Volume XXVI, Nr. 3, 2020

ISSN 2065-376X

MEDICINE IN EVOLUTION

medicineinevolution.umft.ro

CENTER OF PROMOTING HEALTH EDUCATION AND MOTIVATION
FOR PREVENTION IN DENTISTRY
CENTER FOR CONTINUOUS MEDICAL EDUCATION

REDUCE ŞI AJUTĂ LA PREVENIREA PROBLEMELOR GINGIVALE ÎN 4 SĂPTĂMÂNI PENTRU A ÎNTRERUPE CICLUL GINGIVITEI

IULIE 2010 IANUARIE 2011 IULIE 2011

IANUARIE 2012 IULIE 2012 IANUARIE 2013

blend-a-med Oral-B CLINIC LINE GUM PROTECTION SYSTEM

Recomandați Sistemul blend-a-med Oral-B Clinic Line Gum Protection
Este dovedit clinic că reduce si ajută la prevenirea problemelor gingivale în 4 săptămâni pentru a ajuta pacienții să întrerupă ciclul gingivitei. Sistemul combină acțiunea chimică puternică a fluorurii de staniu stabilizate, suplimentată de apă de gură, cu acțiunea mecanică a periuței de dinți Pro-Flex, suplimentată de ață dentară, facând din acesta completarea perfectă a tratamentului din cabinetul dumneavoastră.

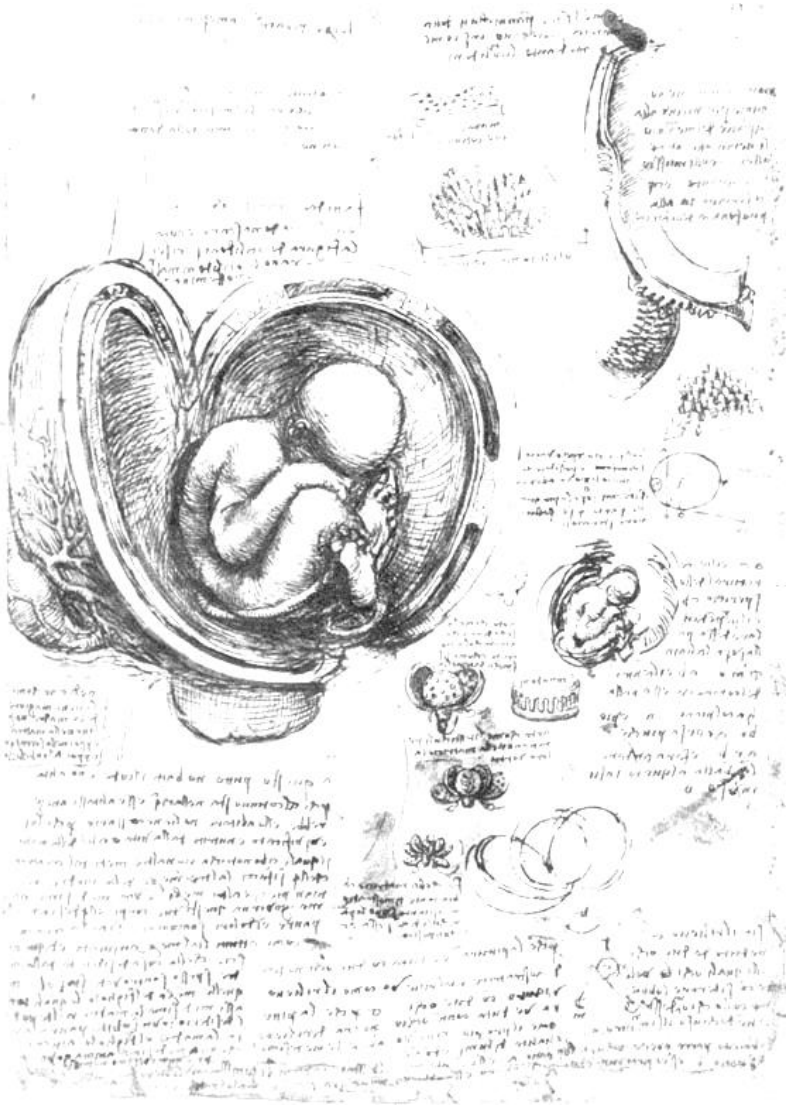
P12125.01

Continuă îngrijirea ce începe în cabinetul dumneavoastră

blend-a-med RESEARCH CENTERS Oral-B

Volume XXVI, Nr. 3, 2020, Timişoara, Romania
ISSN 2065-376X

MEDICINE IN EVOLUTION



CENTER OF PROMOTING HEALTH EDUCATION AND MOTIVATION FOR PREVENTION IN DENTISTRY
CENTER FOR CONTINUOUS MEDICAL EDUCATION

medicineinevolution.umft.ro

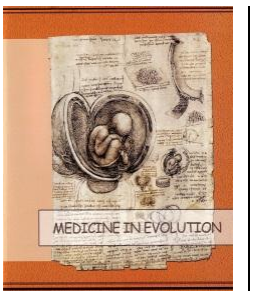
Journal edited with the support of

Printed at: WALDPRESS, Timisoara,
17 Brandusei Street,
Phone/Fax: 0040256422247

Edited at: EUROSTAMPA, Timisoara
26, Revolutiei 1989 Street,
Phone: 0040256204816

EDITORIAL BOARD

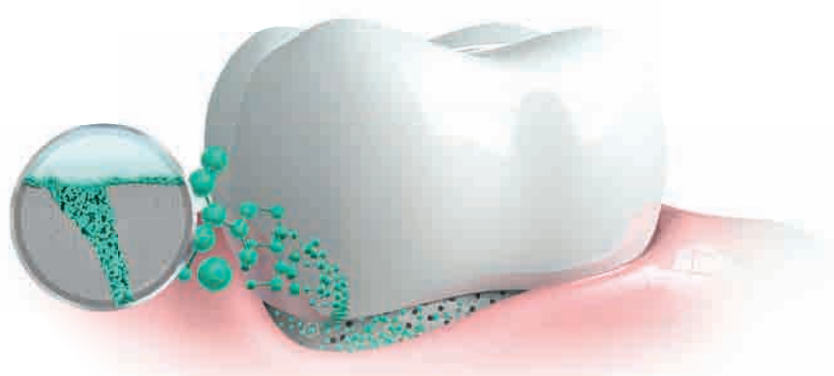
CONTENTS



ARTICLES

Noul elmex®SENSITIVE PROFESSIONAL cu tehnologia PRO-ARGIN

Calmarea **imediată* și de durată** a durerii din sensibilitatea dentară[1,2]



- În contact cu saliva, se formează un strat bogat în calciu, care obturează instant[1,*] tubulii dentinari deschiși
- Stratul rămâne intact în timp, chiar după expunerea la acizi, asigurând calmarea de durată a durerii din sensibilitatea dentară[2,3]

93% dintre pacienți confirmă calmarea durerii din sensibilitatea dentară[4]

93% dintre pacienți — confirmă reducerea hipersensibilității dentinare[4]

91% dintre pacienți — apreciază recomandarea medicului stomatolog[4]

Calmarea imediată* a durerii din sensibilitatea dentară[1]

Calmarea imediată* și de durată începe cu recomandarea dumneavoastră**

*Pentru calmare imediată, aplicați direct cu degetul pe dintele sensibil și masați ușor pentru 1 minut;
**Doar în legătură cu pasta de dinți

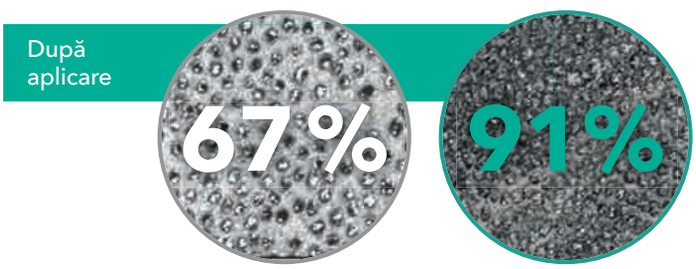
Referințe: 1. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123 –130; 2. Docimo R, et al. J Clin Dent. 2009;20(Spec Iss): 17– 22.; 3. Report Deon Hines-0003, 2016; 4. Studiu Ipsos cu privire la utilizarea produsului elmex® SENSITIVE PROFESSIONAL Repair & Prevent, efectuat în Polonia, rezultate după 2 săptămâni de utilizare, cu 325 de participanți (2017).

elmex® SENSITIVE PROFESSIONAL realizează obturarea superioară a tubulilor dentinari în comparație cu tehnologiile concurente[1,2,*]

Studiul 1[1,*]

Tehnologia cu fluorură de staniu/ fluorură de sodiu

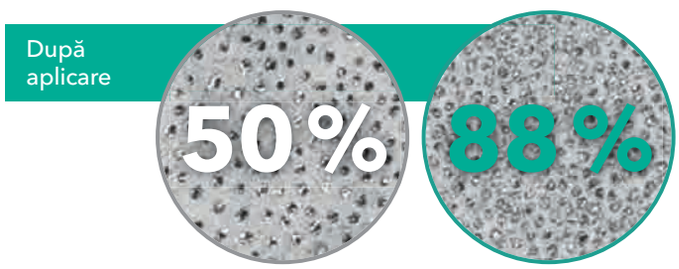
Tehnologia PRO-ARGIN



Studiul 2[2,*]

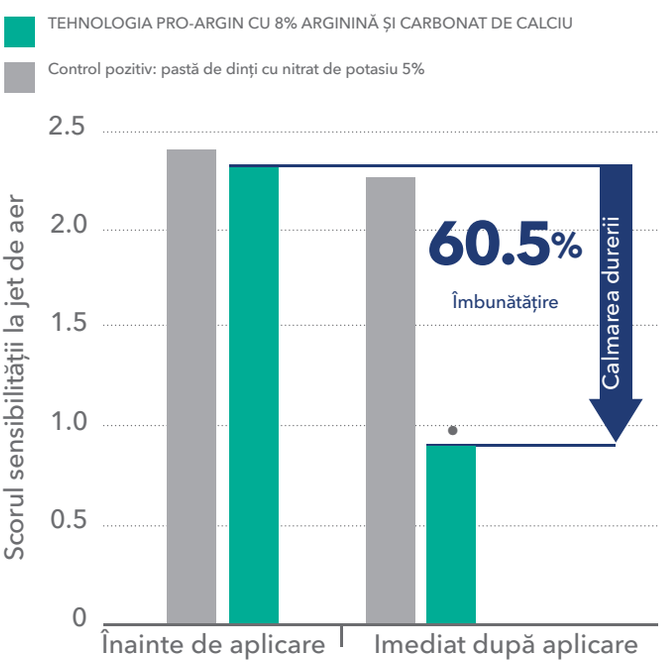
Tehnologia Novamin/ fluorură de sodiu

Tehnologia PRO-ARGIN



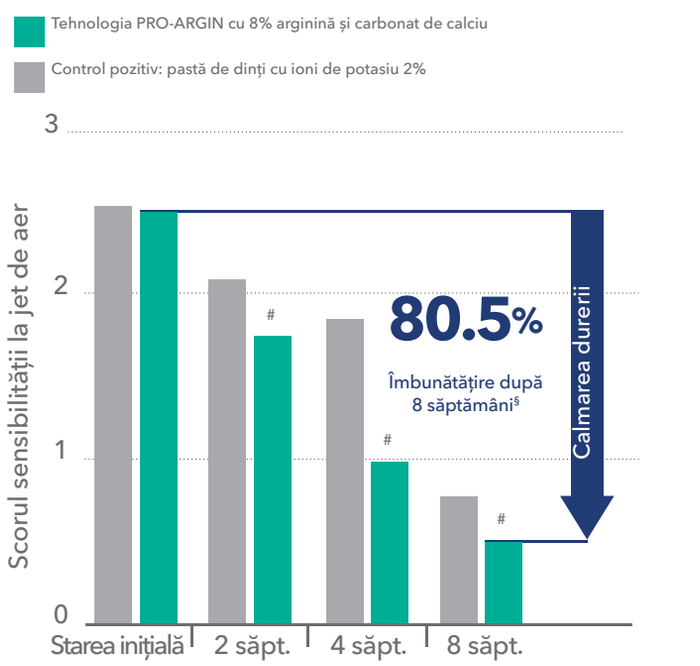
elmex® SENSITIVE PROFESSIONAL oferă calmare semnificativă imediată** și de durată a durerii din sensibilitatea dentară[3,4]

Calmarea semnificativă a durerii din sensibilitatea dentară instant[3,‡,**]



‡ În comparație cu starea inițială (sunt prezentate doar datele relevante)
• Semnificativ statistic (p<0,001)

Calmarea semnificativă de lungă durată a durerii din sensibilitatea dentară după 2, 4, și 8 săptămâni de utilizare[4,§,&]



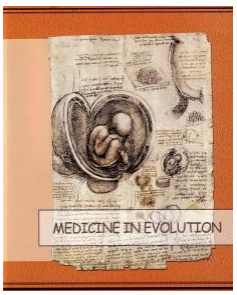
§ În comparație cu starea inițială
& În comparație cu o pastă de dinți comercială desensibilizantă, ce conține 2% ioni de potasiu și 1450 ppm de fluor (NaF)
# Semnificativ statistic (p<0,05)

*Studiu in vitro, imagini reale de microscopie confocală după 5 aplicări (p<0,05%);
**Pentru calmarea imediată aplicați direct pe suprafața sensibilă și masați ușor cu vârful degetului timp de 1 minut.

Referințe: 1. Hines D, et al. Poster acceptat, July 2018 IADR. Colgate- Palmolive Company 2018.; 2. Hines D, et al. Poster #0742, March 2018 AADR. Colgate-Palmolive Company 2018.; 3. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123 –130; 4. Docimo R, et al. J Clin Dent. 2009; 20(Spec Iss): 17– 22.

elmex®
SENSITIVE PROFESSIONAL

Associated Protein Rich Plasma (PRP) technique in traumatic meniscus tear and cartilage lesion



Cojocaru D.G.[1], Hogea B.G.[1,2], Florescu S.[1,2], Patrascu Jr. J.M.[1,2], Patrascu R.[2], Patrascu J.M.[1,2]

[1]*Department of Orthopedics and Trauma SCJUPBT*
[2]*Victor Babes University of Medicine and Pharmacy, Eftimie Murgu Sq., 300041 Timisoara, Romania*

Correspondence to:
Name: Dan G. Cojocaru
Address: I. Bulbuca no 156, Orthopaedics and Traumatology II Department
Phone: +40 721667798
E-mail address: dr_cojocaru@yahoo.com

Abstract

The most common acute knee disorders in the emergency department are cartilage and meniscus injuries, anterior cruciate ligament tears and MCL elongations. But even more important is that most of these lesions occur in the active population, both physically and socially, therefore the socio-economic impact of these diseases is huge. Beside the classic treatment methods, lately a new adjuvant procedure brings favorable results: Protein Rich Plasma. PRP is a relatively new technique, introduced around 2000 in orthopedics and traumatology practice, but generally accepted after 2010. It is based on the ability to repair of thr growth factors stored in blood platelets. We will analyze the follow-up patients with meniscal and cartilaginous lesions, the treatment options and their association, as well as the evolution with or without treatment.

Keywords: meniscus tears, cartilage lesion, protein rich plasma, orthobiologic treatment

INTRODUCTION'

Knee joint injuries are the most common presentations in emergency services worldwide in active patients aged 21-55 years. In the USA alone, this type of presentation remains represented by a number of 660K patients / year (2012, Bret E. Gage et co. In Academic Emergency Medicine). Over time, the therapeutic conduct has been adapted to a better understanding of the pathophysiology, biomechanics of the knee joint and new therapeutic discoveries. At present, the therapy applied 20 years ago is no longer relevant, which means an adaptation of the all trauma centers to the general trend of treatment.

Studies show that women are more likely to suffer knee injuries compared to men, with some studies advancing 2-8% more than men. This is related to decreased muscle balance between the quadriceps and the Hamestring complex, increased levels of estrogen and relaxin, including even certain anatomical differences, such as increased pelvic width in women.

An adequate treatment is considered the one after which, the well-being returns, the mobility is the one before the trauma, and the integration of the patient in the ecomic and social life is complete. This is not easy to obtain and is usually the result of a combination of treatments.

It should also be taken into account that most of these patients are active people, prolonged immobilizations or long-term treatments that decrease muscle mass are not preferred.

MATERIAL AND METHODS

A number of 45 patients between May 2019- May 2020, who suffered acute injuries of the knee joint, meniscus or cartilage lesion or a combinations of this types of injuries. Patients with ACL and MCL lesions were excluded. The suspected diagnosis in the emergency room was confirmed or refuted by MRI, performed in an imaging center with a 1.5 Tesla device. The ratio women per men was 1.1 / 0.9, and the average age was 39 years.

After clinical and imaging evaluation, it was observed that some patients had combinations of the 2 types of lesions and not just single lesions. Therefore a third group of patients was considered.

At the end the 3 groups of patients were divided differently:

21 patients with meniscal injuries

8 patients with cartilaginous lesions in various degrees

16 patients with combined lesions

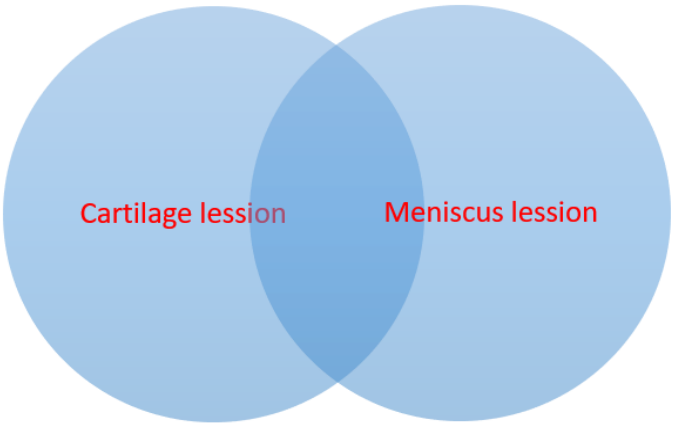


Figure 1. Type of lession

Type of treatment:

- Surgical: partial arthroscopic meniscectomy - for buckle handle meniscal lesions
- PRP: for cartilaginous lesions less than 1 cm and for degenerative, radial or longitudinal meniscal lesions
- Combined. applied for traumatic meniscal injuries with surgical treatment in which cartilaginous lesions have been identified intraoperatively.

Partial arthroscopic meniscectomy was performed only on patients with bucket handle meniscal tear. We really believe in the importance of the meniscus and its influence on the biomechanics of the knee. Moreover, the recommendations of the latest studies recommend a great precaution for meniscectomy, the lack of the meniscus being a predisposing factor for secondary osteoarthritis. At the same time, it has been proven that approximately 70% of the adult population has meniscal lesions that do not show clinical manifestations.

Some of our patients in the study treated surgically also discovered to had cartilaginous lesions. When these lesions were deep enough and affected the subchondral bone, limited chondroplasty was performed in the affected area.

PRP procedure involves the body's ability to self-repair capacity by using growth factors stored in platelets. Being a procedure that uses the body's healing capacity, the quality of the blood used is very important. Therefore, the patients in our study were educated that 5 days before performing the procedure to avoid NSAIDs, have a very good body hydratation and avoid fatty foods. Harvesting was done using 10 ml specimens, and the product resulting from centrifugation was 5 ml, with a growth factor concentration of 5-6 X compared to normal blood concentration. After infiltration patients were advised to avoid drugs or local procedures that inhibit inflammation.

Combined procedures, fisrt partial arthroscopic meniscectomy and PRP therapy procedures was performed after 30 days from surgery.

Table I. Type of lession

Lession	Meniscal lession	Cartilage lession	Combined lession
Patients no	21	8	16
Total	45		

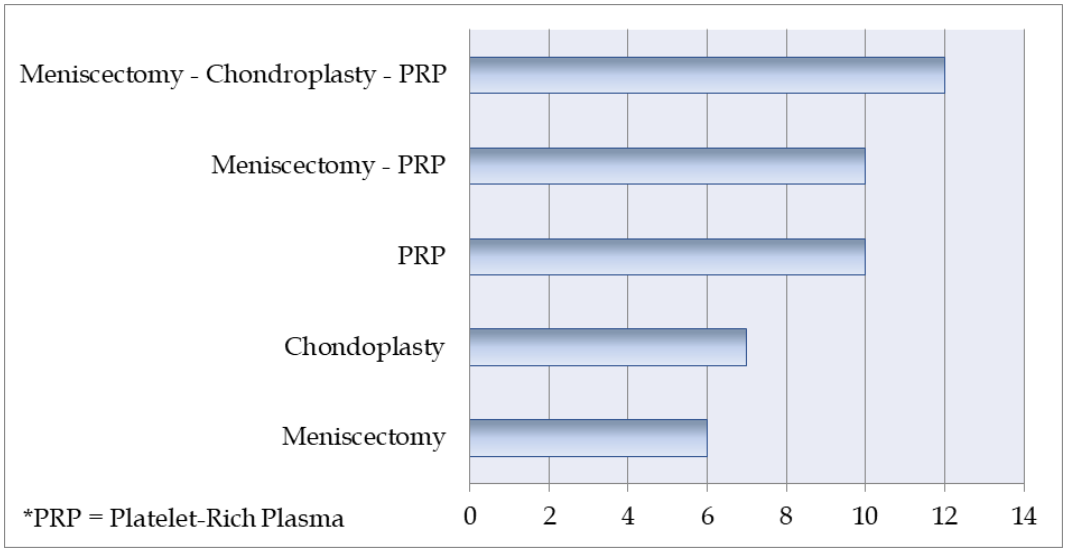


Figure 2. Procedures

RESULTS

For a correct evaluation of the knee, we use functional orthopedic scores (Lysholm and WOMAC) associated with the patient's individual perception of mobility and knee function at the end of treatment.

In case of patients with partial arthroscopic meniscectomy, we noted great results in 85% of cases, with a good degree of pacient satisfaction, associated with improved mobility, decreased pain and local edema at approximately 30 days postoperatively.

For patients with meniscal lesion and conservative treatment represented by of PRP technique as the single method of treatment, the degree of satisfaction was approximately 40% overall. The pain decreased by about 60% but the discomfort continued at 30 days post-therapy especially in case of large movements, also mobility was improved by about 50% and there was a decrease in local edema but not completely.

In case of patients with chondral defects less than 1 cm for whom the treatment was decided by the PRP technique as a treatment, the results were exceptional in proportion of 90%. In these patients, mobility increased considerably and the pain almost became non-existent after 30 days. Resumption of activity at high intensity was allowed 45 days after the last infiltration. In the 10% in which the result was satisfactory, it was considered that probably the lesions detected on MRI investigation were much more extensive than initially thought.

In the case of patients with meniscal lesions for whom partial arthroscopic meniscectomy was performed and intraoperatively cartilaginous lesions less than 1 cm were discovered, it was decided that postoperatively at 30 days to be used as additional treatment 3 infiltrations with PRP at a distance of 10 days. Following the evaluation, it was observed that the general degree of satisfaction was 75%, in most cases the patients resumed their active life, achieving approximately performances similar to the situation before the trauma.

DISCUSSIONS

Meniscal injuries caused by trauma are a cause of joint discomfort with secondary decrease in regular physical activity. The indication for partial surgical resection must remain reserved for these types of lesions, being recommended their preservation if this is possible (small radial and longitudinal lesions). The use of the PRP technique as an associated therapy, brings an additional clinically proven benefit. For cases in which only the conservative treatment of meniscal lesions is decided, it should be mentioned that the best results are obtained with a subsequent joint reeducation program secondary to PRP injection. However, if the result obtained only by PRP treatment, without surgery, is not satisfactory, partial arthroscopic meniscectomy should be considered.

Cartilaginous lesions respond very well to PRP therapy, especially in cases of defects less than 1 cm without severe damage to the subchondral bone.

CONCLUSIONS

The use of intraarticular PRP injections brings a definite benefit to traumatic injuries following arthroscopic surgical treatment or as a single therapy. In order for a great results, a well-documented selection and treatment of patients must be made. It should not be considered that one of the procedures replaces the other, the best results are obtained when for each specific injury a singular or associated treatment is decided, depending on the specificity of the trauma and the particularity of the case.

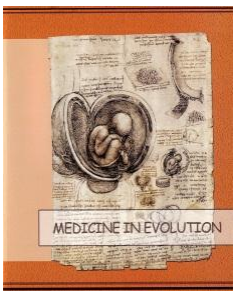
We recommend using the PRP injection technique for minor meniscal lesions, for cartilaginous defects under 1 cm and in which the subchondral bone is not affected. For major meniscal lesions associated or not with superficial cartilage lesions, we recommend

arthroscopic meniscectomy, chondroplasty, followed by additional PRP after 30 days from surgery, as well as joint reeducation after 10 days to the last infiltration.

REFERENCES

1. D.E Anderson, MB Rose, Arthroscopic mechanical chondroplasty of the knee is beneficial for treatment of focal cartilage lesion in the absent of concurrent pathology. Orthopaedics Journal of Sport Medicine, 24 May 2017, https://journals.sagepub.com/doi/full/10.1177/2325967117707213
2. A.J Scillia, KT Aune, Return to play after chondroplasty of the knee, The American Journal of Sport Medicine, 8 Jan 2015, https://journals.sagepub.com/doi/abs/10.1177/0363546514562752
3. J.M Fox, ME Blazina, Multiphasic view of medial meniscectomy, The American Journal of Sport Medicine, 1 May 1979, https://journals.sagepub.com/doi/abs/10.1177/036354657900700303
4. N.C Small, Complications in arthroscopic surgery performed by experienced arthroscopists, The Journal of Arthroscopic and Related Surgery, 1988, https://www.sciencedirect.com/science/article/abs/pii/S0749806388800306
5. J.C Riboch, AK Tilton, Meniscal allograft transplantation in the adolescent population, The Journal of Arthroscopic and Related Surgery, June 2016, https://www.sciencedirect.com/science/article/abs/pii/S0749806315009822
6. The basics science of PRP: what clinicians need to know, SP Arnoczky et co, Sports Medicine and Arthroscopy Review, Dec 2013, Volume 21, https://journals.lww.com/sportsmedarthro/Abstract/2013/12000/The_Basic_Science_of_Platelet_rich_Plasma__PRP___.2.aspx

Negative pressure wound therapy in orthopaedic surgery



Hogea B.G.[1,2], Voiculescu M.A.[1], Sandesc M.A.[1,2], Misca L.C.[1], Patrascu J.M.[1,2], Patrascu R.[2], Ilie C.A.[2], Patrascu Jr. J. M.[1,2]

[1]*Department of Orthopedics and Trauma SCJUPBT*
[2]*Victor Babes University of Medicine and Pharmacy, Eftimie Murgu Sq., 300041 Timisoara, Romania*

Correspondence to:
Name: Mihai Alexandru Sandesc
Address: Street Orhideea 12A,Town Giroc, County Timis, Country Romania
Phone: +40 744300745
E-mail address: sandesc.mihai@umft.ro

Abstract

Negative wound pressure therapy (NPWT) is a popular method used for the management of acute and chronic wounds. Its use is widespread in surgical specialties that use NPWT to varying degrees as part of their arsenal against high-risk wound injuries. The use of this method in orthopedics is diverse and includes: management of acute traumatic wounds, wounds resulting from surgical therapy, management of bedsores, management of infected or dehiscent wounds.

This paper is based on a retrospective study in order to provide current information related to the use of negative pressure therapy in orthopedic pathology, to report the effectiveness of this method of treatment depending on the duration of wound healing, the success rate of surgery and reducing complications.

Keywords: Negative pressure therapy, orthopedic pathology, support in wound treatment

INTRODUCTION

NPWT was originally promoted in 1989 by Charker et al. [8] which described a suction drainage system used in the management of skin incisions and fistulas. The system described by them was different from the devices used today, because it used as an absorbent material for filling the wound, cotton gauze, connected to a vacuum that sucks at pressures of 60-80 mmHg. Charker et al. considered that their system is effective in promoting fluid drainage, helping to form granular tissue and reduce skin lesions.

Despite the growing popularity of NPWT in the last two decades, there are a small number of studies proving the superiority of this method. Also, wound healing is a complex process, affected by both local and systemic factors, related to the general condition of the patient, so the results after using NPWT vary from one patient to another.

Aim and objectives

The aim of this study is to highlight the effectiveness of NPWT negative pressure wound therapy as a therapeutic aid, a viable solution in the modern treatment of complicated wounds in orthopedic surgery.

The main objective of this study is to use NPWT for the curative purpose of complicated wounds. Although the exact mechanism of NPT's action on wound healing is still an active area of research, evidence suggests that it is achieved by removing edema, increasing blood circulation, reducing bacterial bio-load, ensuring a moist wound healing environment and increasing granulation and tissue formation.

MATERIAL AND METHODS

This study was performed at the Orthopedics - Traumatology Clinic II of SCJUPBT and is a retrospective observational study. The study aims at the use of NPWT in orthopedic pathology performed on hospitalized and treated cases, for a period of 2 years, between January 1, 2017 and January 1, 2019, in the Orthopedics-Traumatology II clinic within the County Emergency Clinical Hospital "Pius Brânzeu" Timisoara, the studied group being represented by 28 patients. The study includes patients belonging to both sexes, with both urban and rural backgrounds, and ages between 18 and 82 years, who underwent surgery. In carrying out the paper, a series of correlations were followed, the most important being the following:

- Personal data of patients (sex, environment of origin)
- The type of trauma suffered and the anatomical location;
- NPWT and open fractures;
- NPWT and arthroplasties complicated by infections;
- The degree of complications and the duration of treatment

RESULTS

Regarding the distribution of cases by sex and environment of origin, we can see a predominance of male patients(16 out of a total of 28) from urban areas. In this category are included both cases of implants / prostheses complicated with infections, as well as patients who suffered soft tissue injuries with a lack of substance or open fractures.

Table I. Distribution of cases according to the type of lesions

	Cases	Value
Dehiscent wounds	5	17,8%
Open fractures	6	21,4%
Infected wounds	7	25%

	Cases	Value
Post-arthroplasty hip infection	3	10,7%
Post-knee arthroplasty infection	3	10,7%
Wounds with a great lack of substance	4	14,2%

With reference to the distribution of cases according to the type of lesions (fig. IV) for which NPWT was used, an approximately uniform distribution could be observed, most cases being cases complicated by infections or cases with an increased risk of complication by infections; we can observe a slight predominance of NPWT use in postoperatively infected wounds and in the case of open fractures. Arthroplasties complicated by infections can also be classified as infected wounds.

Table II. Correlation between the type of lesion and the anatomical location

Location	Arm	Forearm	Hip	Thigh	Knee	Calf	Ankle	Total
Wound with great lack of substance	0	1	0	0	0	3	1	5
Open fractures	1	0	0	2	0	2	2	7
Infected wounds	1	0	1	2	1	0	1	6
Dehiscent wounds	0	0	1	1	2	0	0	4
Post-arthroplasty hip infection	0	0	3	0	0	0	0	3
Post-knee arthroplasty infection	0	0	0	0	3	0	0	3
Total	2	1	5	5	6	4	4	28

Table III. Distribution of horses for which NPWT was used and location

Location	Number of cases	Value
Upper limb	3	10,7 %
Lower limb	25	89,2 %

In terms of anatomical location, most cases for which NPWT was used were located in the lower limb, totaling 25 cases out of the 28 studied. Most cases were located in the knee (6 cases) and hip (5 cases) and included both arthroplasties complicated by infections, dehiscent wounds or infected wounds. At the level of the leg, the wounds with a great lack of substance predominated, and at the level of the ankle, most of the cases were of open fractures for which NPWT was used. It should be noted that the indication for the use of secondary suture or grafting as a complementary technique in the management of these lesions is based on several factors. For the management of lesions with large defects of substance, with the exposure of bone or ligaments or for the management of open fractures, grafting was mainly used as a complementary technique. Also for some cases of complicated arthroplasties due to infection, grafting was used because after extensive debridement, the resulting defect was too large to allow secondary suturing, especially at the knee where the skin tensions are very high. In most infected wounds and for all cases of dehiscent wounds, the secondary suture was used as the final procedure in the management of these patients.

Table IV. The relationship between the complementary procedure used and the severity of the injuries

		Secondary suture	Skin graft/Flap	Total
Type of Wound	Wounds with a great lack of substance	0	3	3
	Open fractures	2	4	6
	Infected wounds	6	1	7

		Secondary suture	Skin graft/Flap	Total
	Post-arthroplasty hip infection	2	1	3
	Post-knee atroplasty infection	2	2	4
	Dehiscent wounds	5	0	5
Total		17	11	28

The duration of use of NPWT in orthopedic practice in the present study was between 7 and 28 days, depending on the type of lesions and their size. The lesions for which NPWT was used for a longer period were arthroplasties complicated by infections and lesions with residual infections. For the rest of the cases of open fractures, dehiscent wounds or wounds with severe lack of substance, the duration of use of NPWT did not exceed 14 days. The average duration of use of NPWT in orthopedic practice was about 17 days.

Table V. Duration of use of NPWT depending on the type of lesions

		Duration of NPWT use					
		7 days	10 days	14 days	21 days	28 days	Total
Type of Wound	Wounds with a great lack of substance	2	1	1	0	0	4
	Open fractures	0	4	2	0	0	6
	Infected wounds	0	1	0	4	2	7
	Post-arthroplasty hip infection	0	0	0	1	2	3
	Post-knee atroplasty infection	0	0	0	1	3	4
	Dehiscent wounds	2	1	1	0	0	4
Total		4	5	4	5	6	28

Frequency of complications

Of the 28 cases included in this study and for the management of which NPWT was used, only 3 cases presented complications, the complications being of a septic nature. The 3 complicated cases were represented by a case with open fracture and a case with a wound with a lack of large substance, in both cases being present bone exposure and massive contamination. Following antibiotic therapy and prolonged use of NPWT, the evolution of the 3 cases was favorable.

Table VI. Frequency of complications following the use of NPWT

	Cases	Value
Without complications	25	89,2%
Septic complication	3	10,8%
Total	28	100%

Table VII. Distribution of complications depending on the type of lesions

		Without complications	Septic complication	
Tipul plăgii	Wounds with a great lack of substance	2	1	3
	Open fractures	4	1	5
	Infected wounds	6	0	6
	Post-arthroplasty hip infection	3	0	3
	Post-knee atroplasty infection	3	0	3
	Dehiscent wounds	4	0	4
Total		22	2	24

DISCUSSIONS

Limb wounds are usually associated with significant loss of soft tissue, exposure of the implant, bones, tendons and are difficult to treat, the risk of complications being high. Favoring local vascularization and rapid formation of granulation tissue are essential for the uncomplicated healing of these lesions. In fact, lesions in the lower limbs often require the use of skin grafts or flaps to cover defects, and in order to use these complementary methods of treatment we need the presence of granulation tissue, the use of NPWT speeding up this process.

Use of NPWT in infected wounds - According to the study by Stannard et al. [8] the risk of complications from infections in patients treated with NPWT is 5 times lower than the risk of infection in patients who have used conventional dressings. Sinha et al [1] also reported a significant reduction in positive bacterial cultures in patients receiving NPWT. Although these results are encouraging, there are divided opinions regarding the ability of NPWT to reduce the bacterial load on the lesions.

Use of NPWT in open fractures - In the case of open fractures, there is a very high risk of complications due to infections or of not consolidating, which causes a high morbidity. According to the literature, in the case of open tibial fractures there is an 8-12% risk of complications from infections.

Blum et al. [2] conducted a retrospective study comprising 229 cases of open tibial fractures, of which 72% of cases benefited from NPWT and 28% of cases of conventional dressings. They found a significantly lower infection rate in the NPWT group (8.4%) compared to the conventional bandage group (20.6%). They also noted that NPWT reduces the risk of deep infections by 80%.

In the present paper we recorded the use of NPWT in 6 cases of open fractures, of which only 2 cases had complications. In a study published by Stannard et al. [8] performed on 62 patients with open fractures and who aimed to compare the results after management with NPWT (37 patients) or with classic sterile dressings (25 patients), the following results were obtained: - incidence rate of complications in patients receiving conventional dressings was much higher than the rate of complications in patients receiving NPWT; - in the group of patients who benefited from conventional dressings, 2 cases of acute infections and 5 cases of infections with delayed evolution were reported; - in the group of patients who benefited from NPWT, 2 cases of infections with delayed evolution and no case of acute infections were reported.

Use of NPWT in complicated arthroplasties - Intraarticular septic foci continue to be a serious problem in orthopedic surgery and are extremely difficult to treat due to the poor penetration of antibiotics at this level.

Kelm et al. [5] reported the eradication of infections that complicated arthroplasties in 9 of the 10 cases included in the published study. Also, Kirr et al. [4] reported favorable results in 3 cases of infected hip arthroplasties benefiting from NPWT. In another study published by Ludeman et al. [3] involving 17 patients who received NPWT, persistence of infection or reinfection was reported in 8 of the 17 cases (47%).

Clinical Case

Use of NPWT 14 days associated with soft tissue coating as a complementary technique; 23-year-old patient; the victim of a road accident; open fracture Gustilo Anderson III A left ankle; in the following images (fig. X, XI, XII, XIII) the evolution of the case is presented.

Figure 1. The appearance of the lesion after the physico-chemical wound therapy

Figure 2. Application of NPWT

Figure 3. Appearance at 21 days of NPWT use

Figure 4. The final result after complementary surgical treatment

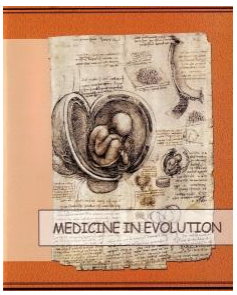
CONCLUSIONS

- Wound healing is a complex process, affected by both local and systemic factors, related to the general condition of the patient, so the results after using NPWT vary from one patient to another;
- To identify the independent effect of NPWT, a large number of cases are needed to reduce the effect of patient-dependent factors;
- There are a small number of studies proving the superiority of NPWT in terms of efficacy compared to the use of conventional dressings;
- The main mechanisms by which NPWT acts are: -macrodeformation; -microdeformarea; -removal of fluids (drainage); -modifying the healing environment;
- Side effects of NPWT use are: -hemostasis; - modulation of inflammation; - stimulating cell division, differentiation and migration; - stimulation of angiogenesis; -stimulating the formation of granulation tissue; - changes in microbiosis
- The general interpretation of the various studies is that NPWT can promote healing by modulating inflammation and cell migration, while inhibiting epidermal development and maturation;
- Following the use of NPWT, it was observed the reduction of the wound dimensions and the acceleration of the granulation tissue formation, thus being possible to cover with grafts or flaps the defects as early as possible

REFERENCES

1. Hinman CD, Maibach H. Effect of air exposure and occlusion on experimental human skin wounds. Nature. 1963;200:377–378.
2. Ingber DE, Levin M. What lies at the interface of regenerative medicine and developmental biology? Development. 2007;134(14):2541–254.
3. Kairinos N, Solomons M, Hudson DA. Negative pressure wound therapy I: the paradox of negativ-pressure wound therapy. Plast Reconstr Surg. 2008;123:589–598.
4. Lancerotto L, Bayer LR, Orgill DP. Mechanisms of action of microdeformational wound therapy. Semin Cell Dev Biol. 2012;23(9):987–992.
5. Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. Ann Plast Surg. 1997;38(6):553–562.
6. Orgill DP, Bayer L, Neuwalder J, Felter R. Microdeformational Wound Therapy—A New Era in Wound Healing. Business Briefing: Global Surgery—Future Directions. 2005:1-3.
7. Schaum KD. A new Medicare part B wound care policy. Adv Skin Wound Care. 2001;14(5):238–240.
8. Winter GD. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. Nature. 1962;193:293–294.

Review
Is air pollution involved in the epidemiological manifestation of viral infections with respiratory tropism?



Ştefan I.M.[1], Bădiţoiu L.M.[2], Popovici E.D.[2], Pavel R.[2], Anghel M.[2], Anghel A.[3]

[1]*Resident doctor epidemiology, Emergency "Pius Brînzeu" Hospital, Timişoara*
[2]*Department of Epidemiology, "Victor Babes" University of Medicine and Pharmacy, Timişoara, Romania*
[3]*Department of Biochemistry, "Victor Babes" University of Medicine and Pharmacy, Timişoara, Romania*

Correspondence to:
Name: Stefan Irina-Maria
Address: Timişoara, Cicio Pop street, No. 2, 2-nd floor
Phone: +40 744476380
E-mail address: popescuirina5@gmail.com

Abstract

The article is a synthesis of current information from the literature regarding the link between chemical atmospheric pollutants and their impact on respiratory tract infections. The viruses most frequently involved in respiratory pathology are represented by: respiratory syncytial virus, adenoviruses, influenza virus, parainfluenza, rhino - and enteroviruses, after the SARS epidemic, MERS and COVID-19 pandemic, coronaviruses are etiological factors with significant fatality. The most common air pollutants are: PM10, PM2.5, NO, NO_2, SO_2, and soil level O_3. Increased levels of these pollutants influence the incidence and fatality of viral respiratory infections. Unlike PM10, which remains confined to the upper respiratory tract, PM2.5 penetrates deep into the respiratory tree, causing local inflammatory reactions and impaired defense mechanisms. Also, the increase in susceptibility to respiratory infections is correlated with long-term exposure to high concentrations of: NO, NO_2, SO_2 and O_3.

Keywords: atmospheric pollution, COVID-19, respiratory infection

INTRODUCTION

Air, the main component of the atmosphere, the gaseous shell that surrounds the Earth, is made of nitrogen (79.2%), oxygen (20.8%) and in an insignificant proportion, carbon dioxide, ammonia and water vapor. [1] It is considered a polluting factor, any substance in the air in excess, which could cause environmental disturbance, human discomfort and health disorders. Physical, chemical and biological pollution are known. The main air pollutants are: nitrogen oxides (nitrogen monoxide NO and nitrogen dioxide NO_2), suspended particles (as main representatives PM10, PM2.5), sulfur dioxide (SO_2) and ground-level ozone. (O_3).

