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Contents

ORIGINAL ARTICLES

PASTES BASED ON ROYAL JELLY, AN ALTERNATIVE FOR THE MINIMALLY INVASIVE TREATMENT OF PULPITIS (HISTOPATHOLOGICAL EXPERIMENTAL DATA)

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Abstract

INTRODUCTION: Royal jelly is the most interesting of trio product that comes from bees. The 10-hidroxydeconoic acid is a precious ingredient of royal jelly that exerts antibacterial and antitumoral activiity. It looks like a white-yellow cream, with a pH around 4-4.5.

The purpose was to define through histopathological slides, the anti-inflammatory and regenerative actions of pastes based on royal jelly in vital amputations in the near future.

MATERIAL AND METHODS: In 16 patients, aged from 35-60 years, there were planned extractions of 16 teeth for orthodontics and prosthetics purposes. Patients were treated at the University Clinic of Aldent, Tirana. The teeth were divided into three groups, and were treated with pastes based on royal jelly. Pulpal disease diagnosis was acute partial pulpitis based on clinical data. the coronary vital amputation was used, removing the inflamed pulp and aiming at a well-preserved retention of the radicular pulp.

RESULTS: From clinical examination, patients did not express concerns (as pains, pulsations, reactions on percussion or changes to the surrounding tissues) in conjunction with treated teeth. **Conclusions:** After 30 days of treatment, by the histopathologic examination a normal pulp was observed, with well expressed odontoblasts proliferation, a cell proliferation and lack of inflammation at the 1st group.

KEYWORDS: anti-inflammatory, propolis, regeneration, royal jelly, vital amputation.

Introduction

The propolis is a mixture of substances and other elements as: tannin, propolis resin, wax of bees, essence, pollen, different vitamins, microelements, etc. (Ahn, et al. 2009; Li-Chang Lu et al, 2005). Being a secondary product of bees, it is known for his antitumoral, antioxidant, antimicrobial, anti-

inflammatory, and immunomodulatory effects (Al-Shaher, et al. 2004***; Scazzocchio***, et al. 2006***)***.

A well-kept hive can produce during 5-6 months of spring-summer seasons about 500 grams of royal jelly. This is the most interesting of trio product that comes from bees.

Royal jelly is one of the most valuable products of the bee hive. It is excretion of hypopharynx glands and jaw of bees. The precious ingredient of royal jelly is 10-hidroxydeconoic acid which exerts antibacterial and antitumoral activities (Baker, et al. 1990; Hattori, et al. 2007***)***.

It contains 70% water, 30% dry substance, from which 15% are proteins, 12% carbohydrates and 3% lipids, it contains enzymes, cholinergic factors, vitamin B1, B2, B6, PP, Biotin, B5, B9, B12, inositol. Minerals and 28 oligoelements**.** Antitoxic factors, antibiotic (roializina), a growth factor that is biopterin, and antitumoral cytostatic factor (neopterin), etc. (Bonomi 1983; Fernandes Júnior et al 2003; Xiao et al, 1995).

It is a stimulant that facilitates the cellular metabolism, strengthens the immune defense and resistance to stresses, operating in depression and fatigue **(Table.1).** It looks like a white - yellow cream, perfumed and with a pungent flavor. Its PH is 4-4.5.

Table 1. Properties of royal jelly.

General revitalizer	Stimulate the level of anti-anemic senile (aging)	Stimulant of appetite	Stimulant of humor	Equilibrator of neurovegetative and psychological systems
Immunomodulatory (incentive to anticorps)	Antibacterial	Anti-hypertensive	Antiviral	Antitoxic

Aim

To define through histopathological slides, the anti-inflammatory and regenerative actions of pastes based on royal jelly, in vital amputations in the near future.

Materials And Methods

In 16 patients, aged from 35-60 years, including 7 women and 9 men, extractions of 16 teeth were planned for orthodontics and prosthetics purposes. For the interventions, a sub-agreement with the patients was done, on the method of treatment and dental extractions. Patients were treated at the University Clinic of Aldent, Tirana. The teeth were divided into three groups, where were treated with pastes based on royal jelly **(Table.2).** Pulpal disease diagnosis based on clinical data was acute partial pulpitis. As a technique, we used the coronary vital amputation removing the inflamed pulp and aiming at the well-preserved retention of the radicular pulp. Anesthesia in all cases was infiltrative with 4% Articaine. Cavity was opened by traditional techniques.

Table 2. Therapeutic pastes taken in treatment based on royal jelly.

