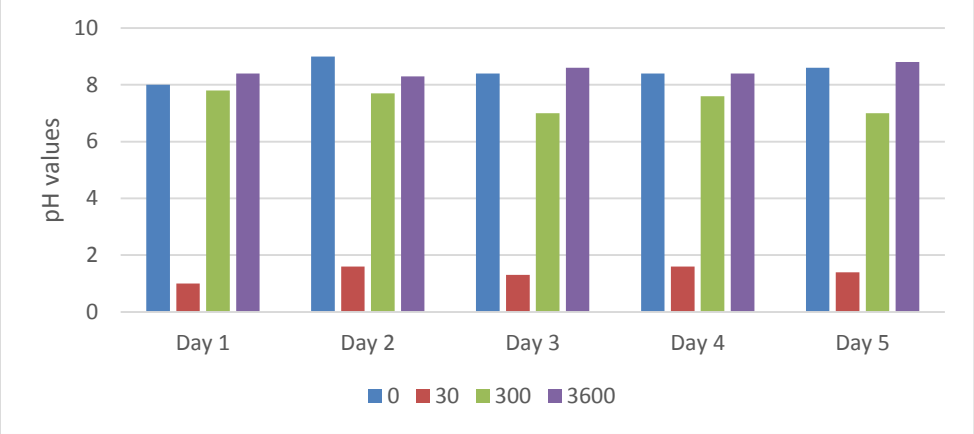


Figure 2. pH – values measured before, after 30s, 300s and 3600s the application of citric acid solution

This study was developed on SKH1 mice in order to evaluate the effects of citric acid solution used in dentistry field. After topical application on the dorsal side of the mice were measured physiological skin parameters by means of a non-invasive techniques, mexametry.

Macroscopic evaluation indicated the presence of redness and skin dryness but the values of erythema were almost normal (figure 3). During the five days of the experiment was observed minor changes in the values of erythema. In the first day these values are increased after which it stabilizes at an intermediate value.

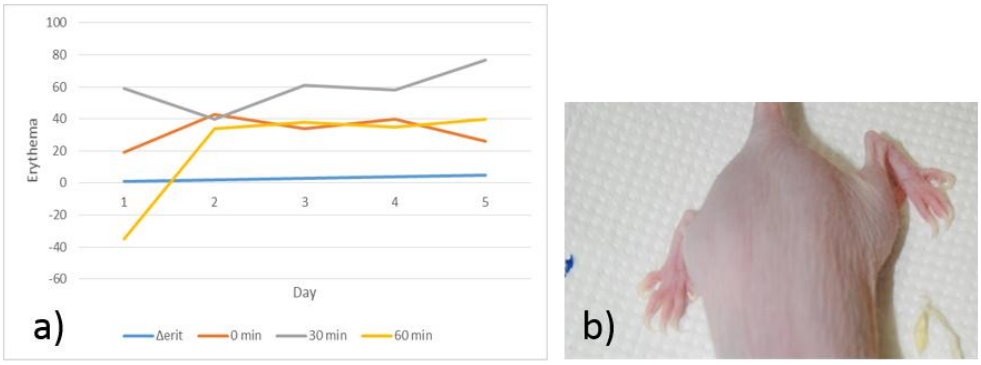


Figure 3. Measurements of erythema values before the application, at 30 min and 60 min, after application of the citric acid solution (a) and toxicological aspect of SKH1 mice after topical exposure to citric acid solution

CONCLUSIONS

These preliminary results showed that oral application of the citric acid solution leads to a drastic decrease of the pH but this is a transient state because the value returns to the normal in less than 5 minutes. The topical application of the compound have no cutaneous signs of toxicity, erythema values were higher only in the first day as compared to control group, after which there is a stabilization of these values without affecting the animal.

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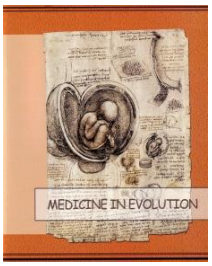
EFFICIENCY OF ORAL HEALTH EDUCATION METHODS AT CHILDREN FROM RURAL AREA



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ABSTRACT

Introduction: *Children who suffer from poor oral health are 12 times more likely to have restricted-activity days than those who do not. More than 50 million school hours are lost annually because of oral health problems which affect children's performance at school and success in later life. The aim of this study was to evaluate the effectiveness of experiential learning (EL) or traditional lecturing (TL) school-based oral health education on improving the oral health knowledge, attitude and behavior as well as oral hygiene, gingival health and caries of 6-8 year-old children in Romania rural area.*

Methods: *In one group, students received a classroom-based EL (n=117) and in the other group, students received only a single TL (n=107).*

Results: *Oral health knowledge improved in both groups in a statistical significant level at 6 months for EL group, 82 students answered correctly after six months comparatively with 11 at baseline p = 0.036. Concerning dental caries it significantly increased in both groups (EL group p = 0.023, TL group p = 0.030) at 6 months.*

Conclussions: *Experimental program was found more successful than traditional in oral hygiene improvement. Both oral health education programs improved the oral health knowledge, attitude and behavior of children.*

Key words: *children, education methods, dental health education, dental public health services; oral hygiene*

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INTRODUCTION

Children who suffer from poor oral health are 12 times more likely to have restricted-activity days than those who do not (*1*). More than 50 million school hours are lost annually because of oral health problems which affect children's performance at school and success in later life (*2*).

Schools provide an important place for promoting health, as they reach over 1 billion children worldwide and, through them, the school staff, families and the community as a whole. (3) Health promotion messages can be reinforced throughout the most influential stages of children's lives, enabling them to develop lifelong sustainable attitudes and skills. Poor oral health can have a detrimental effect on children's quality of life, their performance at school and their success in later life (4).

Oral disease can lead to pain and tooth loss, a condition that affects the appearance, quality of life, nutritional intake and, consequently, the growth and development of children. The burden of oral disease is considerable. Tooth decay and gum disease are among the most widespread conditions in human populations, affecting over 80% of schoolchildren in some countries (5–7). The prevalence of other oral disorders such as dental erosion and enamel defects is rising (6, 7). Many children have experienced oral trauma.

Dental disease is one of the most costly diet- and lifestyle related diseases (8, 9). The cost of treating dental decay alone could easily exhaust a country's total health care budget for children (10). However, the cost of neglect is also high in terms of its financial, social and personal impacts (11). Many oral health problems are preventable and their early onset reversible. However, in several countries a considerable number of children, their parents and teachers have limited knowledge of the causes and prevention of oral disease (12–15).