SUSPENSED PARTICLES - represents a complex mixture of solid and liquid particles, of various sizes, the most important being PM 10 (with a diameter between 2.5 and 10 μg / m^3), respectively PM 2.5 (with a diameter less than 2.5 /μg / m^3). These particles come from natural sources (volcanic eruptions, rock erosion, soil particle suspension, sandstorms, aerosolization of pollen or sea waves) or from anthropogenic sources (road traffic, both by burning fossil fuels and by friction produced at the level of tires, agricultural and industrial activity - especially in the chemical and extractive fields, waste treatment). Larger diameter particles tend to remain in the nasal mucosa, while smaller ones slightly exceed this filter and reach the respiratory shaft, causing changes in respiratory cilia motility, inflammatory reactions and exacerbation of chronic lung pathology. In order to prevent harmful effects on human health and / or the environment, reference intervals are established, with internationally accepted values. Thus, according to law no. 104/2011, in force in Romania, the daily limit value for PM 10 is 50 μg / m^3, the annual limit value 40 μg / m^3, while the limit value established as a target until January 1, 2020, is 20 μg / m^3 . For PM 2.5, the annual limit value is 25 μg / m^3 and the limit value set as a target until January 1, 2020, is 20 μg / m^3. [1, 2, 3, 4, 5]

OZONE is a stifling smell gas, very reactive, and oxidizing. If at atmospheric level 15-40 Km above earth's shell, offers protection against UV, ground level ozone represents a pollution factor. It is produced by chemical reactions between the sun's rays and organic gases, nitrogen oxides, emissions from power plants, refineries, chemical plants or road traffic. It has an irritating effect on the nasopharyngeal mucosa, produces pulmonary pathological changes even after the disappearance of acute symptoms, so it is considered to be a risk factor for asthma and a worsening factor for chronic lung disease. According to the Romanian legislation, the threshold alert is 240 μg / m^3 for a period of 3h consecutive, the information threshold at 180 μg / m^3, for a period of 1h, and the target value is considered 120 μg / m^3. [1, 2, 3, 4, 5]

NITROGEN OXIDES - The main nitrogen oxide present in the air is represented by nitrogen dioxide (NO2), with a toxicity 4 times higher versus nitrogen monoxide (NO). The main source comes from the burning of fossil fuels, from industrial activity, from electricity production or from engine emissions. They are responsible for the formation of smog, reduced visibility in urban areas, the greenhouse effect and the appearance of acid rain. Increases the incidence of chronic respiratory pathology (asthma, chronic bronchitis), increases the number of hospitalizations and the death rate from lung pathology. The limit values for NO_2 are set at 200 μg / m^3 for the hourly limit, 40 μg / m^3 for the annual limit and 30 μg / m^3 - the critical level for vegetation protection. The alert threshold value was set at 400 μg / m^3, measured for 3 consecutive hours, at representative points, on an area of at least 100 km^2 or for an entire area. [1, 2, 3, 4, 5]

SULFUR DIOXIDE - is a colorless gas, with a pungent, bitter, non-flammable odor, water soluble, resulting from the burning of sulfur. It comes from natural sources - phytoplankton, bacterial fermentation in swampy areas, volcanic eruptions by oxidation of sulfur-containing gases, but also from burning fossil fuels in electrical installations, the activity of oil refineries, cement factories or materials processing industry. Short-term

exposure to high concentrations of sulfur dioxide causes severe respiratory distress, and long-term exposure predisposes to respiratory tract infections. It especially affects extreme ages and aggravates pre-existing lung diseases. It also potentiates the harmful effects of ozone. The hourly limit value for the protection of human health is set at 350 μg / m^3, the daily limit value at 125 μg / m^3 and the critical level for the protection of vegetation at 20 μg / m^3. The alert threshold, of 500 μg / m^3, is determined by measuring for 3 consecutive hours in representative points, on an area of at least 100 km^2 or on an entire area. [1,2,4,5]

Aim and objectives

This article aims to synthesize the knowledge from the specialized literature regarding the link between atmospheric chemical pollutants and their impact on acute viral respiratory infections.

Acute respiratory tract infections are a major cause of morbidity and mortality today, among all age groups and in all geographical regions. Pneumonia, one of the lower respiratory tract infections, affects 450 million people annually, with 3 million deaths worldwide in 2016, 2.56 million deaths in 2017, making it the 4th leading cause of death in world and the pathology transmissible with the highest fatality (for 2016). [6,7] Its incidence is 5 times higher in developing countries versus developed ones and from an etiological point of view, viral pneumonia occupies a main place , with approximately 200 million cases annually.

The relationship between air pollution and respiratory pathology was investigated before the current pandemic of COVID 19, in an attempt to quantify the correlation between pollution and morbidity / mortality from respiratory infections.

Since 2003**, Cui.Y. et** al studied the relationship between Air Pollution Index (API) - derived from CO, NO_2, O_3, PM, SO_2 concentrations and fatality from SARS, highlighting that the population in areas with moderately high Air Pollution Index has a risk of death from SARS with 84 % higher versus those living in regions with low API [RR = 1.84; 95% CI: 1.41-2.40], and the population in areas with high API has a double risk of mortality from SARS versus those living in areas with low API (RR = 2.18; 95% CI: 1.31 -3.65). [9]

In a study conducted in 6 cities in Italy, in the period 2001-2005**, Faustini. A et al**. demonstrated that a 10 μg/m^3 increase in PM 10 values leads to an increase in hospitalizations for respiratory diseases by 0.59% [90% CI: 0.10-1.08%] and outpatient mortality by 3.95% [90% CI: 1.53-6.43%], while a 10 μg/m^3 increase in NO_2 values determined an increase of 1.19% [90% CI: 0.23-2.15 %] of hospitalization following respiratory pathology. [10] Similarly, in a 2016 study by **Carugo. M et al**, in the Lombardy region, Italy, known for its high degree of pollution, showed that an increase of 10 μg / m^3 in PM 10 and NO_2 values corresponds to an additional mortality of 1.64% [90% CI: 0.56 -2.72] for PM 10 and 0.46%, respectively [IC 90%: - 1.23-2.18] for NO_2. [11]

Reilly.P.J in 2018 also identifies a correlation between elevated levels of air pollutants and acute respiratory distress syndrome (ARDS), when exposure to air pollutants occurs over a longer period of time. [12]

In a study conducted between 2010-2017 on a cohort of 19,093 patients at the Hospital in Antwerp, the province with the highest concentrations of PM 2.5 in Belgium, **Annik De Weerdt et al** reported an association between the duration of artificial ventilation and exposure to air pollutants, 10 days before admission. [13]

After the previous flu pandemic, a study conducted in Asia in 2010 by the team led by **P.S. Chen** identified higher concentrations of the 2009 AH_1N_1 virus in the air during sandstorms, when concentrations of air pollutants - PM2.5, PM10 and CO, were higher than on clear days. [14]

Wei Su and colleagues reported in 2019 in Jinan, China, a correlation between increased concentrations of air pollutants (PM2.5, PM10, SO_2, CO) and increased risk of developing influenza-like illness (ILI). Thus, a 10 μg/m^3 increase in PM 2.5 concentration was

associated with an increase in the relative risk of developing ILI of 1.01 [95% CI: 1.01-1.02], for an increase of 10 μg/m^3 of PM 10 concentration, RR increased by 1.001 [95% CI: 1.00-1.01] and for a similar increase in SO_2 concentration, increased by 1.00 [95% CI: 1,0003-1.0012]. The increase in O_3 values was negatively correlated with ILI [RR 0.99; 95% CI: 0.98-0.99]. [15] Also, in 21 cities in China, in the period 2013-2014, the 10 PM μg / m3 increase in PM2.5 was associated with an increase of 1,010 [95% CI: 1,003-1,018] per day and 1,006 [95% CI: 1,000.11.012] at three days, of the number of cases of measles, determined by a morbilivirus also transmitted by air. [16]

The positive correlation with the high concentrations of PM10, PM2.5, NO_2, NO, respectively the negative correlation with O_3 were also relevant in the study of **Wang.L et al,** from 2019. [17] In the same year, **C. Liu et al** , analyzed the degree of air pollution and mortality recorded in 652 cities in various regions, predominantly in the northern hemisphere. A significant, positive association was observed between PM10 and PM2.5 concentrations and overall mortality. An increase of 10 μg / m3 of the PM10 / PM2.5 concentration was correlated with an increase of 0.47% [95% CI: 0.35-0.58], respectively of 0.74% [95% CI: 0.53-0.95] of daily mortality attributable to respiratory pathology. [18]

Another category of studies focuses on the etiopathogenic mechanisms through which air pollutants can intervene in respiratory pathology**. Wei Su et al** show that the presence of suspended particles facilitates and increases the ability of the virus to attach to respiratory epithelial cells, and to penetrate deep into the respiratory tree. It also decreases the ability of macrophages to phagocytose the virus, increasing the susceptibility to infection. [15]

In an experimental study**, Jeong.S** and his team inoculated intratracheally with the pollutant factor PM 2.5 to a group of mice, and found a significant increase in the number of macrophages and neutrophils in lung tissue at 24 hours after exposure. Persistence of leukocyte infiltrate in the lungs and liver has persisted for at least two days after exposure. [19] Jie Yang et al report a significant increase in IL4, TGF-β1 and TNF-α (tissue and serological levels), an increased number of inflammatory cells, intracellular edema and significant tissue damage in a group of mice exposed to high concentrations of air pollutants (PM2.5, CO, NOx) versus the control group in the laboratory. [20]

In a recent mini-review, from 2020**, Cao Y et al** describes the harmful effects of pollutants on hair epithelial cells, changes in their structure, which leads to reduced mucociliary clearance, a factor determined in the occurrence of respiratory pathology. It also reports the correlation between prolonged exposure to high concentrations of NO_2 and increased susceptibility to respiratory infections, with the risk of developing permanent lung damage. [2]

The emergence of the new SARS-COV2 coronavirus had as its starting point the city of Wuhan, Hubei Province, China, from where it spread very quickly around the world, with the onset of the COVID-19 pandemic in 2020. The speed of spread, the existence of seemingly random outbreaks, raised the issue of identifying the factors that led to this evolution, as well as the role of air pollution. Multiple recent studies have shown that pollution influences the transmission of SARS-COV2 coronavirus, but also sensitizes the respiratory tree by acting on protective mechanisms. The investigation of the pollution level of the regions with the highest number of cases, respectively the involvement of geographical factors were discussed especially in the Asian and Italian articles, in an attempt to identify the factors that led to the fulminant evolution of COVID 19.

Z.Yongjian et al (China, 2020) identify a positive correlation between the increase of O_3, CO, NO_2, PM10 / PM2.5 concentrations and the number of COVID-19 cases, simultaneously with a negative correlation between the number of cases and the SO_2 concentration, possible due to the virulicidal effect of SO_2. Thus, for a 10 μg / m^3 increase in PM2.5 values, an increase of 2.24% [95% CI: 1.02-3.46] was reported in the number of confirmed daily cases, for PM10 an increase of 1.76% [95% CI: 0.89-2.63], for O_3 4.76% [95%

CI: 1.99-7.52], and for NO_2 6.94% [95% CI: 2.38-11 51]. Instead, an increase in SO_2 values by 10 μg / m^3 determined a decrease in the number of daily illnesses by 7.79% [95% CI: -14.57-1.01]. [21]

Xiao Wu Ms et al (USA, 2020) investigating 98% of the population of 3000 counties, reports that an increase of 1 μg / m^3 in the long-term mean concentration of PM2.5 is associated with an increase in the mortality rate through COVID-19 with 15% [95% Cl: 5-25]. [22]

Travaglio. M et al (UK, 2020) in a study on the correlation of NO_2 and NO concentrations with the fatality rate of SARS COV2 cases, identified a correlation between increased NO_2 and NO concentration and the severity of disease forms in England, as well as a inversely proportional relationship between O_3 concentration and severity of COVID-19 forms, respectively with the number of deaths. [2. 3]

Conticini. E et al (Italy, 2020), in a study that hypothesizes that exposure to high concentrations of polluted air for a longer period, leads to a weakening of the defenses respiratory mechanisms, reports a correlation on the value of Air Quality Index (which measures the degree of pollution) and the number of deaths through Covid-19, in the regions of Lombardy and Emilia Romagna. After the onset of the pandemic, there was a decrease in pollution levels, but the number of cases did not decrease in direct proportion to them, which shows that the effects of pollution on the respiratory system are long-lasting and cannot be reversed in the short term. [24]

Fattorini. D et al (Italy, 2020) evaluated the distribution of air pollutants (PM2.5, PM10, NO_2, O_3) for 4 years and highlighted the high level of pollution in northern Italy, as well as the correlation between the degree of pollution and the number of cases of COVID-19 in 71 provinces in Italy. [25].

Ogen Y et al (Italy, 2020) highlighted two European areas that showed significantly increased concentrations of NO_2 between January and February 2020: northern Italy and the region of Madrid, Spain. Also these 2 regions presented the highest number of cases: Lombardy 2168 cases, Emilia Romagna 531 cases, Piedmont 175 cases, Veneto 115 cases, Madrid 498 cases, at the date of the study. Fatality was 12% for the regions of Lombardy and Emilia Romagna, compared to 4.5% in the rest of Italy. By March 2020, a total of 4443 deaths were caused by SARS COV2 in these two countries, and a percentage of 83% (3701 cases) of these deaths were recorded in areas where the NO_2 concentration exceeded 100 μmol / m2 . Both regions are surrounded by mountain ranges, and low airflows cause a concentration of NO_2 close to the earth's surface. Topographic conditions together with atmospheric ones prevent the dispersion of air and pollutants, which can cause respiratory diseases. [26]

Setti.L et al (Italy, 2020) after an analysis of 34 PM10 samples taken from the industrial region of the province of Bergamo, showed that the viral RNA of SARS-COV2 may exist associated with PM10 in the external environment, and in conditions of atmospheric stability and high concentrations of PM10, can lead to the appearance of clusters. [27]

CONCLUSIONS

In the current pandemic context, it becomes imperative to identify any risk factor and try to annihilate it, in order to control and subsequently stop this exacerbated manifestation of the epidemiological process. The most common air pollutants are PM10, PM 2.5, NO, NO_2, SO_2, and soil level O_3. Increasing the values of these pollutants, even by a few units, has a decisive influence on the incidence and fatality of viral respiratory infections.

Suspended particles with a large diameter, PM 10, remain largely confined to the nose and throat, while particles with a smaller diameter of PM 2.5 penetrate deep into the respiratory tract, affect mucociliary clearance, allowing agents to penetrate. deep tracheobronchial infections and increases the ability of the virus to attach. It also causes local inflammatory reactions, with a decrease in the body's natural protection capacity against

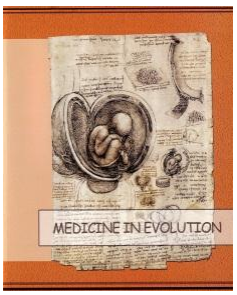
inhaled infectious agents. The presence of NO and NO_2, SO_2, O_3 in high concentrations and long-term exposure are correlated with increased susceptibility to respiratory infections.

REFERENCES

1. Munteanu, Mioara Dumitraşcu, Romeo-Alexandru Iliuță. Ecologie şi protecția calității mediului. Editura Balneară, Bucureşti, 2011, 16-18 Available from: http://bioclima.ro/ ECO.pdf
2. Yu Cao, Miao Chen, Dan Dong, et al. Environmental pollutants damage airway epithelial cell cilia: Implications for the prevention of obstructive lung diseases. Thorac cancer 2020; 11: 505-510.
3. Junfeng Zhang, Yongjie Wei, Zhangfu Fang. Ozone Pollution: A Major Health Hazard Worldwide. Front Immunol 2019; 10: 2518.
4. Gerard Hoek, Ranjini M Krishnan, Rob Beelen, et al. Long-term air pollution exposure and cardio-respiratory mortality: a review. J. Environ. Health, 2013, 12: 43.
5. ARPM Galati, Raport privind starea mediului, 2011;17-61. Available from: http://www.anpm.ro/documents/19877/2250777/CAP+2+CALITATEA+AERULUI.pdf/a486481c-b771-4259-acda-678dbe7b8ea8
6. Bernadeta Dadonaite, Max Roser, Pneumonia. Our World In Data, 2011, Available from: https://ourworldindata.org/pneumonia.
7. *** Epidemiology of pneumonia, 2020. Available from: https:// en.wikipedia.org/wiki/ Epidemiology_of_pneumonia.
8. Monika Jevsnik, Tina Ursic, Nina Zigon, et al. Coronavirus infections in hospitalized pediatric patients with acute respiratory tract desease. BMC Infect. Dis; 2012; 12:365.
9. Yan Cui, Zuo-Feng Zhang, John Froines, et al. Air pollution and case fatality of SARS in the People's Republic of China: an ecolgic study. J. Environ. Health; 2003, Available from: https://doi.org/10.1186/1476-069X-2-15.
10. Annunziata Faustini, Paola Colasis, Gilberto Berti. Air pollution and multiple acute respiratory outcomes. Eur Respir J, 2013; 42:304-313.
11. Michele Carugo, Dario Consonni, Giorgia Randi, et al. Air pollution exposure, cause specific deaths and hospitalizations in a highly polluted Italian region. Environ.Res; 2016, 147: 415-424.
12. John. P. Reilly, Zhiguo. Zhao, Michael G.S. Shashaty, et al. Low to Moderate Air Pollutant Exposure and Acute Respiratory Distress Syndrome after Severe Trauma. Am.J. Respir Crit Vare Med; 2019, 199: 62-70.
13. Annick De Weerdt, Bram G. Janssen, Bianca Cox et al. Pre-admission air pollution exposure prolongs the duration of ventilation in intensive care patients. Intensive Care Med; 2020,.Available from: https://doi.org/10.1007/s00134-020-05999-3.
14. Pei-Shih-Chen, Feng Ta Tsai, Chien Kun Lin, et al. Ambient Influenza and Avian Influenza Virus during Dust Storm Days and Background Days. Environ. Health Perspect.; 2010, 118(9): 1211-1216.
15. Wei Su, Xiuguo Wu, Xingyi Geng, et al. The short term effects of air pollutants on influenza-like illness in Jinan China. BMC Public Health; 2019; 19: 1319.
16. Gongbo Chen, Wenyi Zhang, Shanshan Li, et al. Is Short-Term Exposure to Ambient Fine Particles Associated With Measles Incidence in China? A Multy –City Study. Environ. 2017, 156: 306-311.
17. Lijun Wang, Ju Wang, Xiaodong Tan, et al. Analysis of Nox Pollution Characteristics in the Atmospheric Environment in Changchun City. Atmosphere; 2019; 11:30.
18. C. Liu, R. Chen, F. Sera, et al. Ambient Particulate Air Pollution and Daily Mortality in 652 Cities. N. Engl. J. Med; 2019, 381 (8): 705-715.
19. Soi Jeong, Sang A Park, Inwon Park, et al. PM2.5 Exposure in the Respiratory System Induces Distinct Inflammatory Signaling in the Lung and the Liver of Mice. J. Immunol. Res.; 2019, Available from: https://doi.org/10.1155/2019/3486841.
20. Jie Yang, Yi Chen, Zhi Yu, et al. The influence of PM 2.5 on lung injury and citokines in mice. Exp. Ther. Med.; 2019, 18: 2503-2511.

21. Zhu Yongjian, Xie Jingu, Huang Fengming, et al. Association between short-term exposure to air pollution and COVID-19 infection: Evidence from China. Sci. Total Environ; 2020, 727, .Avaliabe from:https://doi.org/10.1016/j.scitotenv.2020.138704.
22. Xiao Wu MS, Rachel C, Nethery PhD, et al. Exposure to air pollution and COVID-19 mortality in the United States. medRxiv; 2020, Available from: DOI: 10.1101/2020.04.05.20054502.
23. Marco Travaglio, Rebecka Popovic, Yizhou Yu, et al. Links between air pollution and COVID-19 in England. medRxiv; 2020,. Available from: https:// doi.org/10.1101/2020.04.16.20067405.
24. Eduardo Conticini, Bruno Frediani, Dario Cro. Can atmospheric pollution be considered a co factor in extremely high level of SARS-CoV-2 lethality in Northen Italy?. Environ. Pollut; 2020, 261, Avaliable from: https://doi.org/10.1016/j.envpol.2020.114465.
25. Daniele Fattorini, Francesco Regoli. Role of the atmospheric pollution in the Covid-19 outbreak risk in Italy. medRxiv; 2020, Available from: https://doi.org/10.1101/2020.04.23.20076455.
26. Yaron Ogen. Assesing nitrogen dioxide (NO2) levels as a contributing factor to coronavirus (COVID-19) fatality. Sci. Total Environ; 2020, 726, Available from: www.doi:10.1016/ j.scitotenv. 2020.138605.
27. Leonardo Setti, Fabrizio Passarini, Gianluigi De Gennaro, et al. SARS-Cov-2 RNA Found on Particulate Metter of Bergamo in Northen Italy: Fist Preliminary Evidence. medRxiv; 2020, Available from: https://doi.org/10.1101/2020.04.15.20065995.

A newborn case of double-male syndrome (48, XXYY)



Pop L.E.[1], Vasile G.[2], Miclea D.[3], Pantelemon C.[4]

[1]*Pediatric Cardiology Department, Emergency Institute for Cardiovascular Diseases and Heart Transplant, Targu Mures*
[2]*Neonatology Department, Emergency County Hospital, Cluj-Napoca*
[3]*Genetics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca*
[4]*Neurosciences Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca*

Correspondence to:
Name: Pop Lacramioara-Eliza
Address: 50 Gheorghe Marinescu Street, postal code 540136, Targu Mures, Romania
Phone: +40 744181011
E-mail address: lacri_lizy@yahoo.com

Abstract

Introduction: The 48, XXYY syndrome represents a chromosomal aneuploidy which consists in the presence of an extra X and Y chromosome in males. It has an incidence of 1/18 000 to 1/40000 male births. For a long time 48, XXYY syndrome was considered as a variant of Klinefelter syndrome, but nowadays it represents a distinct disorder due to associated comorbidities: mental retardation and psychiatric disorders.

Case presentation: We report the case of a newborn boy, who presented at physical examination craniaofacial dysmorphism consisting of hypertelorism, enlarged bitemporal diameter, flat occiput, downwardly and oblique displaced ears, short lingual frenulum and clinodactyly of the fifth digit. Transfontanelar ultrasound identified two left choroid plexus cysts of 0,4/0,4 cm. Abdominal and cardiac ultrasonography showed no abnormalities. The karyotype analysis revealed a chromosomal aneuploidy with 48, XXYY formula.

Conclusion: We reported a case of 48, XXYY syndrome diagnosed immediately after birth, a rare disorder with approximately 100 cases reported in literature to date and to the best of our knowledge, the first reported case in Romania.

Keywords: 48, XXYY syndrome; brain development; Klinefelter's syndrome; sex chromosomes aneuploidy

INTRODUCTION

The 48, XXYY syndrome represents a chromosomal aneuploidy which consists in the presence of an extra X and Y chromosome in males. It was first described by Muldal *et al* in 1960 as the double-male syndrome [1]. It has an incidence of 1/18 000 to 1/40000 male births [2]. The 48, XXYY aneuploidy is not inherited, it is a sporadic mutation with a very low risk of recurrence. For a long time 48, XXYY syndrome was considered as a variant of Klinefelter syndrome, but nowadays it represents a distinct disorder due to associated mental retardation and psychiatric disorders [3].

CASE REPORT

I. Anamnesis: We report the case of a newborn boy, second child in the family, born from non- consanguineous parents, both healthy, with no history of genetic problems in the extended families. There was no prenatal suspicion of the malformation on fetal ultrasonography. The pregnancy had a normal evolution, followed by an uneventful birth. He was delivered vaginally at term, in cephalic presentation, with an Apgar score of 10 and with a birth weight of 3500 g.

II. Clinical examination data: At physical examination he presented normal weight, length, cranial and thoracic circumference dimensions. Normal male genitalia were observed. The patient presented craniofacial dysmorphism (fig. 1) consisting of hypertelorism, enlarged bitemporal diameter, flat occiput, downwardly and oblique displaced ears, short lingual frenulum and clinodactyly of the fifth digit (fig. 2).

Figure 1. Craniofacial dysmorphism consisting of hypertelorism, enlarged bitemporal diameter, flat occiput, downwardly and oblique displaced ears

Figure 2. Clinodactyly of the fifth digit

III. Laboratory data: There were no pathological findings regarding laboratoy data.

IV. Additional paraclinical investigations: Transfontanelar ultrasound identified two left choroid plexus cysts of 0,4/0,4 cm and no other modifications - ventricular system with normal dimensions, brain mass without pathological changes, corpus callosum present, normal subarachnoid and interhemispheric space (fig. 3). Abdominal and cardiac ultrasonography showed no abnormalities. Because of the observed characteristics - craniofacial dysmorphism, bone and brain malformation, a genetic consult was requested, and the doctor suspected a genetic malformation. The karyotype analysis using lymphocytes from peripheral blood was performed. The result revealed a chromosomal aneuploidy with 48, XXYY formula.

V. Treatment and evolution: No treatment was needed and the newborn had a normal evolution in the neonatal period.

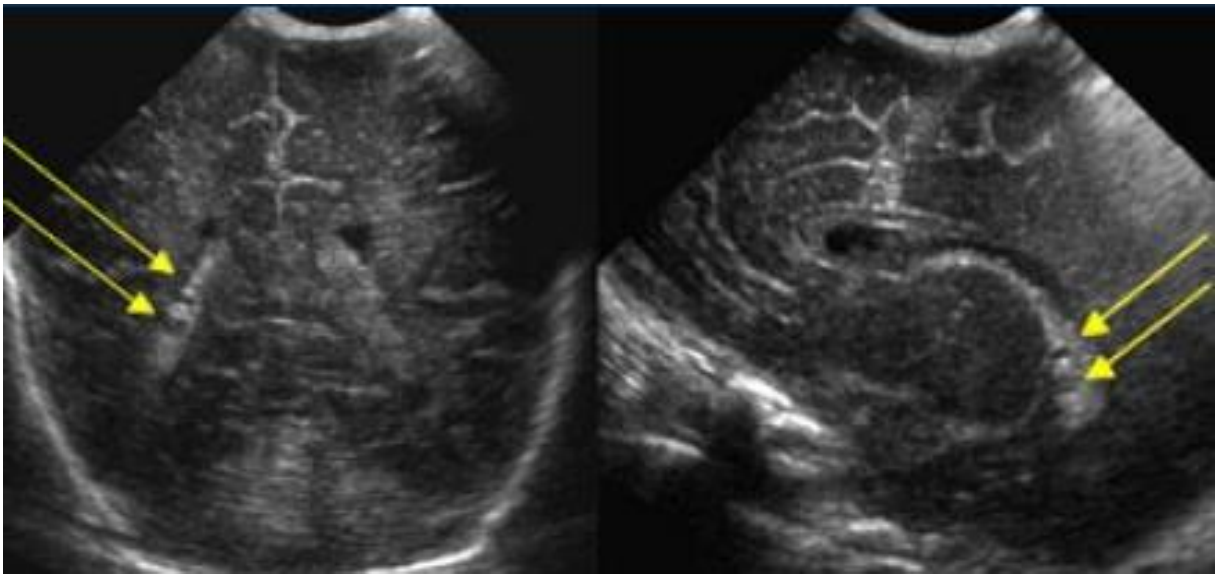


Figure 3. Transfontanelar ultrasound showing two left choroid plexus cysts

DISCUSSIONS

The aneuploidy is secondary to nondisjunction of chromosomes during mitosis of a normal egg or has parental origin - oocyte or spermatozoid with supernumerary X and Y chromosomes. Most of the published articles mentioned the parental origin of the triploid gamete (XYY) [4,5,6,7,8].

There are different types of aneuploidy described: sex chromosomes trisomy like Klinefelter syndrome (47, XXY) and Jacobs syndrome (47, XYY) [9], tetrasomy like 48, XXXY and 48, XXYY syndromes and pentasomy like 49, XXXXY syndrome [10]. They are considered variants of Klinefelter syndrome because of the presence of an extra X chromosome which associate testicular dysgenesis and phenotypical characteristics, but they differ from patients with Klinefelter syndrome due to psychological disorders associated. It is important to make this differentiation because there are a variety of behavioural, learning disabilities and emotional problems that are unique to patients with 48, XXYY syndrome that may be better addressed with more targeted therapies [3].

Patients with 48, XXYY karyotype formula present hypergonadotropic hypogonadism [10] which results in small testicles, delayed puberty with underdeveloped or absent secondary sexual characteristics, infertility, tall stature and abdominal adiposity [11]. Skeletal deformities are also described in these patients, the most common being clinodactyly of the fifth digit, as in our case. Other bone malformations reported were: radio-ulnar synostosis, osteoporosis, hyperostosis, pseudoepiphysis, cleft palate, hip dysplasia, clubfoot,

kyphoscoliosis [11]. Patients with 48, XXYY syndrome present congenital malformations such as heart defects, kidney dysplasia, inguinal hernia and cryptorchidism [10]. In our case, screening performed for these malformations revealed none. Other problems like seizure disorders, intention and postural tremor, strabismus, constipation, dental problems, asthma/reactive airway disease, food/environmental allergies and recurrent otitis are common in these patients [10]. Studies [12,10] reported that 48, XXYY syndrome associate endocrine disorders such as hypergonadotropic hypogonadism and acromegaloidism. Patients have a normal life expectancy, but they need regular medical follow-up for somatic and psychiatric disorders. Testosterone replacement therapy is recommended at puberty and fertility procedures in adulthood.

There are specific brain abnormalities in patients with 48, XXYY syndrome: grey and white matter volume changes, larger lateral ventricular volumes, colpocephaly and abnormalities of the corpus callosum [13]. A quantitative and qualitative brain anatomy study in adult males with 48, XXYY karyotype [14], demonstrated that brain volume of the patients is smaller compared with controls and frequently have anatomical anomalies like excess of grey and white matter in parietal lobes versus temporal and frontal lobes. Brain imaging performed in our case was comparable with that of normal new-borns regarding ventricular system and corpus callosum. There are no pediatric studies of brain anatomy in 48, XXYY syndrome, therefore we did not have comparison values for the grey and white matter volumes. Brain malformations associate psychiatric disorders such as autism, attention-deficit/hyperactivity disorder, anxiety, depression, aggressivity and mood disorders [14]. Neurological and psychiatric problems are the main feature which distinguish it from patients with Klinefelter syndrome who have a normal neurocognitive development.

This gonosomal aneuploidy is exceptionally discovered during childhood because there are no specific modifications, only mild craniofacial dysmorphism and some minor bone malformations, like in our case. A few studies reported prenatal diagnosis due to low maternal serum alpha-fetoprotein level or modifications on fetal ultrasound like hydramnios and bilateral club feet [15]. In our case, no modifications were observed on maternal level of hormones or on ultrasonography during pregnancy. Just one case confirmed in the neonatal period was reported [16] due to its phenotypical characteristics: ambiguous genitalia. Most cases are diagnosed after puberty because of the phenotypical similarities to the Klinefelter syndrome or due to variable developmental, cognitive, behavioural and physical abnormalities.

48, XXYY syndrome is considered a rare disorder because approximately 100 cases have been reported to date. To the best of our knowledge, this is the first reported case in Romania.

CONCLUSIONS

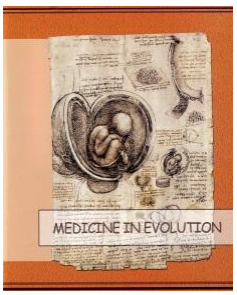
The reported case was a newborn with phenotypical and brain malformations which lead to an early diagnosis of a rare gonosomal aneuploidy: 48, XXYY syndrome.

REFERENCES

1. Muldal S, Ockey C, Thompson M, White L. 'Double male'– a new chromosome constitution in the Klinefelter syndrome. Eur J Endocrinol. 1962;39(2):183–203.
2. Liu DF, Zhao LM, Hong K, et al. Fertility achieved through in vitro fertilization in a male patient with 48,XXYY syndrome. Asian J Androl. 2018;20(2):208-209.
3. University of California - Davis - Health System. Characteristics, Treatment Options For XXYY Syndrome. ScienceDaily. 24 August 2008. Available from: https://www.sciencedaily.com/releases/2008/08/080822180744.htm

4. Rinaldi A, Archidiacono N, Rocchi M, Filippi G. Additional pedigree supporting the frequent origin of XXYY from consecutive meiotic non-disjunction in paternal gametogenesis. J. Med. Genet. 1979;16(3):225–226.
5. Balsera AM, Estévez MN, Beltrán EB, Sánchez-Giralt P, García LG, Moreno TH, García de Cáceres M, Carbonell Pérez JMC, Gómez EG, Rodríguez-López R. Distinct mechanism of formation of the 48, XXYY karyotype. Mol. Cytogenet. 2013;6(1):25.
6. Iitsuka Y, Bock A, Nguyen DD, Samango-Sprouse CA, Simpson JL, Bischoff FZ. Evidence of skewed X-chromosome inactivation in 47, XXY and 48, XXYY Klinefelter patients. Am. J. Med. Genet. 2001; 98:25–31.
7. Leal CA, Belmont JW, Nachtman R, Cantu JM, Medina C. Parental origin of the extra chromosomes in polysomy X. Hum. Genet. 1994;94(4):423–426.
8. Lorda-Sanchez I, Binkert F, Maechler M, Robinson WP, Schinzel AA. Reduced recombination and paternal age effect in Klinefelter syndrome. Hum. Genet. 1992;89(5):524–530.
9. Sood B, Clemente Fuentes RW. Jacobs (XYY) Syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557699/
10. Tartaglia N, Ayari N, Howell S, D'Epagnier C, Zeitler P. 48,XXYY, 48,XXXY and 49,XXXXY syndromes: not just variants of Klinefelter syndrome. Acta Paediatr. 2011;100(6):851-860.
11. Alhalabi MM, Alhalabi M. 48, XXYY syndrome presenting with long-term infertility and newly observed neck deformities: a case report. J Med Case Rep. 2020;14(1):58.
12. Zantour B, Sfar MH, Younes S, Alaya W, Kamoun M, Mkaouar E, et al. 48XXYY syndrome in an adult with type 2 diabetes mellitus, unilateral renal aplasia, and pigmentary retinitis. Case Rep Med. 2010 [2010 Aug 17]. Available from: https://europepmc.org/article/med/20827436#impact
13. Hanley AP, Blumenthal JD, Lee NR, et al. Brain and behavior in 48, XXYY syndrome. NeuroImage Clinical. 2015;8:133–139.
14. Tartaglia N., Davis S., Hench A., Nimishakavi S., Beauregard R., Reynolds A., Fenton L., Albrecht L., Ross J., Visootsak J., Hansen R., Hagerman R. A new look at XXYY syndrome: medical and psychological features. Am. J. Med. Genet. 2008;146A:1509–1522.
15. Kemeny S, Pebrel-Richard C, Gouas L, et al. Prenatal ultrasound diagnosis of a 48,XXYY syndrome. Morphologie. 2013;97(317):65-67.
16. Messina MF, Aversa T, Mamì C, et al. Ambiguous genitalia in a 48, XXYY newborn: a casual relationship or a coincidence?. J Pediatr Endocrinol Metab. 2013;26(9-10):921-923.

Decreasing the risk of complications in Covid-19 patients by prescribing substitute erythropoietin



Pirvu R.[1], Parlatescu I.[2], Ciobanu O.[3], Tiliscan C.[4], Moroti Constantinescu R.[4]

[1]*Dentist, Bucharest*
[2]*Oral Medicine Department, Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest;*
[3]*Endocrinology Department, University Emergency Hospital Elias, Bucharest;*
[4]*Infectious Diseases Department, Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest*

Correspondence to:
Name: Parlatescu Ioanina
Address: Bucharest, Str. Eforie, 4-6, Floor 3, Room 18
Phone: +40 724950104
E-mail address: ioanina.parlatescu@umfcd.ro

Abstract

Substitutes of erythropoietin (Epo) prescribed to the Covid-19 confirmed patients might increase blood oxygenation. A higher benefit will be in comorbidities patients because Epo may lower complications such as acute respiratory distress syndrome, myocardial dysfunction, and acute kidney injury. Exogenous erythropoietin downregulates inflammatory T cells and myeloid cells responses and reduces endothelial activation. Multiple myeloma patients treated with recombinant human erythropoietin (rHuEpo) for anemia have a prolonged survival rate. In inflammatory response, Epo level drops, hepcidin level increases and affects the iron metabolism by blocking iron inside macrophages. A low dose of rHuEpo administered three times a week is enough for healthy moderate athletes to maintain an increased hematocrit and maximum oxygen volume approximately 5% –10% above the initial levels. Under these circumstances adding rHuEpo may improve the blood oxygenation level from 5% to 20%, which is a great contribution in tissular oxygen levels and enhance humoral immunity.

Keywords: erythropoietin, organ protection, lower complications, Covid-19

INTRODUCTION

Erythropoietin (Epo) is the hormone responsible for the production and regulation of red blood cells [1]. Epo is produced especially in the kidney, but in many other tissues. And also it has been synthesized as recombinant human erythropoietin (rHuEpo) to treat different conditions. These are anemia caused by hematologic disorders or induced by renal failure and lately there were conducted different studies that prove its effectiveness in organ protection, including brain and heart [2]. The recent study rewarded at Nobel 2019 has discovered how cells sense and adapt to oxygen availability[3]. Our understanding of how oxygen levels affect cellular metabolism and physiological function increased, leading the way for new ways to fight anaemia, cancer and other diseases, including Covid-19 [2].

Aim and objectives

This article aims to show the importance of Epo as adjuvant to the Covid-19 treatment and prevention of its severe and critical forms, by increasing blood oxygenation, humoral immunity, and organ protection. This may lead to a lower rate of complications in most of the patients, also in individuals with comorbidities such as diabetes or heart diseases.

MATERIAL AND METHODS

1. Epidemiology and clinical features of critically ill Covid-19 patients

Most severe and critically ill patients are over 50 years old, and about 30% to 50% of Covid-19 patients have chronic comorbidities. At about 7 days (5-12 days) from the initial symptoms a "cytokine storm" and respiratory failure may appear. Also patients may develop hypoxemia without signs of respiratory distress. These patients could associate also, other organ dysfunction [2].

The number of persons infected with Sars-CoV exceeded fourteen million Covid-19 cases worldwide on July 18, 2020; the case fatality rate across 210 countries and territories was 5·2% [4]. WHO-China Joint Mission of 55 924 laboratory-confirmed cases in China were appreciated severe in 13.8% cases (dyspnoea, respiratory rate ≥30 breaths per min, oxygen saturation ≤93%, the partial pressure of arterial oxygen to fraction of inspired oxygen ratio 50% within 24–48 h) and 6.1% were classified as critical (respiratory failure, shock, and multiple organ dysfunction or failure)[5].

The Covid-19 critically ill patients, mainly elderly, with more comorbidities such as hypertension and diabetes, carry a higher risk than non-critically ill patients [2]. Fever, cough, fatigue, and dyspnoea are some of the nonspecific symptoms [6,2]. The time from the first symptoms to the development of pneumonia is approximately 5 days [2,6] and the medium time from symptom burst to severe hypoxemia and intensive care unit (ICU) admission is around 5–12 days [6]. Most of the patients have pathognomonic bilateral opacities on thoracic CT and chest radiography [6].

The most common complication in 60%–70% of patients admitted to the ICU is acute hypoxaemic respiratory failure sometimes complicated with severe hypercapnia, with acute respiratory distress syndrome (ARDS), followed by shock (in 30% cases), myocardial dysfunction (20%–30%), arrhythmia (44%), and acute kidney injury (10%–30%) [2,6]. Although, elderly patients might develop hypoxemia without respiratory distress.

2. Inflammatory response and Epo activity

Epo and cytokines

Erythropoiesis is controlled by different regulators, such as Interleukin 3, granulocyte-macrophage colony-stimulating factor, and also stem cell factors that play regulatory functions in the early stages of erythropoiesis. Erythropoietin is the main positive acting factor in the last steps of erythrocyte production in mammals. Epo is specific for erythroid

progenitor cells and has only a small effect on other cells. The target cells for Epo are the progenitors of erythroid (BFUe and CFUe), therefore Epo acts on these via Epo-specific surface receptors. Epo induces proliferation and differentiation of erythroid progenitors eventually leading to reticulocytes. During this process, certain conditions are required to allow this differentiation: progenitors must be enough, the bone marrow environment must be normal, and nutrients such as folic acid, vitamin B12, and especially iron must be available.

Elementary iron is an absolute necessity for adequate hemoglobin formation. In a normal adult, without any stimulation, the bone marrow synthesizes 4 x 10^{14} hemoglobin molecules per second, each molecule containing four iron atoms, which corresponds approximately to 20 mg iron [2]. Conversely, erythropoiesis is negatively regulated by several cytokines. These are cytokines derived from macrophages, inclusive tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and growth transformation factor-β (TGF-β). All these factors are enhanced in inflammatory conditions and are involved in the pathogenesis of chronic anemia. TNF-α has an inhibitory effect on erythroid progenitors either directly or mediated by interferon-β (INF-β). IL-1 inhibits erythropoiesis in vivo in mice and in vitro in humans. Also, inhibition is direct or mediated by INF-β. TGF-β induces anemia when injected into mice and inhibits colony formation by erythroid progenitors. IL-6 injected in vivo induces anemia, but the inhibitory effect on erythropoiesis in vitro is unclear. These cytokines affect the metabolism of iron by blocking iron inside macrophages [5]. Inflammatory cytokines induce iron metabolism. In all types of infectious or inflammatory disorders a significant hypoferremia is observed despite adequate iron deposits. Probably this is a consequence of the unparalleled release of iron from the monocyte-macrophage system. The iron is not available because of its sequestration in the monocyte-macrophage system. Activated macrophages and granulocytes synthesize lactoferrin, a protein which is competing with transferrin for iron-binding [2]. More IL-1 has been reported to increase ferritin production, and this extra ferritin could act as an iron trap that might otherwise be available for erythropoiesis. Impairment of iron metabolism certainly contributes to erythropoiesis affected in inflammation, but it is not its most important contribution [2]. Experimental animal studies have confirmed the relationship between inhibition of erythropoiesis and inflammatory cytokines. Chronic TNF-a injection in animals produces a high development of anemia [2]. This effect is specific to erythroid because neither granulocytes nor platelets are affected. Similarly, infections of TGF-β in mice induce a severe and progressive manifestation of manifested erythropoiesis by a decrease in the reticulocytes number and spinal cord erythroblasts. The effect seems to be indirect and mediated by TNF.