Group 1	Group 2	Group 3
Zinc oxide + royal jelly	Zinc oxide + royal jelly + 5% propolis dissolved in propylene glycol	$Ca(OH)_2$ + royal jelly + 5% propolis dissolved in propylene glycol

Pastes were prepared ex-tempore, thanks of royal jelly obtained from the pipe (Fig.1) in the mashed consistency, applicable in the cavity. Hemorrhage was banned in most of cases with saline. After drying the cavities under sterile conditions, application of paste was done without pressure on the radicular pulp in a thickness of 2 mm, and then the setting the layer of cement without pressure above, and in the end the definitive composite fillings.

Patients were observed for a period of 30 days. After 30 days the extractions of teeth were done and fixed in 10 % formalin. EDTA was used as a decalcificator, in the Histopathologic Laboratory of the QSUT "Hospital Center University of Mother Teresa" Tirana, whereas stains Hematoxylin - Eosin and Masson's Trichrome were used. Slides were performed longitudinally and transversally as well.

Fig. 1: Pipe with royal jelly, used for the preparation of pastes

Results Of The Treatment

During clinical examination, patients did not complain of any concerns (as pains, pulses, reactions on percussion or changes to the surrounding tissues) in relation to treated teeth.

In 1st group, as the intention was to see the action of royal jelly mixed with an indifferent powder, but not radiotransparent such as zinc oxide, after 30 days of treatment and observation, by histopathologic examination,it was noted a normal pulp with well expressed odontoblasts proliferation, and in the pulp it was noticed a cell proliferation and lack of inflammation (Fig.2,3,4).

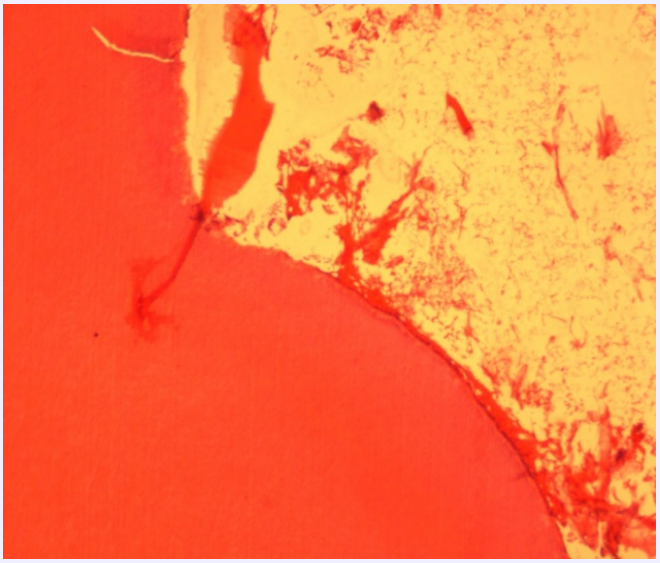

Fig.2: Longitudinal slide of pulp with Hematoxylin-Eosin stain, normal histological structure.

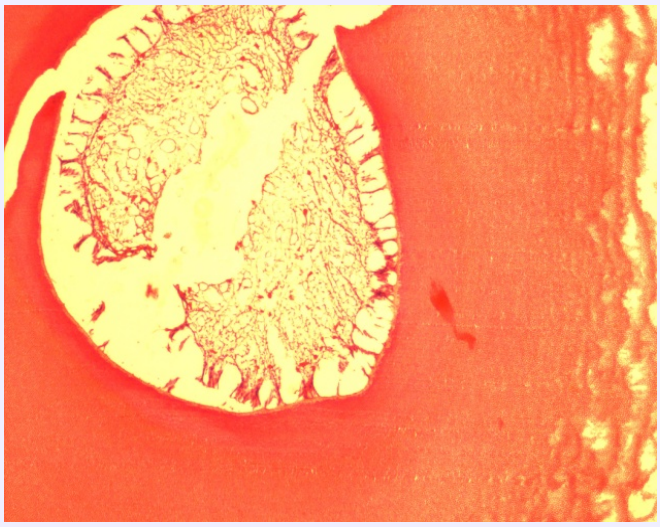

Fig.3: Transversal slide of pulp with Hematoxylin-Eosin stain after treatment with Zinc Oxide + royal jelly.