The limited information available on the oral health status of rural children in the Romania makes it difficult to devise policy strategies to address perceived problems.

The need for the promotion of oral health in schools is evident and it can easily be integrated into general health promotion, school curricula and activities. Children can be provided with skills that enable them to make healthy decisions, to adopt a healthy lifestyle and to deal with conflicts. Healthy behaviors and lifestyles developed at a young age are more sustainable.

Experiential learning is a educational approach where learning comes through experience [16-18]. It has been used effectively in enhancing knowledge and improving attitude in health education [19,20] and recently was introduced in oral health education with promising results [21-25]. However, these programs have used isolated experiential learning techniques lacking the benefit of a comprehensive experiential learning program [21-25]. Furthermore, all the studies reported have tested the short-term effect of these programs and in a limited sample [21-25].

The aim of this study was to evaluate the effectiveness of experiential learning (EL) or traditional lecturing (TL) school-based oral health education on improving the oral health knowledge, attitude and behavior as well as oral hygiene, gingival health and caries of 6-8 year-old children in Romania rural area.

METHODS

The study was concerned on the effectiveness of two methods of dental health education (DHE) for improving oral hygiene knowledge and habits was evaluated among children between 6 and 8 years from Arad County rural area. Children from elementary schools from 10 villages of Arad County were assigned to one of two groups. In one group, students received a classroom-based experimental DHE lesson (*n*=117) and in the other group, students received only a single traditional DHE lesson (*n*=107). Data regarding oral health knowledge, attitude and behavior were collected via questionnaires applied before and after 6 months from the DHE. The experimental DHE lesson was contained for the children 6-7 years old: picture books, slides, video, puppet shows, food pyramid,, fishing games, jigsaws, different teeth puzzles, drawing/exercise sheets with theme teeth, oral hygiene, nutrition, shape and function of models and for the children between 7 and 8 years slides, videos, fishing games, food, leaflets, models with theme: dentitions function and structure of teeth, body/oral consciousness, on nutrition, trauma, caries process. The traditional DHE lesson was made by a Power Point Presentation and a discussion group about oral hygiene. Data regarding dental plaque, gingivitis and caries were collected by clinical examination made before and made after 6 months from the DHE. Two calibrated pediatric dentists examined the students using a periodontal probe and artificial light. They followed: dental plaque recording for modified hygiene index (HI), the simplified gingival index (GI-S) was used for gingivitis and DMFT, based on BASCD criteria, for dental caries.

RESULTS

One hundred and seventeen school children were allocated to the EL group and one hundrend and seven to the TL group. Power analysis was 81% at $\alpha = 0.05$. Sample's socioeconomic data are presented in Table 1.

Table 1. Demographic data for the EL and TL groups and for the total sample

	EL group (n=117)	TL group (n=107)	Total (n=224)
sex	female – 67 (57,26%)	female -55 (51,4%)	122 (54,46%)
	male- 50 (42,745)	male- 52 (48,6%)	102 (45,54%)
low incomes	67 (57,26%)	64 (59,81%)	131 (58,48%)
medium incomes	36 (30,71%)	34 (31,775%)	70 (31,25%)
high incomes	14 (11,96%)	9 (8,41%)	23 (10,26%)

At baseline the two intervention groups did not present any statistical significant differences. Descriptive results at baseline for each group are presented in Table 2.

Descriptive results from the questionnaire and the clinical examination at 6 months as well as differences detected within the same group are presented in Table 2. Within the same group, oral health knowledge improved in both groups in a statistical significant level at 6 months for EL group, 82 students answered correctly after six months comparatively with 11 at baseline $p = 0.036$. At TL group the difference was lower than the EL group, $p = 0.05$) (Table 2). About oral health behavior we detected improvement in both groups at 6 months, but statistical significance was found just at the EL group (p=0,05).

Changing attitude about the oral health is difficult and is the aim at every oral health promotion campaign. With our intervention we obtained that at the both groups, but just at EL group was statistical significant (p=0,044) (Table 2).

Regarding the clinical results, oral hygiene was improved for the EL group at 6 months while it was decreased for the TL group but the changes were non statistical different within the same group. Gingivitis increased within the same group for both groups at 6 months in a non statistical significant level (Table 2). Concerning dental caries it significantly increased in both groups (EL group p = 0.023, TL group p = 0.030) at 6 months. The six year molar is affected at 25,64% at baseline, including the four already missing sis year molar at the EL group, but after six month that was reduced to 10,71%, but this difference was not statistical significant. The same situation was at the TL group but the problems at six year molar was reduced from 37,38% to 23,8%.

Table 2. Questionnaire scores and clinical examination data at baseline, 6 months

		EL		TL	
		baseline n=117	6 month n=112	baseline n=107	6 month n=105
Questionarire	behavior	16	76	15	58
	attitude	17	83	18	68
	knowledge	11	82	10	71
Clinical evaluation	Bacterian plaque	83	49	81	63
	missing teeths	11	11	9	9
	six year molar decay	26	8	32	19
	six year molar missing	4	4	6	6
	dentar carries	54	21	64	32
	gingivitis	17	6	18	12

After DHE interventions, students in both groups displayed improved knowledge for most oral hygiene questions (e.g., when should you throw your toothbrush away?). However, for several questions, a significantly higher proportion the students from the EL group displayed improved knowledge compared to students receiving TL. These items included: awareness that cavity prevention and removal of germs are two purposes of oral hygiene; and knowledge that teeth help people to eat and talk.