Epo and organ protection

A surprising finding in neural protection research on Epo was that carbamylated Epo, which does not bind to the canonical Epo receptors (EpoR) and transduce the signaling cascade mediated by JAK2-STAT5 also showed neuroprotective effects [7]. Carbamylated Epo also has the cardioprotective effect of Epo. Asialoerythropoietin failed to increase erythropoiesis, but conferred protection of neurons in vivo [7]. The pleiotropic effect of Epo is mediated by non-canonical heteromultimeric composed of EpoR and common β subunit, the common granulocyte-macrophage subunit colony-stimulating factor, IL-3 and IL-5 receptors in injuries of spinal cord. It should be noted, however, that β subunit is not always necessary to transduce Epo signals to protect against apoptosis [7]. Subsequent characterization of the structure of the mediating cellular receptor pleiotropic effects of Epo in non-hematopoietic cells are required.

The protection of organs by non-erythropoietic Epo derivatives led the investigators to delimit the areas of protection of tissues from Epo to amino acids that form the aqueous face of the B helix [8]. Pyroglutamate the helix B surface peptide was effective in ameliorating multiples organ failure in a pattern of hemorrhagic shock [7].

New mechanisms elucidated of tissue protection through Epo. Hu et al. proves that Klotho is protective; the effect against oxidant-induced cytotoxicity is partially mediated by one year increasing the endogenous expression of the classical EpoR [9]. While erythropoiesis is stimulated by canonical EpoR homodimer, the Epo protective effects on tissues are mediated by a "tissue-protective" heterodimeric receptor [9].

RESULTS

1. Epo activities in several diseases

Epo and malaria

During the sanguine stage of malaria a reduction of circulating hemoglobin should be noticed. Also, tissue hypoxia should elevate the levels of Epo, but the clinical evidence for appropriately raised levels of Epo is contradictory.

Studies from Thailand and Sudan have suggested that Epo concentrations, even if raised, were still very low for the degree of anemia [10]. An experimental research in murine malaria suggests that exogenous Epo can improve the integrity of the blood-brain barrier, downregulate T cells and myeloid cells inflammatory responses and reduce endothelial activation [10].

Anemia in chronic diseases and inflammatory response

rHuEpo is available for the anemia treatment secondary to renal failure [11], and also other causes such as solid and hematological neoplasms. Besides its erythropoietic action, Epo has been shown to have pleiotropic effects [11]. These results were supported by the demonstration of Epo receptors on non-hematopoietic tissues and different cells. It has been previously observed that patients with multiple myeloma (MM) treated for anemia have demonstrated a prolonged survival rate [12]. Using a mouse model, it has been demonstrated that Epo has a mediated anti-myeloma effect. Additional studies have shown that many of the cellular immune deficiencies commonly encountered in MM and myelodysplastic syndromes patients are corrected in patients treated with rHuEpo for anemia [13].

Hepcidin is the primary regulator of iron availability to developing C- reactive protein (CRP) and is increased in chronic inflammatory conditions including anemia of chronic disease /anemia of inflammation (ACD/AI). If the hepcidin levels increase, the iron will remain blocked in the cells thus it will lead to anemia. The anemia of chronic illnesses was initially associated primarily with infectious, inflammatory, or neoplastic disease, but it has been shown that conditions including obesity, diabetes mellitus, congestive heart failure, severe trauma, and other forms of acute or chronic immune activation produce ACD [14]. Administration of Epo or other darbepoetin (erythropoiesis-stimulating agent) may have an antiinflammatory effect and reduce hepcidin expressions. Both agents have been used for the treatment of ACD/AI for many years.

Patients with inflammatory conditions treated with antitumor necrosis factor TNF (antibody) or anti-IL-6 antibody show reductions of inflammatory markers, such as IL-6, hepcidin, and/or CRP, which correlate with the improvement of anemia. The measurement of Epo concentration is indicated for ACD/AI patients who show symptomatic anemia and / or anemia that does not improve after the treatment of the main disorder with or without iron supplements. In these casehats, it suggests a low level of Epo is due to a continuous inflammatory block or perhaps a more serious kidney disease than previously considered.

A very high Epo level suggests insufficient erythropoiesis due to bone marrow disease, such as myelodysplastic syndrome.

The elevated levels of IL-6 and TNF-α are imposing a higher dose for darbepoetin in patients with kidney disease.

Recent evidence suggests that vitamin D can suppress hepcidin (14). Anemia and vitamin D deficiency sometimes coexist and enhancement of vitamin D deficiency can

improve anemia in a certain percentage of people; this is believed to function by direct inhibiting of hepcidin formation by the active vitamin D [15,14].

2. Prophylactic effects of Epo (erythropoietin enhances oxygenation)

A multicentric study demonstrated that erythropoietin enhances oxygenation [16]. It proved that in critical ischemic and hypoxic surrounding tissue, using Epo as pretreatment improves tissue infusion and oxygenation in vivo. This effect can be attributed to Nitric Oxide (NO)-dependent vasodilatory and anti-inflammatory actions on the altered vascular endothelium. We thought about the effect of recombinant human erythropoietin on the microcirculation and oxygenation of critical ischemic tissue and how to elucidate the role of nitric oxide endothelial synthase in the protection of erythropoietin mediate tissue. A critically ischemic hypoxic area and some other island flaps were dissected from the back skin of an anesthetized Syrian male golden hamster which has been infused through a collateralized vasculature. Before ischemia, animals received a shot of epoetin beta at a dose of 5,000 U / kg body weight with (n = 7) or without (n = 7) NO block synthase with 30 mg/kg body weight L-NUME (methyl ester hydrochloride N5-nitro-L-arginine). Animals treated with saline as a control group (n = 7). After 5 hours of collateralization, ischemic tissue damage was characterized by severe hypoperfusion and inflammation, hypoxia, and accumulation of apoptotic cell nuclei. Pre-treatment of erythropoietin increased arteriolar and venous blood flow by 33% and 37%, respectively ($P<0.05$) and attenuated leukocytic inflammation around 75% ($P<0.05$). In addition, the partial pressure of oxygen in the ischemic tissue increased from 8.2 to 15.8 mmHg ($P<0.05$), which was in parallel with a 21% increased density of patent capillaries ($P<0.05$) and a 50% reduced number of apoptotic cells ($P <0.05$). Improved microcirculation and oxygenation were associated with a 2.2-fold ($P<0.05$) increase in endothelial NO synthase protein expression. Interestingly, N5-nitro-L-arginine methyl ester hydrochloride totally eliminated all the beneficial effects of Epo as pretreatment [16]. It has been proven that both Epo mRNA and proteins are found in the brains of a variety of mammals, including humans. The Epo receptor is widely expressed in most types of brain cells, including neurons, endothelial cells, microglial cells, and astrocytes. **Table 1** provides an overview of the cellular sites of Epo and EpoR expression in the central nervous system [17].

Table 1. Sites of Epo and EpoR expression in the central nervous system in humans, modified after[17]

	Epo		Epo receptors	
Cell types	In vitro	In vivo	In vitro	In vivo
Neurones	-	+	+	+
Astrocytes	+	+	+	+
Microglial cells	-	NA	+	NA
Endothelial cells	NA	?	+	+

+, expression detected, NA Not analyzed, ? not proven

A clinical evidence is stated in a recent study that analyzed the improvement of Epo treatment for neonates with moderate to severe hypoxic-ischemic encephalopathy. Epo reduces the risk of MRI brain injury, cerebral palsy, and moderate to severe cognitive impairment [18]. The evidence is limited to suggesting its role as an adjunct to hypothermia. Higher power studies are underway to overcome this limitation [18]. The usage of Epo substitutes athletes to improves their performance by enhanced oxygen level.There has been confirmed that a low dose of rHuEPO (20 IU/ kg body weight) administered three times a week is enough for healthy moderate athletes to maintain an increased Hct and maximum oxygen volume (approx 5% –10% above the initial levels), which can be achieved in itself within 3-4 weeks of a more aggressive dose (50 IU/kg body weight, three times a week). During submaximal exercise with intensities of up to 81% of baseline maximum oxygen volume was generally lower thereafter administration of r-HuEpo [19].

3. Humoral immunity improvement in vaccination associated with rHuEPO treatment

Humoral immunity can also be enhanced by rHuEpo treatment. A benefic effect on cell-mediated immunity and humoral immunity (hepatitis B vaccine) was produced by RhuEpo treatment, and also in response to the seasonal flu vaccine. In a study on hematologic patients, three groups of individuals received the flu vaccine [20]. These were divided as follows: healthy controls, hematological patients with no rHuEpo (NoEpo group), and rHuEpo treated hematological patients for their anemia (Epo group). The anti-influenza Ab titer was measured (complement fixation test) from blood samples taken before and about 3 to 4 weeks, 7–8 weeks and 4 months after vaccination. Nine healthy subjects were compared with 17 NoEpo and 17 patients with Epo. The average ages were 59.5, 61.3, and 73.1 years, and in those the Epo patients were older. In the healthy group, the percentage of those who support only a partial (double) response, a strong response (four times larger), and an ensemble the response (partial and strong responses combined) were 31.6%, 57.9%, and 89.5%, respectively.

In the NoEpo group, the values were 35.3%, 17.6%, and 52.9%, respectively. In the Epo group the results were similar to those of healthy controls: 23.5%, 58.8%, and 82.4%, Epo vs. NoEpo [20].

Hematological patients (NoEpo group) respond poorly to the influenza vaccine compared to healthy subjects and rHuEpo treatment is associated with an improved immune response to influenza vaccine in patients with hematologic with similar titers in those of healthy subjects [20].

4. Effects of EPO substitutes on blood viscosity

Previous study suggests that in hemodialysis patients rHuEPo enables raising the hematocrit to 0.35 with correction of bleeding time without causing intravascular hemostatic activation. Shortening of bleeding time with rHuEPo appears to be due to the hemostatic effects of an increased number of red blood cells, rather than changes in intrinsic platelet function [21] .

Because of the known side effect of rHuEpo in blood coagulation, a study performed a prospective, placebo-controlled, randomized, double-blind trial to determine the effects of intravenous rHuEpo of 200 U/kg daily for 3 consecutive days. They recorded measures of platelet, endothelial cell activation, soluble Fas ligand, and peripheral blood mononuclear cell expression of angiogenesis signaling proteins in 44 subjects with acute myocardial infarction (AMI) treated with aspirin and clopidogrel after successful percutaneous coronary intervention.

The study that evaluated safety and efficacy markers relevant to the biological activity of rHuEpo in patients with AMI concluded that short-term administration of rHuEpo did not alter markers of platelet and endothelial cell activation thrombosis associated. It did increase expression of angiogenesis signaling proteins in peripheral blood mononuclear cells when compared with placebo. Erythropoietin attenuates myocardial lesions and improves ventricular performance after experimental ischemic injury [22]. Recent evidence shows that severe coronavirus disease 2019 (Covid-19) may be complicated with coagulopathy as disseminated intravascular coagulation, which has a rather prothrombotic character with high risk of venous thromboembolism. Present recommendations suggest that all hospitalized COVID-19 patients should receive thromboprophylaxis, or full therapeutic-intensity anticoagulation if such an indication is present [23]. The American Society of Hematology recommends for all hospitalized Covid-19 patients thromboprophylaxis with low molecular weight heparin or fondaparinux (suggested over unfractionated heparin to reduce contact), unless bleeding risk is higher than the thrombosis risk. Despite the lack of present published research, the protocols concerning the strategy for thromboprophylaxis are based on previous clinical experience [24]. In conclusion, the risk of thrombosis as a possible side effect of

rHuEpo therapy can be abolished by the prophylactic use of anticoagulant therapy among Covid-19 patients.

DISCUSSIONS

Dosage of Epo substitute

In a safety and pharmacokinetic study, Wu et al. [25] compared four different Epo dosing regimens (250, 500, 1000 and 2500U/kg, six doses, 48-hour intervals) to 24 newborns who experienced hypothermia for hypoxic-ischemic encephalopathy. The authors found that Epo 1000 U/kg/dose produces optimal neuroprotective levels comparable to animal models, while 500 U/kg/dose produces insufficient neuroprotective levels and 2500U/kg/dose produces levels that exceed the optimal neuroprotective range by approximately three times. Also, in another pharmacokinetic study, the authors found that weekly administration of darbepoetin α produces sufficient serum Epo concentrations. Zhu et al. evaluated two different Epo dosing regimens, 300 U/kg/dose, and 500 U/kg/dose, and found similar clinical effects. Similarly, no differences were observed with the two dosing regimens of darbepoetin α in the study by Baserga et al [26]. It is not known whether the clinical implications are substantial with a high dose (1000 U/kg/dose) compared to the lower dose (300–500 U/kg/dose). Further research is needed to understand the ideal dose at which maximum clinical benefit with minimal or no side effects is seen, as well as a head-to-head comparison of the efficacy of αbepoetin with Epo [18]. Routine use of sc rather than iv rHuEpo to manage anemia in hemodialysis patients could increase survival and reduce hospitalizations for cardiovascular complications by minimizing the dosage of rHuEpo [27].

Epo and Covid-19

A recent article presents the improved results after using rHuEpo in a Covid-19 patient with associated anemia [28]. The patient was 80 years old and had a medical history of Alzheimer's disease and depression. Adjuvant to the Covid-19 treatment protocol, the detected anemia was treated with transfusion of one unit of packed red blood cell (1 day) and rhEpo (300 IU/kg divided into 5 doses of 4000 IU subcutaneous injections in days 1,3,5 and 7). The lab data improvements are presented in **Table 2**[28].

Table 2. Blood tests results in a Covid19 patient with anemia and rhuEpo treatment from[28]

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Hb(g/dl)	5.2	6.7	7.9	8.2	7.6	8.6	8.5	9
Neutrophil/uL	5562	5776	7331	9718	6020	6288	4482	4590
Lymph (uL)	333	933	958	893	809	748	588	648
Neutr/Lymph	16.7	6.19	7.65	10.88	7.44	8.41	7.62	7.08

CONCLUSIONS

Substitutes of Epo administered to Covid 19 patients may have a beneficial impact in blood oxygenation in addition to the severe inflammatory reaction due to viral replication. Also, it demonstrated the role in the protection of organs, including brain in brain hypoxia, heart protection in cardiac ischemia and renal protection.

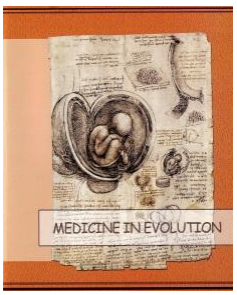
The medical dosage is established based on kg/body, type of the Epo substitute selected and the route of intravenous or subcutaneous administration. The subcutaneous route has greater benefits and low risks. Regarding the thrombosis risk, it can be overcomed by the use of thromboprophylaxis therapy among Covid-19 patients.

REFERENCES

1. Eckardt KU, Kurtz A. Regulation of erythropoietin production. Eur J Clin Invest. 2005;35:13-9.
2. Xie J, Tong Z, Guan X, Du B, Qiu H, Slutsky AS. Critical care crisis and some recommendations during the COVID-19 epidemic in China. Intensive Care Med. 2020:1-4.
3. European researchers among 2019 Nobel Prize laureates. 2019; Available from: https://ec.europa.eu/info/news/european-researchers-among-2019-nobel-prize-laureates-2019-oct-16_en.
4. Worldometer. Coronavirus Case. 2020 [cited 2020 18 July]; Available from: https://www.worldometers.info/coronavirus/.
5. Organization WH, Organization WH. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19). Geneva; 2020.
6. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020.
7. Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, et al. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science. 2004;305(5681):239-42.
8. Brines M, Patel NS, Villa P, Brines C, Mennini T, De Paola M, et al. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proceedings of the National Academy of Sciences. 2008;105(31):10925-30.
9. Nangaku M. Tissue protection by erythropoietin: new findings in a moving field. Kidney Int. 2013;84(3):427-9.
10. Roberts DJ. Anemia in malaria. 2019.
11. PRUTCHI-SAGIV S, MITTELMAN M, NEUMANN D. Erythropoietin—A Hematopoietic Hormone with Emerging Diverse Activities. Handbook of Biologically Active Peptides: Elsevier; 2006. p. 1393-400.
12. Mittelman M, Zeidman A, Kanter P, Katz O, Oster H, Rund D, et al. Erythropoietin has an anti-myeloma effect–a hypothesis based on a clinical observation supported by animal studies. Eur J Haematol. 2004;72(3):155-65.
13. Prutchi-Sagiv S, Golishevsky N, Oster HS, Katz O, Cohen A, Naparstek E, et al. Erythropoietin treatment in advanced multiple myeloma is associated with improved immunological functions: could it be beneficial in early disease? Br J Haematol. 2006;135(5):660-72.
14. Camaschella C, Weiss G, Means Jr RT. Anemia of chronic disease/anemia of inflammation.
15. Perlstein TS, Pande R, Berliner N, Vanasse GJ. Prevalence of 25-hydroxyvitamin D deficiency in subgroups of elderly persons with anemia: association with anemia of inflammation. Blood, The Journal of the American Society of Hematology. 2011;117(10):2800-6.
16. Nairz M, Schroll A, Moschen AR, Sonnweber T, Theurl M, Theurl I, et al. Erythropoietin contrastingly affects bacterial infection and experimental colitis by inhibiting nuclear factor-κB-inducible immune pathways. Immunity. 2011;34(1):61-74.
17. Marti HH. Erythropoietin and the hypoxic brain. J Exp Biol. 2004;207(18):3233-42.
18. Razak A, Hussain A. Erythropoietin in perinatal hypoxic-ischemic encephalopathy: a systematic review and meta-analysis. J Perinat Med. 2019;47(4):478-89.
19. Russell G, Gore CJ, Ashenden MJ, Parisotto R, Hahn AG. Effects of prolonged low doses of recombinant human erythropoietin during submaximal and maximal exercise. Eur J Appl Physiol. 2002;86(5):442-9.
20. Oster HS, Prutchi-Sagiv S, Halutz O, Shabtai E, Hoffman M, Neumann D, et al. Erythropoietin treatment is associated with an augmented immune response to the influenza vaccine in hematologic patients. Exp Hematol. 2013;41(2):167-71.
21. Gordge M, Leaker B, Patel A, Oviasu E, Cameron J, Neild G. Recombinant human erythropoietin shortens the uraemic bleeding time without causing intravascular haemostatic activation. Thromb Res. 1990;57(2):171-82.
22. Tang Y-D, Hasan F, Giordano FJ, Pfau S, Rinder HM, Katz SD. Effects of recombinant human erythropoietin on platelet activation in acute myocardial infarction: results of a double-blind, placebo-controlled, randomized trial. Am Heart J. 2009;158(6):941-7.
23. Kollias A, Kyriakoulis KG, Dimakakos E, Poulakou G, Stergiou GS, Syrigos K. Thromboembolic risk and anticoagulant therapy in COVID-19 patients: Emerging evidence and call for action. Br J Haematol. 2020.

24. HEMATOLOGY ASO. COVID-19 and VTE/Anticoagulation. Available from: https://www.hematology.org/covid-19/covid-19-and-vte-anticoagulation.
25. Hu M-C, Kuro-o M, Moe OW. Klotho and kidney disease. Journal of nephrology. 2010;23(Suppl 16):S136.
26. Baserga MC, Beachy JC, Roberts JK, Ward RM, DiGeronimo RJ, Walsh WF, et al. Darbepoetin administration to neonates undergoing cooling for encephalopathy: a safety and pharmacokinetic trial. Pediatr Res. 2015;78(3):315-22.
27. Wright DG, Wright EC, Narva AS, Noguchi CT, Eggers PW. Association of erythropoietin dose and route of administration with clinical outcomes for patients on hemodialysis in the United States. Clin J Am Soc Nephrol. 2015;10(10):1822-30.
28. Hadadi A, Mortezazadeh M, Kolahdouzan K, Alavian G. Does recombinant human Erythropoietin administration in critically ill COVID-19 patients have miraculous therapeutic effects? J Med Virol. 2020.

Clinical Forms of Manifestation of Human Trichinellosis in Brasov County, for a Period of 30 Years



Dobrescu C.E., Nemet G.C.

Transilvania University of Brasov, Faculty of Medicine

Correspondence to:
Name: Nemet Codruța
Address: 28 Fagetului Street, Block 1, 6th flat, Brasov, Brasov County, Romania
Phone: +40 723611488
E-mail address: codruta_nemet@yahoo.com

Abstract

Our professional interest in human trichinellosis evolution lies for a period of 30 years (1983-2013). In this study we have included 1112 cases of human trichinellosis recorded in that period of time. The aim of the study was: the symptoms and clinical signs correlated with the results of laboratory tests and epidemiological investigations could lead the physician to suspect the trichinellosis. Thus would shorten the patient route by various medical specialties until diagnosing the trichinellosis and would hasten the establishment of antiparasitic treatment. Severe symptoms did not hasten accurate diagnosis of trichinellosis owing to frequent misdiagnosis. High numbers of eosinophil cells was found consistently in all forms of the disease, which confirms the value of this investigation in the diagnosis of trichinellosis. We emphasize the importance of performing dynamic eosinophilia and dysproteinemia tests for the diagnosis of the disease and its follow-up evolution.

Keywords: human trichinellosis, incubation period, disease form, eosinophilia values

INTRODUCTION

Trichinella infection in humans is strongly associated with the consumption of raw or undercooked meat; thus, cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products play an important role in the epidemiology of the disease. Overall, domestic pork and related products remain the most important source of Trichinella infection in humans, especially when pigs are raised under free-ranging or backyard production conditions [1,2,3]. In Romania, the highest prevalence of trichinellosis in humans occurred in the Transylvanian region, where the local ethnic group maintains the food habit of raw meat consumption [4,5].

Brasov County, located in Transylvania - mountain district in which one of the main occupations of rural inhabitants is farming, offers animal and human trichinellosis a wide field of development. Unlike counties in the south and east of the country, where people eat more vegetables, the population of Brasov county is consuming meat, mainly pork, but also beef, poultry, game, processed in different ways: roasted, boiled, fried smoked, brine, in some cases insufficient or incorrectly cooked [4].

Aim and objectives

The symptoms and clinical signs correlated with the results of laboratory tests and epidemiological investigations could lead the physician to suspect the trichinellosis and thus would shorten the patient route by various medical specialties until diagnosing the trichinellosis and would hasten the establishment of antiparasitic treatment.

MATERIAL AND METHODS

Our professional interest in human trichinellosis evolution lies for a period of 30 years (1983-2013). In this study we have included 1112 cases of human trichinellosis recorded in that period of time in Brasov County, Romania. We mention that currently in our country, the diagnosis of human trichinellosis is based on clinical diagnostic elements (fever, edema, myalgia), case medical history, epidemiological, epizootological investigations, and laboratory guidance tests. Specific anti-parasite IgM and IgG immunoglobulins are currently limited in practice, because they are chargeable, and are not reimbursed by health insurance.

Patient definition. The patient calls many doctors of various specialties until the suspicion of trichinellosis, thus increasing the time from the disease onset (end of incubation) to the diagnosis of trichinellosis.

Laboratory procedures. To guide the trichinellosis diagnosis, the eosinophilia value in dynamic, hypoproteinemia values and low A/G ratio are used.

Outbreak definition. A disease outbreak is the occurrence of cases of disease in excess of what would normally be expected in a defined community, geographical area or season. An outbreak may occur in a restricted geographical area, or may extend over several countries. It may last for a few days or weeks, or for several years.

Epidemiological investigation definition. Epidemiological investigations are usually conducted in outbreak situations. The main reasons for conducting an epidemiologic investigation are: to determine the cause of an outbreak, and to implement control measures to prevent additional illness.

Focus of the study. Laboratory and paraclinical examinations, more or less specific can support the clinical suspicion of trichinellosis. We wondered if a minimum of laboratory tests in which prevails eosinophilia along with hypoproteinemia, low A/G ratio, and hyperleukocytosis are helpful in guiding clinical diagnosis to a parasitosis, along with epidemiological anamnesis can suspect the trichinellosis and hasten the antiparasitic therapy.

RESULTS AND DISCUSSIONS

The incubation period is the date between the consumption time of pork infected with Trichinella spiralis and the time when the first signs of disease appear, that actually disturbs the patient. The incubation period for trichinellosis lasts from 2 to 50 days, depending on the number of infective larvae ingested, with a greater number of larvae corresponding to a shorter incubation period. The length of the incubation period is also generally believed to be predictive of the clinical severity of the disease [6].

On the studied cases the average incubation is 17.25 days, the data were obtained from epidemiological investigations carried out for each case of trichinellosis. Allowing an infection to go undetected for that long, because of an incorrect diagnosis, led to late start of treatment for trichinellosis with the possibility of the disease to become chronic. We mention that in the asymptomatic forms (35 cases) we could not calculate the incubation period because the patients could not specify the date of onset due to the lack of the subjective symptoms.

Correlating the incubation with the form of the disease in the mild one (451 cases), the average incubation is 17 days; the early signs are hardly appreciated by patient as signs of the disease. In moderate forms (713 cases) it is 15 days, but unlike the mild one the clinical signs are more obvious alerting the patient and sending him towards the doctor. In moderate-severe forms of the disease (32 cases), average incubation is 21 days, higher value because although there were clinical signs in the context of disease the clinical diagnosis was not directed toward the parasitosis, but towards other diseases. In the severe forms (47 cases) the average incubation was 16 days; this may have been due the patient's preexisting diseases overlapping trichinellosis. In a retrospectiv analysis of Romanian patients, severe symptoms did not hasten accurate diagnosis of trichinellosis owing to frequent misdiagnosis.

Mild and severe forms of the disease prevail in urban areas, and the asymptomatic, moderate and moderate-severe in rural areas. In adults were recorded more fervently moderate, moderate-severe and severe forms of the disease, while in children were prevailing asymptomatic and mild forms, because they consume much smaller quantities from the infected meat. In the case of women there are frequently moderate and severe forms of disease, and in men the asymptomatic and mild forms, which could prove damaging effects of alcohol on the number of Trichinella larvae in the intestinal phase of the infection.

We investigated the result of another parameter: the interval between falling ill to the accurate diagnosis of the disease; the average time interval between the date of illness and the detection of trichinellosis in days. It changed for the better over the years, from 16.42 days in 1985 to 5.41 in 1990, rising to 8.63 days in 1992. Decreasing the time from the first signs of illness until the correct diagnosis of trichinellosis in humans results in the immediate establishment of an etiological treatment, symptomatic pathogenic and promptly, with beneficial repercussions on future evolution of the disease [7].

The symptoms of patients with trichinelosis: 80% of patients had fever (39 to 40 degrees Celsius), 96% had fatigue that lasts at least 10 days after disease onset. Muscle pains were present in 90% of patients, with various manifestations. In mild cases the muscle pain had low intensity, patients not giving them too much attention, while in the moderate, moderate-severe and severe form of the disease were a cardinal symptom; muscle were swollen, painful, with difficulty in walking, swallowing (5%) or while breathing (5%). Muscle weakness has been noted by all patients who accused myalgia during the disease (90%). Facial (88%) and palpebral edema (83%) were commonly reported, including in mild forms of the disease with fever and myalgia being symptom that can facilitate clinical diagnosis of trichinellosis. Lower limb edema was reported only in the moderate forms of the disease (42%). Rash was seen in 37% of the studied patients. Allergic skin manifestations always came with changes to the mucous membranes: oral-pharyngeal enanthema, conjunctivitis, conjunctival chemozis.

Laboratory diagnosis. High numbers of eosinophil cells was found consistently in all forms of the disease (89.35%) which confirms the value of this investigation in the diagnosis of trichinellosis, especially in asymptomatic and mild forms of the disease. We can notice the pathognomonic value of eosinophils cells, joined by low levels of total protein, low A/G ratio, and low levels of transaminases which warns of a possible liver damage in trichinellosis – a manifest disease (figure 1). Hyperleukocytosis is found in a much lower number of patients (270), recording elevated values in all severe forms of the disease, inconsistent to those with mild, moderate, and asymptomatic forms. Hypoproteinemia appears in all forms of the disease, totalizing a large number of patients in medium, moderate-severe and severe forms of the disease. Low A/G ratio is found in 212 investigated patients, being more frequent in the medium forms of the disease and signaled in nearly all moderate-severe forms. Hypoproteinemia and low A/G ratio have a great practical importance, drawing the attention to a possible depletion of liver function in human trichinellosis, especially in those with moderate-severe and severe form of the disease, often associated with marked edema as objective sign. Lower ALT values are found in all forms of the disease including the asymptomatic and mild forms.

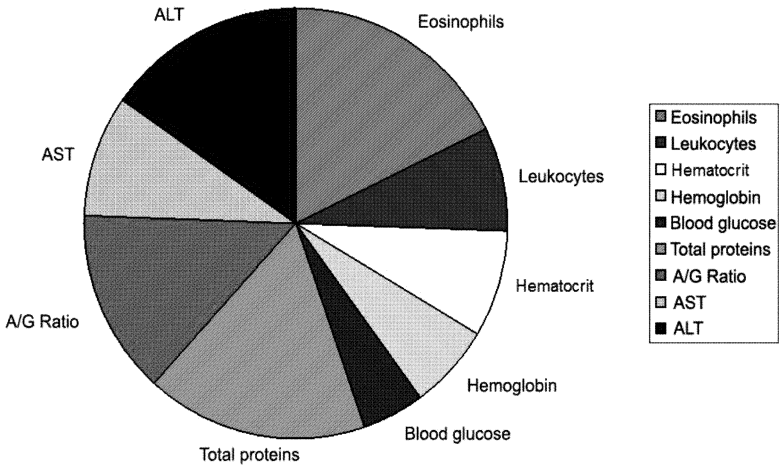


Figure 1. The share of pathognomonic values of laboratory tests

CONCLUSIONS

The incubation period for human trichinellosis does not register significant variations in the number of days depending on the form of the disease also due to the active detection in the disease outbreaks.

Severe symptoms did not hasten accurate diagnosis.

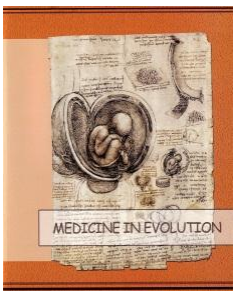
Considering that in Romania the serological diagnosis of trichinellosis is not a common practice, we emphasize the importance of performing dynamic eosinophilia and dysproteinemia tests for the diagnosis of the disease and its follow-up evolution.

REFERENCES

1. Dupouy-Camet J. Trichinellosis: a worldwide zoonosis. Vet Parasitol. 2000; 93:191–200.
2. Dupouy-Camet J, Kociecka W, Bruschi F, Bolas-Fernandez F, Pozio E. Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin Pharmacother. 2002; 3(8):1117-1130.
3. Gottstein B, Pozio E, Nöckler K. Epidemiology, Diagnosis, Treatment, and Control of Trichinellosis. Clin Microbiol Rev. 2009; 22(1):127–145.

4. Blaga R, Durand B, Antoniu S, Gherman C, Cretu C.M, Cozma V, Boireau PA. Dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits. Am. J. Trop. Med. Hyg. 2007; 76:983-986.
5. Dupouy-Camet J. Trichinellosis: still a concern for Europe. Euro Surveill. 2006; 11(1):5.
6. Pozio E, Gomez Morales MA, Dupouy-Camet J. Clinical aspects, diagnosis and treatment of trichinellosis. Expert Rev Anti Infect Ther. 2003; 1(3):471-482.
7. Nemet C, Dobrescu C, Balescu A, Idomir M, Buvnariu L, Rogozea L. Retrospective epidemiological survey of diagnostic errors and their implications in the development of human trichinellosis in Braşov County-Romania over a period of 10 years. Vet Parasitol. 2013; 194(2-4):139-141.

Evaluation of the cytotoxic and anti-angiogenic potential of flavone apigenin using the B164A5 mouse melanoma cell line



Ghiţu A.[1], Avram Ş.[1], Pavel I. Z.[1], Scurtu A.-D.[1], Minda D.[1], Danciu C.[1], Tigmeanu C.V.[2]

[1]*Department of Pharmacognosy, "Victor Babeş" University of Medicine and Pharmacy, 2, Eftimie Murgu Square, 300041 Timişoara, Romania*
[2]*Department I, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania*

Correspondence to:
Name: Pavel Ioana Zinuca
Address: Department of Pharmacognosy, "Victor Babeş" University of Medicine and Pharmacy, 2, Eftimie Murgu Square, 300041 Timişoara, Romania
Phone: +40 762659981
E-mail address: ioanaz.pavel@umft.ro

Abstract

Aim and objectives: The present study was designed in order to evaluate the cytotoxic and anti-angiogenic potential of apigenin using the B164A5 mouse melanoma cell line. Material and methods: The effect of apigenin against B164A5 mouse melanoma cell line was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Lactate dehydrogenase (LDH) assays. For the determination of the anti-angiogenic potential CAM assay was performed. Results: At 72h post-stimulation, Api provoked a dose-dependent decrease of melanoma cells viability. Significant statistic results were found at the concentrations of 10 μM 30μM and 60 μM for reduction of proliferation, respectively 30μM and 60 μM for cytotoxicity. Data from this study showed that at both tested doses Api reduced the migration potential of melanoma cells. Conclusions: Apigenin induced, dose-dependent antiproliferative, cytotoxic and anti-angiogenic effects on B164A5 cells. The compound represents a promising candidate for further studies of experimental animal models of melanoma.

Keywords: apigenin, B164A5 mouse melanoma cell line, antiproliferative, cytotoxic, antiangiogenic effects.

INTRODUCTION

Although used since ancient times, in recent years medicinal plants and natural compounds have been in the spot light of the medical research due to the continued development of physico-chemical and analytical methods that allowed a comprehensive qualitative and quantitative characterization of various plant extracts. Along with this aspect, countless studies attest the therapeutic value of natural compounds for the treatment and/or prevention of a wide range of both acute and/or chronic pathologies [1, 2].

Nowadays, over 50% of current medication derives from natural compounds [3] , and globally more than 80% of the substances used in various pathologies such as cardiovascular, immune, bacterial or viral diseases have a vegetal source. For neoplastic diseases, substances of plant origin used as such or in the form of chemical derivatives account for more than 60% [1].

Carcinogenesis is an accumulation of genetic and epigenetic anomalies leading to cell alteration. As a treatable pathology, but with very rapid progress most of the time, the stage of the disease and the type of cancer are very important [4]. According to the latest WHO statistics, cancer is one of the most common causes of death in recent years [5]. The latest statistics show that there are around 9.6 million deaths and 18 million new diagnoses [6].

Skin cancer is one of the most common types of cancer. It is frequently classified into four categories: basocellular cancer, squamous cell cancer, actinic keratosis and melanoma. Due to its fast and high capacity of metastasis, melanoma represents one of the most aggressive type of cancer [7].

Among years an increased number of phytochemicals have represented scaffolds for the development of anti-cancer drugs. Apigenin (4,5,7– trihydroxyflavone) belongs to the class of flavonoids, a very broad therapeutic family, widely studied, which includes numerous therapeutic substances, currently used in medicine for various ailments (8).

Apigenin has a multitude of pharmacological properties, of which: vasoprotective, hypotensitive, antibacterial, antiviral, immunosuppressive, anti-angiogenic, hemostatic effects and antitumor potential [8, 9]. Apigenin has also anti-inflammatory, antimutagenic, antioxidant effects, regulates the cell cycle, activates the apoptotic process and has an antiproliferative role in case of some cancer cell lines including: cervical and ovarian cancer, breast, neck and head cancer, colorectal, lung, skin and prostate cancer [10].

Matricaria chamomilla L. is one of the most important sources of apigenin [11] [5010-5320 mg / 100 g dry product] [12]. This phytocompound can be also found in important amounts in Apium graveolens L., Rosmarinus officinalis L., Petroselinum crispum L., Camelia sinensis L. Piper nigrum L. [8], Carum carvi L. [13], Mentha longifolia L. [14], Scutellaria barbata L. [15], Lycopodium clavatum L. [16] and Euterpe oleracea L. [17].

So far, multiple specialized studies have proved the antiproliferative, proapoptotic and cytotoxic action of apigenin. Experimental tests with apigenin have been shown to be effective in vitro in case of several cancer cell lines including: breast cancer [MCF-7] [18], [HER2] [19], gastric carcinoma [SGC-790] [4], thyroid carcinoma [BCPAP], prostate cancer [DU145] [20], lung carcinoma [A549], human colon cancer [HCT116], ovarian cancer [A2780] [21], human melanoma [A375 and C8161] [22], murine melanoma [B16F10] [23].

Aim and objectives

Since data from the literature present informations regarding B16F10, the high metastatic melanoma cell line, the aim of this study was to evaluate the antiproliferative, cytotoxic and anti-angiogenic potential of flavone apigenin using the B164A5 mouse melanoma cell line. B164A5 is one of the most widely used cell line for the murine melanoma model, being well accepted by the C57BL6J mouse strain [24].

MATERIAL AND METHOD

Cell Culture

The murine melanoma cell line B164A5 (code no. 94042254) was purchased from Sigma-Aldrich (Germany). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, Germany), supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, Germany) and 1% penicillin/streptomycin mixture (Pen/Strep, 10,000 IU/mL; Sigma-Aldrich, Germany). The cells were maintained in standard conditions (humidified atmosphere with 5% CO2 and 37°C).

MTT Assay

The effect of Api on B164A5 murine melanoma cells viability was evaluated by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The method was conducted as previously described [27]. Briefly, 1 × 104 cells/well were seeded in 96-well culture plates and allowed to adhere overnight. The second day, the cells were stimulated with different concentrations of Api (1, 3, 10, 30 and 60 µM) and incubated for 72h. The Control group is represented by cells treated with the solvent dimethyl sulfoxide (DMSO). After the 72h incubation period, the cells were treated with 10 µL of 5 mg/mL MTT solution from the MTT kit (Sigma-Aldrich) and incubated for an additional 3h. The obtained formazan crystals were dissolved in 100 µL of lysis solution provided in the MTT kit. The absorbance was determined at 570 nm with a microplate reader (BioRad, xMark Microplate Spectrophotometer).

LDH Assay

The cytotoxic effect of Api on B164A5 murine melanoma cells was determined by means of Lactate dehydrogenase (LDH) assay (Thermo Fisher Scientific). The protocol was applied as previously described [27]. Briefly, 1 × 104 cells/well were seeded in 96-well culture plates and allowed to adhere overnight. The second day, the cells were stimulated with two concentrations of Api (30 and 60 µM) and incubated for 72h. After the incubation period, 50 µL from each well was transferred into a 96-well culture plate and mixed with 50 µL of reaction mixture. The plate was incubated for 30 minutes at room temperature and then 50 µL of the stop solution was added into each well. The level of LDH release in the medium was measured at 490 nm and 680 nm using a microplate reader (BioRad, xMark Microplate Spectrophotometer).

Tumor angiogenesis evaluation on the chorioallantoic membrane (CAM) using melanoma B164A5 cells

The CAM assay makes use of fertilized chicken (Gallus gallus domesticus) eggs, according to an adapted technique [25, 26], that involves incubation at controlled 37°C and 50% humidity. On the third, and fourth day of incubation, 4-5 ml of albumen were removed and a window was cut on the upper side of the eggs.

Subsequently, the B164A5 mouse melanoma cells, cultured according to the above described protocol, were inoculated using concentrations of 105 cells/5µl, on top of the developing membrane on day 10 of incubation (0H) [25]. Apigenin in concentration of 30 µM, 60 µM and solvent control DMSO 1% were applied in volumes of 5 µl and was repeated for 48h. The treated CAMs were daily monitored, and in ovo photographs were registered.

The evaluation was performed by means of a stereomicroscope (Discovery 8 Stereomicroscope, Zeiss) and relevant images were registered using the Axio CAM 105 colour, Zeiss digital camera and processed by Zeiss ZEN software, ImageJ and GIMP.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). The statistical analysis was performed by means of GraphPad Prism 5. Comparison among groups was performed using One-way ANOVA followed by Dunnett's post-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

RESULTS

MTT Assay

The effect of Api was assessed on a murine melanoma cell line and compared to Control group (cells that were stimulated with the solvent DMSO). In Figure 1 is represented the effect of Api after a stimulation period of 72h. Api produced a dose-dependent decrease of tumor cell viability, the most significant effects were obtained at 30 and 60 µM (cells viability was for 30 µM 76 ± 1.7 % vs. Control and for 60 µM it was 57.8 ± 1.8 % vs. Control)

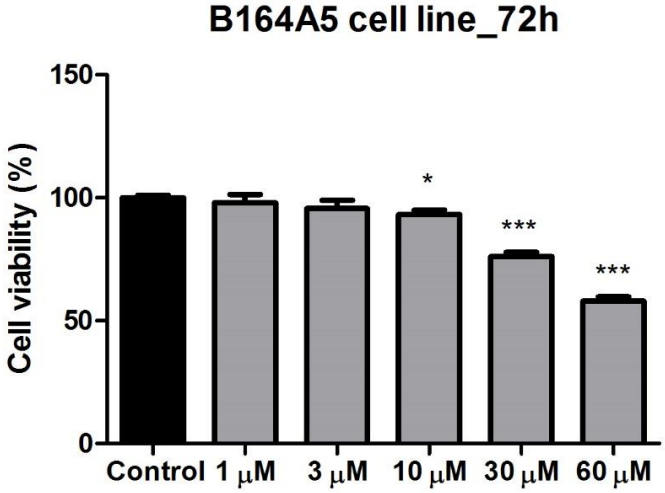


Figure 1. B164A5 murine melanoma cells viability after 72h stimulation with Apigenin (1, 3, 10, 30 and 60 µM)

Determination of the Cytotoxic Potential by LDH Release

The cytotoxic potential of Api was evaluated using two concentrations that proved to be the most efficient in decreasing tumour cells viability. At 72h post-stimulation, at both tested doses of Api there was a significand release of lactate dehydrogenase compared to DMSO groups. At 30 µM Api produced a cytotoxicity rate of 13.3 ± 1.7% and at 60 µM the cytotoxicity rate was slightly higher 15.1 ± 1.8% (Figure 2).