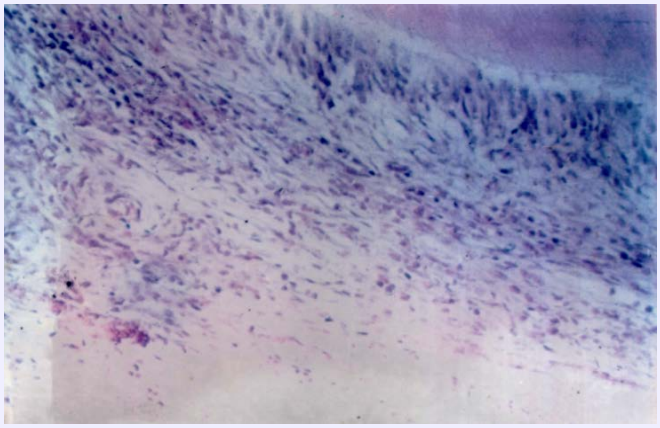

Fig.4: Apical pulp - odontoblasts borderless with a regular palisades placement, blood capillary and normal pulp in the apex. Hematoxylin-Eosin stain.

In 2nd group, treated with the paste based on oxide zinc + royal jelly + 5% propolis dissolved in propylene glycol, normal pulp was observed after 30 days of treatment, odontoblasts borders in 50% of cases, while in the rest of cell structure fibrocytes and fibrotic tissue prevailed, with calcic clusters in spherical shapes (Fig.5,6,7).

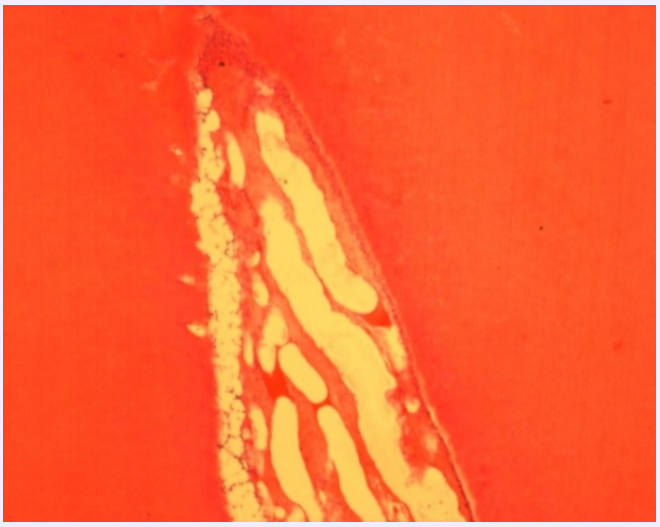

Fig.5: Normal pulp after 30 days of treatment of paste based on ZnO + royal jelly + 5% propolis dissolved in propylene glycol. Hematoxylin-Eosin stain.

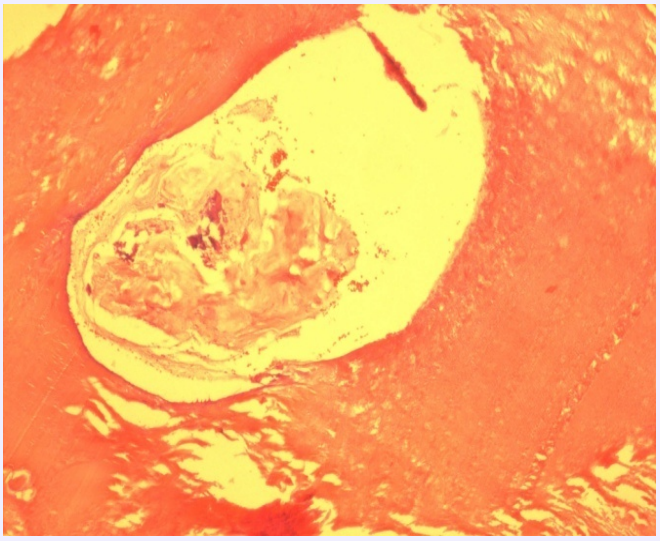

Fig.6: It was noted in this transverse slide, the presence of fibrosis and calcification of the pulp. Hematoxylin-Eosin stain.

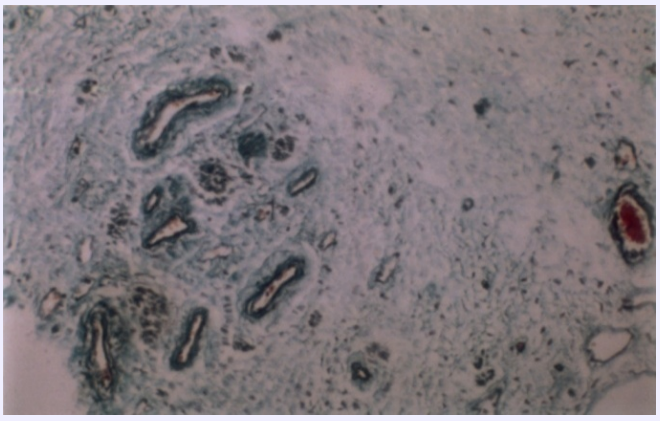

Fig.7: Neurovascular bundles of radicular pulp, after 30 days of treatment. Coloration with Masson's Trichrome.