The children six- seven years old prefer puzzles, puppet shows, picture books, comparatively with children seven- eight year old which prefer more fishing games, puppet shows and video. If the age of students is higher than they prefer for learning the modern technology methods and the interactive games. (see figure 1)

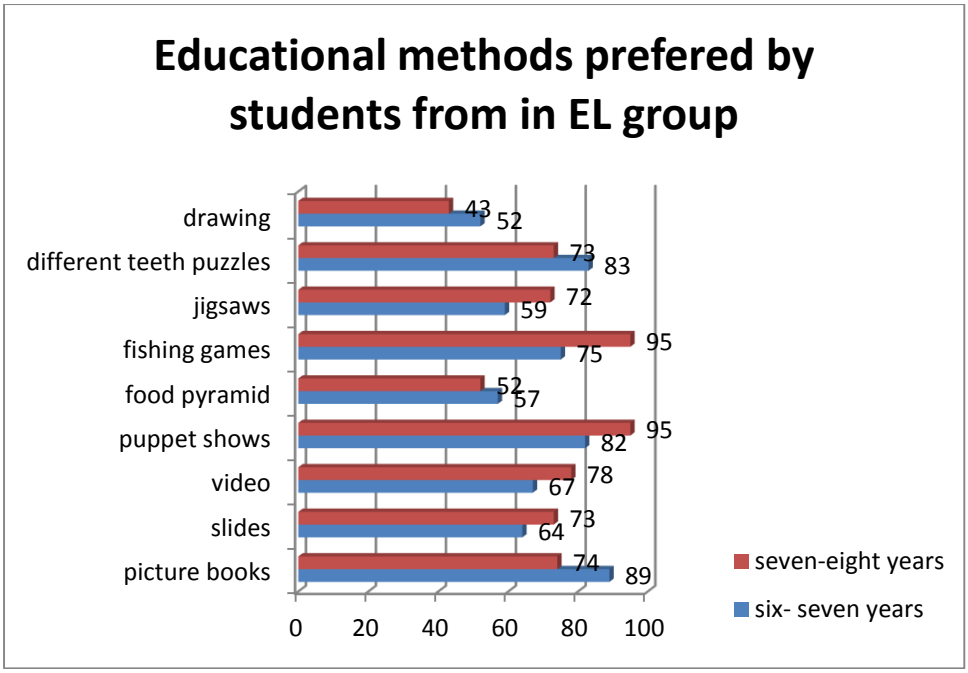

Figure 1. Educational methods prefered by students from in EL group

DISCUSSIONS

Results of the present study suggest that experiential learning is an effective school based oral health education method for short term and can improve the oral hygiene in primary school children. School based oral health education programs in the past using traditional lecturing had been found effective in improving knowledge but not for changing attitude. Students at 6- 8 years oral were not able to positively impact their oral health behavior, and that we are not sure that is correctly quantified. Usually a dentist or a dental hygienist when applies the oral health education program at the school want to change the dental hygiene and the health behavior. This is the first study to use experiential learning for a comprehensive school based oral health education program in rural area. Additionally, this program exploits the teacher who is most suited to implement such a program since he has daily contact with the students and is experienced in teaching skills. In order for teachers to be able to apply successfully an experiential learning program, related training is necessary [4], thus, a day seminar was given to the participating teachers.

Regarding clinical data, experiential learning group showed improved oral hygiene as previously found when using experiential learning techniques. However, the improvement was temporary which is in accordance with the findings of other studies [21-24]. In the traditional lecturing group, no statistically significant improvement has been found even for the short term, suggesting that experiential learning is a more effective method for oral hygiene instruction.

CONCLUSIONS

Experimental program was found more successful than traditional in oral hygiene improvement. Both oral health education programs improved the oral health knowledge, attitude and behavior of children.

This oral health education program was effective in establishing good oral health habits among

preschool children and in increasing oral health knowledge of their parents, in conjunction with supervised daily tooth brushing with fluoridated toothpaste, which could reduce the development of new dental caries in preschool children in rural area.

Children residing in rural areas have less access to and utilization of dental care compared to children residing in urban areas. Moreover, poor rural children display less utilization of dental services than poor urban children. Differences in the sum of decayed and filled primary teeth and the sum of decayed, missing, and filled permanent teeth were not significant.

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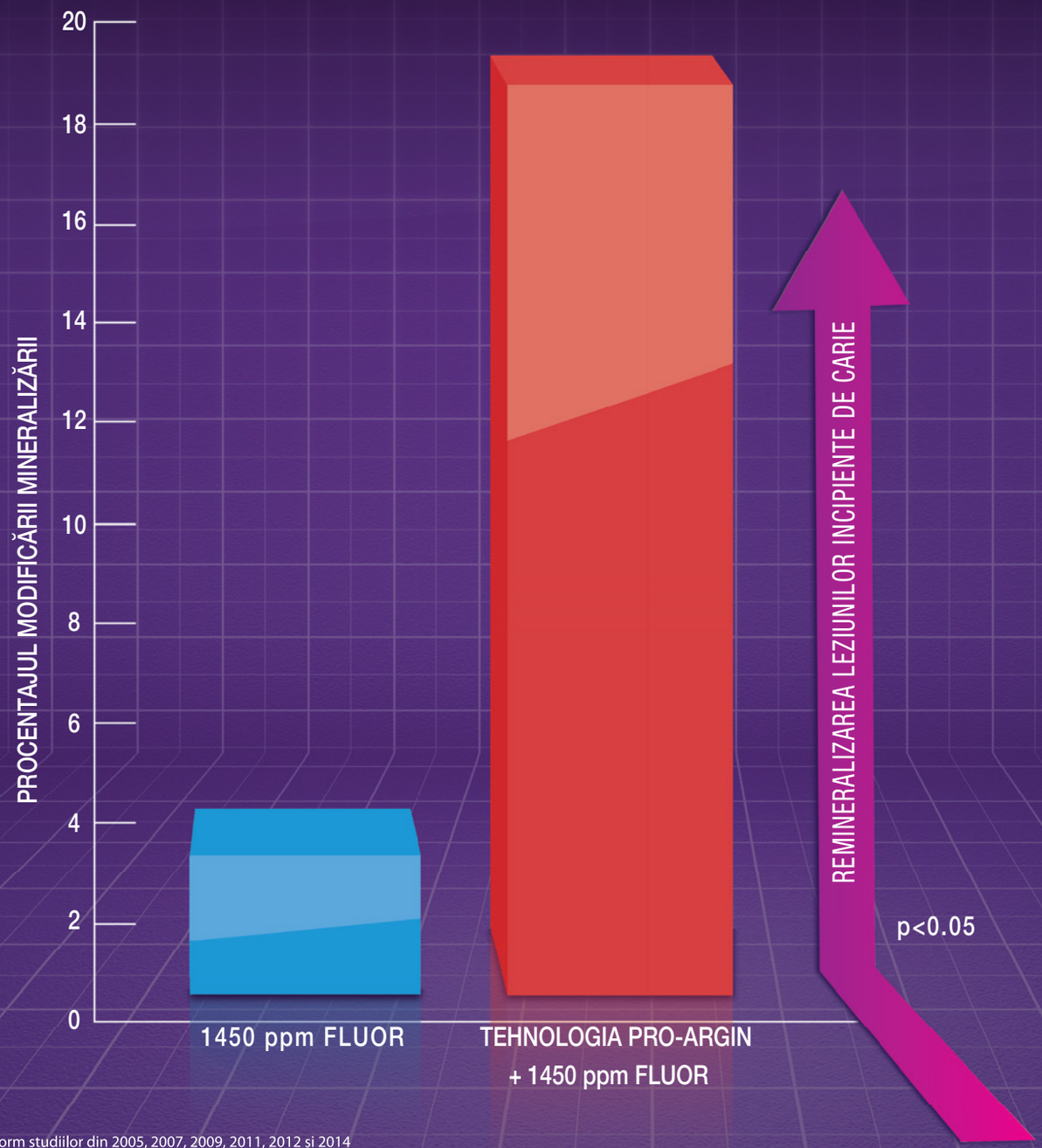
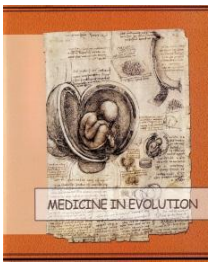