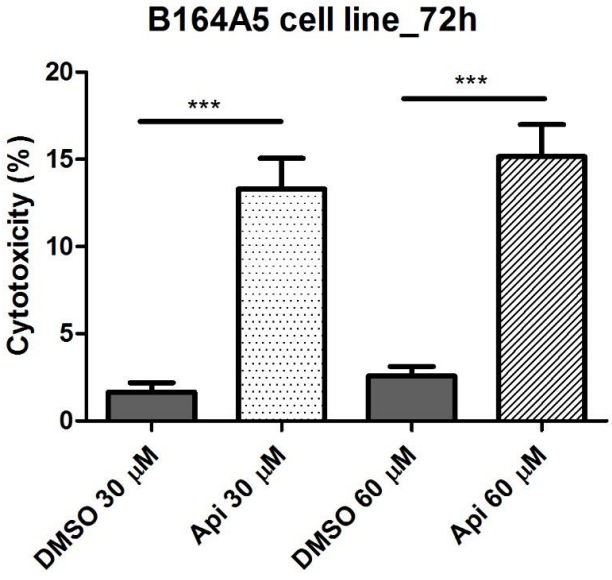


Figure 2. The cytotoxic effect of Apigenin and DMSO (30 and 60 µM) on B164A5 murine melanoma cell line at 72h post-stimulation

Chorioallantoic Membrane Assay (CAM)

The evaluation of the specimens was performed 48 hours post inoculation of the mouse melanoma cells on top of the developing CAMs and after the administration of two doses of test solutions. The specimens exposed to only DMSO showed a more compact tumor area, adherent to the inner surface of the application ring, next to several areas of migrated cells outside the ring, displaying an intense angiogenic reaction.

In contrast, when treated with Api, the compact tumour areas were reduced and a more scattered display of the cells was observed. For both concentrations, Api reduced the migration potential of melanoma cells, and only a few dispersed cells were observed in the outer proximity of the application ring. The number of pigmented cells was also reduced, with a slightly higher degree of pigmentation induced by Api at the higher tested concentration (60 µM).

When analysing the impact induced by the samples on the tumor angiogenesis process, there were some differences between the tested samples. Api in concentration of 30 µM caused a spokes wheel angiogenic reaction converging toward the application ring, while inside the ring the capillaries had a fine aspect and an ununiform distribution. Meanwhile, the higher concentration of Api, induced a higher vascular reaction inside the ring, but still vessels were displayed in an irregular pattern (Figure 3).

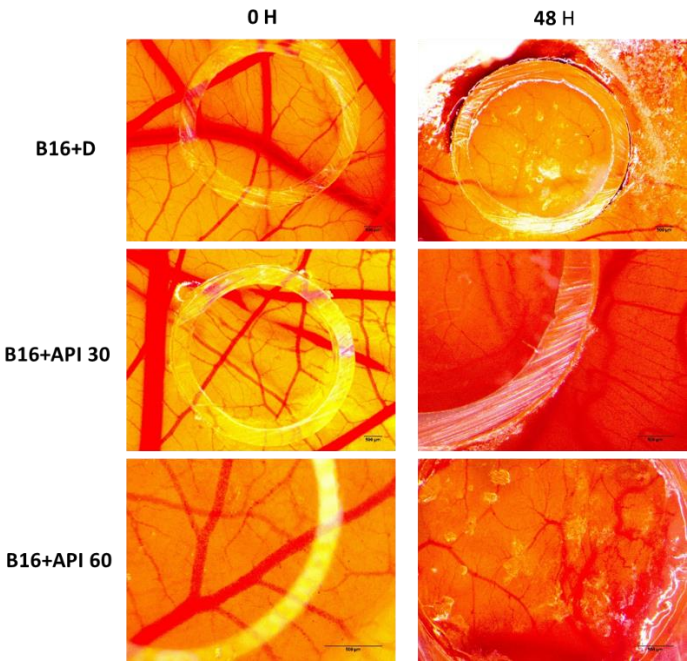


Figure 3. Apigenin treated B164A5 melanoma cells on CAM: apigenin 30, 60 µM and DMSO 1% as solvent control, 0 H and 48 H post inoculation; in ovo, stereomicroscopy photographs, scale bar 500 µm

DISCUSSIONS

In a previous published paper by our group [27] it was shown that Api had an antiproliferative and proapoptotic effect against a human melanoma cell line (A375 cells). Comparing the data obtained in the present study with the data obtained on A375 cells, we can indicate that Api has a more potent effect on the human melanoma cell line than on the murine one.

Cao et al. indicated that the flavonoid apigenin reduced A375 human and B16F10 murine melanoma cells migration and invasion in a dose dependent manner [23]. In another paper Caltagirone et al. showed that Api inhibited B16-BL6 murine melanoma growth. Furthermore, the authors indicated that Api inhibited lung colonization of B16-BL6 cells in an in vivo model [28].

As previously showed by our group [27] Api at both concentration of 30 and 60 μM reduced the development of A375 human melanoma cells, while reducing the angiogenesis reaction. Here we explored the behaviour of a murine melanoma cell line, namley B164A5, when exposed to Api in vivo using the CAM assay. We noticed that in the case of these cells too, the tumor formation process was impaired, by means of reducing cell adherence and by inducing a dysregulated vessel plexus. The migration potential of the cells was reduced in this in vivo model. A number of recent publications explore the antimetastatic potential of apigenin by inhibiting both upstream and downstream aberrations of the STAT-3 pathway, including the decrease of VEGF, HIF-1, MMP-2, MMP-9 and oncogenes Src and JAK 2 [23, 29, 30]. Also a possible melanogenic effect was observed here, more intensively at the higher tested concentration, confirming the tyrosinase inhibition shown by others [31].

Another group conducted by Xu et al. performed a series of assays to examine the influence of apigenin on the cell proliferation and apoptosis of A375, A2058 and RPMI-7951 melanoma cells. It was obtained that apigenin suppressed the propagation of all three melanoma cell lines, in a concentration-dependent manner, maximum effect being at the concentration of 60 μM and that apigenin treatment resulted in significant increases in the percentages of apoptotic populations in A375, A2058 and RPMI-7951 cells. Also, the group investigated the influence of apigenin on the tumorigenesis of melanoma in vivo, generating melanoma xenograft mouse models using the B16-F10 melanoma cell line derived from the C57BL/6 strain. It turned out that apigenin treatment significantly suppressed the growth of B16-F10 xenograft tumours [32].

In a similar approach Ye et al. demonstrated that apigenin presents potent melanogenic activity in murine melanoma B16 cells without affecting proliferation [33]. In the study designed by Nasr Bouzaiene et al., it was observed that incubation of B16F10 mouse melanoma cells with apigenin-7-glucoside in the dose range [10-50 μM] inhibited cell proliferation in a dose-dependent manner [34].

CONCLUSIONS

The in vitro evaluation of Api effects against B164A5 murine melanoma cells indicated that the compound decreased tumour cells viability and had a cytotoxic effect especially at the highest doses tested (30 and 60 μM).

It was observed a reducing effect of the angiogenesis process at the concentration of 30 μM at a higher extent then at 60 μM. Both concentrations reduced the migration potential of the cells, while at 60 μM a stronger melanogenic effect was observed. As far as we know no other study was performed on the CAM assay using the highly metastatic B164A5 cells for the evaluation of apigenin.

The study concludes that apigenin induced, dose-dependent antiproliferative, cytotoxic and anti-angiogenic effects on B164A5 cells. The compound represents a promising candidate for further studies of experimental animal models of melanoma.

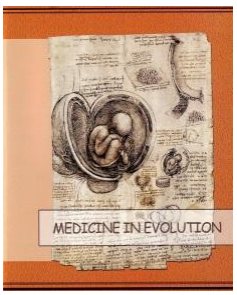
REFERENCES

1. Mateescu I, Paun LA, Popescu S, Roata G, Sidoroff M. Medicinal and aromatic plants - a statistical study on the role of phytotherapy in human health. In 2014.
2. Chinembiri T, du Plessis L, Gerber M, Hamman J, du Plessis J. Review of Natural Compounds for Potential Skin Cancer Treatment. Molecules. 2014 Aug 6;19(8):11679–721.
3. Pan Y, Birdsey RA, Phillips OL, Jackson RB. The Structure, Distribution, and Biomass of the World's Forests. Annu Rev Ecol Evol Syst. 2013 Nov 23;44(1):593–622.
4. Shukla S, Gupta S. Apigenin: A Promising Molecule for Cancer Prevention. Pharm Res. 2010 Jun;27(6):962–78.

5. Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances. 2015 Dec;33(8):1582–614.
6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018 Nov;68(6):394–424.
7. Tipuri de cancer de piele | Euromelanoma [Internet]. [cited 2020 Jun 29]. Available from: https://www.euromelanoma.org/romania/informa%C8%9Bii-generale/tipuri-de-cancer-de-piele
8. Ali F, Rahul, Naz F, Jyoti S, Siddique YH. Health functionality of apigenin: A review. International Journal of Food Properties. 2017 Jun 3;20(6):1197–238.
9. Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: anti-cancer effects and mechanisms of action. Cell Biosci. 2017 Dec;7(1):50.
10. Madunić J, Madunić IV, Gajski G, Popić J, Garaj-Vrhovac V. Apigenin: A dietary flavonoid with diverse anticancer properties. Cancer Letters. 2018 Jan;413:11–22.
11. Jo F, Nl F, Lp de O, Ca E, Gr P, Lc da C, et al. Matricaria recutita and its Isolate-Apigenin: Economic Value, Ethnopharmacology and Chemico-Biological Profiles in Retrospect. Research & Reviews: Journal of Pharmacognosy and Phytochemistry. 2016 Oct 27;4(4):17–31.
12. Hostetler GL, Ralston RA, Schwartz SJ. Flavones: Food Sources, Bioavailability, Metabolism, and Bioactivity. Adv Nutr. 2017 May;8(3):423–35.
13. Sen P, Sahu P, Haldar R, Sahu K, Prasad P, Roy A. Apigenin Naturally Occurring Flavonoids:Occurrence and Bioactivity. UK Journal of Pharmaceutical Biosciences. 2016 Dec 1;4:56.
14. Farzaei MH, Bahramsoltani R, Ghobadi A, Farzaei F, Najafi F. Pharmacological activity of Mentha longifolia and its phytoconstituents. Journal of Traditional Chinese Medicine. 2017 Oct 1;37(5):710–20.
15. Yao H, Li S, Hu J, Chen Y, Huang L, Lin J, et al. Chromatographic fingerprint and quantitative analysis of seven bioactive compounds of Scutellaria barbata. Planta Med. 2011 Mar;77(4):388–93.
16. Das S, Das J, Paul A, Samadder A, Khuda-Bukhsh AR. Apigenin, a bioactive flavonoid from Lycopodium clavatum, stimulates nucleotide excision repair genes to protect skin keratinocytes from ultraviolet B-induced reactive oxygen species and DNA damage. J Acupunct Meridian Stud. 2013 Oct;6(5):252–62.
17. Brunschwig C, Leba L-J, Saout M, Martial K, Bereau D, Robinson J-C. Chemical Composition and Antioxidant Activity of Euterpe oleracea Roots and Leaflets. Int J Mol Sci. 2016 Dec 29;18(1).
18. Scherbakov AM, Andreeva OE. Apigenin Inhibits Growth of Breast Cancer Cells: The Role of ERα and HER2/neu. Acta Naturae. 2015;7(3):133–9.
19. Ai X-Y, Qin Y, Liu H-J, Cui Z-H, Li M, Yang J-H, et al. Apigenin inhibits colonic inflammation and tumorigenesis by suppressing STAT3-NF-κB signaling. Oncotarget. 2017 Nov 21;8(59):100216–26.
20. Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: anti-cancer effects and mechanisms of action. Cell & Bioscience [Internet]. 2017 [cited 2020 Jun 28];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5629766/
21. Stacks NM. Apigenin and Naringenin: Natural Sources, Pharmacology and Role in Cancer Prevention. 2015.
22. Zhao G, Han X, Cheng W, Ni J, Zhang Y, Lin J, et al. Apigenin inhibits proliferation and invasion, and induces apoptosis and cell cycle arrest in human melanoma cells. Oncol Rep. 2017 Apr;37(4):2277–85.
23. Cao H-H, Chu J-H, Kwan H-Y, Su T, Yu H, Cheng C-Y, et al. Inhibition of the STAT3 signaling pathway contributes to apigenin-mediated anti-metastatic effect in melanoma. Sci Rep. 2016 Feb;6(1):21731.
24. Danciu C, Oprean C, Coricovac DE, Andreea C, Cimpean A, Radeke H, et al. Behaviour of four different B16 murine melanoma cell sublines: C57BL/6J skin. Int J Exp Pathol. 2015 Apr;96(2):73–80.
25. Avram S, Coricovac D-E, Pavel IZ, Pinzaru I, Ghiulai R, Baderca F, et al. Standardization of A375 human melanoma models on chicken embryo chorioallantoic membrane and Balb/c nude mice. Oncology Reports. 2017 Jan;38(1):89–99.

26. Ribatti D, Vacca A, Roncali L, Dammacco F. The chick embryo chorioallantoic membrane as a model for in vivo research on angiogenesis. Int J Dev Biol. 1996 Dec;40(6):1189–97.
27. Ghiţu A, Schwiebs A, Radeke HH, Avram S, Zupko I, Bor A, et al. A Comprehensive Assessment of Apigenin as an Antiproliferative, Proapoptotic, Antiangiogenic and Immunomodulatory Phytocompound. Nutrients. 2019 Apr 16;11(4):858.
28. Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, et al. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. Int J Cancer. 2000 Aug 15;87(4):595–600.
29. Fang J, Zhou Q, Liu LZ, Xia C, Hu X, Shi X, et al. Apigenin inhibits tumor angiogenesis through decreasing HIF-1α and VEGF expression. Carcinogenesis. 2007 Oct;28(4):858–64.
30. Shankar E, Goel A, Gupta K, Gupta S. Plant Flavone Apigenin: an Emerging Anticancer Agent. Current Pharmacology Reports. 2017 Dec 14;3(6):423–46.
31. Ye Y, Wang H, Chu JH, Chou GX, Yu ZL. Activation of p38 MAPK pathway contributes to the melanogenic property of apigenin in B16 cells. Vol. 20, Experimental Dermatology. 2011. p. 755–7.
32. Xu L, Zhang Y, Tian K, Chen X, Zhang R, Mu X, et al. Apigenin suppresses PD-L1 expression in melanoma and host dendritic cells to elicit synergistic therapeutic effects. J Exp Clin Cancer Res. 2018 Dec;37(1):261.
33. Ye Y, Chou G-X, Wang H, Chu J-H, Yu Z-L. Flavonoids, apigenin and icariin exert potent melanogenic activities in murine B16 melanoma cells. Phytomedicine. 2010 Dec;18(1):32–5.
34. Nasr Bouzaiene N, Chaabane F, Sassi A, Chekir-Ghedira L, Ghedira K. Effect of apigenin-7-glucoside, genkwanin and naringenin on tyrosinase activity and melanin synthesis in B16F10 melanoma cells. Life Sciences. 2016 Jan;144:80–5.

Preparation and characterization of ear drop solutions based on herbal extracts



Marin A., Marin A.M., Mot I.C., Horhat D., Trandafir C., Guran K., Chioreanu A., Balica N.C., Poenaru M.

"Victor Babes" University of Medicine and Pharmacy, Faculty of Medicine, 2nd Eftimie Murgu Sq., Timisoara-300041, Romania

Correspondence to:
Name: Balica N.
Address: Bd. Revolutiei 6., Timisoara
Phone: +40 746288006
E-mail address: balica@umft.ro

Abstract

Otitis is an infection in the ear caused by bacteria that causes pain and discomfort. If not treated in time, long-term complications can occur. An ear infection affects the middle ear; they are more common in children. Unlike the synthetic drugs, the herbal products have a complex composition, being complex mixtures of bioactive compounds. Aim and objectives: The main aims of this research were to develop a novel ear drop solution based on different phytocompounds and to characterize it by specific methods. Material and Methods: Mixed solutions based on Aloe vera and Tea tree extracts have been obtained and they have been characterized by pH measurements, UV-Vis spectroscopy, *in vitro* cytotoxicity and efficacy evaluations. Results and Conclusion: The present results indicate the obtaining of an ear drop solution that can be used in further clinical trials.

Keywords: Aloe vera, mesenchymal stem cells, MTT technique, otitis, pH, Tea tree, UV-Vis

INTRODUCTION

The ear has a very important role in terms of communication and socialization with other people, while also having a function in balance and spatial direction. The acoustic-vestibular apparatus has several segments, namely: a peripheral segment consisting of the outer ear, the middle ear and the inner ear; an intermediate segment represented by the acoustic and vestibular pathway; a central segment represented by the cortical and subcortical hearing centres and the balance centres. The peripheral auditory system is divided into three components, namely: the outer ear made up of the earlobe and the external auditory canal; the middle ear made up of the tympanic membrane, the ossicular chain, the muscles of the middle ear and the pneumatized portion of the mastoid; the inner ear located at the level of the temporal rock that is divided in the vestibule, the system of the vestibular semi-circular canals and the cochlea defined as the auditory organ. On the other hand, the middle ear is made up of the eardrum, the Eustachian tube, and the mastoid cells, while the inner ear is located at the level of the temporal rock and has two parts (one for the acoustic organ and one for the vestibular organ). It consists of multiple interconnected channels that are generically called labyrinths [1-3].

Aim and objectives

Ear pain is a painful sensation with auricular location; it can be felt as pressure, stabbing, stinging, throbbing, etc. and it may have variable intensity. Ear pain often scares the patient, who goes to the doctor. Of course, there may be mild, transient pain that resolves without treatment, but a persistent pain that does not yield to symptomatic treatment or that is accompanied by other manifestations such as fever, dizziness, balance disorders, ear discharge, should worry us and make us see a doctor [4]. There are three major groups of otitis, corresponding to the location of inflammation: external, medium and internal. Otitis externa is the inflammation of the earlobe and / or external auditory canal (the canal leading to the eardrum). Otitis media is the inflammation of the eardrum and of the tympanic cavity (a small chamber, located behind the eardrum, normally full of air, which in otitis media fills with fluids - serum, mucus or pus). Otitis media, less common, includes inflammation of the cochlear and vestibular labyrinth [5].

Man has always used plants for healing and almost as soon as he learned to write he recorded descriptions of their healing properties in different "handbooks on plants". The first known data was written almost 5,000 years ago during the Chinese Emperor Chi'en Nung; it was called Pen Tsao and it contains the descriptions of the medicinal uses of over 300 plants. By 2000 BC, the ancient Egyptians used plants in medicine, cosmetics and embalming; Greeks and Romans have perfected some of these techniques and developed new ones of their own [6]. They learned about their studies from the writings of Hippocrates in the fifth century BC and from the books "De Materia Medica" by Dioscorides and "Naturalis Historia" - 37 volumes by Pliny the Elder (both from the first century AD).

This paper describes the obtaining and the preliminary characterization of mixed solutions based on Aloe vera and Tea tree extracts, that can be used in the otitis treatment.

MATERIAL AND METHODS

The obtaining of extracts: Vegetal material (leaves of Aloe vera and Tea tree - *Melaleuca alternifolia*) was kindly donated by our colleagues from the Biology Dept. of West University Timisoara; they have previously analysed and labelled the samples. The material was rapidly dried at 90 ^{o}C for 48 hours and the dried material was deposed in paper boxes, in darkness, at room temperature.

The phytocompounds from this vegetal material were extracted using 70 % ethanol (dried material/ solvent ratio = 1:5, w/v) for 12 hours. Then, the mixture was filtered and centrifuged at 800 rpm for 15 minutes. The two extracts were concentrated using a rotavapor at 85 ^{o}C until constant weight. This procedure was repeated two times in order to obtain different extracts - samples Ex_1 and Ex_2 (Table 1).

Table 1. The ratios between the raw materials

Sample code	Dried vegetal material, mg		Solvent
	Aloe vera	Tea Tree	70 % ethanol, mL
Ex_1	5	10	75
Ex_2	10	5	75

Preparation of the ear drop solution: Aqueous solutions (1:100, w/v) of vegetal extracts were prepared to evaluate their properties, their toxicity, and their efficacy; a sterile saline solution (9 mg/mL NaCl) was used as the solvent. Polypropylene sterile vials containing these solutions were kept at room temperature before any evaluation [7].

Characterization of the extracts: The pH values of the samples based on the ear drops solutions were determined using a HI 2221 (Hanna Instruments, USA) with a combined electrode (a glass electrode and a calomel reference electrode) at 25 oC. Three standard buffer solutions (pH=4.50, 7.00, and respectively 9.50, at 25°C) were previously used to calibrate the instrument. The electrode was rinsed repeatedly with distilled water and dried prior to pH measurement.

The presence of the main phytochemicals was controlled using a UVi Line 9400 (SI Analytics, Germany); the main components of Aloe vera extract (phenols, around 70 %) have been determined at 765 nm according to O.A. Wintola et al. [8], while myrtenal from Tea tree extract have been determined at 255 nm according to G. V. Buxton et al. [9].

In vitro evaluations: Bone marrow was obtained from patients admitted to The County Clinical Hospital Timisoara (Romania), who have been submitted for bones' surgery; the protocol was previously reviewed and approved by our Ethical Committee. The volunteers were informed about the goal of this study and they signed an Informed Consent according to the Helsinki Declaration. 10.0 mL bone marrow, as source for mesenchymal stem cells (MSCs), was diluted with phosphate-buffered saline (PBS), centrifuged, and placed in a proliferation medium (Dulbecco modified Eagle's medium - high glucose, with 4.5 mg/mL glucose, L-glutamine, and sodium bicarbonate) supplemented with 10 % Fetal calf serum (FCS), 10 ng/mL fibroblast growth factor, and 2% mixture of penicillin/streptomycin in plastic culture plate specific for adherent cell culture. These were incubated at 37 oC and the medium was replaced after 48 hours with a fresh one; the plates were washed after 7 days using PBS and the medium was replaced every 4 days; the cells were passed until they reached 90 % confluence. The culture plate was washed again with PBS and preheated Trypsin-EDTA was added to act on the incubated MSCs after that the microscope was used to observe their separation. The cells were counted using Trypan Blue as vital dye and depending on their number, there were distributed and reinoculated in other culture to ensure optimal proliferation according to M.F. Munteanu et al. [7].

Efficacy evaluation: The murine macrophage cell line RAW 264.7 was achieved from the American Type Culture Collection (ATCC, USA). The cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 5.5 % heat-inactivated FCS, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a 5 % CO2 incubator at 37 °C. The viability of cells treated with the samples was evaluated by the MTT technique (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay). This method assesses the activity of mitochondrial dehydrogenase from metabolically viable cells. The effect can be quantified through a colorimetric reaction in which the MTT (yellow compound) is reduced by viable cells to formazan (dark blue compound) according to a previous study [10].

Statistics: All the measurements from this research were done in triplicate for each sample; the results were expressed as mean ± standard error. Paired Student's t tests or One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between different experimental and blank groups. $p < 0.05$ was considered statistically significant; *, ** and *** indicate $p<0.05$, $p<0.01$ and <0.001.

RESULTS AND DISCUSSIONS

The pH of samples that are biomedical applications is a very important parameter because it may modify the therapeutic activity, solubility, stability and comfort to the patient [11]. The following pH values of the solutions have been found: 6.83±0.09 (Ex_1), and 6.88±0.22 (Ex_2). The present values are proper for solutions with a possible application as otitis treatment.

Table 2 describes the results of the UV-Vis characterization. Different absorption levels were found for the investigated compounds (phenols from Aloe vera and respectively myrtenal from Tea tree extract).

Table 2. The absorption of investigated phytocompounds

Sample code	Absorption values	
	Phenols	myrtenal
Ex_1	0.82 ± 0.06	1.37 ± 0.11
Ex_2	1.21 ± 0.14	0.54 ± 0.09

The molar extinction coefficient for the oxidized Alamar Blue at 570 and 600 nm, respectively the molar extinction coefficient for the reduced Alamar Blue at the same wavelengths and the absorbance of tested cells were used based on a formula that was described in the literature [12]. Figure 1 presents the cytotoxicity evaluations of the samples tested on MSCs at 24- and 48-h.

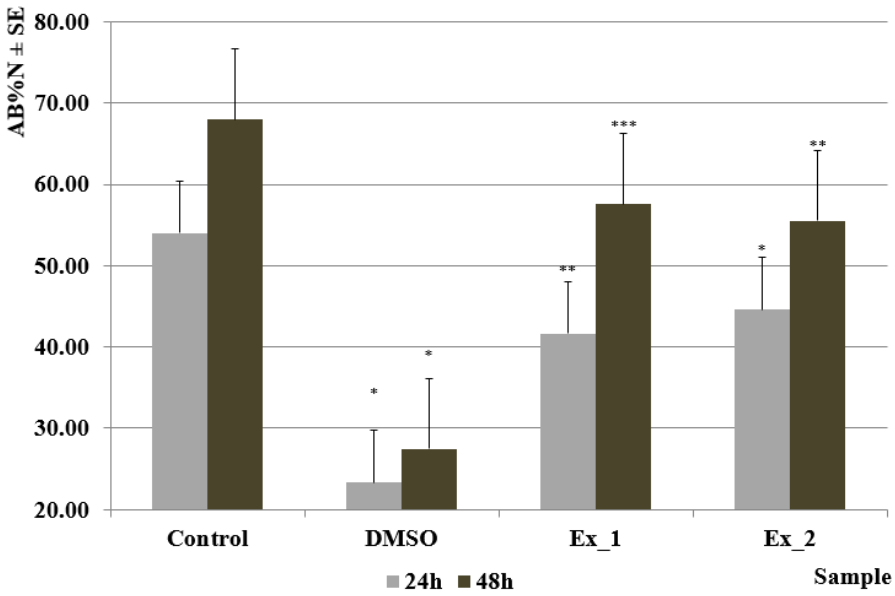


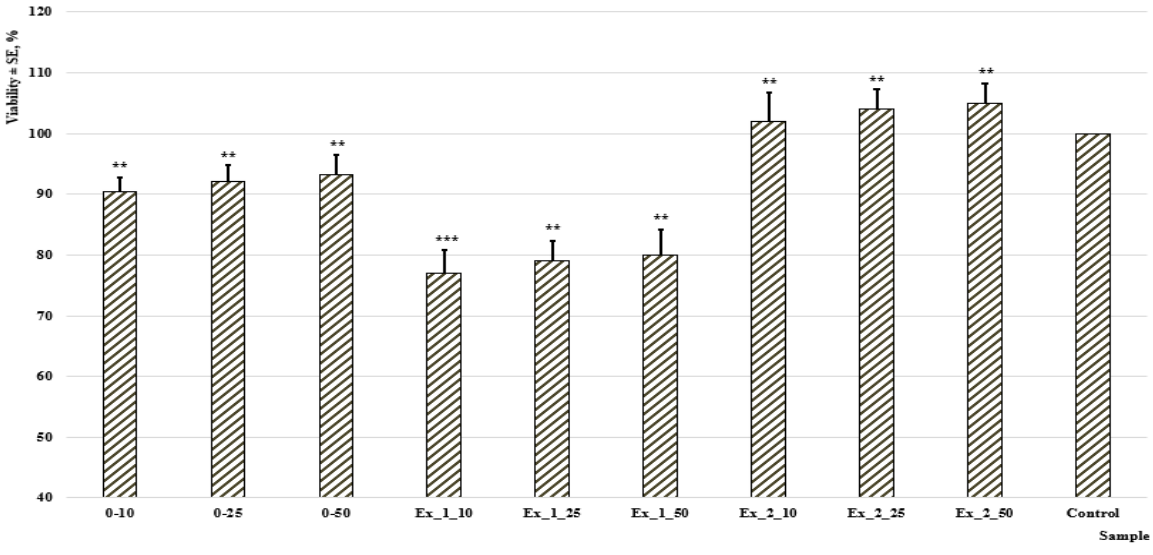
Figure 1. The cytotoxicity potential of the tested samples compared to control and DMSO

The results from the *in vitro* evaluation (Figure 1) show that the tested extracts had no major toxic activity on MSCs. After 24 and 48 h, the first extract (with an increased amount of tea tree leaves) reduced the viability of cells with around 15 % compared to control, but the best results of cells' viability were obtained in the case of the second extract.

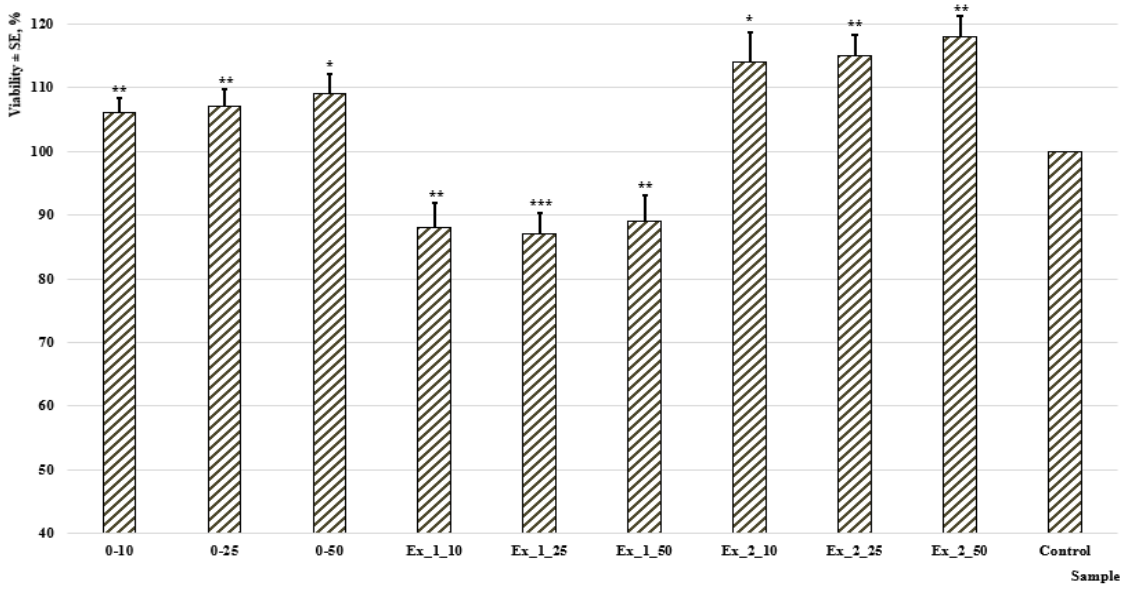
The popular murine macrophage cell line, RAW 264.7, is often used to initially screen natural products for bioactivity and to predict their anti-inflammatory effects [13]. The cells were stimulated with different volumes of tested compounds (10, 25 and respectively 50 µl). Figure 2 shows that the second extract (with an increased quantity of Aloe vera) presents the best anti-inflammatory effect compared to control sample and to the other sample.

The viability of the RAW 264.7 cells was greater after the treatment with the second extract, versus control. By contrast, in the case of RAW cells treated with the first extract, the numbers were reduced (Figure 2A and B). These results indicate that the second extract induced RAW 264.7 cell proliferation, whereas the first extract probably had the opposite effect, suggesting that these increases may indicate increases in antigen presenting cells and enhanced cellular immunity.

On the other hand, it is important to mention that both samples based on these extracts indicate that the obtained effects are directly proportional to the concentration of samples.



A)



B)

Figure 2. The efficacy of the tested samples as anti-inflammatory agents at (A) 48-h and (B) 72-h.

CONCLUSIONS

Otitis media can affect people of any age. Its typical symptoms include pressure sensation, ear pain and hearing loss; the existing treatments are effective with a good chance of cure, but also with the help of some natural methods. In this study, two different samples based on herbal extracts (Aloe vera and Tea tree) have been obtained and characterized. The comparative analysis revealed that the best effects were obtained in the case of the sample with an increased amount of Aloe vera. The present results indicate that an ear solution based on a mixture of Aloe vera and Tea tree extract can be used in the management of otitis, that continues to affect millions of people around the world annually.

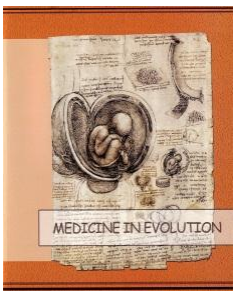
COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Jones SM, Jones TA, Mills KN et al. Anatomical and Physiological Considerations in Vestibular Dysfunction and Compensation. Semin Hear. 2009; 30(4): 231-241. doi:10.1055/s-0029-1241124.
2. Physiopedia contributors. Vestibular System. Available from: https://www.physio-pedia.com/index.php?title=Vestibular_System&oldid=230178. Last accessed: July 29, 2020.
3. Casale J, Browne T, Murray I, et al. Physiology, Vestibular System. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532978. Last accessed: July 30, 2020.
4. Bakaletz LO. Otitis Media. Available from: https://www.ncbi.nlm.nih.gov/ books/NBK2482. Last accessed: July 30, 2020.
5. Klein JO. Otitis Externa, Otitis Media, and Mastoiditis. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2015; 767-773.e1. doi:10.1016/B978-1-4557-4801-3.00062-X.
6. Gadea S, Vatca S, Vatca A. The use of medicinal plants in human civilisation. Agiculture – Science and Practice. 2016; 3-4: 46-50.
7. Munteanu MF, Ardelean A, Borcan F et al. Mistletoe and Garlic Extracts as Polyurethane Carriers – A Possible Remedy for Choroidal Melanoma. Curr. Drug Deliv. 2017; 14(8): 1178-1188.
8. Wintola OA, Afolayan AJ. Phytochemical constituents and antioxidant activities of the whole leaf extract of Aloe ferox Mill. Pharmacogn Mag. 2011; 7(28): 325-333. doi:10.4103/0973-1296.90414.
9. Buxton GV, Salmon GA, Williams JE. The Reactivity of Biogenic Monoterpenes towards OH. And SO4-. Radicals in De-Oxygenated Acidic Solution. J. Atmosph. Chem 2000; 36: 111-134.
10. Popovici RA, Vaduva D, Pinzaru I, et al. A Comparative Study On The Biological Activity Of Essential Oil And Total Hydro-Alcoholic Extract Of Satureja Hortensis L. Exp. Ther. Med. 2019; 18: 932-942.
11. Banker GS, Siepmann J, Rhodes C. Modern Pharmaceutics, 4th ed. Boca Raton: CRC Press; 2002.
12. McBride J, Ingram PR, Henriquez FL et al. Development of colorimetric microtiter plate assay for assessment of antimicrobials against Acanthamoeba. J. Clin. Microbiol. 2005; 43(2): 629-634.

Chronic inflammation – the link between periodontal and cardiovascular disease



Motoc G.[1], Popa A.R.[2], Sava-Rosianu R.[3], Cosoroaba R.[3], Marian R.[3], Tigmeanu C.V.[3], Alsaeyd Ahmad Mhd Kher[3], Motoc O.[4]

[1]*Faculty of Medicine and Pharmacy from the University of Oradea, Doctoral School of Biomedical Sciences, Oradea, Bihor County, Romania*
[2]*Clinical Emergency Hospital of Oradea, Internal Medicine-Diabetes Department, Oradea, Bihor County, Romania*
[3]*I Department, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania*
[4]*Faculty of Medicine and Pharmacy from the University of Oradea, Oradea, Bihor County, Romania*

Correspondence to:
Name: Ruxandra Sava Rosianu
Address: Splaiul Tudor Vladinirescu nr. 14A, University of Medicine and Pharmacy "Victor Babes" Timisoara
Phone: +40 740315848
E-mail address: savarosianu@yahoo.com

Abstract

Coronary heart disease (CHD) is the leading cause of death and morbidity in many developed countries. Many risk factors for CHD have been identified, but a significant proportion of CHD is not explained by traditional risk factors. Recently, several lines of evidence have implicated chronic inflammation etiologically in CHD and cardiovascular disease (CVD). Periodontal disease is a chronic gram-negative anaerobic infection of the tooth-supporting structures. Periodontal disease is associated with elevations of several markers of chronic inflammation, and because of evidence implicating chronic inflammation in the etiology of CHD, an etiologic relationship between periodontal disease and CVD has been hypothesized. For these reasons, there has been strong interest in evaluating whether periodontal disease is independently associated with CVD.

Keywords: periodontal disease, cardiovascular disease, coronary heart disease, chronic inflammation, oral health

INTRODUCTION

Periodontitis is a family of diseases that affect dental supporting tissues, caused by infections sustained by periodontal pathogens such as Porphyromonas gingivalis, Prevotella intermedia, Tannarella forsythia, and Aggregatibacter actinomycetmcomintans, which lead to soft and hard tissue destruction, dental mobility, and the loss of dental elements [1]. Susceptibility to these diseases is highly variable and depends on host responses to periodontal pathogens. Although bacteria cause plaque-induced inflammatory periodontal disease, the progression and clinical characteristics of these diseases are influenced by both acquired and genetic factors that can modify susceptibility to infection [2].

Periodontal disease is a chronic gram-negative anaerobic infection of the tooth-supporting structures with an estimated prevalence of as high as 75% in adults in the US, among whom approximately 20–30% have severe forms of the disease [3-5] Alveolar bone resorption is both a measure and a consequence of severe periodontal disease. Common signs of periodontal disease that are identified by dentists and may be noted by primary care providers include: tooth loss, gingivitis with gum inflammation and bleeding, excess tartar, infection, decay, tooth mobility, and gum recession with bone loss. [boala paro]

Periodontitis depends on host responses to periodontal pathogens. The initial increased presence of neutrophils at the site is followed by the release of cytokines by neutrophils and macrophages; the chemical mediators released include tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and prostaglandins.

The inflammatory process includes the stimulation of fibroblasts by IL-1 and the secretion of matrix metalloproteinases (MMP), of which collagenase is the most prominent, by polymorphonuclear neutrophils. MMPs are responsible for increased collagen breakdown, and TNF-α is primarily responsible for increased osteoclast activity resulting in bone resorption. T-lymphocytes secrete receptor activator of nuclear factor kappa-B ligand (RANKL), which is involved in osteoclast activity and, therefore, bone resorption [6].

Periodontitis has also been associated with elevations in circulating levels of IL-6 and C-reactive protein (CRP). IL-6 is an important pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection. It is produced by a variety of cells, such as monocytes, fibroblasts, osteoblasts, and vascular endothelial cells, in response to inflammatory challenges. Moreover, it is widely accepted that IL-6 induces CRP production.

In addition, a significant overexpression of IL-21, IL-1β, IL-17, and IL-23p19 has been detected in tissues affected by periodontal disease compared with healthy gingival tissues. In particular, IL-21 is overexpressed in chronic periodontitis gingival tissues and is correlated with the clinical parameters of periodontal destruction and with pro-inflammatory cytokines [10]. A negative modulatory role of IL-4 and IL-13 in osteotropic cytokine production could be a mechanism that plays an important inhibitory role in inflammation induced periodontitis. In facts the activation of STAT6 by IL-4 and IL-13, through type 2 IL-4 receptors, seems to inhibit the production of IL-11 and leukemia inhibitory factor stimulated by IL-1β and TNF-α in human gingival fibroblasts [7].

Also, IL-10 and tumour growth factor-β1 (TGF-β1) are down-regulated in periodontal lesions. Generalized aggressive periodontitis subjects are characterized by a higher IL-1β/IL-10 ratio than are periodontal healthy subjects, suggesting an imbalance between pro- and anti-inflammatory cytokines in generalized aggressive periodontitis. IL-10 is also associated with periodontal health and seems to be a regulator of inflammation and alveolar bone loss in periodontal diseases. It might be involved in controlling the inflammatory process at periodontal healthy sites [7].

Coronary heart disease (CHD) is the leading cause of death and morbidity in many developed countries. Worldwide, CHD kills more than 7 million people each year [8]. Many risk factors for CHD have been identified, but a significant proportion of CHD is not

explained by traditional risk factors. Recently, several lines of evidence have implicated chronic inflammation etiologically in CHD and cardiovascular disease (CVD) [9].

Aim and objectives

In this systematic review, we evaluate the epidemiologic literature evaluating the possible link between periodontal disease and associated measures of oral health, and CHD. Identifying individuals at higher risk for CVD than predicted by traditional risk factors could facilitate more aggressive treatment of risk factors known to decrease CHD in high-risk individuals, such as those with hyperlipidaemia.

MATERIAL AND METHODS

CARDIOVASCULAR DISEASE AND PERIODONTITIS

Cardiovascular diseases are a leading cause of morbidity and mortality in developed countries. The disease process that underlies the majority of cardiovascular events is atherosclerosis, an inflammatory disease of the blood vessel wall. The earliest physical evidence of atherosclerosis are fatty streaks, which are typically present in childhood.

In the presence of arterial endothelial dysfunction, which is involved in the initiation and progression of atherosclerosis, these early lesions progress through to complex atheromatous lesions in adulthood, finally resulting in occlusion, plaque rupture and ischaemic events [10].

Periodontal disease is inflammation of the tissues surrounding teeth and results from a complex interplay between bacteria and host risk factors such as long-term smoking, poor oral hygiene, poorly controlled diabetes, stress and genetic predisposition [11]. Not only have periodontal organisms adapted to survive within an environment that is constantly besieged by host defences, but they flourish in the presence of inflammation, enabling their capacity to invade host tissues and gain direct access to the circulation [12]. Repeated bacteremias and endotoxemias are characteristic of periodontal infection, and periodontal organisms have been found to co-localise within atheromatous plaques [13]. The constant exposure of the vasculature to these pathogens provides an opportunity for endothelial inflammatory activation and functional impairment. Clinically, periodontal disease manifests as deepening of the epithelial attachment around teeth, loss of periodontal attachment and, ultimately, tooth loosening.

Periodontal disease has been associated with atherosclerosis [14], cardiovascular disease [15], diabetes [16], pre-term low birth weight [17], stroke [18], and premature death [19]. Accordingly, periodontal disease may account for a portion of the risk for cardiovascular disease via a shared pathogenic underlying inflammatory response [10].

Treating periodontal disease results in a functional improvement in cardiovascular status [20-23]. These studies are consistent with the concept that periodontal disease may be an important source of infectious and inflammatory vascular stress, and that periodontal therapy may be of particular clinical relevance in populations with high prevalence of both periodontal disease and cardiovascular disease.

HYPERTENSION AND PERIODONTITIS

Hypertensive patients suffering from metabolic syndrome show increased oxidative stress and compromised antioxidant activity in plasma and cells [24,25]. In addition, obesity and overweight are strictly related to hypertension. In fact, weight loss determines a diminished blood pressure independent from sodic diet [95]. Moreover, hyperglycemia and hypertension are strictly related. Hyperglycemia provokes an increased stimulation of a sympathetic nervous system that causes vasoconstriction and increased sodium reabsorption with consequent water attraction and insurgence of hypertension, which damages the endothelium integrity of vessels [26]. Augmented endothelial permeability allows the passage of lipoproteins and platelet-derived growth factors (PDGF), which give rise to the

proliferation of muscular smooth cells in the intima, which occludes vessel lumen and causes emboli, hypoxia, and consequent cellular death [27].

It also seems that periodontitis can influence some types of hypertension [28]. Several studies have taken into consideration the relationship between hypertension and periodontitis, although an association between periodontal disease measures and incident hypertension in cohort studies has not yet been evidenced. In a sample of 31,543 participants of the Health Professionals' Follow-Up Study, based on a prospective cohort of 40- to 75-year-old men at baseline, with no prior hypertension history and complete baseline information on oral health, an incidence of 10,828 cases of hypertension over 20 years of follow-up was identified, with no significant association between incident hypertension and periodontal disease [29].

Although statistical evidence is lacking, a clinical relation between high blood pressure and aggressive periodontitis has been deduced, as patients with poor oral hygiene have higher blood pressure problems than do healthy subjects with good oral hygiene condition [30].

Regarding the biological mechanism of this relationship, a recent study evaluated endothelial function in patients with periodontitis. Circulating levels of CRP and IL-6 were significantly higher in the periodontitis subjects with hypertension, than in the control group. Periodontal therapy seems to reduce serum concentrations of CRP and IL-6 [31].

PERIODONTAL DISEASE AND METABOLIC SYNDROME

As already mentioned, metabolic syndrome is a syndrome characterized by several signs that together seriously compromise the health of an individual. It is clear that the common denominator of the member pathologies of Metabolic syndrome is oxidative stress and the consequent hyperinflammation that primes chain interactions and leads to grave systemic complications, such as CVD, or local complications, such as periodontitis. Metabolic syndrome allows a pro-oxidative state in periodontal tissue, altering antioxidant defense mechanisms. This adversely affects tissular response against bacterial plaque attack.

On the contrary, periodontitis, being a great source of oxidative markers, promotes the onset of insulin resistance and metabolic syndrome in a vicious circle [32]. Chronic inflammation during old age periodontitis causes increased neutrophil defense activity, which involves increased oxidative activity, resulting in peroxidation and oxidative stress. In fact, both metabolic syndrome and periodontitis show increased serum rates of oxidative stress markers [33,34]. Regarding the oxidative stress markers found in periodontitis, individuals with periodontal disease exhibit a significant increase in the activities of oxidative stress markers. The increase in glutathione peroxidase may represent possible antioxidant compensation in detoxification reactions of organic peroxides produced during oxidative stress in gingival tissue. Since glutathione S-transferase (GST) has a direct role in the neutralization of hydroperoxides derived from the lipoperoxidation processes, increases in GST activities are probably related to the oxidative stress caused by the periodontal inflammatory process. GST comprises a group of enzymes that are also able to detoxify a variety of compounds, including xenobiotics derived from pathogenic microorganisms. Hence, increases in GST activities are excellent indicators of endogenous detoxification from exogenous sources. Myeloperoxidase activity in gingival tissue has shown a significant increase in patients with periodontal disease when compared with controls: this seems indicative of a chronic inflammatory process also reflected at a systemic level. A significant increase in oxidized glutathione (GSSG) concentrations has been detected in periodontitis patients, which is a clear biomarker of oxidative stress detected in inflammatory processes linked to periodontitis.

Consistent with the results for GSSG, tissue lipoperoxidation, measured as thio-barbituric acid reactive substances, seems to increase in the gingival tissue of periodontitis [35]. Periodontal diseases seem related to pathologies and conditions characterized by high

oxidative stress and by the presence of AGE, such as diabetes and physiologic aging. AGEs are able to favour chemotaxis and the production of proinflammatory mediators, to inhibit fibroblasts and osteoblasts, and to accelerate periodontal damage directly or binding their receptors RAGE [36]. Periodontitis is strictly correlated to hyperglycemia; in fact, it is also considered the sixth complication of diabetes mellitus [37]. Predialysis and hemodialysis in chronic kidney diseases are also associated with a higher prevalence of severe periodontitis compared with healthy individuals.

Chronic kidney failure is a clinical syndrome due to the slow, progressive, and irreversible loss of the glomerular filtration rate, and may be associated with several oral manifestations, such as xerostomia, uremic stomatitis, and periodontitis, diagnosed as clinical attachment loss.

Recent studies have shown an association between high levels of CRP and IL-6 and periodontitis, an association that decreases after periodontal treatment. Due to this association with the systemic inflammatory response, chronic periodontitis has recently been included as a non-traditional risk factor for chronic kidney failure [38].

In synthesis, metabolic alterations related to metabolic syndrome component diseases cause an augmented response to bacterial plaque, which favours periodontitis insurgence. It has been pointed in many studies out how periodontal treatment can reduce inflammatory mediators related to endothelial and cardio-circulatory dysfunctions [39]. A very recent work reported a real relationship between periodontitis and Metabolic syndrome, especially in women, while abdominal obesity was the largest contributory factor in both genders [40]. On the contrary, another new work about metabolic syndrome and periodontal diseases and caries did not find a strong association between metabolic syndrome and periodontal infections [41].

CONCLUSIONS

The role of dentists in the diagnosis, therapy, and management of cardiovascular patients is fundamental, but an improvement of collaboration among dentists, cardiologists, endocrinologists, nutritionists, etc., is needed.

REFERENCES

1. Andriankaja OM, Sreenivasa S, Dunford R, DeNardin E: Association beteen metabolic syndrome and periodontal disease. Aust Dent J 2010, 55:252–259.
2. Armitage GC: Diagnosis of periodontal disease. J periodontology 2003, 74:1237–1247.
3. Brown LJ, Brunelle JA, Kingman A. Periodontal status in the United States. J Dent Res. 1996; 75:672–83.
4. Douglass CW, Jette AM, Fox CH, et al. Oral health status of the elderly in New England. J Gerontol. 1993, 48:M39–46.
5. Papapanou PN. Periodontal diseases: epidemiology. Annals of Periodontology. 1996;1:1–36. In 5/5/2005
6. Di Paola R, Briguglio F, Paterniti I, Mazzon E, Oteri G, Militi D, Cordasco G, Cuzzocrea S: Emerging role of PPAR-β/δ in inflammatory process associated to experimental periodontitis. Mediators Inflamm 2011, 7:87–159.
7. Souza PP, Palmqvist P, Lundberg P, Lundgren I, Hänström L, Souza JA, Conaway HH, Lerner UH: Interleukin-4 and interleukin-13 inhibit the expression of leukemia inhibitory factor and interleukin-11 in fibroblasts. Mol Immunol 2012, 49(4):601–610.
8. Mackay J, Mensah GA. The Atlas of Heart Disease and Stroke. Geneva: WHP; 2004.
9. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Eng J Med. 2000, 342(12):836–43.

10. Pepine CJ: The effects of angiotensin-converting enzyme inhibition on endothelial dysfunction: potential role in myocardial ischemia. Am J Cardiol 1998, 82(10A):23S-27S.
11. Van Dyke TE, Sheilesh D: Risk factors for periodontitis. J Int Acad Periodontol 2005, 7(1):3-7.
12. Offenbacher S, Elter JR, Lin D, Beck JD: Evidence for periodontitis as a tertiary vascular infection. J Int Acad Periodontol 2005, 7(2):39-48.
13. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ: Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000, 71(10):1554-1560.
14. Fifer KM, Qadir S, Subramanian S, Vijayakumar J, Figueroa AL, Truong QA, Hoffman U, Brady TJ, Tawakol A: Positron emission tomography measurement of periodontal (18)f-fluorodeoxyglucose uptake is associated with histologically determined carotid plaque inflammation. J Am Coll Cardiol 2011, 57(8):971-976.
15. Katz J, Marc H, Porter S, Ruskin J: Inflammation, periodontitis, and coronary heart disease. Lancet 2001, 358(9297):1998.
16. Thorstensson H, Hugoson A: Periodontal disease experience in adult long-duration insulin-dependent diabetics. J Clin Periodontol 1993, 20(5):352-358.
17. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S: Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG 2006, 113(2):135-143.
18. Joshipura KJ, Hung HC, Rimm EB, Willett WC, Ascherio A: Periodontal disease, tooth loss, and incidence of ischemic stroke. Stroke 2003, 34(1):47-52.
19. Soder B, Jin LJ, Klinge B, Soder PO: Periodontitis and premature death: a 16-year longitudinal study in a Swedish urban population. J Periodontal Res 2007, 42(4):361-366.
20. Seinost G, Wimmer G, Skerget M, Thaller E, Brodmann M, Gasser R, Bratschko RO, Pilger E: Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. Am Heart J 2005, 149(6):1050-1054.
21. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, Suvan J, Hingorani AD, Vallance P, Deanfield J: Treatment of periodontitis and endothelial function. N Engl J Med 2007, 356(9):911-920.
22. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS: Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J 2006, 151(5):977-984.
23. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS: Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J Dent Res 2005, 84(3):269-273.
24. Abdilla N, Tormo MC, Fabia MJ, Chaves FJ, Saez G, Redon J: Impact of the components of metabolic syndrome on oxidative stress and enzymatic antioxidant activity in essential hypertension. J Hum Hypertens 2007, 21(suppl.1):68–75.
25. Chaves FJ, Mansego ML, Blesa S, Gonzalez-Albert V, Jimenez J, Tormos MC, Espinosa O, Giner V, Iradi A, Saez G, Redon J: Inadequate cytoplasmic antioxidant enzymes response contributes to the oxidative stress in human hypertension. Am J Hypertens 2007, 20(supp 1):62–69.
26. Scott CL: Diagnosis, prevention, and intervention for the metabolic syndrome. Am J Cardiol 2003, 92:35i–42.
27. Arthur H, Friedlander DMD, Jane Weinreb MD: Metabolic syndrome: pathogenesis, medical care and dental implications. Clinical pactice 2007, 138:179–187.
28. Angeli F, Verdecchia P, Pellegrino C, Pellegrino RG, Pellegrino G, Prosciutti L, et al: Association between periodontal disease and left ventricle mass in essential hypertension. Hypertension 2003, 41:488–492.
29. Rivas-Tumanyan S, Spiegelman D, Curhan GC, Forman JP, Joshipura KJ: Periodontal disease and Incidence of Hypertension in the Health Professionals Follow-Up Study. Am J Hypertens 2012, 25(7):770–776. 100.
30. Rivas-Tumanyan S, Campos M, Zevallos JC, Joshipura KJ: Periodontal Disease, Hypertension and Blood Pressure Among Older Adults in Puerto Rico. J Periodontol 2012.
31. Higashi Y, Goto C, Jitsuiki D, Umemura T, Nishioka K, Hidaka T, Takemoto H, Nakamura S, Soga J, Chayama K, Yoshizumi M, Taguchi A: Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. Hypertension 2008, 51(2):446–453.
32. Chapple IL, Brock GR, Milaward MR, et al: Compromised GCF total antioxidant capacity in periodontitis. Cause or effect? J Clin Periodontol 2007, 34:103–110.

33. Brock GR, Matthews JB, Butterworth CJ, Chapple ILC: Local and systemic antioxidant capacity in periodontitis and health. J Clin Periodontol 2004, 31:515–521.
34. Montebugnoli L, Servidio D, Miaton RA, Prati C, Tricoci P, Melloni C: Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factors. J Clin Periodontol 2004, 31:25–29.
35. Borges I Jr, Moreira EA, Filho DW, de Oliveira TB, da Silva MB, Fröde TS: Proinflammatory and oxidative stress markers in patients with periodontal disease. Mediators Inflamm 2007, 4:57–94.
36. Alikhani M, Alikhani Z, Boyd C, MacLellan CM, Raptis M, Liu R, et al: Advanced glycation endproducts stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. Bone 2007, 40:345–353.
37. Offenbacher S, Madianos PN, Champagne CM, Southerland JH, Paquette DW, Williams RC, et al: Periodontitis-atherosclerosis syndrome: an expanded model of pathogenesis. J Periodont Res 1999, 34:346–352.
38. Vilela EM, Bastos JA, Fernandes N, Ferreira AP, Chaoubah A, Bastos MG: Treatment of chronic periodontitis decreases serum prohepcidin levels in patients with chronic kidney disease. Clinics (Sao Paulo) 2011, 66:657–662.
39. Tonetti MS, D Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al: Treatment of periodontitis and endothelial function. N Engl J Med 2007, 356:911–920.
40. Chaffee BW, Wetson SJ: Association between chronic periodontal disease and obesity: a systematic review and meta-analysis. J Periodontol 2010, 81:1708–1724.
41. Timonen P, Niskanen M, Suominen-Taipale L, et al: Metabolic syndrome, periodontal infection and dental caries. J Dent Res 2010, 89:1068–1073.
42. Ciprian Pasca, Emil Urtila, Angela Codruta Podariu, Ruxandra Sava-Rosiuanu, Ioana Mihaela Citu, Adelina Ramona Berari, Teodora Eva Lupulescu, Atena Galuscan, Daniela Jumanca, Ramona Amina Popovici, "Correlation between periodontal disease and acute miytocondrial infarctation", Medicine in Evolution, vol XX, nr.2, 2014, p 294- 300;
43. Ovidiu Motoc, Angela Codruța Podariu, Atena Găluşcan, Daniela Jumanaca, Ruxandra Sava Roşianu, Anca Porumb, Florina Andrica, Ramona Amina Popovici, "The alterations of salivary glands secretion in diabetes mellitus", Medicine in Evolution, vol XXI, nr.1, 2015, p 169-174

Meine Zukunft in Deutschland.

Der Start als Zahnarzt in Deutschland beginnt mit uns.

Ob eigene Praxis oder angestellter Zahnarzt
Mit unseren Kenntnissen und Erfahrungen stehen wir Ihnen in allen Fragen zur Verfügung. Wir helfen Ihnen beim Umgang mit Behörden, dem Erlangen einer Kassenzulassung, der Standort- und Praxissuche, der Beschaffung des Startkapitals und Finanzierungen jeglicher Art.

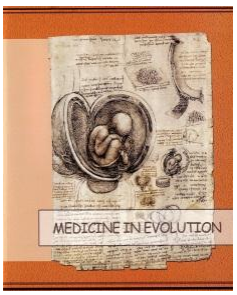
Als führendes Dental-Labor in Deutschland in den Bereichen **CNC-Fräsung**, **digitale Fertigung**, **3D-Druck** und **Intraoral-Scannen** bieten wir ein großes und hoch- modernes Portfolio an dentalen Leistungen an. Nehmen Sie Kontakt zu uns auf. **Wir kümmern uns um Ihre Formalitäten.**

HARTL GmbH
DENTALE TECHNOLOGIEN

Renthof 1
34117 Kassel
Deutschland

Fon: +49 561 - 560 24
+49 561 - 570 390
Email: hartl@web.de

Fluoride prophylaxis through nutrition



Cuc A.[1], Porumb A.[1]*, Sava-Rosianu R.[2]

[1]Department of Dental Medicine, Faculty of Medicine and Pharmacy, University from Oradea, Romania
[2]I Department, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania

Correspondence to:
Name: Porumb Anca
Address: Department of Dental Medicine, Faculty of Medicine and Pharmacy, University from Oradea, Romania Decembrie 1st Square no.10, 410068, Oradea, Bihor county, Romania
Phone: +40 726286237
E-mail address: anca.porumb@yahoo.com

Abstract

The large-scale incidence of tooth decay, the onset of dental cavities from early age, the increase of the disease rate, the multiplication of cases with multiple and galloping tooth decay, the failure of therapeutic procedures to achieve a significant reduction of morbidity, all of the aforementioned make tooth decay prophylaxis an issue of public health concern. This involves the onset of complex primary and secondary prophylaxis programmes, both at collective and individual level. The lack of a sufficient intake of natural fluoride has led to the need to supplementing with fluoride from natural sources to an effective dose, by fluoridation of water, table salt, and fluoridation of various foods.

Keywords: prophylaxis, fluoride, water, salt

INTRODUCTION

Fluoride ion is a trace element present in nature and never found in a free state, its intake through food ranging between 0.2 and 0.5 mg. The amount absorbed is dependent on both the intake and the solubility of the compound in which it is present. In addition to the intake, the age factor is another variable i.e. the new-borns and infantsretain 75% of the intake, while thetoddlers and preschoolers children retain 50%, the storage of fluoride being usually done in the hard tissues [bone and teeth] and in the kidneys.

While the bone remains for the rest of its life dependent on the excess fluoride, the tooth enamel has from a more limited period of dependency to fluoride.

The distribution of fluoride in tooth enamel is uneven, the concentration at surface of tooth being ten times higher than at the amelodentinal junction.

In the end, teeth prone to cavities would not depend entirely on higher fluoride concentrationinteeth, but on the concentration of fluoride in the most superficial layer of the surface enamel [according toFiru and Zarnea, cited by Elvira Cocarla]. [1,2]

Fixation of fluoride in the dental tissues is carried out by ion exchange, the fluoride being able to replace the –OH and –CO3 groups in the apatite crystals, thus resulting influorapatite which isfar less soluble in the acid environment.

Exogenously administered, fluoride can also contribute to the post-eruptive maturation of tooth enamel and its remineralization following acid attack. By decreasing the viscosity of saliva, it also contributes to reducing the dental plaque formation.

The antimicrobial effect is another local mechanism of the caries-preventive action of fluoride.

Aim and objectives

Specific prophylaxis means include fluoride prophylaxis, in addition to sealing dental cracks and dimples.

If the sealing of dental cracks and dimples is a procedure that can be done exclusively in specialized dental offices, fluoride prophylaxis can be done in two ways: by exogenous, and by endogenous administration.

MATERIAL AND METHODS

In this paper we aim to review and make a comparison between the main means of endogenous fluoride administration.

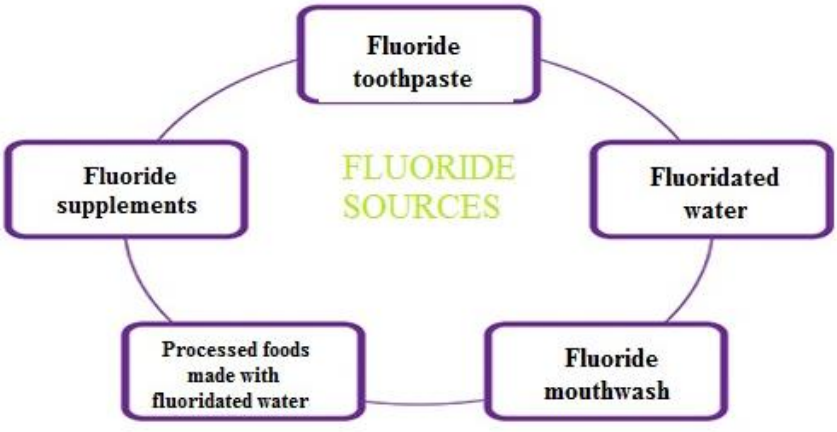


Figure 1. Fluoride sources

RESULTS AND DISCISSIONS

The natural waters in Romania, especially in the western area of the country, have, with a few exceptions, low fluoride levels, and the social and economic condition of the majority of the population do not favour expensive dental treatments. Widespread awareness among the population of the benefits of fluoridated water consumption and raising awareness among thegovernmental institutions on the benefits of this caries-preventive method could be a real method for Romania to reduce the level of decay index in the long run.

Moreover, the people diet in this area of the country is low in fluoride i.e. 0.3-0.5 mg. Children are first to benefit from fluoride intake, during the formation of primary and permanent teeth, until around the age of 14.

The addition of fluoride can be done through water, fluoride tablets, milk, salt, and bread [3]:

In the following, we will present the pros and cons of each fluoridation method.

1. **Water fluoridation:**

The main advantages of water fluoridation are [1,2]:

- Its addressability to all members of the community, regardless of age or social and economic status;
- Continuous exposure, both during pre- and post-eruptive phase, at an exactly calculated concentration of fluoride;
- High effectiveness, since it isa collective prophylactic measure,
- Long-term administration possibilities.
- Lower costs,
- Accurate and easy dosing.

Figure 2. Fluoride water

The disadvantages of water fluoridation are [1,2]:

- First of all, it requires special installations operated by qualified personnel, as well as periodic controls thereof,
- Only people who have a access to a centralized water source benefit from this method,
- There is a variation in terms of individual consumption, since the consumption of fluoridedepend on water consumption,
- Unnecessary loss of fluoride in industrial installations.

In some areas, drinking water is deficient in fluoride, and fluoridation of water should be at the core of prophylaxis programs. When deciding on prophylactic measures within a certain water, the data on concentration of fluoride in drinking áter should be already available.

We present some examples below:

- In several European countries: following a study conducted by Rock et all., cited by Elvira Cocarla, in two cities in England one benefiting from fluoridated water, and the other city – the control one – without benefiting from fluoride in water, the DMF [decay-missing-filled] indices were compared. The finding was that the morbidity was 2.5 times higher in the city with non-fluoridated water. Also, the index of extractions was 4 times higher in the same city. The same studies show that in areas where the level of fluoride is low, the following results were obtained: 50% of first permanent molars are decayed one year after the eruption and,by the age of 12, only 10% are still intact. In areas with fluoridated water, the results are satisfactory, the morbidity in permanent primary molars being low and the index extraction has been reduced by up to 75%. [1,2]
- In the USA, 50% of the population benefits from this method. One method used in this country was to fluoridate water supplied in schools. According to a study conducted by Horowitz et al., and cited by Elvira Cocarla, there was a 40% reduction in the incidence of dental decay, and a maximum protection after 12 years of implementing this method, as well as a progressive benefit. The disadvantage of this method is the late onset of fluoride administration, when the first permanent molars and permanent incisors can no longer benefit from it. The recommended doses of fluoride are increased due to consumption during school days only. [1,2]
- In Romania, in TarguMures, this method was applied and consisted in increasing the concentration up to **1 mg. Fluoride/ litter** by the addition of sodium silicofloride or sodium fluoride. Following the related studies, according toBenedek and Csongor, cited by Cocarla E. [1,2], by comparative data collected from children living in ClujNapocaand TarguMureş, on decay morbidity, they showed a decrease in intensity indices, although fluoridation was not continuous due to technical considerations.

For a good efficiency of this method it is recommended to use the method since child birth. Should the method is applied after teeth eruption, the outcomescan be observed 2-3 years after application.

2. Fluoride tablets

Fluoride tablets [supplements] are effective during the formation and mineralization of dental hard tissues. They contain sodium fluoride and are administeredto children until the age of 14. To be effective, it should be started with the administration to pregnant women and continued to be administeredto breast-feeding mothers.

The right daily dose and age for administration of fluoride tablets are presented in the table below:

Table 1. Daily dose of fluoride tablets

AGE	**DAILY DOSE**
0- 2 years old	1x1 tablet of 0.25 mg
2-4 years old	2x1 tablet of 0.25 mg
4-6 years old	3x1 tablet of 0.25 mg
over 6 years old	4x1 tablet of 0.25 mg or 1x1 tablet of 1 mg
During pregnancy	1 x tablet of 1 mg

Advantages of this method:

- It allows facile application,
- It ensures an exact dosage, depending on age.

Disadvantages of this method:

- It is difficult to perform a regular administration,
- The individual consent of potential beneficiaries is required,
- It requires a strict supervision to prevent overdoses,
- It is an expensive method.

Figure 3. Enamel dystrophies

3. Salt fluoridation

It is successfully practiced in several countries i.e. Switzerland, France, Hungary, Costa Rica, Mexico, Spain, and recently in Romania.

This method is applied where drinking water sources are poor in fluoride, mountainous areas or when water fluoridation specific sanitary policies are lacking.

Disadvantages of table salt method:

- Impossibility of individual dosing,
- It cannot be applied to the new-borns, infant and toddlers whose diet must be low in salt,
- There are large variations in terms of salt use habits,
- Its clinical efficacy is lower in comparison with other methods.

Thesalt is commercially available in packs of 1 kg, each containing 200 mg of sodium fluoride and 10 mg of iodine. In our country, iodized salt has been used to treat endemic goiter, and overthe past years fluoridated salt has been launched on the market. It was found that an average consumption of 6g of salt would provide the necessary fluoride amount to the human body. [1,2].

4. Milk fluoridation

Milk is the basic component of a baby's diet.

Milk fluoridation method is practiced in several countries i.e. Hungary, Bulgaria, Russia, China, England, Scotland.

Advantages of this method:

- It is a method recommended for children.

Disadvantages of this method:

- Implementation of a milk distribution system in schools and kindergartens, as well as the monitoring of milk consumption,
- Possibility of inactivation of fluoride ions by casein.

It is recommended to consume a glass of milk with 0.5 mg fluoride for about 1 year, the addition of fluoride being 2 mg / litter of milk. [1,2]

5. Fluoridation of bread

Before baking, the bread is soaked with a fluoride solution. No special packaging is required [1,2]

Disadvantages of this method:
Consumption rates vary from one individual to another.

CONCLUSIONS

Both the lack of fluoride in the human diet in the western part of the country, and the sources of drinking water poor in fluoride impose the need to supplement the fluoride intake. The additionif fluoride can be madethrough water, milk, bread, and salt.

When prophylactic measures are to be taken for that purpose, the data on concentration of fluoride in water and food must be available, this indicator presenting variable levels from one country area to another.

Caries preventive doses of fluoride is 1 mg Fluoride per day and represents the amount that has the maximum caries-preventive effect, with a minimum risk to onset fluorosis. It is generally considered that an intake of 0.1 mg F/ Kg prevents any side effects.

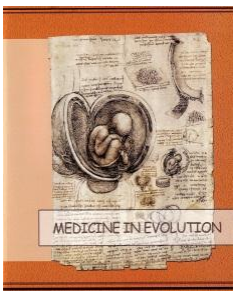
Although in the developed countriestooth decay indices have registered significant reductions as a result of the widespread use of fluorinated products, in our country these indices still have extremely high values.

REFERENCES

1. Laslau M – Notiuni de igiena şi ecologie a mediului, Editura Universitatii din Oradea, 2008, pg. 87-89.
2. Cocarla E: Stomatologie pediatrica, Editura Medicala Universitara ,, Iuliu Hatieganu,, Cluj-Napoca, 2000, pg. 186-189.
3. Cocarla E: Pedodontie, Litografia UMF Cluj-Napoca, 1992, pg. 112-115.
4. Burt B.A., Ismail A.I.: Diet, Nutrition and Food Cariogenity- J. Dent. Res., 1986, 65, pg. 1475-184.
5. Bratu, D. , Ardelean -Leretter, M.C. , Colojoara , Rominu, M.- Dinţii umani permanenţi; , Editura Signata , Timişoara , 1991.
6. Cocarla E – Ortodonţie, Litografia UMF Cluj-Napoca, 1995, pg. 100-101, 172-175, 182-183, 186.
7. Zarnea L – Pedodonţie, Editura Didactică şi Pedagogică, Bucureşti, 1993, pg. 34, 50, 57.
8. Bareliuc L, Neagu N – Embriologie umană normală şi patologică , Editura medicală , Bucureşti, 1987 , pg. 91,103, 119.
9. Popescu A: Biochimie specială pentru stomatologie, Editura Didactică şi Pedagogică , Bucureşti, 1981, pg. 9-22.
10. Lazar Tr. C : Biochimie odontologică , Litografia IMF Cluj- Napoca, 1976, pg. 20-21.
11. Farmer E.D., Lawton P.W., Stone's oral and dental diseases [5th ed.], E&S Livingstone Ltd, Edinburgh, Scotland ,1966.
12. Davidean C , Grivu, O. – Dinţii umani, Editura Waldpress, Timişoara, 2006, pg. 7-8
13. Wenzel A. - A review of dentists use of digital radiography and caries diagnosis with digital systems. Dentomaxillofac.Radiol.,2006 Sept;35[5]:307-14.
14. http://www.copilul.ro/pediatrie/stomatologie-copii/Administrarea-fluorului-pentru-dinti-sanatosi-a3451.html
15. http://jurnalul.ro/viata-sanatoasa/sanatatea-familiei/administrarea-suplimentara-de-fluor-argumente-pro-şi-contra-574058.html
16. http://www.sfatmedical.ro/Diete_şi_nutritie/Vitamine_şi_minerale/Fluorul
17. http://www.desprecopii.com/info-id-254-nm-Fluorul-prieten-sau-dusman.htm
18. http://www.parinti.com/modules.php?name=Forums&file=viewtopic&t=19&highlight=zymafluor
19. http://www.colgate.ro/app/CP/RO/OC/Information/Articles/Oral-and-Dental-Health-Basics/Checkups-and-Dental-Procedures/Fluoride/article/What-is-Fluoride.cvsp
20. http://revistaoralfarm.wordpress.com/2012/04/30/fluorul-prieten-sau-dusman
21. http://www.welldental.ro/press/ZYMAFLUOR%20prospect.pdf
22. http://www.desprecopii.com/info-id-7553-nm-Fluorul-la-copii-Pro-sau-Contra.htm

23. http://www.copilul.ro/pediatrie/stomatologie-copii/Administrarea-fluorului-pentru-dinti-sanatosi-a3451.html
24. FleancuA, Neamtu ML: Metode cu aplicare sistemicăşilocală de prevenţie a carieidentare, AMT, vol II, nr. 3, 2009, pag. 103
25. Anamaria Bica, Ruxandra Sava Roşianu, Roxana Oancea, Ramona Amina Popovici, Daniela Jumanaca, Atena Găluşcan, Alexandra Podariu, Angela Codruța Podariu, ”Oral hygiene behavior among children of 9-10 age old. case study of three schools from Timisoara, Romania”; Medicine in Evolution, vol XXI, nr.2, 2015, p 283- 287;
26. Anamaria Bica, Ruxandra Sava-Rosianu, Roxana Oancea, Amina Ramona Popovici, Daniela Jumanca, Atena Galuscan, Alexandra Sabina Podariu, Angela Codruta Podariu, ”Nutrition behavior of children from primary school. case study of three schools from Timisoara, Medicine in Evolution, vol XXI, nr.3, 2015, p 370-.376;
27. Podariu, A.S., Podariu, A.C., Popovici, R.A. (2017). Communication Strategy on Oral Health Education for Adolescents. Revista de Cercetare si Interventie Sociala, 58, 68-80.
28. Popovici, R.A., Podariu, A.C., Anculia, R.C., Serafin, A.C., Tigmeanu, C.V., Krems, C.D., Levai, M.C. (2017). Accessibility to Dental Health Care: Risk Factor in Oral Health at Rural Area Community. Revista de Cercetare si Interventie Sociala, 59, 48-61.

The importance given to anamnesis in the dental office: a self-evaluation questionnaire



Balean O.[1], Oancea R.[1], Matichescu A.[1], Sava-Roşianu R.[1], Podariu A.C.[1], Jumanca D.[1], Chioran D.[2], Focht D.[1], Găluşcan A.[1]

[1]*Department of Preventive Dentistry, Community and Oral Health, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Sq., Timisoara, 300041, Romania*
[2]*Department of Department of Anesthesiology and Oral Surgery, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Sq., Timisoara, 300041, Romania*

Correspondence to:
Name: Oancea Roxana
Address: Splaiul Tudor Vladimirescu nr. 14A
Phone: +40 721335788
E-mail address: roancea@umft.ro

Abstract

The anamnesis is the first and one of the most important stages in the clinical examination and represents the totality of the information obtained by the doctor in order to establish the diagnosis The purpose of this study is to assess the importance given by dentists to the patient's medical history, given that many of the general conditions and their treatments can negatively interfere with dental treatment. To conduct this study, a questionnaire was applied that included 10 closed questions to assess how the dentist gives due importance and how he realises the patient's medical history. The way of making the medical history differs from doctor to doctor both in terms of its frequency and in terms of its attainment and storage. A well-conducted anamnesis, with special attention to the patient's health problems and his particularities, an adequate attitude to his psychological pattern, are some of the conditions of a quality treatment.

Keywords: anamnesis, dentist, treatment.

INTRODUCTION

Among the medical branches, dentistry occupies a well-defined place, with a significant importance in the terms of workload, time and value of the results obtained, with a logical classification in the wider sphere of general health of patients, this is way it appears normal and necessary to remove the therapeutic maneuvers specific to this field from the area of random and empirical, followed by their thorough and rigorous substantiation on modern scientific bases. The dental treatment plan must be individualized, in relation to the general medical conditions of the patient, whose presence can be established using data that the doctor can obtain through a judicious anamnesis and a rigorous, objective, complex and complete clinical examination. There is a need for a multidisciplinary therapeutic approach, working in a medical team, delegating certain responsibilities to a specially trained and competent staff, collaborating with the general practitioner and other specialties (as appropriate), which manages the evolution of patients' general ailments. [1]

Anamnesis varies in their dept and focus. Systemic, familiar, dental, present, sexual, social (occupation, drug use) are types of anamnesis.[2] Taking a medical history in dentistry is an important tool in the detection of medical problems of patients. Assessment of the patient's general health before any dental approach is necessary to identify any systemic conditions, which may affect treatment procedure and allow the dentist to evaluate the risks and reduce possible complications. The purpose of dental treatment is to satisfy patient's needs. The success of the treatment is built upon careful medical anamnesis taken with a clinical examination for diagnosis of the problem that has been presented. Anamnesis is not only a wise approach to medical history but also, it is essential for the establishment of a successful dentist-patient relationship. If dentist cannot take a detailed anamnesis, an accurate diagnosis cannot be formulated and dental treatment may increase the risk of acute medical complications. Therefore, well-kept medical history is important for ethical and legal aspects in dentistry. Dentists always must avoid harm to the patient and establish a balance with the helpful principles. This situation is not just a legal responsibility, but also an ethical principle. The well-compatibility between dentists, patients and their relatives protects the dentist from possible difficulties. The purpose of this article is to describe the significance level of taking anamnesis among dentists and getting the knowledge, attitudes and behaviors, and to emphasize the importance of dental education with a specific questionnaire. [1]

The assessment of the patient's general health before any dental approach is necessary to identify any systemic condition, which may affect the treatment procedure and allow the dentist to assess the risks and reduce possible complications. The purpose of dental treatment is to meet the needs of the patient. The success of the treatment is based on a careful medical history, through a detailed clinical examination, to diagnose the problem presented. The anamnesis is not only a wise approach to the medical history, it is essential for establishing a successful relationship between dentist and patient. If the dentist cannot make a detailed history, an accurate diagnosis cannot be made and the dental treatment may increase the risk of acute complications. Therefore, the well-established medical history through the correct and careful anamnesis, is important for ethical and legal aspects in dentistry. Dentists should always avoid exposing risks to the patient and strike a balance with useful principles. This situation is not only a legal responsibility, but also an ethical principle. Compatibility between dentists, patients and their relatives protects the dentist from possible ethical difficulties. [2]

The dental treatment of a patient seems to be a common thing, as well as the diagnosis of most oral diseases presented by him, but in reality any patient, especially those with special needs, the elderly, anxious patients, with behavioral deviations, etc. , can always become the victim of incidents, accidents, complications of treatment, if the dentist ignores the individualized aspect of his behavior in relation with the patient. [3] Prevention of any discomfort during treatment becomes a achievable necessity through thorough clinical

examination, done with tact and intelligence, through a set of questions as simple and explicit as possible, which are able to highlight possible health problems of the patient and allow the correct diagnosis of the condition.

The assessment of medical risks in patients with special medical needs with various systemic diseases, multiple disorders or severe physical and / or mental disabilities, requires the dentist, in addition to thorough medical and pharmacological knowledge, to collaborate on the basis of medical letters with the patient's current doctor.[4] With these patients we need a thorough knowledge of the oral impact of their underlying disease and must provide dental care that does not adversely affect their overall health. Adequate means and staff are needed to provide specific and comprehensive management in these cases. Before starting any dental treatment, we need a correct history, medical records and multidisciplinary interconsultation reports to know as much as possible about the patient's medical history. In this context, the hospital environment offers the advantage of access to electronic medical records and data referring to any additional tests that may have been performed. The medical risk should also be assessed before initiating any treatment in these patients. In this regard, we use the ASA scoring system developed by the American Society of Anesthetics This classification takes into account 6 scores depending on the patient's underlying disease. In the present days, patients who present in the dental office may be in a preventive treatment that it is not of interest to the dentist or patient, but which may have a major impact in the context of routine treatment. [4,5] The treatment of osteoporosis with bisphosphonates, although not considered by the patient as a direct-link treatment to the oral cavity, can lead to huge losses in the oro-dental area if not correctly evaluated. Oral bisphosphonates are often recommended for the treatment and prophylaxis of osteoporosis in women, but harmful interference with tooth extraction is often overlooked. [6] The medical dosage of bisphosphonates in the medical evaluation of the patient must be very well known and controlled. [7] Anticoagulants are very often administered in various cardiovascular pathologies. These are common among the population and can cause complications during dental treatment. The large number of drugs that are recommended to prevent or maintain the well-being of patients sometimes dramatically interfere with dental work. [8,9]

The purpose of this study is to evaluate the importance given by dentists in western Romania to the anamnesis of the patient or his medical history, given that a large part of general ailments and their treatments can negatively interfere with dental treatment.

MATERIAL AND METHODS

I addressed a questionnaire to all dentists participating in a conference in western Romania. Out of the total number of participants, only 267 dentists agreed to answer the questions. 2% of the questionnaires were excluded due to partial completion or multiple checking of the answers, the study being a transversal one of correlational, observational type.

The questionnaire includes 10 closed questions to evaluate how the dentist gives due importance and anamnesis of the patient before any dental treatment, but also the connection he has with the patient's general doctor for medical consultation.

The questionnaire was validated by a study on a group of 30 dentists, the value of the Cronbach alfa index being 0.936, which is a good value compared to the threshold required (0.700) to validate the application of this questionnaire.

The questionnaire was distributed by a single person, and the subjects were not allowed to consult any information during its completion.

RESULTS

The anamnesis differs from doctor to doctor, regarding its details. Only 86.3% perform a direct and detailed discussion necessary to prepare a complete medical history. The completion of the file with all the details obtained from the patient's medical history is performed by 74%, the rest notes only the main ailments. When asked if a detailed history of the patient is made at the beginning of each session, the relative frequency of answers was 83.2% positive and 16.8% negative. In this study group, only 31.3% performed the anamnesis at each meeting, taking into account the possible changes in the health status of patients between sessions.

These percentages suggest that the anamnesis is performed carefully (74%) only at the first visit to the dentist, although the patient's health may change during the treatment period.

The interdisciplinarity of dentist-family doctor is a priority for 59.9%, the remaining 40.1% considering the data about the family doctor unimportant. Even if approximately 60% of the surveyed dentists write down the number and name of the family doctor, only 33.2% contact him. This highlights the dentist's lack of interest in the interdisciplinary treatment of patients. Although the relationship between the dentist and the family doctor should be open, only 42.4% of family doctors give data about patients to dentists, while 57.6% of dentists fail to obtain data from them.

Patients' medical histories should be recorded to maintain the details provided over time, but there are 22.1% of dentists who maintain the information provided only in verbal form.

Completing a guided questionnaire on devices and systems is beneficial for the patient to remember all the ailments he suffers from, so 71% of dentists apply a standard form. The use of standardized forms for medical conditions is important because it provides questions related to all devices and systems, the statement is at their own risk, but at the same time the doctor must ensure that the patient understood all the questions and provided the correct answers.

Table 1. Results obtained in the evaluation questionnaire (percentage/number)

QUESTION	YES %	NO %
At the beginning of each intervention do you perform the detailed anamnesis of the patient?	**83,21% (218)**	**16,79% (44)**
Is the anamnesis performed at each session or only at the initial session?	**31,30% (82)**	**68,7% (180)**
Is the anamnesis made following direct discussions with the patient?	**86,3% (226)**	**13,7% (36)**
Is the result of the anamnesis recorded in the file with all the details or with only those that are considered relevant?	**74,05% (194)**	**25,95% (68)**
Do you ask for the family doctor's name and phone number?	**59,92% (157)**	**40,08% (105)**
Do you contact the family doctor?	**33,21% (87)**	**66,79% (175)**
Does the family doctor give you information from patients' medical letters?	**42,4% (111)**	**57,6% (151)**
The anamnesis is a verbal information	**36,64% (96)**	**63,36% (166)**
Is the anamnesis a written information ? (the doctor writes down all the information provided by the patient in the file)	**77,86% (204)**	**22,14 (58)**
Does the patient complete a detailed questionnaire regarding his state of health (guided answers on devices and systems)?	**70,99% (186)**	**29,01% (76)**

DISCUSSIONS

The importance of making a detailed anamnesis is very high, the whole therapeutic act depends on its proper preparation. The patient's history should be carefully prepared and updated at each session. [10,11] In this study, 16.8% of dentists do not perform the anamnesis and only 31.3% perform it at each session, taking into account any changes in the health of patients between sessions. The patient's history is obtained following direct discussions with him. In this study, the vast majority of dentists want to get all the necessary details, observations that they can, which through a questionnaire, can be omitted. The patient's file should not only contain the main conditions, because the details can be forgotten. Interdisciplinarity is the key to achieving an appropriate treatment plan, a fact that is aware of only about 60% of dentists in the study group, who are asking patients for the name and contact details of the family doctor. Of those who retain the doctor's data, only half contact him to discuss patients' health problems and the implications of these conditions. The foundations of interdisciplinarity must be laid by both sides, dentists and family doctors. In this study only 42% of family doctors want to provide data on the health of patients.