* Conform studiilor din 2005, 2007, 2009, 2011, 2012 și 2014

**Cantore R, Petrou I, Levander S, et al. J Clin Dent. 2013; 24 (Spec Iss AA): A32-A44.



PHYTOTOXICITY ASSESMENT OF POLYPHENOLIC DRY EXTRACTS FROM INDIGENOUS *RUBUS IDAEUS* L. (RASPBERRY) AND *BETULA PENDULA* ROTH. (BIRCH) LEAVES

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ABSTRACT

Aim and objectives. *The aim of our research was the cytotoxic evaluation (that forsees possible side/toxic effects, especially genotoxic) of polyphenolic dry extracts from Betula pendula Roth. (birch) and Rubus idaeus L. (raspberry) leaves, in order to obtain a pharmaceutical formulation with potential antidiabetic activity.* ***Material and methods.*** *The material consisted of birch (BE) and raspberry (RE) leaves dry extracts, which were standardized in polyphenols. Triticum assay was used to evaluate the cytotoxic properties of both extracts. Tannic acid was employed as a positive control. Results were statistically assessed using Kruskal-Wallis and Mann-Whitney tests.* ***Results and discussions.****The extracts showed cytotoxic activity at 0.20-0.06% BE and 0.08% RE, simultaneously with changes of kariokynetic film. Birch extract showed stronger inhibitory activity upon root elongation at 0.10% and 0.06% as compared to tannic acid.* ***Conclusions.*** *Both extracts showed cytotoxic properties at high concentrations, due to their chemical composition, rich in phenolic compounds.*

Key words: *cytotoxic, birch leaves, raspberry leaves, kariokynetic film*

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INTRODUCTION

The interest for complementary therapies, such as the use of botanical"food supplements"in different diseases (metabolic, cardiovascular, respiratory etc.) has significantly increased throughout the world. Although improperly considered safe, these supplements, can bring about serious side/toxic effects as well as botanical-drug interactions. The quality and safety of botanical supplements hang on different factors such as: the raw material (misidentification, other part of plants, contamination with pesticides etc.), its manufacture (standardized extracts, solutions) etc. *In vitro* (microorganisms, cell lines) and *in vivo* (animal models) assays are used for evaluating the safety of botanical supplements. Although *in vitro* assays aren't able to entirely predict *in vivo* toxicity, they remain a useful tool for screening of the potential toxicity of food supplements [1].

Birch leaves (*Betulae folium*) are a rich source of: flavonoids (1-3% - rutin, hyperoside, quercitrin, astragalin, isoquercitrin), phenolcarboxylic acids (chlorogenic, caffeic, ferulic, *p*-coumaric, gallic), tannins (1-9% - pedunculagin, casuarictin, tellimagrandin), proanthocyanidins, triterpenic compounds (3% - betulinic acid, betulin), mineral elements (manganese, zinc, vanadium, potassium, selenium etc.), essential oils and fatty acids.

Raspberry leaves (*Rubi idaei folium*) have high amounts of: gallo- and elagotannins (2-10% - sanguiin H6, lambertianin C,D), flavonoids (0.45-1.05% - rutin, hyperoside, astragalin, tiliroside), phenolcarboxylic acids (caffeic, chlorogenic, ferulic, *p*-coumaric) and mineral elements (vanadium, selenium, zinc) [2,3]. Both herbal drugs are claimed for their anticancer, anti-inflammatory, antibacterial and antioxidant properties [2].

Aim and Objectives

The aim of our study was the cytotoxic evaluation of birch (*Betula pendula* Roth.) and raspberry (*Rubus idaeus* L.) leaves dry extracts upon roots of wheat germ (*Triticum vulgare* Mill.), in the perspective of obtaining a pharmaceutical formulation with potential use as an adjuvant therapy for the treatment of type 2 diabetes mellitus.

MATERIAL AND METHODS

Material. Birch leaves were collected in May 2011, from Morăreşti, Argeş county, while raspberry leaves were harvested in April 2011, from Jilava, Ilfov district. Leaves were dried in shadow and stored in laboratory conditions. Herbarium voucher samples were deposited in the Department of Pharmacognosy, Phytochemistry, Phytotherapy, School of Pharmacy, Bucharest.

Preparation of dry extracts. For *Betulae folium dry extract* (BE), 300 g of powdered birch leaves were heated twice under a reflux condenser with 40% ethanol (v/v) for 30 min., using a herbal drug/solvent ratio of 1:20 (1st extraction) and 1:10 (2nd extraction). For *Rubi idaei folium dry extract* (RE), 300 g of powdered raspberry leaves were heated twice on a reflux condenser with 20% ethanol, for 30 min., using a herbal drug/solvent ratio of 1:30 (1st extraction) and 1:15 (2nd extraction). After cooling, the solutions were evaporated under reduced pressure (Buchi R 210-215 rotary evaporator) and then freeze-dried (using a Christ Alpha 1-2/B Braun, Biotech-International lyophylizator) to yield ~ 50 g of extracts (DER 1:6).

Method. The cytotoxic activity was investigated using Gr. Constantinescu method (*Triticum* assay), which is used to determine the maximum dilution of extractive solutions, that depending on exposure time, influence root elongation and kariokynetic film [4]. Research was conducted on roots of wheat germ (*Triticum vulgare* Mill., *Boia* type, SC Adaflor, Tulcea district, Romania). Cytotoxicity of both extracts was compared to that of tannic acid, used as a positive control and well-known for its antiproliferative properties [5].