The way of making the anamnesis differs, some of the dentists perform an interrogation, and the information obtained is noted in the file with all the details or only those that they consider relevant. Other doctors apply a questionnaire, and others consider that only verbal communication is sufficient, without noting the data received.[12] Completing a guided questionnaire on devices and systems is beneficial for the patient to remember all the ailments he suffers from, so 71% of dentists apply a standard form.

The variety of patients who come to us for a specialized treatment is very large, age, sex, degree of medical training, eating habits, profession, general pathology they present, being just some of these variables. Each patient is a specific entity, and the permanent renewal of knowledge in the medical, biological, pharmacological, rapidly changing and permanent treatment methodology, its local and general implications, the patient's physical and biological condition, his individualized behavior, according to its education and other variables, requires individualized management.[12]

Risk assessment in patients with dental conditions is of the utmost importance in routine practice in the office. The aim of our work was to assess the risk of general accidents before, during or after the usual dental treatment, which is difficult to achieve due to the multitude of factors that must be taken into account.

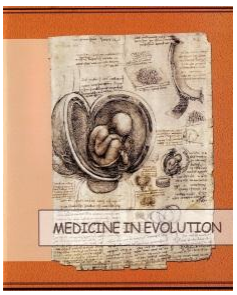
CONCLUSIONS

A well-conducted anamnesis, with special attention to the patient's health problems and his particularities, an attitude adequate to his psychological pattern, are some of the conditions of a quality treatment. The patient's health correlates with his quality of life and has a major role on a personal level, on the decision on the treatment plan and is a tool for understanding and outlining the clinical practice of the success of the therapeutic act. Most dentists in western Romania (71%) prefer the standardized questionnaire to investigate the patient's medical history, but there are still a large number of doctors (16.8%) who do not perform the patient's medical history or do not perform it systematically. Although most doctors (86.3%) state that they perform the anamnesis through a direct discussion WITH THE PATIENT, then 70.99% state that the patient completes the medical questionnaire, so the doctor is based on the patient's medical knowledge and at the same time only 33.3% of doctors dentists contact the patient's family doctor for interdisciplinary consultation of the patient and verification of medical data. Dentists in western Romania (74%) say that they make a detailed history of the patient only at the first visit to the dentist's office without taking into account the fact that over time the state of health may change.

REFERENCES

1. Dana Cristina Bodnar, Mihai Burlibaşa, Ileana Ionescu, Ioana Suciu, Complicaţii generale apărute în cabinetul stomatologic în timpul tratamentelor odontale uzuale analiză statistică, Revista Română de Statistică, 2014 2:33-44
2. Beste Inceoglu, Elif Naz Yakar, Naile Cura, Hakan Eren, Sebahat Gorgun, Importance of taking Anamnesis in Dentistry and Assessment of Knowledge and Attitudes of Dental Students: Journal of Contemporary Dentistry, May-August 2014;4(2):87-91
3. Afşin H, Soysal Z, Çağdır S, Gökdoğan M, Altuğ M, Çelik S. Dişhekimliğinde Anamnez. 1st ed. Nobel Tıp Kitabevi, 2006
4. Javier Silvestre-Rangil, Francisco-Javier Silvestre, Fernando Espín-Gálvez: Hospital dental practice in special patients Med Oral Patol Oral Cir Bucal. 2014 Mar 19 (2):e163-169
5. Borromeo, GL, Ahmad, MS, Buckley, S, et al. Perception of Special Needs Dentistry education and practice amongst Australian dental auxiliary students. Eur J Dent Educ. 2018; 22: e321– e326
6. R. Graham, G. Russell, Bisphosphonates: The first 40 years, Bone 2011; 49(1):2-19
7. Reszka A.A. Bisphosphonate Mechanisms of Action. In: Adler R. (eds) Osteoporosis. Contemporary Endocrinology. Humana Press 2010 pages 443-468
8. Cédric Mauprivez, Roman Hossein Khonsari, Omar Razouk, Patrick Goudot, Philippe Lesclous, Vianney Descroix, Management of dental extraction in patients undergoing anticoagulant oral direct treatment: a pilot study, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 2016, 122(5):146-155
9. Andrade Natália Karol de, Motta Rogério Heládio Lopes, Bergamaschi Cristiane de Cássia, Oliveira Luciana Butini, Guimarães Caio Chaves, Araújo Jimmy de Oliveira, Lopes Luciane, Bleeding Risk in Patients Using Oral Anticoagulants Undergoing Surgical Procedures in Dentistry: A Systematic Review and Meta-Analysis, Frontiers in Pharmacology, 2019, 10:866-868
10. DE MEDEIROS, Glaucia Helena Faraco; BRÜNING, Vanessa. Continued medication use in dentistry: the importance of dental records. Journal of Research in Dentistry, 2017 4(3), 81-85,
11. HanganuB, ManoilescuIS, VelnicAA, IoanBG. Physician-patient communication in chronic diseases. Med. Surg. J.-Rev. Med. Chir. Soc. Med. Nat., Iaşi2018; 122(3): 417-424
12. Veltrini VC, Capelozza ALA, Damante JH. Evaluation of health questionnaires used in dentistry. Special Care in Dentistry 2002; 22(6):221-225

Assessing patients' perceptions on dental esthetics



Kui A.[1], Buduru S.[1], Ismail A.[3], Jiglău-Labuneț A.[4], Negucioiu M.[1], Buzatu R.[2]

[1]*Department of Dental Prosthetics, Faculty of Dental Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*
[2]*Department of Dental Aesthetics, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania*
[3]*Dental Medicine Student, Faculty of Dental Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*
[4]*Department of Dental Materials, Faculty of Dental Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

Correspondence to:
Name: Smaranda Buduru
Address: 32 Clinicilor Street, Cluj Napoca, Cluj
Phone: +40 264597844
E-mail address: smarandabuduru@yahoo.com

Abstract

Aims and objectives. This survey was conducted in order to evaluate the factors that influence patient's satisfaction regarding overall dental aesthetics, and if the opinions differ according to demographic factors such as age, education, or gender. Material and methods. A questionnaire was formulated in order to assess the patients' opinion on dental aesthetics, which included 10 statements, as well as some social – demographic questions. The distribution of the questionnaires was done by email and direct distribution. Results. The results revealed that respondents consider dental aesthetics of highest concern; however perceptions of dental aesthetics vary, especially according to age and level of education. The results also emphasise the fact that many patients were concerned about restoring function when it comes to dental treatments. Conclusion. When planning treatments, dentists should consider, together with the patients, the aesthetic objectives in addition to function, structure and biology.

Keywords: dental aesthetics; patient's perception; functional rehabilitation

INTRODUCTION

In contemporary dentistry, various parameters, such as teeth's shape and colour, or dental arches shape are related to smile aesthetics. In addition, prior to undertaking aesthetic restorative dentistry, the dentist should establish a treatment plan which will provide functional rehabilitation and meet patient's expectations as well [1, 2].

From the dentist point a view, an organised and systematic approach is required in order to assess, diagnose and solve aesthetic issues predictably. Tooth colour is obviously an essential parameter for the final result, but aesthetic treatment planning should take into consideration several parameters in order to obtain a pleasant composition of the smile, such as tooth proportion, tooth-to-tooth proportions, marginal edge positioning, correct placement of the contact areas; in other words to create a result which combines various aesthetic elements in relation to functional principles [3].

From the patients' standpoint, usually the appearance of teeth is related to both cultural factors and individual preferences, meaning that someone's opinion of a visual experience can be pleasant or unpleasant, and what is considered "beautiful" in one culture may be "ugly" in another [4,5,6].

Studies related to this topic emphasise discrepancies between the treatment needs perceived by the patients and those assessed by the clinicians. Often, aesthetics is more important than function in patients' opinion, or the upper anterior teeth ("social six") seem to be of paramount importance compared to the loss of lateral teeth [2,7]. In modern society, smile has a great impact; in this context, if a patient's smile is ruined by different dental pathologies, then he/she can experience loss of self-esteem or even impairment of the overall physical and mental health [8].

Aim and objectives

As the aesthetic demands seem to increase in dentistry, we conducted this survey in order to evaluate the factors that influence patient's satisfaction regarding overall dental aesthetics, and if the opinions differ according to demographic factors such as age, education, or gender.

MATERIALS AND METHODS

A questionnaire was formulated in order to assess the patients' opinion about dental aesthetics. The survey was first pilot tested among five subjects, in order to validate the clarity of the questions, the response options and to estimate the time needed for completion. After making the necessary modifications, 10 statements were included in the survey, apart from the social – demographic questions. The survey design was approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy of Cluj Napoca.

The first section of the survey included general questions, regarding gender, age, education and occupation. The second section included ten statements, assessing the respondent's point of view on dental aesthetics. The ten statements used in the survey were: (1) **Perfect white teeth look natural**; (2) **Perfect white teeth look unnatural**; (3) **Teeth which are not perfectly white look more natural**; (4) **The most important property of teeth is their function**; (5) **The most important property of teeth is their appearance**; (6) **Teeth slightly worn look more natural**; (7) **The appearance of teeth has a great affect on a person's general appearance**; (8) **The appearance of teeth is the most important aspect of dental treatments**; (9) I **wish to have natural looking teeth in accordance with my age**; (10) **I am pleased with the appearance of my teeth**. The possible answers for each statement were **(**a) strongly disagree; (b) slightly disagree; (c) slightly agree; (d) strongly agree; (e) I do not know.

The distribution of the questionnaires was done by email (using Google Forms web site) and direct distribution, on patients receiving treatment in Prosthodontics Department, in University of Medicine and Pharmacy of Cluj Napoca.

Data analysis

Answers obtained from direct distribution were extracted and entered into the Statistical Package for Social Sciences software 22.0 (SPSS, Chicago, Il). The data obtained from the web-survey were downloaded from the Google Forms web site as a Microsoft Excel (Microsoft Corp., Redmond, WA) file. After recording the variables, the answers were exported to SPSS and merged to the direct distribution database.

In a primary stage, a descriptive analysis was performed. In order to determine statistical differences regarding the social-demographic factors, the Chi-Square test was performed. It also includes the McNemar test for the significance of modifications. Statistically significant differences were those with $p \leq 0.05$.

RESULTS

108 subjects responded initially to the survey, but after validating the data, answers obtained from 92 people were statistically analysed. The social-demographic characteristics of survey participants are summarised in table 2.

Out of the 92 patients, 51 were women and 41 men; 25 respondents were aged between 15-24 years, 22 between 25-34 years, 12 subjects between 35-44 years, 12 between 45-54 and 21 subjects aged over 54 years old. 41% of the respondents had a university degree, while 39% were high-school graduated (table 1).

Table 1. Description of the respondents participating in the survey

Variables	*Age, years*					*Gender (F/M)*		*Level of education*			*Occupation*				
	15-24	25-34	35-44	45-54	>54	Females	Males	High-school graduated	University degree	Master/PhD degree	Services	Agricultural	Student	Retired	Different industries
Number of participants (N) and %	25 (27.2%)	22 (23.9%)	12 (13%)	12 (13%)	21 (22.8%)	51 (55.4%)	41 (44.6%)	36 (39.1%)	41 (44.6%)	15 (16.3%)	24 (26.1%)	22 (23.9%)	16 (17.4%)	8 (8.7%)	22(23.9%)
Total	92 (100%)														

Regarding the first statement, 88% of the respondents agree with the fact that perfect white teeth are beautiful. 71.7% agree with the fact that "teeth which are not perfectly white look more natural", while 89.1% believe that the most important property of teeth is their function. Also, 70.1% of the respondents believe that "teeth slightly worn look more natural" and 98.9% of the respondents believe that "the appearance of teeth has a great affect on a person's general appearance" (fig.1). Regarding the statement no. 8, 41.3% agree, 51.1% of the respondents disagree that "the appearance of teeth is the most important aspect of dental treatments".

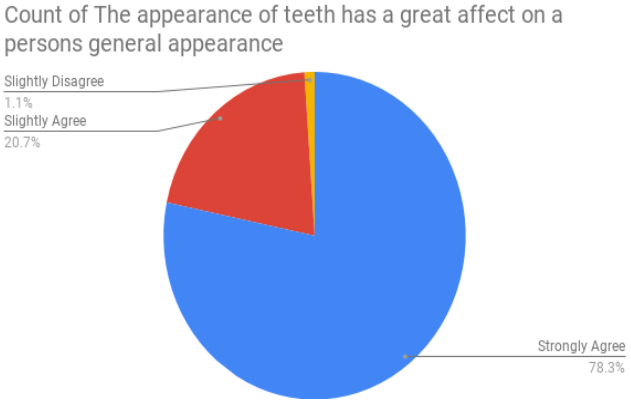


Figure 1. Distribution of answers on statement no.7

For the last two statements, 81.5% of the respondents wish to have natural looking teeth in accordance with age (fig.2), and 79.4% are pleased with the appearance of their teeth.

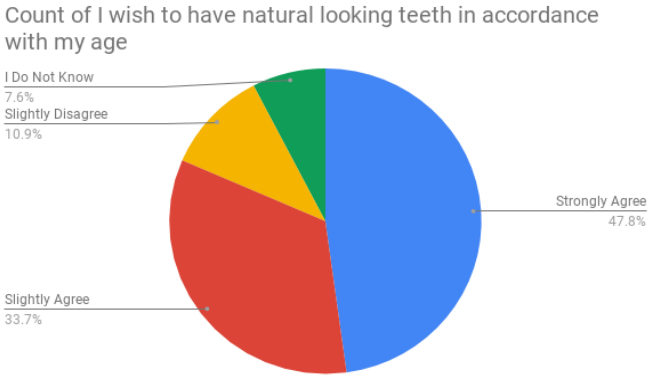


Figure 2. Distribution of answers on statement no.9

Pearson Chi-square test revealed several associations between social-demographic factors and the answers choose for the statements. Taking into consideration age and the answers for the ten statements, a significant association was observed for the statements no. 3 ($\chi 2=29.667$, df=16, p=0.020), statement no.6 ($\chi 2=57.648$, df=16, p=0.000), statement no.7 ($\chi 2=46.159$, df=8, p=0.000) statement no. 8 ($\chi 2=37.365$, df=16, p=0.002) (fig.3) and statement no. 9 ($\chi 2=37.365$, df=16, p=0.002).

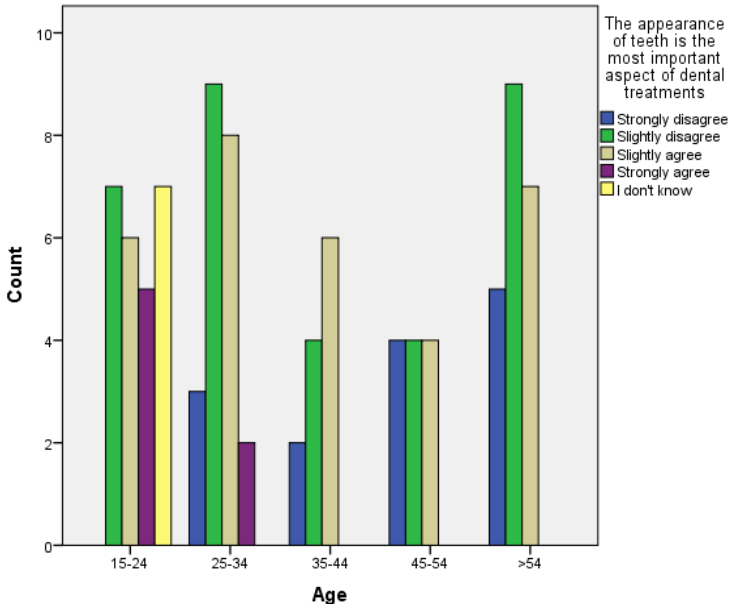


Figure 3. Distribution of answers on statement no.8 in correlation with age

Statistical analysis revealed no significant associations between gender and the choices for the ten statements ($p>0.05$). Considering occupation as a variable and the answers given to the ten questions, Pearson Chi Square revealed a significant association for statement no. 6 ($\chi2=49.143$, $df=16$, $p=0.000$) (fig.4), for statement no. 8 ($\chi2=29.563$, $df=16$, $p=0.020$) and for statement no. 9 ($\chi2=21.901$, $df=12$, $p=0.039$).

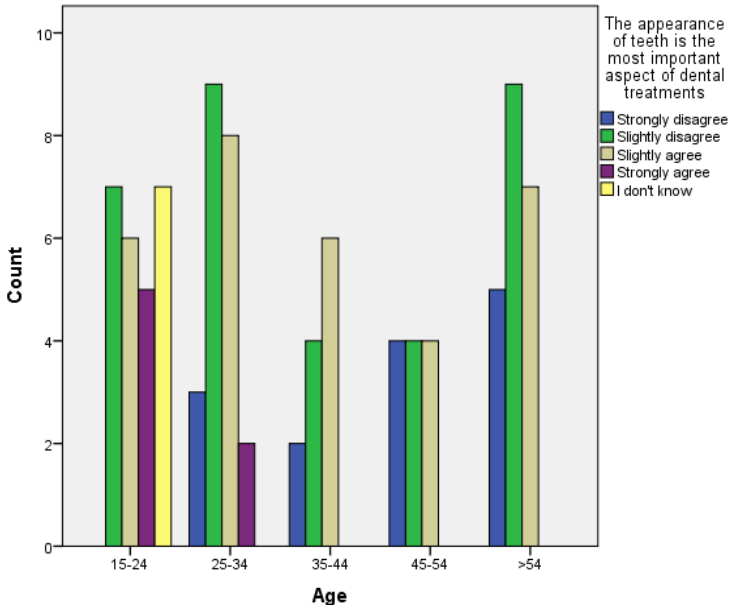


Figure 3. Distribution of answers on statement no.8 in correlation with age

Statistical analysis revealed no significant associations between gender and the choices for the ten statements ($p>0.05$). Considering occupation as a variable and the answers given to the ten questions, Pearson Chi Square revealed a significant association for statement no. 6 ($\chi2=49.143$, $df=16$, $p=0.000$) (fig.4), for statement no. 8 ($\chi2=29.563$, $df=16$, $p=0.020$) and for statement no. 9 ($\chi2=21.901$, $df=12$, $p=0.039$).

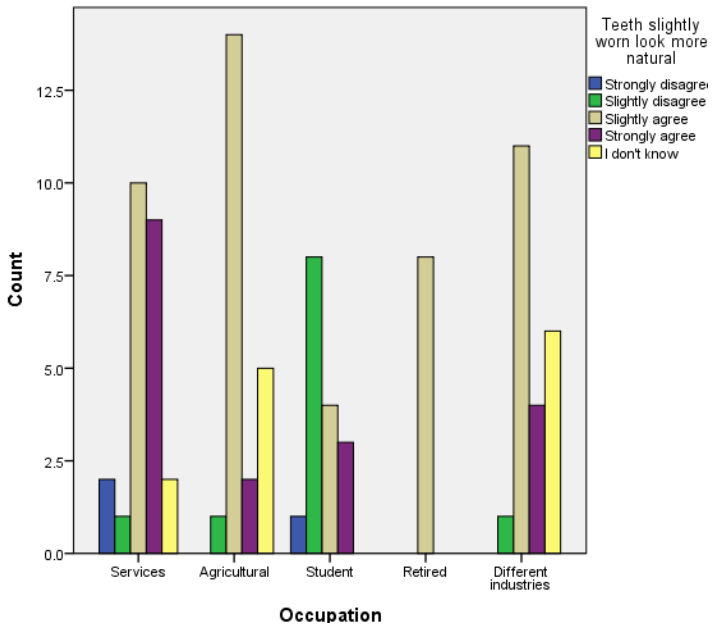


Figure 4. Distribution of answers on statement no.6 in correlation with occupation

Assessing any association between education and the answers chosen for the ten statements, Person Chi Square revealed a significant statistical result for statement no.1 ($\chi 2=18.265$, df=8, p=0.019).

DISCUSSIONS

In clinical practice it is very difficult to achieve a pleasing aesthetic result without having a clear idea of what the patient desires and what his or her expectations and aesthetic goals are. This is why all dental therapies should be based upon a combination of dentists knowledge of aesthetics criteria, and listening to the patient's desires [3, 9, 10].

The aim of this study was to assess the patients' opinion regarding dental aesthetics and if there is any correlation between social demographic variables and the patients' preferences.

Previous studies concluded that people who were not dentists had a stronger preference for white teeth than dentists did [1]. In our study, the answers for statements no. 1 ("very white and straight teeth are beautiful") and no.2 ("very white teeth look unnatural") revealed that the vast majority of respondents consider also the 'Hollywood smile' as the benchmark for dental beauty, but in the same time the respondents generally have clear knowledge of their aesthetic preferences (81.5% of respondents agreed with the second statement). The responses showed that the majority of subjects accept that there is a distinction between aesthetic smiles and extremely white teeth. The statistical analysis also revealed a significant association between the level of education and the answers for statement no. 1. Subjects having high education level strongly agreed with the fact that perfectly white teeth look beautiful.

Regarding statement no. 3 ("teeth which are not perfectly white look more natural") only 19.5% disagreed and 8.7% were unsure. The answers revealed the fact that perfectly white teeth are not necessarily the most natural and aesthetic way to restore teeth. This notion is also backed up by the responses from statement 6, which stated that "having slightly worn teeth is natural", as 70.7% of people agreed. The responses to both these statements suggest the level of understanding that respondents might have over dental aesthetics, regarding the fact that successful aesthetic treatments are not reliant on having the perfect straight white smile, rather they should provide a unique, individualized smile adapted to the patient's wishes and aesthetic demands. Also, Pearson Chi Square test revealed that retired subjects agreed that having slightly worn teeth might offer a more natural look.

Statement 7 was that "the appearance of teeth has a great affect on a person's general appearance". Only 1% slightly disagreed with this statement. This is a clear indicator of the important role dental aesthetics plays in a person's life. The answers also reveal that regardless of patient's feelings about the perfect smile, having an aesthetic smile seems to be very important. A significant association has been observed between age range (15-24 years) of the respondents and strongly agreeing with the statement.

The answers to statement no. 8 ("the appearance of teeth is the most important aspect of dental treatments") indicate similar opinions to the responses given to statements 4 and 5. Although aesthetics are important, function of teeth should not be sacrificed purely for aesthetic gain, and there is more to a successful dental treatment than just focusing on dental aesthetics. Also, Pearson Chi Square test revealed a significant association between this statement's answers and age. Subjects aged between 15-24 years and 25-34 years old strongly agreed with this statement (fig. 3). This is in accordance with other studies' results, as young people tend to perceive aesthetics as being much more important than function, when it comes to dental treatments [9, 10].

Overall, respondents aged 55 years old or above were more likely (compared to younger people) to be satisfied with their dental appearance. This aspect has also been observed in others studies [11, 12, 13] and reveal that the appearance of their teeth is not as important in older individuals than in younger ones [9, 11].

For statement 9 ("I wish to have natural looking teeth in accordance with my age") only 10.9% slightly disagreed, and 7.6% were unsure. This again shows that patients place a higher precedence on natural looking teeth rather than overly white and straight teeth. Statement 10 asked the subjects if they are pleased with the appearance of their teeth. 79.4% of patients agreed with this statement, with only 19.6% disagreeing and the last 1% unsure.

Statistical analysis revealed no significant differences between males and females when it comes to their answers in the survey, as well as regarding the occupation of the respondents.

These results reveal that patients consider dental aesthetics of highest concern; however perceptions of dental aesthetics vary, especially according to age and level of education. The results also showed the fact that many patients might be concerned about function rehabilitation when it comes to dental treatments.

CONCLUSIONS

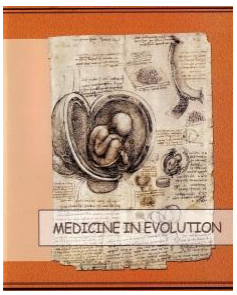
When planning treatments, dentists should consider, together with the patients, the aesthetic objectives in addition to function, structure and biology. This study emphasises the importance of communication between dentists and patients in the aesthetic dental treatment planning process. The use of an aesthetic self-evaluation questionnaire merged with an in-office professional assessment questionnaire will lead to a successful aesthetic treatment, pointing out from the beginning the expectation that patients have in mind.

REFERENCES

1. Samorodnitzky-Naveh G, Geiger SB, Levin L. Patients ' satisfaction with dental esthetics. J Am Dent Assoc 2007; 138(6):805–8. Available from: http://dx.doi.org/10.14219/jada.archive.2007.02692.
2. Thiyagarajan A, Kumar D. Dental Esthetics : Perception from Future Dental Professionals. Acta Sci Dent Sci. 2018;2(4):3–5.
3. Freedman G. Contemporary Esthetic Dentistry. Elsevier Mosby; 2012. 100–1005 p.

4. Aldajani Q, Alsalman A, Alkhamees A, Alabdulaziz M, Madi M. Comparison of Maxillary Anterior Dentogingival Esthetics Perception between Dentists and Saudi Lay Population in Eastern Province of Saudi Arabia. Ecronicon. 2020;19(3):1–11.
5. Tortopidis D., et al. "Evaluation of the relationship between subjects' perception and professional assessment of esthetic treatment needs". Journal of Esthetic and Restorative Dentistry 19 (2007): 154-163.
6. Hassel AJ., et al. "Selfrating of satisfaction with dental appearance in an elderly German population". International Dental Journal 2008; 58: 98-102.
7. Akeel R. Attitudes of Saudi male patients toward the replacement of teeth. J Prosthet Dent 2003;90(6):571-7.
8. Ingber FK. You are never fully dressed without a smile. J Esthet Restor Dent 2006;18(2):59-60
9. Vallittu P., Vallittu AS., Lassila V. Dental aesthetics - a survey different groups of patients of attitudes in. J Dentirsty. 1996;24(5):335–8.
10. Lajnert V, Pavic D, Grzic R, Kovac Z, Pahor D, Kuis D, et al. Influences of age and maxillary anterior teeth status on patient ' s satisfaction with dental appearance and tooth colour. Gerodontology. 2012;29:674–9.
11. Tin-oo MM, Saddki N, Hassan N. Factors influencing patient satisfaction with dental appearance and treatments they desire to improve aesthetics. BMC Oral Health [Internet]. 2011;11(1):6. Available from: http://www.biomedcentral.com/1472-6831/11/6
12. Akarslan Z, Sadik B, Erten H, Karabulut E: Dental esthetic satisfaction, received and desired dental treatments for improvement of esthetics. Indian Journal of Dental Research 2009, 20(2):195-200.
13. Alkhatib M, Holt R, Bedi R: Age and perception of dental appearance and tooth colour. Gerodontology 2005, 22(1):32-36

A model of experiential learning for teenagers' habits related to dental services utilization and smoking



Cărămidă M.[1], Dumitrache M.A.[1], Sfeatcu R.[1], Mihai C.[2], Ilici R.R.[3], Oancea R.[4]

[1]*Oral Health and Community Dentistry Department, Faculty Of Dental Medicine, "Carol Davi-la" University of Medicine and Pharmacy, Bucharest, Romania*
[2]*Preventive Dentistry Department, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[3]*Technology and Dental Materials Department, Faculty of Dental Medicine, "Carol Davila" Uni-versity of Medicine and Pharmacy, Bucharest, Romania*
[4]*Preventive, Community Dentistry and Oral Health Department, Faculty Of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

Correspondence to:
Name: Ilici Roxana Romanita
Address: Technology and Dental Materials Department, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, Eforie Street no. 4-6, district 5, 50037
Phone: +40 723 602 179
E-mail address: roxana.ilici@umfcd.ro

Abstract

Oral health promotion among adolescence is necessary due to the vulnerability specific to their age, such as the debut of smoking habits and the neglecting the dental services utilization. Taking into consideration the tendency of teenagers to be reluctant to conventional education, experiential learning is a more appropriate method for this target group since the participants are engaged in interactive activities with respect to a learning circle "Do", "Observe", "Think", "Plan", as described by David Kolb. The present paper describes the experiential learning activities used for oral health education of a group of 13-17 years-old adolescents in Bucharest, Romania enrolled in a 2-year longitudinal study as part of "Com4you" oral health promotion program. The activities described were part of the third lesson offered during the program and this last one approached as oral health subjects the importance regular visits to the dental office and the oral health risks of tobacco consumption. Students showed interest to both the subjects and the method and the results of the program showed greater changes in the adolescents' oral health-related behavior when this method was used compared to traditional learning.

Keywords: oral health promotion, adolescents' oral health, smoking, dental services use

INTRODUCTION

Oral health-related habits are formed early in life with proper education but while oral hygiene and food consumption behavior are the main subjects approached in oral health education programs offered to toddlers and children, adolescents need to be addressed subjects regarding behaviors often met in this life stage that are either neglected, such as regular dental check-ups and dental service utilization, or tempting and harmful, such as smoking. Most adult smokers start smoking in adolescence and recent studies conducted in Romania showed that around 15% of 13-16 years old teenagers smoke [1,2] and that that the frequency of smokers increased fast from age 13 years to 17 years [3].

Education at this age and, in particular, for the oral health care area tend to have better results if experiential learning is approached, compared to traditional learning [3]. Experiential leaning, developed by David Kolb [4], used the concept of ”learning by doing” in which the students are engaged in experiences (so called the ”Do” phase) then they are invited to evaluate their experience (the ”Observed” phase) and to decide for themselves what could be changed and improved at the previous experience (the ”Think” phase) and also to figure out how to apply the improved future version (the ”Plan” phase) and then the cycle resumes by testing the adapted experience.

The above mentioned learning method was applied in a 2-years longitudinal pilot study in Bucharest, as part of the”Com4You” Oral Health education program, and the results showed improvements in teenagers’ awareness and behavior regarding the dental services utilization compared to control group(in which traditional education with oral presentations), as it follows: the frequency of subjects who visit the dental office regularly, at least once a year, increase from 55% at the baseline to 72,1% at the final of the program (compared to control group with an increase from 48,6% to 51,5%), and, moreover, the frequency of subjects who consider dental visits important for preventive reasons increased from 80,3% at baseline to 96,7% (compared to the control group with an increase from 80% to 82,9%) [5]

METHOD DESCRIPTION

The activities described in the present paper were performed in the “Com4you” Erasmus+ program for Oral Health Education and aimed to engage teenagers in experiential learning in order to increase the level of knowledge, to raise the awareness, and to improve the behavior related to the oral health. The activities were designed by a group of researches and trainers from Oral Health and Community Dentistry Department (“Carol Davila” Medicine and Pharmacy University in Bucharest, Romania) and TES Association, and then performed with a sample of 61 teenagers, 13-17 years old, students at 3 public schools in Bucharest, Romania. The sample group was divided in small sub-groups of 10-15 students who were offered oral health educational lessons in the classrooms, lasting around 30 minutes, using experiential learning and approaching two subjects: smoking and its negative impact on oral health, and the importance of regular visits to the dental office. These lessons were the last from a series of 3 lessons during the two-year period of the Com4you program (between 2014 and 2016), the other two having as themes: oral hygiene and caries-protective diet.

Activity 1. The risks for oral health of tobacco consumption

Aim: To increase the awareness regarding the oral health risk of smoking

Method of education: brainstorming and facilitated discussion

Description of the educational process:

The teenagers are informed by the coordinator of the activity (one member of the research team) that they are invited to an open discussion about the risks of smoking over the

oral health and they are asked to offer examples of modification in the oral cavity that tobacco consumption could induce. The students are encouraged to express their ideas and to offer as many answers as possible. All the answers are written down on a flip chart and discussed, the coordinator facilitates the discussion so that students engaged to offer also arguments and to try to find an explanation for the negative consequences mentioned. Correct answers are pointed out and bad answers are not criticized or deleted but debated under the guidance of the coordinator that directs the discussion to the correct medical explanation. The discussions are concluded by stressing out the severity of the negative effects of tobacco consumption over the whole mouth health. The key messages that should the repeated at the end of the activity should be: tooth discoloration, oral mucosa lesions such as leukoplakia, nicotine stomatitis, periodontal inflammation, poor wound healing, oral cancer, malodor.

Activity 2: Importance of the regular dental check-ups

Aim: Raising the awareness regarding the regular visits to the dental office for prevention and early detection of the disease

Method of education: Questionnaire

Description of the educational process:

The teenagers are informed that they will be given a set of cards with either questions or statements regarding the dental service utilization (Table 1). They are asked to read it and offer a personalized answer to the questions found on the card regarding the individual attitude or habit related to dental visits or decide whether the statement is true or false, in their opinion. Students are offered a couple of minutes to read the cards and prepare their answers and then they are invited to present their questions/statement and their answers to the other members of the group. The coordinator of the activity encourages the participants to offer honest answers accordingly to their opinions and habits. All the answers are discussed, developed base on reasons and explanation from students' point of view, debated, and guided by the coordinator. Proper attitude and habits are pointed out by the coordinator that should facilitate de discussion so that the key message be well understood by the participants: the dental services utilization should be based on prevention, early detection of the disease and treated in a safe and comfortable environment.

Table 1. Questions/Statements

	Question/statement	**Answer**
1.	How often do I need to go to the dentist?	
2.	Why visits to the dentist are important?	
3.	What are the signs that tell me that I should go to the dentist?	
4.	I have no problem with the teeth, must I go to the dentist?	
5.	What happens to me if I go to the dentist?	
6.	What can I do if a tooth hurts?	
7.	I have bleeding gums, just the tooth brushing solves the problem?	T/F
8.	I want to have whiter teeth, will I use sodium bicarbonate?	T/F
9.	With eating an apple, tooth brushing is no longer needed	T/F
10.	Dental floss does not help me to reduce the risk of cavities	T/F
11	Every dental procedure is painful	T/F
12	Hard food such as seeds or nuts reduces tooth decay	T/F
13	Chewing gum reduces tooth decay	T/F
14	My teeth hurt because I fired power	T/F

15	If I brush my teeth properly, there is no need to floss	T/F
16	Scaling and professional brushing at dentist damage my tooth enamel	T/F
17	An expensive toothpaste is much better	T/F
18	If I do not notice any cavity with the naked eye, I do not have to go to the dentist	T/F
19	Hot or cold foods damages my teeth	T/F
20	If my family members don't have dental problems It means that I will not have, either	T/F
21	Treatment of caries detected at early stages is not painful.	T/F
22	Cavities and gum inflammation might be reversible if going to regular check-ups.	T/F
23	Detected in time, decays are reversible by fluoridation and sealing.	T/F
24	Bleeding gums are present because of the incorrect brushing	T/F
25	Regular check-ups can diagnose diseases in their early stage, and treatment is tolerable and cheaper.	T/F

DISCUSSIONS

The aforementioned and described activities intrigued the participants who were interested and engaged in the discussions. However, taking into consideration that these activities were part of the last lesson from the program for which the final assessment of subjects' changes in knowledge, attitude and behavior was performed at only few weeks after the final lesson, and also that habits such as smoking need long periods to change, it would be apposite to assess the long term results as part of a longer follow-up of the study.

CONCLUSIONS

Engaging adolescents in the process of oral health education using experiential learning increases their interest in the subjects' approach and have higher results compared to traditional educational methods. The activities described in the present article are tested in the community and offered as a model for future oral health promotion initiatives targeted to teenagers.

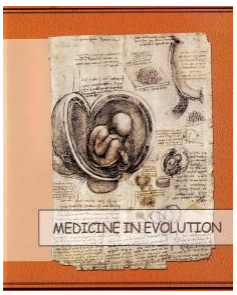
Acknowledgement

The Erasmus+ project ''Youth Community-based Oral Health Learning Model" was cofounded by European Union. The authors also thank Oral-B Romania for support.

REFERENCES

1. FDI / WHO (2005) Tobacco or oral health: an advocacy guide for oral health profession-als. Edited by Beaglehole RH and Benzian HM; FDI World Dental Federation, Ferney Vol-taire, France / World Dental Press, Lowestoft, UK.
2. Sfeatcu R, Cărămidă, Dumitrache MA, Oancea R, Didilescu A. Comparative oral health-related behavior among adolescents of different ages. Int J Med Dent. 2019; 23(4): 508-11.
3. Dumitrache MA, Sfeatcu R, Cărămidă M, Jumanca D, Găluscan A. Smoking behavior among a group of adolescents. A 2-year longitudinal study. Med in Evol. 2020; 26(2): 173-8.
4. Kolb DA. Experiential Learning: Experience as the Source of Learning and Develop-ment. 2nd ed. Englewood Cliffs, NJ: Prentice-Hall; 1984.
5. Sfeatcu R, Dumitrache MA, Cărămidă M, Johannsen A. A pilot study on the effective-ness of a 2-year school-based oral health educational programme using experiential learn-ing among adolescents. Int J Dent Hyg. 2019;17(3):221-8.

Evaluation of fluoride release into saliva after topical application: an in vitro experiment



Funieru C.[1], Oancea R.[2], Cărămidă M.[3], Sfeatcu R.[3]

[1]Preventive Dentistry Department, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Preventive Dentistry, Community and Oral Health Department, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania
[3]Oral Health and Community Dentistry Department, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Correspondence to:
Name: Oancea Roxana
Address: Preventive, Community Dentistry and Oral Health Department, Splaiul Tudor Vladimirescu no. 14A, Timişoara, Romania
Phone: +40 721335788
E-mail address: roancea@umft.ro

Abstract

Fluoride is the most important element in caries prevention and control. Topical fluoride therapies, self-applied or professional procedures, are frequently used such as dentifrices, mouthwashes, varnished, gels etc. The most important thing for a topical fluoride therapy irrespective of type of substance, method, concentration or frequency of application is fluoride concentration in oral fluid or in dental plaque. This study is an in vitro experiment developed on extracted teeth. Teeth were fluoridated with tin fluoride gel (0.4%) after they were cleaned with an ultrasonic scaler, water and immersed in a hypochlorite solution for 10 minutes. Afterward, the concentration of fluoride released from the teeth enamel was measured at 24, 48, and 72 hours using HI-729 Fluoride Low Range Handheld Colorimeter device. The amount of fluoride released between 48 hours and 24 hours from fluoridation (0.22 – 0.37 ppm) was a higher than the one released between 72 and 48 hours from fluoridation (0.15 – 0.18 ppm). The amount of fluoride released from the teeth enamel decreases in time after the topical fluoridation.

Keywords: fluoride release, topical applications, in vitro evaluation

INTRODUCTION

The use of fluorides is the most common and effective method for caries prevention. The fluoride is involved in mineralisation, maturation and preeruptive stages of tooth development, improves enamel remineralisation and has antibacterial effect. Fluoridation can be systemic (water, salt, milk) or topical (self-applied or professional procedures) [1,2,3]. Fluoride is present in saliva and on teeth surfaces after topical therapies were used. Thereby, the fluoride will be adsorbed on the enamel surface, it will reduce the demineralization when the pH rises above the critical level (5.5), and forms fluorhydroxyapatite. The fluoride can also interfere with the physiology and several bacterial mechanisms, such as enzymes activity (ex: glycolytic enzymes, H+ATPases). This is one of the fluoride antibacterial effects [4].

Different types of fluoride agents are used and reported to be effective in preventing dental caries, namely remineralsing early caries lesions and arrest dentine caries. There are various methods, concentrations, frequencies of applications and fluoride substances used in the topical therapies such as sodium fluoride, tin fluoride or acidulated phosphate fluoride (APF) [3,5,6].

Fluoride concentration in dental plaque and oral fluid is very important for topical therapies. The remineralization process and the antibacterial effects of fluoride are present when the fluoride concentration is optimal.

Aim and objectives

The scope of this study is to find how fluoride is released from teeth enamel after topical application using an in vitro experiment developed on extracted teeth and in which a special device is used.

MATERIALS AND METHODS

12 extracted teeth, 6 lateral and 6 frontal were used in this experiment. First, the teeth were cleaned using an ultrasonic scaler. All calculus deposits and extrinsic discolorations were removed in order to obtain cleaned enamel surfaces for a maximum fluoride uptake (figure no. 1). Afterward, the teeth were immersed in a hypochlorite solution for 10 minutes. The rationale of this experiment is to evaluate the fluoride released from the enamel only. Therefore, to exclude the cemetum, the teeth roots were covered with nail polish (figure no. 2, a & b).

Figure 1. Cleaning the extracted teeth with ultrasonic scaler

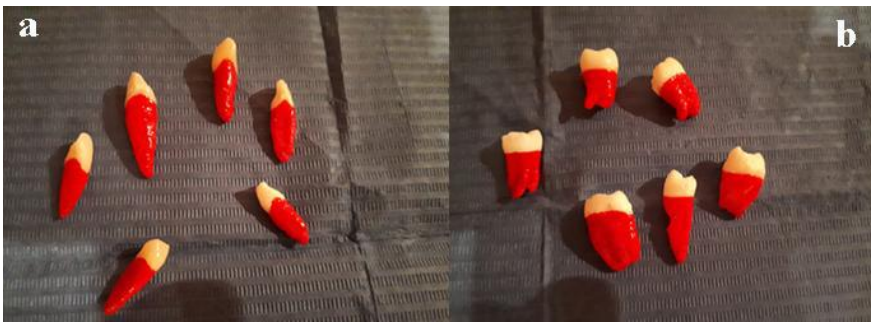


Figure 2. Extracted teeth with roots cover with nail polish; a – frontal teeth, b – lateral teeth

An amount of approximate 0.3 ml of 0.4% tin fluoride gel were put on teeth crowns using a small brush (applicator). After 1 minute, the excess of gel was removed and the teeth were washed with clean water. Then, the teeth were put in two plastic sterile bottles full with deionized water, one for lateral teeth and one for frontal teeth (figure no. 3, a & b, and figure no. 4).