Preparation of tannic acid (positive control) solutions. 0.200 g tannic acid (Riedel de Häen, Germany) were dissolved in 50 mL distilled water and made up to 100 mL (volumetric flask) with the same solvent (SAT_1 = 0.02%). Volumes of 7.5-0.1 mL SAT_1 were diluted to 15 mL with distilled water. The following concentrations were obtained: 0.01%; 0.0066%; 0.0013%; 0.0001% (SAT_2 - SAT_5). Tannic acid concentrations were choosen based on scientific literature data [5].

Betulae folium dry extract (BE) and *Rubi idaei folium dry extract* (RE) concentrations are equivalent to those of tanninc acid and were calculated based on tannins concentration of each dry extract (RE – 22.11 g% tannic acid and BE – 9.92 g% tannic acid).

Preparation of BE and RE solutions. 0.2016 g BE and 0.0859 g RE were dissolved in 100 mL distilled water in a volumetric flask (SBE_1 = 0.2016%; SRE_1 = 0.0859%). Volumes of 7.5; 5; 1 and 0.1 mL SBE_1 and SRE_1 were diluted to 15 mL with distilled water. The following concentrations were obtained: 0.1008%; 0.0672%; 0.0134%; 0.001344% (SBE_2 - SBE_5) and 0.0429%; 0.0286%; 0.0057%; 0.0005% (SRE_2 - SRE_5).

Tannic acid, BE and RE solutions (15 mL) were brought to Petri dishes (d = 50 mm) and kept in contact with 11 germinated wheat caryopses (embryonic root = 1 cm), in the dark, for 5 days. During the whole experiment, the length of embryonic roots was determined by linear method. Results were compared to a negative control, that contained distilled water, instead of extractive solutions. Changes of kariokynetic film for each concentration were investigated after 24 h. Microscopic sections (vegetative cone of an embryonic root of each sample) were stained with a solution of diluted acetic orcein and analyzed at a Labophot 2 Nikon microscope (ocular 40x, 100x).

Statistical analysis. Root elongation of wheat caryopses during 5 days of experiment represent the mean ± SD (n = 11) and was calculated in Microsoft Excel 2007. Results obtained after 72 h of contact between extractive solutions and wheat caryopses have been used for statistical analysis, since they have the advantage of enough contact time and avoid measurement errors, that occur after 96 and 120 hours. Statistical analysis was performed using Minitab 15.1.1.0 (Minitab Inc., 2007) software. Normality distribution was assessed using Ryan-Joiner, Darlington-Anderson and Kolmogorov-Smirnov tests. The statistical analysis used Kruskal-Wallis test (at a 95% confidence interval) to compare root elongation among multiple groups and Mann-Whitney test (at a 99% confidence interval) for pairwise *post hoc* comparisons, as the assumption of normality was not satisfied and the ANOVA could not be applied. The inhibitory effect was determined using the following formula:

$$I_{inh} = 100 - \frac{T-1}{M-1} \times 100$$

Where: T = test solution median, M = control median, 1 = initial value of embryonic roots (cm).

RESULTS AND DISCUSSIONS

During the whole experiment, tannic acid showed a strong inhibitory effect upon root elongation at 0.0200-0.0660%, while at smaller concentrations (0.0013% and 0.0001%) the trend was similar to that of the negative control (fig. 1A). BE and RE showed concentration dependent effects upon root elongation (fig. 1B, C).

For all analysed groups, Kruskal-Wallis test showed significant differences ($p < 0.0001$), so we continued with *post hoc* analysis (between each extract concentration and negative/positive controls) using Mann-Whitney test at a 99% confidence interval (equivalent to a Bonferroni correction of the familywise error rate).

Our results pointed out that BE has a strong (I_{inh} = 100%) and significant ($p < 0.01$) inhibitory effect upon root elongation at 0.2016% and 0.1008% concentration (table I). Birch dry extract had a greater inhibitory effect compared to tannic acid at 0.1008-0.0672%, but results were significant only for 0.1008% concentration ($p < 0.01$). Regarding 0.0134% and 0.0013% concentrations, the extract seemed to stimulate root growth, but the results were not statistically significant compared to the negative or positive controls (table II).

RE had a trend toward inhibition of root elongation at 0.0859% concentration (p = 0.0831, I_{inh} = 47.62%), while for 0.0429-0.005% a stimulatory activity was observed (table III).

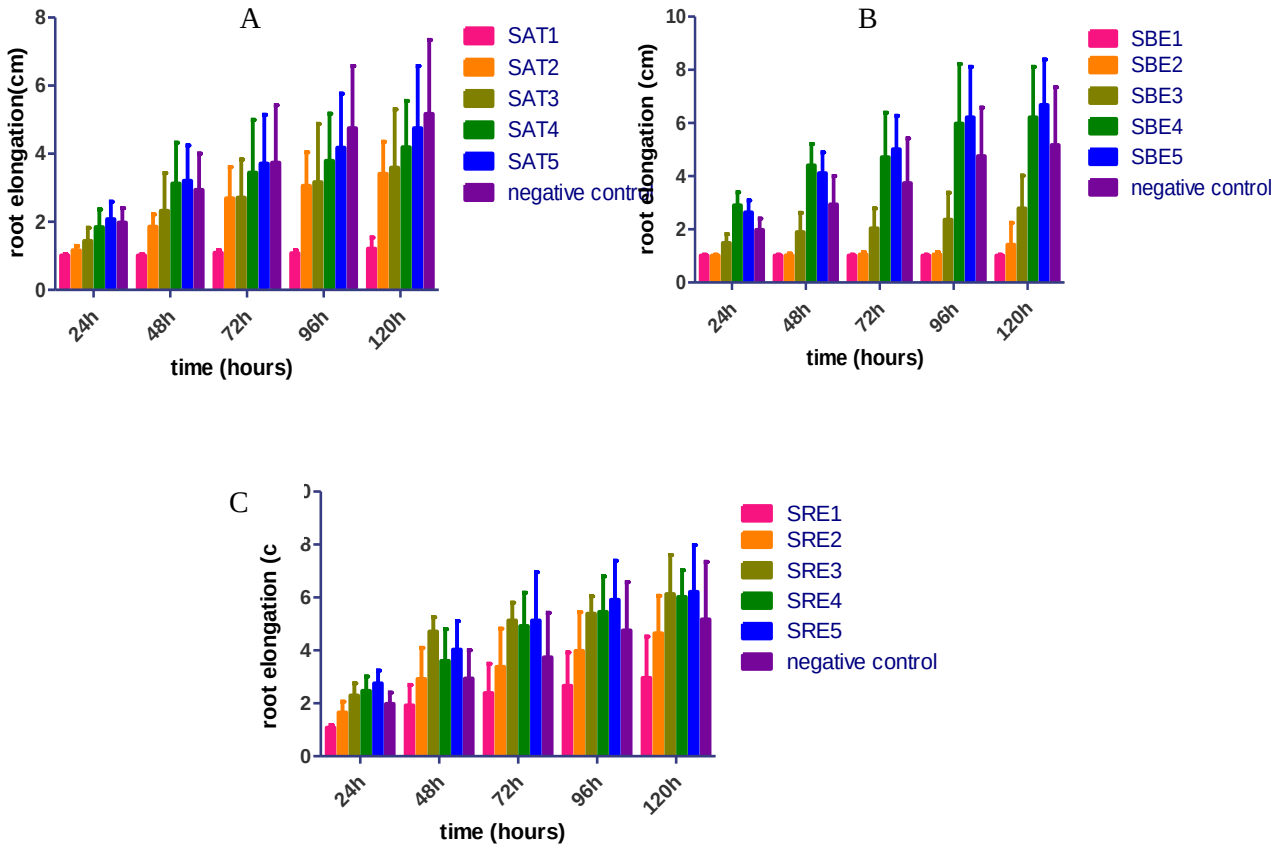


Figure 1. Tannic acid (positive control - A), BE (B) and RE (C) influence upon root elongation during 5 days of experiment

Table I. Statistical comparison between root elongation upon BE treatment vs. negative control

Concentration (%) BE	Root elongation upon BE treatment (median)	Root elongation upon negative control (median)	Difference of medians	99% confidence interval for difference of medians	p value (Mann-Whitney)	Inhibitory effect (%)
0.2016[a]	1.0	3.1	-2.1	(-5.4 – -1.0)	0.0006	**100.00%**
0.1008[b]	1.0	3.1	-2.0	(-5.3 – -1.0)	0.0005	**100.00%**
0.0672[c]	2.1	3.1	-1.2	(-4.5 – 0.2)	0.0180	47.62%
0.0134[d]	4.6	3.1	1.5	(-1.7 – 4.2)	0.0540	-71.43%
0.0013[e]	4.4	3.1	1.4	(-2.1 – 4.0)	0.0403	-61.90%

At all concentration levels, the raspberry dry extract was less inhibitory than tannic acid, although the threshold of significant significance for the differences versus this substance were seen only for 0.0859% and 0.0286% (table IV).

Table II. Statistical comparison between root elongation upon BE treatment vs. positive control

Concentration (%) BE	Root elongation upon BE treatment (median)	Root elongation upon tannic aid (median)	Difference of medians	99% confidence interval for difference of medians	p value (Mann-Whitney)
0.2016[a]	1.0	1.0	0.0	(-0.1 – 0.1)	1.0000
0.1008[b]	1.0	2.6	-1.6	(-2.3 – -0.6)	0.0003
0.0672[c]	2.1	2.45	-0.6	(-2.5 – 1.0)	0.2926
0.0134[d]	4.6	3.7	1.7	(-0.6 – 4.3)	0.0519
0.0013[e]	4.4	4.0	1.0	(-0.8 – 3.8)	0.0397

Table III. Statistical comparison between root elongation upon RE treatment vs. negative control

Concentration (%) RE	Root elongation upon RE treatment (median)	Root elongation upon negative control (median)	Difference of medians	99% confidence interval for difference of medians	p value (Mann-Whitney)	Inhibitory effect (%)
0.0859[a]	2.1	3.1	-1.1	(-4.5 – 1.1)	0.0831	47.62%
0.0429[b]	3.2	3.1	-0.5	(-3.2 – 2.1)	0.6646	-4.76%
0.0286[c]	5.3	3.1	1.9	(-1.2 – 3.2)	0.1033	-104.76%
0.0057[d]	5.0	3.1	1.7	(-1.4 – 3.4)	0.1227	-90.48
0.0005[e]	5.1	3.1	1.6	(-1.7 – 4.5)	0.1556	-95.24

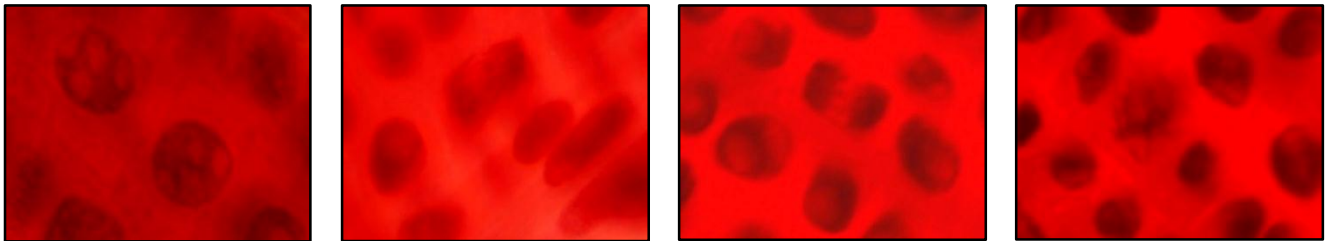
Table IV. Statistical comparison between root elongation upon RE treatment vs. positive control

Concentration (%) RE	Root elongation upon RE treatment (median)	Root elongation upon tannic aid (median)	Difference of medians	99% confidence interval for difference of medians	p value (Mann-Whitney)
0.0859[a]	2.1	1.1	1	(0.1 – 2.9)	0.0029
0.0429[b]	3.2	2.6	0.6	(-1.3 – 2.7)	0.3306
0.0286[c]	5.3	2.5	2.4	(0.6 – 4.0)	0.0019
0.0057[d]	5.0	3.7	1.3	(-0.8 – 3.7)	0.0338
0.0005[e]	5.1	4.0	1.5	(-1.2 – 4.3)	0.1403

Legend: a-e equivalent to 0.02%; 0.01%; 0.0066%; 0.0013%; 0.0001% tannic acid (positive control)

The fact that extracts with an identical contents in tannic acid is less inhibitory than the single substance is likely to be related to the protective effect of other substances accompanying tannins in RE (nutritional or other type of substances acting through a variety of mechanisms).