The fluoride released from the teeth was measured using HI-729 Fluoride Low Range Handheld Colorimeter, Checker®HC device (figure no. 5). The device uses SPANDS method and it has:

- range 0.00 to 2.00 ppm
- ± 0.05 ppm ± 5% of reading
- 0.01 ppm resolution
- light source – LED 575 nm
- light detector – silicon photocell
- environment: 0 to 50°C (32 to 122°F); RH max 95% non-condensing
- 2 sample cuvettes with caps
- reagents for 6 tests (re-ordering information HI-729-26 x 20 tests)
- syringe with tip
- 1 x 1.5V AAA battery

Figure 3. Teeth in plastic containers
a – frontal teeth, b – lateral teeth


Figure 4. Teeth in deionized water

Figure 5. HI-729 Fluoride Low Range Handheld Colorimeter, Checker®HC device

2 ml of special reactive was combined with 8 ml of deionized water for each measure. It was 3 measurements of fluoride concentration: at 24, 48, and 72 hours for both containers.

RESULTS

The results for the 6 measurements were graphically exposed in the figures no. 6 and 7, and in table no. I.

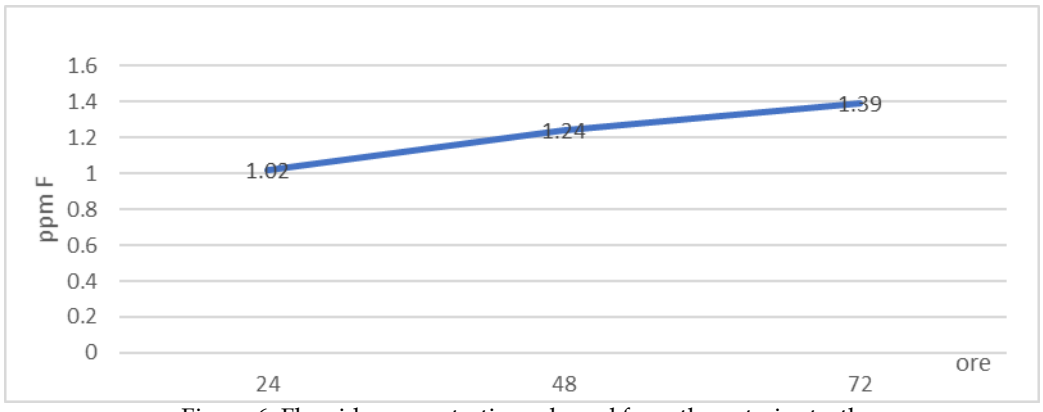


Figure 6. Fluoride concentration released from the anterior teeth

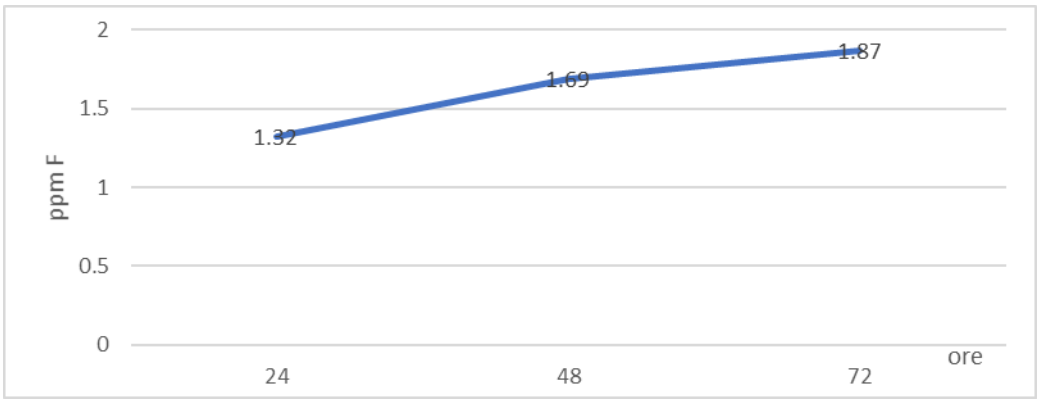


Figure 7. Fluoride concentration released from the posterior teeth

Table I. The amount of fluoride released from the teeth

Teeth	Fluoride released in the first 24 hours	Fluoride released between 48 and 24 hours	Fluoride released between 72 and 48 hours
Anterior	1.02 ppm	0.22 ppm	0.15 ppm
Lateral	1.32 ppm	0.37 ppm	0.18 ppm

DISCUSSIONS

Our study shows that the amount of fluoride released from the frontal teeth is lower than from the lateral teeth, which may seem logical given that they have thinner enamel. On the other hand, the amount of fluoride released from both lateral and frontal teeth is higher at 24 hours after fluoridation compared with that released between 48 hours and 24 hours after fluoridation, which is also higher than the amount of fluoride released in the last 24 hours (at the final point of the experiment – 72 hours after fluoridation). Thus, we can say that the amount of fluoride released from the enamel in a fluid is high at the beginning but decreases in time after the fluoridation.

The major limit of our study is that we used deionized water instead of saliva. Our study is an in vitro experiment and did not include human participants. We had in mind to create a mechanism of fluoride release similar to that in the oral cavity but oral fluoride

kinetics in the oral cavity depends on many factors such as saliva secretion, dietary fluoride sources or topic fluoride therapies [7]. Oral hygiene habit is also very important for fluoride level in saliva, especially brushing time and frequency or the amount of fluoride from the dentifrice, high fluoride toothpaste being considered to be responsible for a high saliva fluoride level as against regular toothpastes [7,8]. All these cannot be reproduced in an in vitro experiment.

Agents used in topical therapy provide different levels of fluoride in saliva. Sodium fluoride and aminofluorides for example, provide high concentration of fluoride in saliva [7]. Fluoride varnishes also release high amounts of fluoride in saliva. One study shows that even was added calcium glycerophosphate in some fluoride varnishes and therefore, the amount of fluoride in saliva increased, this did not lead to greater preventive effect against enamel demineralization [9].

Chewing gum is another donor of fluoride. The amount of fluoride released in saliva by the fluoride-containing chewing gum is almost ten times higher than in case of regular chewing gum [10].

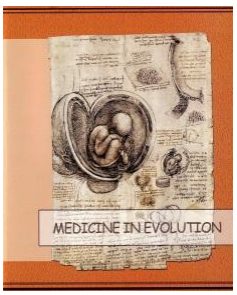
CONCLUSIONS

Our experiment showed that fluoride is released in saliva at 24, 48, and 72 hours after topical fluoride therapy but the rate of release is decreasing. Even so, this is very important because the presence of fluoride in saliva is essential for caries prevention and control.

REFERENCES

1. Wilkins EM, Wyche CJ, Boyd LD. Clinical Practice of the Dental Hygienist. Twelfth Edition. Wolters Kluwer, 2017;Two Commerce Square 2001 Market Street, Philadelphia, USA;593-618
2. Harris NO, Garcia-Godoy F, Nathe CN. Primary Preventive Dentistry, Eighth Edition. Pearson Education Limited 2014;244-300
3. Cuculescu M. Prevenţie primară în carie şi parodontopatii. Editura Didactică şi Pedagogică, R.A. Bucureşti 2010;242-290
4. Kanduti D, Sterbenk P, Artnik B. Fluoride: a review of use and effects on health. Mater Sociomed 2016;28(2):133-137
5. Marinho VCC, Worthington HV, Walsh T, Chong LY. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2015;2015(6):CD002280
6. Gao GS, Zhang S, Mei ML, Lo E C-M, Chu C-H. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review. BMC Oral Health 2016;16:12
7. Naumova EA, Kuehnl P, Hertenstein P, Markovic L, Jordan RA, Gaengler P, Arnold WH. Randomized investigation of the bioavailability of fluoride in saliva after administration of sodium fluoride, amine fluoride and fluoride containing bioactive glass dentifrices; BMC Oral Health 2012;12:3
8. Vincent S, Thomas AM. Fluoride levels in saliva and plaque following the use of high fluoride and conventional dentifrices- a triple blinded randomised parallel group trial. ScientificWorldJournal 2019;2019:1636209
9. Carvalho TS, Peters BG, Rios D, Magalhães AC, Sampaio FC, Buzalaf MAR, Bönecker MJS. Fluoride varnishes with calcium glycerophosphate: fluoride release and effect on in vitro enamel demineralization. Braz Oral Res 2015;29:S1806-83242015000100287
10. Bijella MFTB, Brighenti FL, Bijella MFB, Buzalaf MAR. Fluoride kinetics in saliva after the use of a fluoride-containing chewing gum. Braz Oral Res 2005;19(4):256-260

The relationship between diabetus melitus and periodontal health status



Iova G.[1], Babes A.[2], Ciavoi G.[3], Todor L.[3], Scrobota I.[3]

[1]*PhD student, Faculty of Medicine and Pharmacy Oradea, University Oradea*
[2]*Department of Medical Science, Faculty of Medicine and Pharmacy Oradea, University Oradea*
[3]*Department of Dental Medicine, Faculty of Medicine and Pharmacy Oradea, University Oradea*

Correspondence to:
Name Ciavoi Gabriela
Address: Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, Romania, December 1st Square no.10, 410068 Oradea, Bihor County, Romania
Phone: +40 729.099.622
E-mail address: : gabrielaciavoi@yahoo.com

Abstract

The relationship between diabetes and oral cavity disorders is well known. Until the last decades it was believed that it is unidirectional, respectively diabetes mellitus triggers and favors the occurrence and evolution of periodontal disease. At present, more and more studies show that the relationship is bidirectional, respectively and periodontal disease is a trigger factor and maintains diabetes.More evidence has been made that this connection is present even in the prediabetic patient. this topic, we concluded that no matter what angle the research would have achieved, the unanimous conclusion is that between the two conditions there is a close interdependence, they influence and intercondition each other.

Keywords: diabetus melitus, prediabet, periodontal health, relationship

INTRODUCTION

In the oral cavity there is a balance between the state of the buccal mucosa, respectively of the marginal periodontium and the factors that tend to destabilize this balance. The etiopathogenesis of the periodontal disease has long been discussed in the literature. At the present time, besides the local factors, the general factors are taken into account. Although there have been many studies investigating the link between diabetes and oral health, however, very little is known about the knowledge of patients with periodontal disease in relation to the interdependence and implications of diabetes. Both non-diabetic people and people at risk and diabetics may have limited knowledge about the relationship between diabetes and periodontal disease, and a visit to a family doctor or diabetologist will probably not improve their knowledge in this area. Most family physicians or diabetologists may not provide information on the link between diabetes and periodontal disease. The dentist can provide this information.

For a better understanding of the relationship between diabetes and oral health, awareness of oral health and identification of attitude towards the involvement of the dentist in the screening of diabetes in his patients, the current study wanted to examine two groups, diabetics and nondiabetics. Their knowledge of diabetes and the source of that knowledge. For 2014, it has been estimated that, globally, 422 million adults suffered from diabetes. By comparison, in the year 1980, there were 108 million people with this condition [1]. The recrudescence of the disease has almost doubled since 1980 globally, rising from 4.7% to 8.5% of the adult population. These values reflect an increased incidence of the disease in association with risk factors such as overweight and obesity. Diabetes caused 1.5 million deaths in 2012 and the higher than optimal blood glucose level caused another 2.2 million deaths. Of the total deaths, 43% occurred before reaching the age of 70 [2].

In Romania, the incidence of diabetes is 11.6% among the population aged between 20 and 79 years [3] and 3% in children between 0 and 14 years old [3]. In 2015, the Ministry of Health indicated the number of diabetic patients registered at 999, 192 and the number of persons diagnosed during the same year 2015 was 73,740 (202 new cases per day) [5].According to the data published by the National House of Health Insurance, between 2006 and 2016, the number of people for whom diabetes treatments were settled has doubled and the costs have increased 6 times [1].

Because periodontal disease is associated with diabetes, the utility of screening in the dental office and the reference to primary care aim to introduce the diagnosis for prediabetics and diabetics. Monitoring studies in dental practice were performed on this category of subjects. Studies have estimated that 30% of patients seen in dental practice, older than or equal to 30 years, had dysglycemia [6,7,8].

The study aimed to investigate the relationship that exists between oral cavity health, in particular the degree of periodontal disease and diabetes or even prediabetes. Starting from empirical observations initially, this relationship has been studied by several authors and has shown that this connection exists and the results have shown significant correlations.

ORAL HEALTH

Oral health is a generic term and refers to several factors. In general, when we talk about this aspect, most people understand only problems related to dental caries. We need to clarify from the beginning that oral health also refers to: the pathology of the oral mucosa. , periodontal pathology, TMJ pathology and musculoskeletal pathology. Oral health is the mirror of many internal diseases, often certain general diseases are diagnosed in the first instance by the dentist who observes the signs of the disease in the oral cavity that can signal a diagnosis presumptive. Impaired oral health cannot be seen only as a result of poor oral

hygiene as many people consider. It is the result of many local and general favoring factors that both the physician and the patient must be aware of. Among the local favoring factors we can list: hygiene Defective mouth, smoking, occlusal dysfunctions, vicious habits and among the most significant general factors would be: endocrine disorders, metabolic disorders, drug administration.

Dental plaque (considered a factor determining the onset of periodontal disease) is now considered a localized, chronic oral infection, which activates the host's immune-inflammatory responses both locally and systemically and is also a source of bacteremia. It is known that periodontal disease exerts an important influence on the pathogenesis of numerous systemic diseases, including diabetes mellitus [5].

STUDIES

In the mid-1990s, enough scientific evidence emerged to confirm an association between Diabetus Melitus and periodontitis, which then began to be considered the sixth complication of Diabetus Melitus. Current scientific evidence indicates a two-way relationship between Diabetus Melitus and periodontal disease, whereby Diabetus Melitus is associated with an increased incidence and progression of periodontitis, while periodontal infection is associated with worsening glycemic control in diabetic patients [9]. . This two-way relationship indicates a need to promote oral health in patients with Diabetes Melitus and to implement a common management protocol between endocrinologist and dentist that aims to create adequate conditions for early diagnosis and effective treatment of both diseases. Studies show close links between diabetes sugar and oral mucosal health. Harsas & Colab. [10]. In a study of 197 patients showed significant differences between diabetic patients (97 patients) and nondiabetic patients diagnosed with periodontal disease (97 patients) regarding the severity of periodontal disease. From their studies it turns out that there are significant differences ($p = 0.00$) between the 2 groups of patients tested, so that patients with diabetes have higher values when measuring gingival sacs, loss of gingival attachment and gingival recession. The explanation would be according to the authors the increase in blood sugar that causes microvascular complications it has the increase of the final glycation end product (AGE) in plasma and tissue.

The protein glycation reaction would be responsible for most complications of the diabetic patient (nephropathy, retinopathy, neuropathy). Periodontal infection affects the secretion and synthesis of cytokines, where this condition may increase the response of AGE-mediated cytokines. The formation of AGEs in proteins also leads to the establishment of a cross-linking of collagen with the basement membrane, which leads to a decrease in the interactions of collagen and other components of the matrix, followed by the incidence of damage to the structure and function of the basement membrane. The state of hyperglycemia in patients with T2DM who produce AGE will bind to the AGE receptor (RAGE), leading to additional complications such as the development of vascular damage, increased vascular permeability, increased expression of adhesion molecules and increased migration and activation of monocytes. As a result of this state of stress, which causes disorders of the blood vessels in the periodontal tissue [11].

Another study [12] on 100 diabetic patients found that 97% of diabetic patients had loss of gingival attachment compared with 34% in the non-diabetic control group. Also, severe form of periodontitis was present in 54 % of patients compared to 7% of the non-diabetic control group. There were also significant differences ($p = 0.001$) regarding the correlation of periodontitis severity with glycated hemoglobin and postprandial glycemia.

Maboudi et all. [13,14] starts from the hypothesis that the prediabetic condition can be a triggering or aggravating factor of the periodontal disease. Loe, plaque index and periodontal pocket depth. The sample comprised 20 (19%) men and 88 (81%) individuals with a mean age of 49 years and a mean BMI of 27.5. The average FBS, GTT and HbA1C were 107

MG / DL, 137MG / DL, respectively 5.9%. The clinical evaluation showed 33% of the patients involved with periodontitis. The mean CAL, BOP, PI, PD, GI was 3.7, 0.62, 1.9, 2.1, 1.5, respectively ($p < 0.05$). A significant difference in the periodontal index was found in patients with prediabetes. Moreover, a significant correlation was revealed between the prediabetic condition and the degree of damage to the periodontium. In support of this hypothesis come the studies undertaken by [14], on a batch of 171 prediabetic patients 30-65 years. The association of glucose metabolism and chronic periodontitis was investigated by analyzing multivariable logistic regression and the results revealed significant correlations between the impairment of glucose metabolism level and the number of periodontal disorders, mostly mild and severe [16,15].

Molina et all. 2016 [17] considers and demonstrates the bidirectional relationship between periodontal disease and diabetes. Diabetus Melitus was shown to be a risk factor for the occurrence of periodontal disease and subsequently an inverse relationship was proposed that periodontitis could be a risk factor for glycemic decompensation, as well as to be associated with an increased risk of Diabetus Melitus. The impact of periodontitis on changes in hyperglycaemia was assessed in a 5-year prospective study in 2973 non-diabetic subjects. Subjects with more advanced periodontitis at the start of the study showed absolute increases in blood glucose approximately five-fold higher after 5-year follow-up compared with those who did not have periodontitis at the start of the study (HbA1c change 0.106 ± 0.03% vs. 0.023 ± 0.02%). This was the first study to report that periodontitis predicts increases in HbA1c among people without diabetes[18]. The authors argued that periodontal pathogens can stimulate the production of inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), inducing a state of insulin resistance and systemic inflammation, which has also emerged as a new predictor of incident diabetes. More recently, the same author assessed the relationship between periodontal microbiota and the risk of early Diabetus Melitus in a section study that included 300 adults without DM aged between 20 and 55 years. Although the prevalence ratio for pre- Diabetus Melitus among participants with moderate / severe periodontitis vs. no / mild periodontitis was 1.47 (95% CI 0.78 - 2.74), without statistical significance ($p = 0.23$), higher colonization levels of the specific periodontal microbiota were associated with higher prevalence before Diabetus Melitus in adults without Diabetus Melitus .Current scientific evidence suggests that there is an increased risk of developing Diabetus Melitus in people with severe periodontitis, compared to subjects with good periodontal health or only mild periodontitis. Moreover, studies show that the incidences of macroalbuminuria and end-stage renal disease are increased twice or three times, respectively, in diabetics who also have severe periodontitis. Furthermore, people with Diabetus Melitus and severe periodontitis have three times the risk of cardio-renal mortality compared with those without severe periodontitis[19,20].

Also, Kiran et all [21,22] demonstrated that there is a link between the treatment of periodontal disease and the improvement and periodontal disease. Faria-Almeida et al. [23-26] also demonstrated that periodontal disease treatment significantly improves metabolic control of DM. These authors performed a 6-month controlled clinical study of 20 patients divided into two groups (type 2 diabetes and non-diabetics) with moderate generalized chronic periodontitis. Conventional periodontal scaling and root planning were performed, and the response to this work was compared between groups at 3 and 6 months. An improvement of all clinical variables was observed, without statistically significant differences between them groups, except the depth of the poll. The improvement observed in the blood. HbA1c levels confirmed a positive metabolic response to periodontal treatment, with a lower value for this variable at 3 and 6 months after periodontal treatment.

CONCLUSIONS

Analyzing the ones presented above we can draw the following conclusions:

1. There is a correlation between periodontal disease and diabetes
2. The more diabetes is out of control, the more severe the periodontal disease is.
3. There is a two-way relationship with mutual influence diabetes mellitus-periodontal disease.
4. The prediabetic state is a predictive factor for the occurrence of periodontal disease.

Acknowledgement

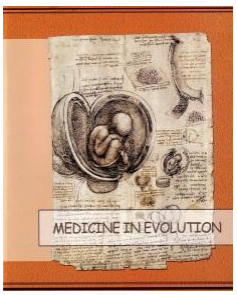
This paper is a result of the research conducted in elaborating the thesis of DMD, PhD student, MS Iova Gilda Mihaela under the supervision of DMC, PhD, MM Babes Petru Aurel.

REFERENCES

1. WHO Global report on diabetes, Geneva, World Health organization, 2016
2. Studiul naţional privind prevalenţa diabetului, prediabetului, supraponderii, obezităţii, dislipidemiei, hiperuricemiei şi bolii cronice de rinichi (PREDATORR), Concluzii prezentate la al 40-lea Congres al Societăţii române de diabet, nutriţie şi boli metabolice, 21-24 mai 2014
3. http://fadr.ro/despre-diabet/diabetul-in-romania
4. Institutul Naţional de Sănătate Publică, Buletin Informativ nr. 12/2015
5. Simpson TC, Needleman I, Wild SH, Moles DR, Mills EJ. Treatment of periodontal disease of glycaemic control in people with diabetes. Chocrane Database Syst Rev. 2010; 5: 714-720
6. Engebretson S, Kocher T. Evidence that periodontal treatment improves diabetes outcomes: a systematic review and meta-analysis. J Periodontol. 2013; 84 (4 Suppl): S153-69
7. Liew AKC, Punmithinont N, LeeYC, Yang J. Effect of non-surgical periodontal treatment on HbA1c: a meta-analysis of randomized controlled trials. Aust Dent J. 2013; 58(3): 350-7
8. Darre L, Vergnea JN, Goury P, Sixou M. Efficay of periodontal treatment on glycaemic control in diabetic patiens: A meta-analysis of interventional studies. Diabetes Metab. 2008; 34(5): 497-506
9. Saravanan AV, Ravishankar PL, Kumar P, Rajapandian K, Kalaivani V, Blaisie Rajula MP. Estimation of serum triglycerides, serum cholesterol, total protein, igg levels in chronicperiodontitis affected elderly patients: A cross-sectional study. J IntSoc Prevent Communit Dent 2017; 7:120-4.
10. Mellitus N. Anindita H., Lessang R., Soeroso Y, AlifaG., Periodontal Status Differences between Chronic Periodontitis Patient with and Without Type 2 Diabetes, Journal of International Dental and Medical Research ISSN Apr 12 2020, vol.12, nr.1/2019, p.175-180
11. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's Clinical Periodontology; 2012.
12. Shah A, Kaushik R M, Kandwal A, Kaushik R. Periodontitis as a complication in type 2 Diabetes mellitus – A hospital based study. SRHU Medical Journal, 2017;1(2):67-
13. Maboudi A., Akha O., Heidari M., Mohammadpour RA., Gheblenama P., Shiva A. Relation between Periodontitis and Prediabetic Condition. J Dent Shiraz Univ Med Sci., June 2019; 20(2): 83-89.
14. Zhang Y., Liu C., Chen B., Associations of impaired glucose metabolism with chronic peridontitis in pre-diabetes patients, Journal of Peking University. Health sciences, 2020, 52(1):71-76
15. Atkinson MA et al. Type 1 Diabetes: New Perspectives On Disease Pathogenesis And Treatment. Lancet 2001; 358: 221-229.
16. Solomon TP1, Haus JM, Kelly KR, Rocco M, Kashyap SR, Kirwan JP. Improved pancreatic beta-cell function in type 2 diabetic patients after lifestyle-induced weight loss is related to glucose-dependent insulinotropic polypeptide,Diabetes Care. 2010 Jul; 33(7):1561-6.
17. Molina C.A., Ojeda L.F., Jiménez M.S., Portillo C.M., Olmedo I.S., Hernández T.M., Moreno G.G., Diabetes and Periodontal Diseases: An Established Two-Way Relationship Journal of Diabetes Mellitus, 2016, 6, 209-229

18. Carranza FA, Newman MG, Takei HH, Klokkevold PR. Carranza's Clinical Periodontology. 10th ed. St. Louis, Mo.: Saunders Elsevier; 2006. 30 p
19. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. J Periodontol. 2015;86(5):611-22
20. Bragger U, Hakanson D, Lang NP. Progression of periodontal disease in patients with mild to moderate adult periodontitis. J Clin Periodontol. 1992;19(9 Pt 1):659-66
21. Heasman L, Stacey F, Preshaw PM, McCracken GI, Hepburn S, Heasman PA (2006) The effect of smoking on periodontal treatment response: a review of clinical evidence. J Clin Periodontol 33, 241-253.
22. Kiran M1, Arpak N, Unsal E, Erdoğan MF. , The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. J Clin Periodontol. 2005 Mar; 32(3):266-72.
23. Tonetti MS, Claffey N (2005) Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. J Clin Periodontol 32, Suppl 6, 210-213.
24. Albandar JM, Streckfus CF, Adesanya MR, Winn DM (2000) Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss. J Periodontol 71, 1874-1881.
25. Ciprian Pasca, Emil Urtila, Angela Codruta Podariu, Ruxandra Sava-Rosiuanu, Ioana Mihaela Citu, Adelina Ramona Berari, Teodora Eva Lupulescu, Atena Galuscan, Daniela Jumanca, Ramona Amina Popovici, "Correlation between periodontal disease and acute miytocondrial infarctation", Medicine in Evolution, vol XX, nr.2, 2014, p 294- 300;
26. Ovidiu Motoc, Angela Codruța Podariu, Atena Găluşcan, Daniela Jumanaca, Ruxandra Sava Roşianu, Anca Porumb, Florina Andrica, Ramona Amina Popovici, "The alterations of salivary glands secretion in diabetes mellitus", , Medicine in Evolution, vol XXI, nr.1, 2015, p 169-174

Study of the influence of juice consumption and eating habits on children's teeth in Rieni, Bihor County



Porumb A.[1], Ratiu C.[1]*, Balint A.M.[2], Ciavoi G.[1]

[1]Department of Dental Medicine, Faculty of Medicine and Pharmacy, University from Oradea, Romania
[2]Student, Department of Dental Medicine, Faculty of Medicine and Pharmacy, University from Oradea, Romania

Correspondence to:
Name: Cristian Adrian Ratiu
Address: Department of Dental Medicine, Faculty of Medicine and Pharmacy, University from Oradea, Romania Decembrie 1st Square no.10, 410068, Oradea, Bihor county, Romania
Phone: +40 741 077 976
E-mail address: ratiu_cristian@yahoo.com

Abstract

The present study included all preschool and school children from Rieni, Bihor County and their parents. The study aims to assess parents' knowledge of oro-dental health, as an integral part of general health, means of prevention and prophylaxis of tooth decay, as well as juice consumption and eating habits of children in Rieni, Bihor Country. As material and method, we proceeded to collect individual opinions, expressed by completing a questionnaire. In conclusion, slow consumption of acidic beverages over a long period of time, especially if drunk in small sips, can create dental erosion, induced by the action of acidic substances. Small amounts consumed between meals, will permanently provide food for cariogenic bacteria, will subject the teeth to long periods of demineralization and will therefore be at the origin of numerous carious processes.

Keywords: juices, children, tooth decay

INTRODUCTION

In dentistry, studies over time show that populations that do not consume carbohydrates are resistant to caries, such as Eskimos. Gustafsson et al found that patients on a high-fat, low-sugar, low-sugar diet had low tooth decay levels. [1-5] He added refined sugar as a supplement to the meal, and the caries rate remained virtually low. However, when the caramels were added between meals, there was a significant increase in the number of new caries. [6-12]

Aim and objectives

The study aims to assess parents' knowledge of oro-dental health, as an integral part of general health, the means of prevention and prophylaxis of dental caries, as well as the consumption of juices and children's eating habits and how the diseases affects the quality of life of children.

MATERIALS AND METHODS

The study took place between 11.04 - 22.04. 2016, including all preschool and school children from Rieni, Bihor County and their parents.

We started by informing the parents and receiving the consent to participate in this study.

We continued by collecting individual opinions, expressed by completing a questionnaire. It consists of 15 questions, to which the answers are preformulated, and the options considered correct will be chosen by the respondent. Through this questionnaire we collected data on the optimal age for the first visit to the dentist, the frequency of brushing teeth, hygiene habits and the use of karyopreventive means, diet and frequency of juice consumption per day.

The questionaire was processed after Guidance for researchers and reviewers, Version 3.2 May 2007, National Research Ethics Service, England, Informed consent to take part in a clinical research study, The Queens Mwdical Center Honolulu Hawaii, The Human Research Protection Program, University of California, San Francisco.

RESULTS

If the number exceeds 4 consumptions per day, the risk of dental erosion induced by the action of acidic substances increases. Most carbonated soft drinks are acidic. Slow consumption of acidic beverages over a long period of time, especially if drunk in small sips, can create tooth erosion, induced by the action of acidic substances. AS A PRACTICAL IDEA, avoid keeping acidic drinks in the oral cavity for too long or avoid drinking them in small sips for a long time or consume them with a straw!!!

DISCUSSIONS

Data processing will be performed by statistical analysis.

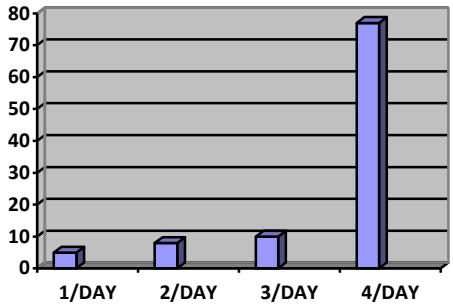


Figure 1. Drinking juice/day

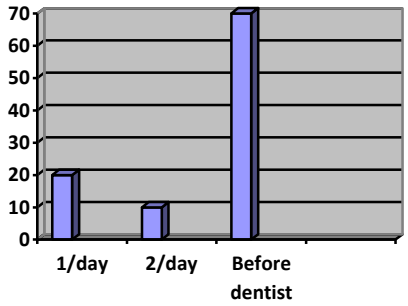


Figure 2. Frecqvency of tooth brushing

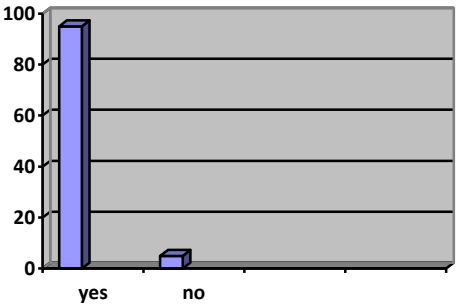


Figure 3. Habits of drinking juices

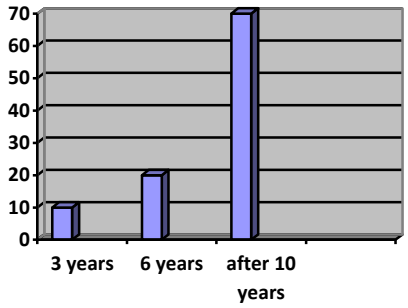


Figure 4. First visit at dentist

CONCLUSIONS

The consumption of sugar in indirect form, such as juices, has increased in the last quarter of a century, with a natural tendency to further increase.

Studies show that if sweets are consumed during main meals, even in large quantities, they do not produce such important shortcomings. Instead, small amounts consumed between meals will permanently provide food for cariogenic bacteria, subject the teeth to long periods of demineralization and will therefore be at the origin of numerous carious processes. [13-14]

The results will allow the dentist to orient the methods and means of information, as well as the prophylaxis actions.

Health education programs conducted in kindergartens and schools aim to train, strengthen or change children's attitudes and behaviors toward their own health. In the present study we wanted to evaluate the attitudes, behaviors, eating habits of children towards various aspects of oral health. [15-19]

The impact of socio-economic factors on children's eating habits was obvious. As we assumed at the beginning of the research, the consumption of juices in children in Rieni is increased, and the frequency with which they consume these drinks especially loved by children is also increased.

In health education programs it is necessary, therefore, to introduce information on prophylactic means, education for a proper diet with a healthy diet, and the frequency of visits to the dentist. Also, the education programs will aim at changing the administration schedule, the sweet drinks can be consumed after the main meals of the day and not at all between meals. [20-25]

The realization of preventive programs is especially important both in kindergartens and in schools.

Partnerships between Mayors and Universities, such as the one between Rieni City Hall and the University of Oradea, Faculty of Medicine and Pharmacy, can create the framework for scientific aspects to be complemented by dental health education programs.

Acknowledgement

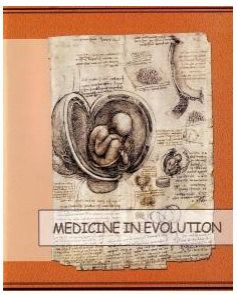
This study is part of contract no. 4 of 29.02./2016 and was financed by Rieni City Hall, in partnership with the University of Oradea.

REFERENCES

1. Dumitrascu L, Dumitrache A, Sfeatcu A. Evaluarea atitudinilor si comportamentelor referitoare la sanatatea orala ale copiilor scolari din Bucuresti, Revista Romana de Medicina Dentara nr.3 din 2010, pg.184-194.
2. Armfield JM, Spencer AJ Water fluoridation and the association of sugar-sweetened beverage consumption and dentak caries in Australian children - America Journal of public health, nr.3, vol 103, march 2013.
3. Sebastian RS, Cleveland LE, Goldman JD, Moshfegh: AJ. Trends in the food intakes of children 1977-2002. Consumer Interests Annual. 2006; 52:433---434.
4. Hector D, Rangan A, Louie J, Flood V, Gill T. Soft Drinks, Weight Status and Health: A Review. Sydney, New South Wales, Australia: NSW Centre for Public Health Nutrition; 2009.
5. Ludwig DS, Peterson KE, Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet. 2001;357(9255):505---508.
6. Sanigorski AM, Bell AC, Swinburn BA. Association of key foods and beverages with obesity in Australian schoolchildren. Public Health Nutr. 2007;10(2):152--- 157.
7. Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a sys- tematic review and meta-analysis. Am J Public Health. 2007;97(4):667---675.
8. Tahmassebi JF, Duggal MS, Malik-Kotru G, Curzon MEJ. Soft drinks and dental health: a review of the current literature. J Dent. 2006;34(1):2---11.
9. Heller KE, Burt BA, Eklund SA. Sugared soda consumption and dental caries in the United States. J Dent Res. 2001;80(10):1949---1953.
10. Ismail AI, Burt BA, Eklund SA. Cariogenicity of soft drinks in the United States. J Am Dent Assoc. 1984;109 (2):241---245.
11. Sohn W, Burt BA, Sowers MR. Carbonated soft drinks and dental caries in the primary dentition. J Dent Res. 2006;85(3):262---266.
12. Marshall TA, Levy SM, Broffitt B, et al. Dental caries and beverage consumption in young children. Pediatrics. 2003;112(3 pt 1): e184---e191.
13. Walker A, Gregory J, Bradnock G National Diet and Nutrition Survey: Young People Aged 4---18 Years. Vol. 2: Report of the Oral Health Survey. London, England: The Stationery Office, 2000.
14. Slater PJ, Gkolia PP, Johnson HL Patterns of soft drink consumption and primary tooth extractions in Queensland children. Aust Dent J. 2010; 55(4):430---435. 26. Food Standards Australia New Zealand. Consumption of Intense Sweeteners in Australia and New Zealand: Benchmark Survey 2003. Evaluation Report
15. Ortiz-Hernández L, Gómez-Tello BL. Food consumption in Mexican adolescents. Rev Panam Salud Publica. 2008;24(2):127---135.
16. R e h m C D , M a t t e T D , V a n W y e G , Y o u n g C , Frieden TR. Demographic and behavioral factors associated with daily sugar-sweetened soda consumption in New York City adults. J Urban Health. 2008;85 (3):375---385.
17. Vereecken CA, Inchley J, Subramanian SV, Hublet A, Maes L. The relative influence of individual and contextual socio-economic status on consumption of fruit and soft drinks among adolescents in Europe. Eur J Public Health. 2005;15(3):224---232.

18. Armfield JM, Spencer AJ. Dental Health Behaviours Among Children. Dental statistics and research series no. 56. Canberra, Australian Capital Territory, Australia: Australian Institute of Health and Welfare; 2012.
19. Gill TP, Rangan AM, Webb KL. The weight of evidence suggests that soft drinks are a major issue in childhood and adolescent obesity. Med J Aust. 2006;184 (6):263---264.
20. Anamaria Bica, Ruxandra Sava-Rosianu, Roxana Oancea, Amina Ramona Popovici, Daniela Jumanca, Atena Galuscan, Alexandra Sabina Podariu, Angela Codruta Podariu, Nutrition behavior of children from primary school. case study of three schools from Timisoara, Medicine in Evolution, vol XXI, nr.3, 2015, p 370-.376;
21. Anamaria Bica, Ruxandra Sava Roşianu, Roxana Oancea, Ramona Amina Popovici, Daniela Jumanaca, Atena Găluşcan, Alexandra Podariu, Angela Codruța Podariu, "Oral hygiene behavior among children of 9-10 age old. case study of three schools from Timisoara, Romania"; Medicine in Evolution, vol XXI, nr.2, 2015, p 283- 287;
22. Daniela Jumanaca, Atena Găluşcan, Angela Codruța Podariu, Ramona Amina Popovici, Roxana Oancea, Ruxandra Sava Roşianu, Dumitrache Mihaela Adina, "Oral health education class in preschool children communities", Medicine in Evolution, vol XXI, nr.2, 2015, p 294- 299;
23. Bica, A., Podariu, A.C., Krems, C.D., Podariu, A.S., Popovici, R.A. (2016). Tooth Brushing Behavior in 6-11 Year Olds Children and the Importance of a Break of Tooth Brush Once a Day in School. Revista de Cercetare si Interventie Sociala, 54, 115-124Podariu, A.S., Podariu, A.C., Popovici, R.A. (2017). Communication Strategy on Oral Health Education for Adolescents. Revista de Cercetare si Interventie Sociala, 58, 68
24. Popovici, R.A., Podariu, A.C., Anculia, R.C., Serafin, A.C., Tigmeanu, C.V., Krems, C.D., Levai, M.C. (2017). Accessibility to Dental Health Care: Risk Factor in Oral Health at Rural Area Community. Revista de Cercetare si Interventie Sociala, 59, 48-61.

Anxiety and Panic Attacks in the Dental Office



Ratiu C.[1], Porumb A.[1]*, Sava-Rosianu R.[2], Tanase A.[2], Ciavoi G.[1]

[1]*Department of Dental Medicine, Faculty of Medicine and Pharmacy Oradea, University Oradea*
[2]*Faclty of Dentistry, University of Medicine and Pharmacy "Victor Babes" Timisoara*

Correspondence to:
Name: Porumb Anca
Address: Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, Romania, December 1st Square no.10, 410068 Oradea, Bihor County, Romania
Phone: +40 726286237
E-mail address: anca.porumb@yahoo.com

Abstract

The patient's anxiety about the dental act is recognized and studied a lot lately. Manifestations of patient anxiety can take different forms, ranging from simple agitation, sometimes accompanied by physiological manifestations to extreme forms of lipothymia or even myocardial infarction. Panic attacks can occur as well, as one of the manifestations of anxiety. The problem that occurs in the dentist's office is the correct and rapid diagnosis of panic attack and establishing a differentiated diagnosis with other conditions that can endanger the patient's life, such as heart attack. Also, the dentist must prevent, know and apply the treatment immediately in case of a panic attack. For these reasons we consider of the utmost importance the approach of this subject in this paper.

Keywords: panic attacks, dental office, emergency

INTRODUCTION

Anxiety can be a very common problem in everyday life. Anxiety is an emotional state characterized by a feeling of insecurity, disorder, diffusion. The term anxiety can refer to the feeling of fear or tension felt by a person, which appear as reactions to the stressful situation or a vague and unpleasant emotional state, accompanied by sometimes horror, distress and feelings of discomfort [1].

Răşcanu and Sava's suggested proposal or definition regarding anxiety, through care I see a feeling of diffuse danger, vaguely specified, with imminent repercussions, there are individual existences [2].

For P. Pichot , anxiety is an emotional state of constant worry, a phenomenological plan, based on three fundamental elements: imminent danger perception, an attitude to be realized in terms of dangerous use and a feeling of disorganization related to the consciousness of a total helplessness regarding the dangerous operation " [3].In addition to anxiously waiting for the reflection of anticipation of unfortunate events, anxiety is accompanied by muscle tension, motor inhibition and, above all, by neurovegetative manifestations, among the cares include: palpitations in repair, without dizziness or fainting, excessive sweating, redness or pallor, dry mouth, feeling of emptiness in the stomach, nausea, limited need for urination [4,5].

Some people experience anxiety as a concern about their own ability to cope with threats, which they perceive as imminent. They can become hypervigilant, thus increasing the level of activation of the nervous system, and thus, the attention paid to the negative valence of environmental stimuli.

Martin (1961) proposes that anxiety reactions be seen as a set of complex neurophysiological responses that must be differentiated, conceptually and operationally, from internal or external stimuli that provoke these responses [5]. Thus, the author emphasizes the importance of identifying and measuring patterns of behavioral and physiological responses associated with anxiety and differentiating between anxiety and other emotional reactions.

S. Freud is, without a doubt, the one who made the most important contribution to understanding the phenomenon of anxiety. Freud says that anxiety does not need a description, everyone has experienced, at some point, this sensation or, rather, this affective condition. Freud defines anxiety as "something felt", an affective state or a fundamentally unpleasant condition [6]. The experiential qualities associated with anxiety, which give it the character of "unpleasant", consist in feelings of apprehension and tension. The physiological and behavioral phenomena that accompany anxiety are essential components of anxiety.

Anxiety is, in Freud's view, a unique combination of unpleasant feelings and thoughts, physiological changes associated with the activation of the nervous system. The physiological changes are similar to those that Darwin associates with fear: accelerated heartbeat, sweating, muscle tension, disturbed breathing. Freud showed little interest in them, considering that feelings of apprehension, tension, and subjective qualities make anxiety unpleasant. Using introspective methods of psychoanalysis, the author tried to discover the historical element of the patient's childhood, which makes the connection between the anxious reactions and the external stressor that evokes them. Finally, he defines the concept of anxiety as an emotional reaction that serves as a signal that indicates the presence of a dangerous situation [7].

In the context of the theory of danger signaling, Freud distinguishes between objective anxiety and neurotic anxiety, a distinction based on the source of danger, a source that may be external to the individual or resulting from repressed impulses of the individual himself [6]. Objective anxiety is triggered when a real danger from the outside world is perceived by the individual as threatening. An essential feature of objective anxiety is that the intensity of the emotional reaction is proportional to the magnitude of the external danger.

Fear is an often unconsciou emotion. It is the emotion specific to danger or the perception of danger. Fear has a great psychological impact: the heartbeat and breathing rate accelerate, the muscles contract and the hands tremble and are cold. [8] Fear refers to an innate, almost biological-based alarm response to a dangerous or life-threatening situation. Anxiety, in contrast, is a more future-oriented and global concern – it is sometimes referred to as panic attacks.

ANXIETY IN DENTAL OFFICE

The fear of the dentist is nothing but an immediate reaction of the patient to what he considers to be a danger, a direct threat. Fear and anxiety can make a person more difficult to treat. Trying to explain the direct causes of dental anxiety, several authors have concluded that, in addition to previous psychotraumatic experience - sometimes even in childhood - in connection with the first visits to the dentist or information contagion from other people (family, acquaintances), There are several major factors as sources of this form of anxiety: fear of pain, anesthesia, noise, smell [9]

The patient views the dental treatment as a threat and will react according to a multitude of factors. These factors can be internal-subjective (personality, previous experience, moment factor, generalized anxiety) or external-objective (stressors from dental office). The stressors that trigger anxiety in the dental office can be: Psychological (pain, fear of contact with a disease, invasion of the intimate space, uncertainty, the presence of companions, certain keywords - needle, injection, blood) and physical (noise, smell, light, color, dust, sight of instruments). [10]

In the dental office there are a series of reactions, coping mechanisms in front of stressors. One of Murphy's laws says that "Under stress, most people choose, from several options, the worst." The most common behavioral reactions encountered in the dental office are: talks a lot, nervous, discontinue treatment frequently, shaking, squeeze the chair or fists, stands up ,closes his/her eyes, orbicularis contraction, raises hand, follows the doctor's movements, avoidance movements, nausea, hitting , crying, panic attack, passing out [11].

PANIC ATTACK

From biological theories, there is a genetic predisposition and disturbance in the functioning of certain neurotransmitter systems in the brain (noradrenergic, serotonergic, dopaminergic, GABA). During panic attack an excessive vegetative reaction, with an increased tonus of sympathetic system is present, and also with increased catecholamine release [12]. Psychological theories speak of separation fears, the austerity of the release of sexual energy, the traumatized trauma, various misconceptions, or irrational thoughts, etc Several studies have shown that the risk of PD is eight times higher in those with first-degree relatives with PD compared to those with no family history [13].

Panic attacks often strike without warning, often without a clear trigger, and can occur when the person is relaxed or even asleep. Panic attacks are common. A panic attack can be a one-off, but usually many people experience repeated episodes over a longer period of life. Among people who have ever had a BP, most have had recurrent BP (66.5, ie 0.5%). Most people recover without treatment. Sometimes recurrent panic attacks are often triggered by a specific situation, in which the person felt in danger before. A panic attack can also occur as part of another disorder, such as panic disorder, social phobia or depression. Patients who experience repeated panic attacks are diagnosed with "panic disorder." [14]

Depending on the relationship between the onset of the attack and the absence or presence of situational triggers, panic attacks can be divided into the following: unexpected (tested) panic attacks in which the occurrence of a panic attack is not related to a situation trigger (occurs spontaneously as a flash) and is the most common type of attack and panic

attack that occurs almost invariably immediately after exposure or anticipation of a triggering situation (e.g., seeing a snake or dog always triggers a panic attack immediately). However, they are not inextricably linked to the trigger and do not need to appear immediately after exposure (for example, a patient who has a panic attack after the extraction is completed, before leaving the office) [15]. There may also be night attacks that are not the subject of this article [16].

Panic attacks are terrible and often confused with medical emergencies. Once medical causes are ruled out, the role of the psychiatrist is to contain the patient's anxiety and provide psychoeducation. Panic attacks can be unique, recurring events or indicate severity in another anxiety disorder.

The panic attack is an emergency in the dental office due to the need for a quick and correct diagnosis. Although it does not endanger the patient's life and in most cases it resolves spontaneously, if the wrong diagnosis of a panic attack is made. endangers the patient's life.

The signs and symptoms of a panic attack may include : hyperventilation, heart racing, chest pain, and trembling, sweating, and dizziness, with a fear of losing control, going crazy, or dying [13]. In the dentist's office, a panic attack can be triggered before the start of treatment, during treatment or after its completion. Many people with panic attacks may have difficulty breathing, sweat profusely, tremble, and feel their hearts pounding. Some people will also experience chest pain and a feeling of detachment from reality or themselves during a panic attack, so they make think they’re having a heart attack. Others have reported feeling like they are having a stroke. Panic attacks can be scary and may hit you quickly. [14].

The patient may have a panic attack from the moment he enters the office or the waiting room. In general, this is triggered by the anticipation of dental treatment, the vast majority of cases having a traumatic history related to the dental act. In the dental office, the patient is generally known to have a history in this regard and knows how to anticipate or can prevent the doctor from the fact that it is a panic attack. Most often the patient treats himself, as he does in other situations. Triggers can be anticipation of treatment, the smell in the office, the noise of the equipment, the presence of certain attendants or other patients in the waiting room. During treatment, the triggering factor can be, against the background of the patient's anxiety, any stress factor in the office from those mentioned above. Some patients manage to manage anxiety during treatment and in the end, on the background of relaxation when they get out of conscious control they make that panic attack [16].

The diagnostic criteria - according to DSM IV TR for a panic attack are [17]:

- a distinct period of intense fear or discomfort, in which four (or more) of the following symptoms appear suddenly and reach maximum intensity within up to 10 minutes;
- palpitations, strong heartbeat or accelerated pulse;
- sweating;
- tremors or trembling of the whole body;
- feeling short of breath or suffocation;
- feeling suffocated;
- chest pain or discomfort;
- nausea or abdominal discomfort;
- sensation of dizziness, instability, "light head" or fainting;
- derealization (feelings of unreality) or depersonalization (detachment from one's self);
- fear of losing control or going crazy;
- fear that he will die;
- paresthesias (numbness or stinging sensations);
- waves of cold or heat.

Not all panic attacks meet these criteria; there are also cases without obvious respiratory manifestations, in which the cardiac ones predominate

Diferential Diagnosys is done with:

- Somatic diseases: heart disease (myocardial infarction, mitral valve prolapse, paroxysmal tachycardia, congestive heart failure, hypertension), endocrine disease (thyrotoxicosis, Addison's disease, Cushing's disease), pheochromocytoma, lung disease (embolism, asthma) with neurological diseases (cerebrovascular, embolism, TIA, Wilson's disease), with other diseases (premenstrual syndrome, temporal arteritis, uremia, systemic infections, SLE, electrolyte disorders, heavy metal poisoning);
- Mental disorders: simulation, false disorders, hypochondriac disorder, schizophrenia, social / specific phobias, post-traumatic stress disorder, depression [18].

The differential diagnosis must be made quickly, in general the patient knows that it is a panic attack and he inform the doctor. If it is the first experience or if the patient's condition deteriorates, the help of an ambulance is requested.

Treatment

- Preventing

Prevention of a panic attack can be done by a correct anamnesis with the identification of patients likely to develop such a reaction. It is also very important to communicate with the patient, identify factors that could trigger the reaction and adapt as much as possible to treatment or treatment approach accordingly**.**

- Emergency treatment [15]:
  - Recongnise the panic attack
  - Deep breathing - While hyperventilating is a symptom of panic attacks that can increase fear, deep breathing can reduce symptoms. Focus on taking deep breaths in and out through the mouth, feeling the air slowly fill chest and belly and then slowly leave them again. Breathe in for a count of four, hold for a second, and then breathe out for a count of four. The patient may be allowed to breathe in a bag.
  - To reduce the stimulus, close your eyes during your panic attack. This can block out any extra stimuli and make it easier to focus on your breathing.
  - Find a focus object, distraction
  - Use muscle relaxation techniques-Think of a nice place [14]

Treatment of panic disorder should in no way be limited to providing first aid during panic attacks (usually by injection of diazepam intramuscularly as an emergency) without planning a targeted and ongoing treatment. The main treatment options are psychotherapy and medications. Combination of them is considered as the most effective treatment [19] Antidepressants acting on the serotonergic system—citalopram, fluvoxamine, fluoxetine, paroxetine, sertraline, the SNRIs venlafaxine and duloxetine, and the TCAs imipramine and clomipramine are effective in treating acute phase of panic disorder [20].

Cognitive-behavioral therapy involves teaching patients to recognize their distorted thinking. The goal is to clarify the patient's misinterpretation of the physical symptoms of panic attacks and act on avoiding behavior by gradually exposing the situations that led to the attack.

Useful relaxation exercises as well as regular breathing exercises, with moderate physical activity, are also useful [21-24].

CONCLUSIONS

Analyzing the above we can draw the following conclusions:

1. Panic attacks do not endanger the patient's life but can be an emergency in terms of establishing the correct diagnosis

2. There are many pathologies with which the differential diagnosis is made, the most common of which is myocardial infarction.

3. In general, the patient knows that it is a panic attack and communicates this to the doctor. In this case, an attempt is made to adapt the treatment to the patient's psychological needs.

4. If it is determined that it is a panic attack, emergency treatment consists of stopping treatment, advising the patient to regulate breathing, distraction, trying to calm him down without minimizing or mocking the patient's symptoms.

5. If there are suspicions related to the diagnosis or the patient's condition does not evolve favorably, call the ambulance services.

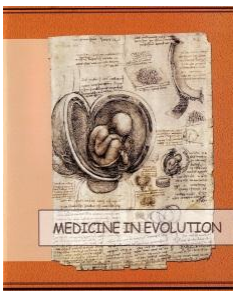
6. The treatment is recommended to be done by the psychiatryst, the patient is advised to follow the treatment recommended by him.

REFERENCES

1. Robu V, Psihologia anxietăţii faţă de testări şi examene. Repere teoretice şi aplicaţii. Iaşi: Editura Performantica, 2011, 322-323
2. Iamandescu IB, Psihologie medicală, Ed. InfoMedica, Bucureşti, 2005, 125-129
3. Răşcanu R., Psihologia sănătăţii: de la credinţe şi explicaţii la sisteme de promovare a ei. În M. Zlate (coord.). Psihologia la răspântia mileniilor., Iaşi: Editura Polirom, 2001,111-124
4. World Health Organization. ICD-10 classifications of mental and behavioural disorder: Clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992, 121-130
5. Stora JB, Stresul, Ed. Meridiane, Bucureşti, 1999, 144-145
6. Sarason, I. G. (1978). The Test Anxiety Scale: concept and research. În C. D. Spielberger & I. G. Sarason (Eds.). Stress and anxiety (Vol. 5, pp. 193-216). Washington, D. C.: Hemisphere Publishing Corp.
7. Bonewski D., Naumovska A., Panic Attacks and Panic Disorder, In book: Psychopathology - An International and Interdisciplinary Perspective,2020
8. T. Newton, K. Asimakopoulou, B. Daly, S. Scambler & S. Scott, The management of dental anxiety: time for a sense of proportion? British Dental Journal 213, 271 - 274 (2012)
9. Paraschiv C, Esanu I, Barbu RM, Anxiety and panic disorders in the dental office, Buletin de Psihiatrie Integrativa,2019, No. 4 (83) /141-145
10. Appukuttan D– Strategies to manage patients with dental anxiety and dental phobia: literature review Clinical, Cosmetic and Investigational Dentistry, 2016, 8:35-50
11. Amy B. Locke, Nell Kirst, Cameron G. Shultz - Diagnosis and Management of Generalized Anxiety Disorder and Panic Disorder in Adults Am Fam Physician. 2015. 1;91(9):617-624.
12. Dresler T et al. Revise the revised? New dimensions of the neuroanatomical hypothesis of panic disorder. Journal of Neural Transmission (Vienna). 2013; 120:3-29
13. Thwaites N, Panick attacs, In book: Psychiatry: Breaking the ICE: Introductions, Common Tasks and Emergencies for Trainees,2015,1447-1450
14. Hill, K. T., & Wigfield, A. (1984). Test anxiety: a major educational problem and what can be done about it. The Elementary School Journal, 85 (1), 105-126.
15. Ronad S, Preventing panic attacks, Nurse Care Open Acces J. 2019;6(5):155–156.
16. T. Newton, K. Asimakopoulou, B. Daly, S. Scambler & S. Scott, The management of dental anxiety: time for a sense of proportion? British Dental Journal 213, 271 - 274 (2012)
17. Fresco, D. M., Heimberg, R. G., Mennin, D. S., & Turk, C. L. (2002). Confirmatory factor analysis of the Penn State Worry Questionnaire. Behaviour Research and Therapy, 40, 313-323.
18. APA. Diagnostic and Statistical Manual of Mental Disorders. 3rd ed. Washington, DC: APA; 1987, 1135-1137
19. Hoge EA, Ivkovic A, Fricchione GL. Generalized anxiety disorder: diagnosis and treatment. BMJ. 2012; 345: e7500
20. Appukuttan D– Strategies to manage patients with dental anxiety and dental phobia: literature review Clinical, Cosmetic and Investigational Dentistry, 2016, 8:35-50
21. Podariu, A.S., Podariu, A.C., Popovici, R.A. (2017). Communication Strategy on Oral Health Education for Adolescents. Revista de Cercetare si Interventie Sociala, 58, 68-80.

22. Ramona Amina Popovici, Doina Onisei, Mihaela Codrina Levai, Angela Codruta Podariu, ”Legislation and management in the dental office”, Medicine in Evolution, vol XXI, nr.3, 2015, p 561-565;
23. Mihaela Codrina Levai, Angela Codruța Podariu, Ramona Amina Popovici, “The principles of basic medical attendance keys for the development of the health system management”, Medicine in Evolution, vol XXI, nr.3, 2015, p 359-.364;
24. Popovici, R.A., Podariu, A.C., Anculia, R.C., Serafin, A.C., Tigmeanu, C.V., Krems, C.D., Levai, M.C. (2017). Accessibility to Dental Health Care: Risk Factor in Oral Health at Rural Area Community. Revista de Cercetare si Interventie Sociala, 59, 48-61.

Success rate of pulp capping in primary and first permanent molars – A retrospective study



Per S.[1], Feraru I.V.[2], Răducanu A.M.[2], Buzatu R.[3], Didilescu A.C.[1]

[1]*Department of Embryology, Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[2]*Department of Paediatric Dentistry, Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[3]*Department of Dento-Facial Aesthetics, Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania*

Correspondence to:
Name: Ion Victor Feraru
Address: Str. Masina De Paine Nr. 4, Bloc OD 32 B ap 41 Bucuresti
Phone: +40 745064014
E-mail address: victor.feraru@umfcd.ro

Abstract

The aim of this study was the evaluation of the success rate of 3 pulp capping treatment techniques and to compare the results obtained in primary (PM) and first permanent molars (FPM). Material and methods: In this retrospective study we collected data from 194 medical records; 330 PM and immature FPM treated by pulp cappings were analyzed. Results: The success rate of pulp capping treatments in PM was 64.5%, while for FPM this counted for 96.6%. The means of survival time in PM were: indirect pulp capping (IPC) – 59.24±3.92 months, stepwise (SW) – 32.55±3.19 months, and direct pulp capping (DPC) – 32.35±6.6 months; for FPM: IPC – 102.47±3.07 months, SW – 57.31±5.18 months, and DPC – 49.46±17.07 months. When we compared the findings between dentitions, we observed that IPC and SW techniques had superior results in FPM than in PM. Conclusions: Based on our results, IPC and SW methods seem to be better choices in terms of preserving pulp vitality in both PM and FPM. Overall, FPM vital therapy was more successful than that of PM.

Keywords: pulp capping, vital pulp therapies, stepwise, indirect pulp capping

INTRODUCTION

Pulp capping is a dental treatment for deep carious lesions without symptoms of pulp degeneration.[1] A deep carious lesion is considered that one reaching the inner third or quarter of dentine. [2] The treatment itself consists in leaving a small amount of demineralized dentine in the most profound region of the cavity that will be covered with a biostimulatory material. [1]

There are 3 types of pulp capping treatment techniques: indirect pulp capping (one step), stepwise caries excavation (two steps), and direct pulp capping with the minimum opening of the pulp chamber. [2]

Currently, some of the most important focus points in scientific research are vital pulp therapy and selective carious removal with a great comeback for the stepwise excavation technique. [2-5] The scientific community and papers encourage the use of pulp capping and vital pulp therapies in general. [1-6]

Pulp capping procedures are indicated both in primary and immature permanent teeth for deep carious lesions with healthy pulp tissue or with reversible inflammation, [1] with the specification that a correct pulp diagnosis is difficult to establish in these teeth.

The aim of this study is to evaluate the success/failure rate of the different types of pulp capping techniques used in paediatric dentistry and to compare the results obtained in primary and immature permanent molars.

MATERIALS AND METHODS

This retrospective study was carried out over a period of two year (2017-2019), at the Paediatric Dentistry Department of the Carol Davila University of Medicine and Pharmacy, Bucharest.

The study was performed on the following samples of patients and teeth:

The patients study sample - Primary Molars contained 96 Romanian children aged between 2-10 years, mean age 5.86±0.18 years. The patients study sample - First Permanent Molars contained 98 Romanian children, aged between 5-10 years, mean age 7.77±0.09 years.

The primary molars study sample (PM-SS) contained 155 primary molars. The first permanent molars study sample (FPM-SS) contained 175 immature first permanent molars.

The molars study samples were divided based on 2 age groups, as follows: PM-SS, 2-6 years (51%, n=79), 6-10 years (49%, n=76); FPM-SS, 5-8 years (48%, n=84), 8-10 years (52%, n=91).

Among the PM-SS, 50.3% (n=78) were treated by indirect pulp capping (IPC), 45.8% (n=71) by stepwise caries excavation (SW) and 3.9% (n=6) by direct pulp capping (DPC). Among the FPM-SS, 75.4% (n=132) were treated by IPC, 22.3% (n=39) by SW and 2.3% (n=4) by DPC.

The inclusion criteria of the participants were: healthy patients without any chronic diseases who received at least one pulp capping treatment in either primary or immature first permanent molars. The inclusion criteria for teeth: all primary and immature first permanent molars which benefited of either indirect (one step/two steps techniques) or direct pulp capping.

The materials used for pulp capping were calcium hydroxide (Life®, SDS Kerr) and glass-ionomer restorative cement (Fuji IX®, GC).

Clinical success criteria were defined as absence of pain or any symptom of pulp alteration, no sign of necrosis or apical periodontitis, and absence of radiographic alterations.

Data were collected from the patients' medical records, without revealing their identity.

The variables extracted from the patients' medical records were the following: age, gender, type of tooth, duration of treatment supervision and time of failure due to pulpal complications.

Data analysis was performed using Stata® 11IC (StataCorp LP) statistical software. The Kaplan-Meier, Mantel-Cox and Pearson chi-squared methods were used to evaluate the mean survival time according to the pulp capping method and treatment's success rate according to several variables. A *p*-value of 0.05 was considered statistically significant.

RESULTS

The distribution of the PM-SS by age revealed that 51% (n=79) of the molars belonged to children aged 2-6yo and 49% (n=76) aged 6-10yo. In the FPM-SS 48% (n=84) of the molars belonged to children aged 5-8yo and 52% (n=91) to children aged 8-10yo.

The distribution of the PM-SS by type of molar was the following: 71 first molars (45.8%) and 84 second molars (54.2%). Distribution of PM-SS by dental arch: 58 superior teeth (37.4%) and 97 inferior teeth (62.6%).

The distribution of FPM-SS by dental arch was the following: 60 maxillary teeth (34.29%) and 115 mandibular teeth (65.71%).

The success rate of the capping treatment in the PM-SS was 64.5% (n=100), while for the FPM-SS success was 96.6% (n=169).

The success rates of the treatment by type of capping in the PM-SS were as follows: IPC 78.2% (n=61), followed by SW 52.1% (n=37), and DPC 33.3% (n=2) (p=0.001). Success rates for the pulp capping methods in the FPM-SS were as follows: 97.7% (n=129) for IPC, 94.9% (n=37) for SW, and 75% (n=3) for DPC (p=0.039).

The success rates in PM-SS for the 2-6 years age group were as follows: IPC 71.8% (n=28), SW 44.1% (n=15) and DPC 3.9% (n=2) (p=0.028). In the 6-10 years age group, the success rates were as follows: IPC 84.6% (n=33) and SW 59.5% (n=22), (p=0.014). In the 5-8 years old age group of the FPM-SS, the following success rates were recorded: 98.3% (n=58) for IPC, 91.3% (n=21) for SW and 50.0% (n=1) for DPC (p=0.004). In the higher age group, almost all treatments were reported as successes: IPC 97.3% (n=71), SW 100.0% (n=16) and DPC 100.0% (n=2) (p=0.777).

When we compared the findings between dentitions, we observed that IPC and SW had better results in permanent than in primary dentition, the differences being statistically significant: p=0,016 (IPC), and p=0,030 (SW), while for DPC the differences were not statistically significant (p=0,850).

There was no statistically significant difference between the success rates of first and second primary molars (64.8% and 64.3%, respectively) (p=0.948).

Statistically significant differences were not recorded between the treatments success rates in maxillary molars and mandibular molars, neither in primary (56.9% vs. 69.1%, p=0.125), nor in permanent teeth (98.3% vs. 95.7%, p=0.355).

The means of survival time for the three treatment methods in PM-SS were as follows: IPC - 59.24±3.92 months, SW – 32.55±3.19 months, and DPC – 32.35±6.6 months. Statistically significant differences were recorded between IPC and SW (p<0.001), as well as between IPC and DPC (p=0.038) (**Fig. 1**).

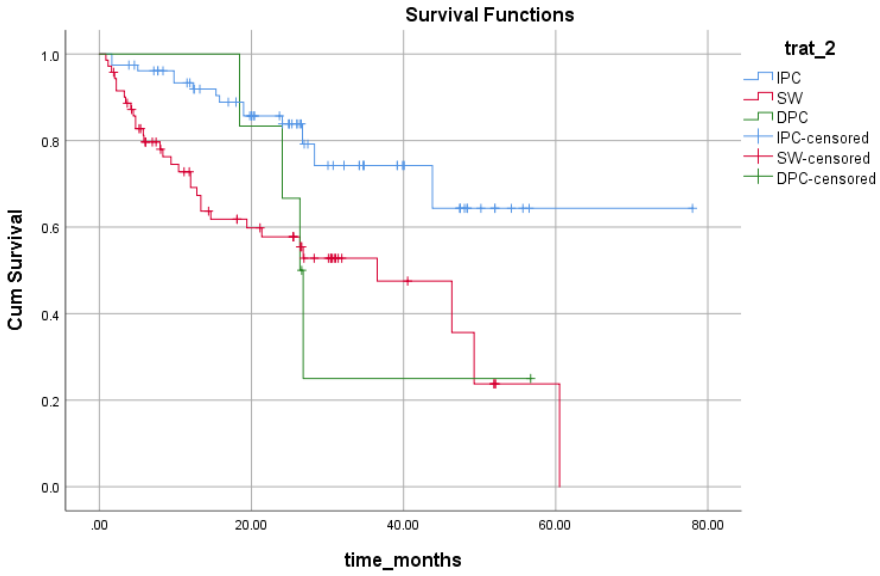


Figure 1. Kaplan-Meier survival curves for the pulp capping methods in primary molars

The mean survival time for the three treatment methods in immature FPM-SS were the following: IPC – 102.47±3.07 months, SW – 57.31±5.18 months, and DPC 49.46±17.07 months. Statistically significant differences were recorded only between IPC and DPC (p=0.027) (**Fig. 2**).

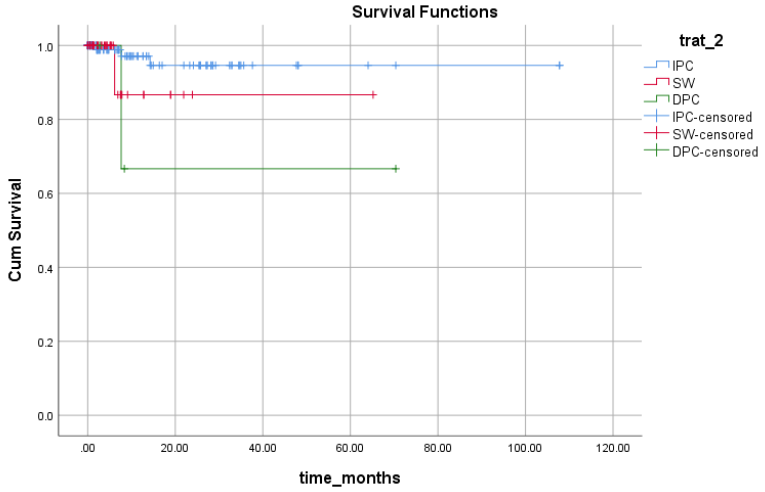


Figure 2. Kaplan-Meier survival curves for the pulp capping methods in immature first permanent molars

The overall success rate of pulp capping was greater in immature FPM-SS (96.6%, n=169) than in PM-SS (64.5%, n=100) (p<0.001). The mean survival time was, as well, greater in immature FPM-SS (99.75±3.24 months) than in PM-SS (43.95±3.69 months) (p<0.001) (Fig. 3).

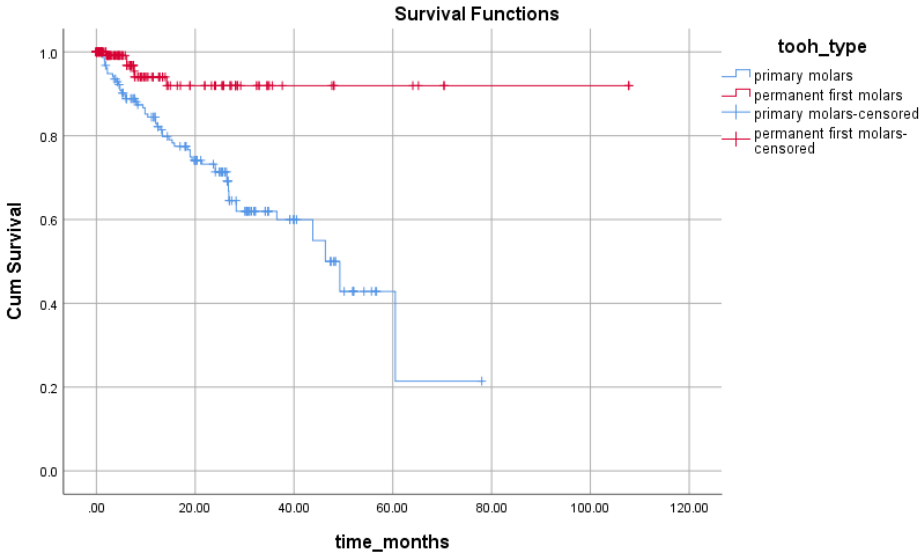


Figure 3. Kaplan-Meier survival curves for pulp capping in primary molars and immature first permanent molars

DISCUSSIONS

Our retrospective study's findings regarding the IPC success rate for PM-SS of 78.2% is lower than that of Al Zayer's, who estimated a 95% rate or that of Coll's systematic review and Meta-analysis (94.4%). [7,8] Still, the difference becomes smaller when comparing only our 6-10 yo group result - 84.6% success rate, a more appropriate comparison with Al Zayer's study that comprised children of 8y 6m mean age. [7] Meanwhile, Casagrande's et al. success rate of IPC in primary teeth, of 78%, is in accordance with the results with our study. [9]

Gurcan et al conducted a prospective clinical trial and evaluated the success rate for IPC. Their study revealed an 84.6% success rate for calcium hydroxide pulp capping. Though their study evaluated both primary and permanent teeth, they concluded that there was no statistically significant difference between primary and permanent teeth regarding success. Our 97.7% success rate for IPC is in discordance to this study. [11]

A success rate for SW technique of 52.1% found in our study is much lower in comparison with that of 90% in Coll's literature review. [10] This can be related to the fact that our research included a great number of very small children, the majority of them being difficult to cope with.

The DPC's success rate resulted in our research, of 33.3%, is lower than that in Coll's systematic review and Meta-analysis, which was 88.8%. [10] The number of DPC on PM-SS evaluated in our study is small, therefore in a larger sample the results might be different. DPC in the FPM-SS registered a success rate of 75%, with higher values recorded in the 8-10 years old age – 100%. Our findings are similar to those of Raedel et al. [12], with a 79.7% success rate, and Auschill et al. with 76.4%. [13]

While our study found no significant difference between the success rates of pulp capping in first and second primary molars, Al Zayer's et al. study found a statistically significant difference and stated that the first molar was 4.4 times more likely to fail than the second primary molar.[7] Casagrande's study did not find a statistically significant difference between the success rate of pulp capping in first and second primary molars but, similarly to the present study, recorded a slightly higher success rate in the first than the second primary molar.[9]

Gruythuysen et al. evaluated both primary and permanent teeth. While the success rate in permanent teeth was similar to ours, the one in the primary dentition was higher. They found no significantly statistical difference between the two dentitions, while the findings in our study favoured the permanent teeth. [14]

CONCLUSIONS

The results of our study suggest that all efforts should focus on minimising the risk of pulp exposure, in both dentitions. As such, when there is great risk of exposure, and complete excavation of the carious lesion cannot be done, stepwise excavation technique can be a viable solution of treatment. This conclusion is supported by the favourable results we found in immature FPM-SS.

IPC and SW methods seem to be better choices in terms of preserving pulp vitality in both PM and FPM. Overall, FPM vital therapy was more successful than that of PM.

Acknowledgement

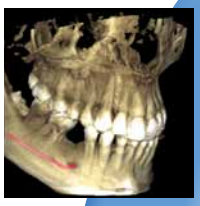
This work was supported by a grant of Ministery of Research and Innovation, CNCS – UEFISCDI, project number PN-III-P4-ID-PCE-2016-0506, within PNCDI III.

REFERENCES

1. ***American Academy of Pediatric Dentistry. Guideline on Pulp Therapy for Primary and Immature Permanent Teeth. Pediatr Dent. 2016;38(6):280-288.
2. Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. Int Endod J. 2019;52(7):949-973.
3. Hilton TJ. Keys to clinical success with pulp capping: a review of the literature. Oper Dent. 2009;34(5):615-625.
4. Smaïl-Faugeron V, Glenny AM, Courson F, Durieux P, Muller-Bolla M, Fron Chabouis H. Pulp treatment for extensive decay in primary teeth. Cochrane Database Syst Rev. 2018;5(5) (online), DOI: 10.1002/ 14651858. CD003220: CD003220.
5. Ha WN, Kahler B, Walsh LJ. Dental material choices for pulp therapy in paediatric dentistry. Eur Endod J 2017; 2(1):1-7.
6. Răducanu AM, Pedodonție-Îndrumar practic, Editura Bren, 2015, 95-96.
7. Al-Zayer MA, Straffon LH, Feigal RJ, Welch KB. Indirect pulp treatment of primary posterior teeth: a retrospective study. Pediatr Dent. 2003;25(1):29-36.
8. Coll JA, Seale NS, Vargas K, Marghalani AA, Al Shamali S, Graham L. Primary Tooth Vital Pulp Therapy: A Systematic Review and Meta-analysis. Pediatr Dent. 2017;39(1):16-123.
9. Casagrande L, Bento LW, Dalpian DM, Garcia-Godoy F, De Araujo FB. Indirect pulp treatmentin primary teeth: 4-year results. Am J Dent 2010;23(1):34-38.
10. Coll JA. Indirect pulp treatmentand primary teeth: is the primary tooth pulpotomy out of date? Pediatr Dent. 2008;30(3):230-236.
11. Gurcan AT, Seymen F. Clinical and radiographic evaluation of indirect pulp treatmentwith three different materials: a 2-year follow-up study. Eur J Paediatr Dent. 2019;20(2):105-110.
12. Raedel M, Hartmann A, Bohm S, Konstantinidis I, Priess HW, Walter MH. Outcomes of direct pulp treatment: interrogating an insurance database. Int Endod J. 2016;49(11):1040-1047.
13. Auschill TM, Arweiler NB, Hellwig E, Zamani-Alaei A, Sculean A. [Success rate of direct pulp treatment with calcium hydroxide]. Swiss Dental J.2003 ;113(9):946-952.
14. Gruythuysen RJ, van Strijp AJ, Wu MK. Long-term survival of indirect pulp treatmentperformed in primary and permanent teeth with clinically diagnosed deep carious lesions. J Endod. 2010;36(9):1490-1493.

ProMax 3D • PlanScan • ProFace

Unique 3D combination for open CAD/CAM



Digital perfection

Planmeca sets new standards with world's first dental unit integrated intraoral scanner for open connectivity to various CAD/CAM systems.

We would like to invite you to explore the dentistry in new dimensions – see the perfect combination of digital intraoral scan, CBVT and 3D facial photo datasets in one 3D image. This digital perfection enables you to study patient's complete anatomy in detail, plan and utilise open interface with modern CAD/CAM systems according to your needs. Now you can be one of the pioneering specialists, whether you are an implantologist, endodontist, periodontist, orthodontist or maxillofacial surgeon. The new era of dentistry is reality. It's your decision.

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555, sales@planmeca.com, www.planmeca.com

PLANMECA

1971 - 2011

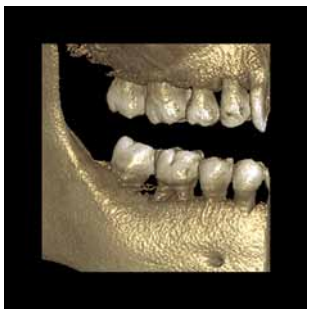
Planmeca ProMax 3D

All volume sizes

The Planmeca ProMax concept offers a full range of imaging volumes providing detailed information on patient anatomy. The comprehensive Planmeca ProMax platform complies with every need in dental radiology, offering digital panoramic, cephalometric, and 3D imaging as well as 3D face photo together with advanced imaging software.

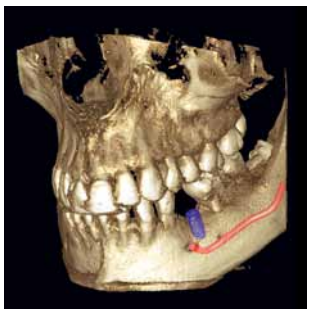
At the heart of the concept is the robotic SCARA technology: the unique robotic arm enables any movement pattern required by existing or future program, eliminating all imaging restrictions. With the Planmeca ProMax concept superior maxillofacial radiography can be performed with a single platform, today and in the decades to come.

All volume sizes



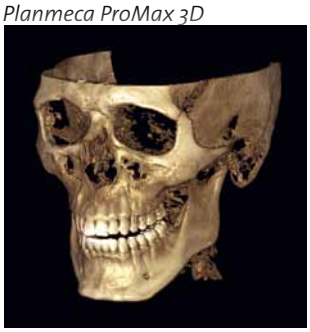
Planmeca ProMax 3D s

Ø42 x 42 mm–90 x 60 x 130 mm



Ø34 x 42 mm–140 x 105 x 130 mm

Planmeca ProMax 3D



Planmeca ProMax 3D Mid

Ø34 x 42 mm–Ø160 x 160 mm

Planmeca ProMax 3D Max

Ø42 x 50 mm–Ø230 x 260 mm

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555
sales@planmeca.com, www.planmeca.com

PLANMECA

Planmeca Romexis

Software refined

Available on the App Store

Planmeca Romexis is the software of choice for all dental imaging purposes. All patient's digital images – intraoral and extraoral X-ray images, 3D volumes, and photographs – are processed and stored in one easy-to-use system. Planmeca Romexis offers a complete set of tools for image viewing, enhancement, measurement, and implant planning, and fully integrates digital imaging with the patient's other clinical data.

Thanks to its powerful printing features, stunning printouts can be produced. Planmeca Romexis provides direct image capture from Planmeca X-ray units, interfaces with 3rd party devices via TWAIN, and is fully DICOM-compatible. Planmeca Romexis is a JAVA software that runs on Windows, Mac OS, and Linux operating systems, and embraces modern IT standards.

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555
sales@planmeca.com, www.planmeca.com

PLANMECA 40 1971 - 2011

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 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.

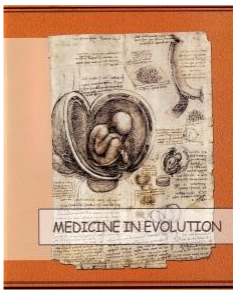
5. ETHICAL ASPECTS

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

6.1. CONTENT OF THE PAPER - INDICATIONS FOR ORIGINAL ARTICLES

Paper title [Book Antiqua 20, bold, left alignment]



Surname N.[1], Surname N.[2] [Book Antiqua, 14, bold]

[1] *Author Affiliation (DEPARTMENT, FACULTY, UNIVERSITY, CITY/COMPANY) [10, italic]*
[2] *Author Affiliation (DEPARTMENT, FACULTY, UNIVERSITY, CITY/COMPANY) [10, italic]*

Correspondence to:
Surname Name: [10, italic]
Address: [10, italic]
Phone: +40 [10, italic]
E-mail address: [10, italic]

Abstract [Book Antiqua, 12, bold, justify alignment]

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.

Keywords: Innovation, technology, research projects, etc. [Book Antiqua 9].

INTRODUCTION [Book Antiqua, 11, bold, left alignment]

Introduction presentation of general aspects, in the context of the approached theme.

Introduction include **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.

There is a limitation of 4/6 pages. All pages size should be A4 (21 x 29,7cm). The top margins should be 2 cm, the bottom, right, margins should be 2cm and left margins should be 2,85 cm. All the text must be in one column and Book Antiqua font, including figures and tables, with single-spaced 10-point interline spacing.

Aim and objectives [Book Antiqua 11, bold italic, left alignment]

The text included in the sections or subsections must begin one line after the section or subsection title. Do not use hard tabs and limit the use of hard returns to one return at the end of a paragraph. Please, do not number manually the sections and subsections; the template will do it automatically.

[Book Antiqua, 11 point, normal, justified alignment].

MATERIAL AND METHODS [Book Antiqua, 11, bold, left alignment]

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). [Book Antiqua, 11 point, normal, justified alignment].

RESULTS [Book Antiqua, 11, bold, left alignment]

Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations. [Book Antiqua, 11 point, normal, justified alignment].

DISCUSSIONS [Book Antiqua, 11, bold, left alignment]

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. [Book Antiqua, 11 point, normal, justified alignment].

CONCLUSIONS [Book Antiqua, 11, bold, left alignment]

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. [Book Antiqua, 11 point, normal, justified alignment].

REFERENCES [Book Antiqua, 11, bold, left alignment]

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.

- Books - Standard format - #. Author of Part, AA. Title of chapter or part. In: Editor A, Editor B, editors. Title: subtitle of Book. Edition(if not the first). Place of publication: Publisher; Year. p. page numbers.
- Journal Articles - Standard format - #. Author of article AA, Author of article BB, Author of article CC. Title of article. Abbreviated Title of Journal. year; vol(issue):page number(s).
- E-Books - Standard format - #. Author A, Author B. Title of e-book [format]. Place: Publisher; Date of original publication [cited year abbreviated month day]. Available from : Source. URL.
- E-Journals - Standard format - #. Author A, Author B. Title of article. Abbreviated Title of Journal [format]. year [cited year abbreviated month day];vol(no):page numbers[estimated if necessary]. Available from: Database Name (if appropriate). URL.

Internet Documents - Standard format - #. Author A, Author B. Document title. Webpage name [format]. Source/production information; Date of internet publication [cited year month day]. Available from: URL. [Book Antiqua, 10 point, normal, justified alignment].

[1] _____
[2] _____
[3] _____

6.2. CONTENT OF THE PAPER - INDICATIONS FOR CASE REPORTS

Content of the paper for case report will respect indications for original articles.

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.

6.3. MEASUREMENT UNITS, SYMBOLS, ABREVIATIONS

All measurements must be expressed in International System (IS) units. Abreviations must be fully explained when first used.

6.4. TABLES

Tables are noted with Roman figures and they will have a brief and concise title, concordant with their content.

6.5. 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.

6.6. EXPLANATIONS FOR DRAWINGS AND GRAPHS

Explanation for drawings and graphs must be clear and in readable dimensions, considering the necessary publishing shrinkage.

6.7. PHOTOGRAPHS

Offer glossy, good quality photographs. Any annotation, inscription, etc. must contrast with the ground. Microphotographs must include a scale marker.

6.8. ILLUSTRATION LEGENDS

Include explanations for each used symbol, etc. Identify the printing method for microphotographs.

7. COPIES FOR PUBLISHING

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.

Papers submitted for publishing will be addressed to:

Prof. Angela Codruta Podariu, DMD, PhD

Journal Medicine in evolution
Department of Preventive, Community Dental Medicine and Oral Health
Splaiul Tudor Vladimirescu no. 14 A
300041, Timişoara
Email: proiectetim@gmail.com

Dana Brehar-Cioflec, MD, PhD

Institute of Public Health *"Prof. Dr. Leonida Georgescu"* Timişoara
Bd. Victor Babeş no. 16
300226, Timişoara
Phone: 0256-492101
Email: danabreharcioflec@yahoo.com

Descoperiți elmex®.
Specialistul de încredere în îngrijire orală.

*Sondaj telefonic reprezentativ privind recomandările de pastă de dinți, organizat de Ipsos pe un eșantion de 300 de stomatologi, în ian-feb 2018.

elmex®
Healthy teeth for life.

Acest număr a apărut cu suportul COLGATE-PALMOLIVE