The inhibitory effect of BE and RE upon root elongation is associated with changes in the kariokynetic film: abnormal shaped and hypertrophied nuclei (fig. 2A, B), bridge anaphases and telophases (fig. 2B, C) and metaphases in tropokinesis (fig. 2D).



A B C D

Figure 2. Kariokynetic film changes induced by BE and RE
A - hypertrophied nuclei, B - bridge anaphases, abnormal shaped nuclei, C – bridge telophases, D - metaphases in tropokinesis

We assume that the chemical composition of both extracts is responsible for these changes, since polyphenols are known for their pro-oxidant effects at high concentrations [6], but other classes of substances might also be involved.

Betulae folium dry extract was standardized in polyphenols: tannins (9.9200 g% tannic acid), flavonoids (13.0880 g% hyperoside), phenolcarboxylic acids (8.0592 g% chlorogenic acid) and proanthocyanidins (2.0144 g% cyanidin chloride). According to our previous results, *Betulae folium dry extract* is also a source of triterpenic saponins (5.1468 g% betulinic acid), well-known for their cytotoxic properties upon normal and cancer cells (B16 - melanoma, HeLa - cervix adenocarcinoma, MCF7 - breast cancer, HT29 - colon cancer) [7]. We hypothesize that the equal/-stronger inhibitory effect of BE (observed for 0.2016%; 0.1008% and 0.0672% concentration) as compared to tannic acid is due to a possible synergism between cytotoxic properties of polyphenols and lupeolic saponins.

Rubi idaei folium dry extract has high amounts of: tannins (23.2308 g% tannic acid), flavonoids (7.6040 g% hyperoside) and phenolcarboxylic acids (5.4642 g% chlorogenic acid). Among raspberry leaf compounds, elagotannins (sanguiin H6, lambertianin C,D) are well-known for their antiproliferative effects towards different cancer cell lines (melanoma, breast and gastric cancers) [8].

CONCLUSIONS

Birch and raspberry leaves dry extracts have cytotoxic properties at high concentrations. Since our goal is to obtain a pharmaceutical formulation with potential use in type 2 diabetes mellitus treatment, further pharmacological studies are needed in order to establish the therapeutic interval, which is safe for long term administration.

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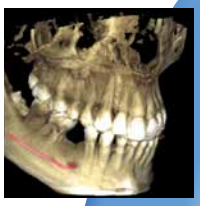

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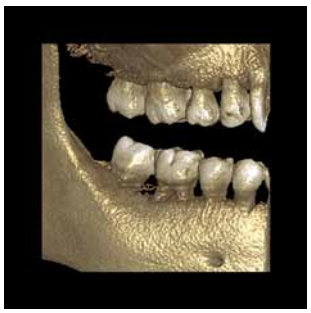

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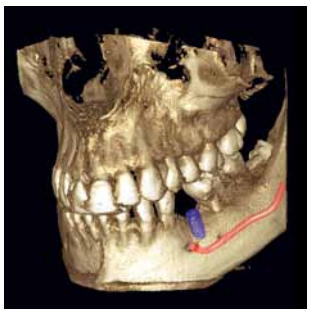
Ø42 x 42 mm–90 x 60 x 130 mm



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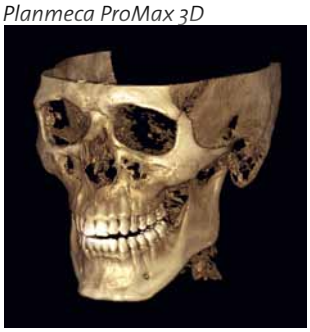

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Planmeca ProMax 3D Max
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INSTRUCTIONS FOR AUTHORS

The journal publishes general reviews, studies and clinical, epidemiological, experimental and laboratory research, clinical case presentation, papers from the history of medicine, reviews, scientific and technical state-of-the-art articles, medical informations and opinions. Only papers which have not been published or sent for publishing in other journals are accepted. The authors are responsable for the opinions expressed in the papers. ***The paper must be edited both in Romanian and in English; the English version will be supervised by our collaborator Dana Brehar-Cioflec, MD, PhD; typed on white A_4 paper (fonts - Times New Roman 12, Romanian characters, line spacing 1.5, upper and lower margins 2cm, left border 3cm, right border 2cm) and on CD, DVD or Memory Stick***.

Manuscripts will not exceed:

- general reviews: 6-8 pages
- studies and researches: 5-7 pages
- case presentations: 2-4 pages
- reviews, scientific and technical state-of-the-art articles, medical informations and opinions: 1-2 pages.

The paper will be edited according to international editing rules for manuscripts. The title will be written in capital characters and it will be followed by the name and surname of the author (authors), followed by their place of work (place where the paper has been elaborated). Studies and researches will be followed by a brief abstract, followed by 3-4 key-words.

The body of the paper will be structured on the following chapters: introduction, aim, objectives, material and method, results and discussions, conclusions. The references will be presented alphabetically and in conformity to the Vancouver Convention, including:

- for articles: name of the authors and surname initials, title of the article in the original language, title of the journal according to the international abreviation system, year of issue, volume, number, pages;
- for books: name of the authors and surname initials, volume, publisher (editors), city of publishing, year of issue.

Citation of references inside the body of the paper will be put between brackets, Harward style (author, year) or Vancouver style (number in square brackets or superscript). Cited reference titles will be selected, maximum 6 for studies and case presentations and 12 for general reviews. Acceptance, rejection or the need of alterations in sent materials, or in inconography, will be comunicated to the authors in due time. For this, the authors will indicate the person and address for corespondence (phone number, e-mail address). Given the less pleasant experience of the editorial board with some articles being rejected because they did not meet publishing criteria, we decided to support those who intend to publish in this journal by detailing the way such a paper should be elaborated, as well as our requirements.

Except some particular aspects concerning this journal, the following details are general requirements asked or imposed by other journals as well. Conditions to be met in order to propose a paper for publishing. The main author has the

responsability to make sure the article has been approved by all the other authors. The journal will have copyright for papers accepted for publishing. The editorial board reservs the right to change the style and dimensions of an article (major changes will be discussed with the main author) and to decide the date of issue.

2. FIRST PUBLICATION

The editorial board will not consider a paper already reported in a published general review or described in a paper proposed to or accepted by another journal. This does not exclude papers which have been rejected by other journals. Also, papers which have been presented at a scientific meeting will be accepted for discussion if they have not been entirely or partially published in a similar publication. „Multiple" publishing of the same study is seldom justified. One of the possible justifications is publishing in a second language but only if the following conditions are met:

- Editors of both journals involved are fully informed;
- Priority of the initial publication will be respected by a minimum publishing interval of two weeks;
- For the second publication, a shortened version will suffice;
- The second version strictly reflects data and interpretations in the first;
- A footnote may state: „This article is based upon a study initially published in [title of the journal]".

3. PATERNITY

Paternity must reflect the common decision of the coauthors. Each author must have participated enough to take public responsability for the content. A paper with collective paternity must have a key person responsable for the article.

4. COPYRIGHT

In order to reproduce materials from other sources, written agreement from the copyright owner must be obtained:

- photographer – for unpublished photographs;
- hospital where the photographer (physician) is employed – for unpublished photographs performed during the employment period;
- initial publisher – for a table, picture or text which have previously been published elsewhere.

5. ETHICAL ASPECTS

Do not use name of patients, initials or hospital observation charts numbers. If a photograph of a body part which could allow direct or deductive recognition of the patient needs publishing, then the paper must be accompanied by the written consent of the patient and clinician, as well.

6. PRESENTING THE MANUSCRIPT

For the journal *„Medicine in evolution"*, the manuscript must be typed double spaced, on white A_4 paper – 210 x 297mm, on one side (2.5cm upper and lower borders, 3cm left and 2cm right border, respectively), in clear characters, no further corrections or addings. It is advisable that articles are presented on CD or other data transfer methods, in Word format, 12 Times New Roman fonts - using Romanian characters – respecting the same page order, accompanied by a printed version. Graphs – black and white or coloured – may be generated in MS Excel or MS Graph, inserted in the body of the paper or presented in a different file.
Infected materials will not be used.

6.1. FIRST PAGE (TITLE PAGE)

Together with the title and names of the authors, the first page must include the affiliation, professional and university degree (if applicable), marked by asterisc for every author; it is advisable to give at least a phone and/or fax number or e-mail address of the first author who may be contacted by the editors for additional recommendations or explanations.

6.2. ABSTARCT OF THE PAPER

6.2.1 Recommendations for original studies

Original studies must include a structured abstarct of maximum 150 words, containing the following titles and informations:

- Aim and objectives;
- Material and methods;
- Results;
- Conclusions;
- Key words: give 3-5 key words;
- The abstract will be translated into an international circulation language.

6.3 CONTENT OF THE PAPER

6.3.1 For original articles

The text will usually be divided into sections:

- Introduction – presentation of general aspects, in the context of the approached theme
- Aim and objectives – Define the aim of the article. Briefly expose the rationale of the presented study or observation. Make strictly pertinent referals and do not exhaustively review the subject. Do not include data or conclusions from the paper.
- Material and methods – Describe the selection of observations or subjects for the experiment (including controls). Identify methods, equipments (with the name and address of the manufacturer in brackets) and give sufficient details on procedures. Give references for the selected methods, including statistical methods; offer details and brief descriptions for previously published methods which are not well known; describe new or

substantially modified methods, justify their use and assess their limitations. Precisely identify all used drugs and chemicals, including generic names, dosage and administration ways. Describe statistical methods with sufficient details for reported results to be verified. Whenever possible, quantify discovered aspects and present them with appropriate measurement indicators for the uncertainty or error of measurement (such as confidence intervals).

- Results – Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations.
- Discussions – Underline new, important aspects of the study. Do not repeat in detail data which have been presented in previous sections. Include implications of revealed aspects and their limitations, including implications for future studies. Connect your observations to other relevant studies. Relate the results to the aim proposed for the study.
- Conclusions – organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support; c) thanks for financial or material support.

6.3.2 Indications for case reports

Themes may be selected from all medical fields. Manuscripts which offer a special gain for daily activity will have priority. The title must be clearly, precisely stated. It may be completed by a subtitle. It is advisable to include in the key words of the title the main message, the special element which may be observed from the case evolution. The content of a case report must be divided into three parts:

- Introduction – It must include a maximum of 15 typed rows (half page). Here, the main medical problem is summarized in order to place the case in a specific domain.
- Case report – It contains essential specific information on the case.
- In order to make a logical, chronological and didactical case report the following 5 chapters are needed:

  I. Anamnesis;
  II. Clinical examination data;
  III. Laboratory data;
  IV. Additional paraclinical investigations;
  V. Treatment and evolution.
- Discussions – The reason for the case report must be stated. The report must be patient-centered. Occasional deviations from typical (characteristic) evolutions, nosologically important facts must be presented in such a manner to expose the clinical picture as completely as possible. The case report must not appear as an appendix of a general review. Dimensions of a case report: maximum 6-8 typed pages, 30 rows of 60 characters/page.

6.4. MEASUREMENT UNITS, SYMBOLS, ABREVIATIONS

All measurements must be expressed in International System (IS) units. Abreviations must be fully explained when first used.

6.5. TABLES

Tables are noted with Roman figures and they will have a brief and concise title, concordant with their content.

6.6. ILLUSTRATIONS

Number all illustrations in Arabic figures in a single succession. Apply a label on the back side of every illustration, containing its number and an arrow indicating the upper side. Coloured illustrations may be accepted but it is the choice of the editors, according to particular technical abilities of each journal issue, or it may involve a fee in special cases.

6.7. EXPLANATIONS FOR DRAWINGS AND GRAPHS

Explanation for drawings and graphs must be clear and in readable dimensions, considering the necessary publishing shrinkage.

6.8. PHOTOGRAPHS

Offer glossy, good quality photographs. Any annotation, inscription, etc. must contrast with the ground. Microphotographs must include a scale marker.

6.9. ILLUSTRATION LEGENDS

Include explanations for each used symbol, etc. Identify the printing method for microphotographs.

6.10. REFERENCES

A numbered list of references must be provided at the end of the paper. The list should be arranged in the order of citation in the text of the publication, assignment or essay, not in alphabetical order(according to the Vancouver rules). List only one reference per reference number. It is very important that you use the correct punctuation and that the order of details in the references is also correct.

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In order to accelerate publishing, the main author will send a set of printed sheets presenting the final version of the paper, as it will appear in the journal. It is really helpful that texts to be also sent on electronic support, diacritic characters mandatory.

8. REJECTION OF PAPERS

If a paper does not meet publishing conditions, whatever these may be, the editors will notify the first author on this fact, without the obligation of returning the material. Original photographs or the whole material will be returned only if the author comes to the editor and takes them.

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