In the 3rd group, treated with paste based on calcium hydroxide + royal jelly + 5% propolis dissolved in propylene glycol, in the longitudinal slide was noticed fibrotic pulp where prevailing fibrocytes after 30 days of treatment, while in the transversal slide was surveyed fibrosis and calcium agglomerations. In one of the cases, in the preparation stained with the Masson's Trichrome we discerned a superficial demineralization lesion and expressed tissue fibrosis. The presence of sclerotic pulp with calcic foci was noticed in this group (Fig.8,9,10,11).

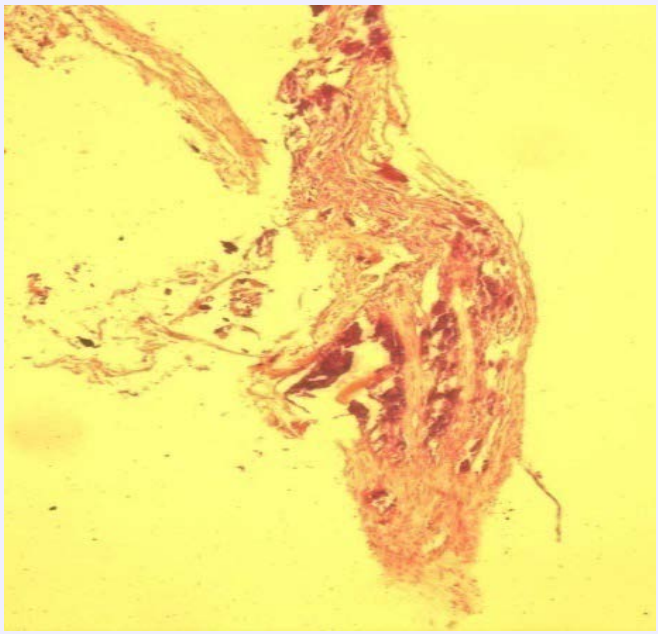

Fig.8: Fibroblastic proliferation, large fibrous and calcic clusters no condensed, after 30 days of treatment with Ca (OH) ☐ + royal jelly + 5% propolis dissolved in propylene glycol.

In 2nd group, treated with the paste based on zinc oxide + royal jelly + 5% propolis dissolved in propylene glycol results were better, while in 3rd group where calcium hydroxide was present the result did not change, but in the pulpal tissue were more present fibrosis, radicular pulpal calcifications and pulpal sclerosis.

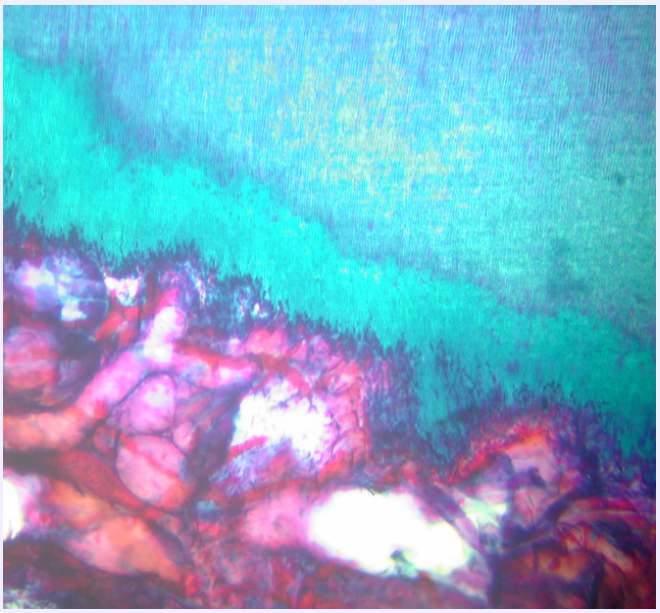

Fig.9: Fibrosis and micro calcic, with Masson's Trichrome stain.

Fig.10: Fibrosis + canalar structure of dentin, after 30 days of treatment. Masson's Trichrome stain.

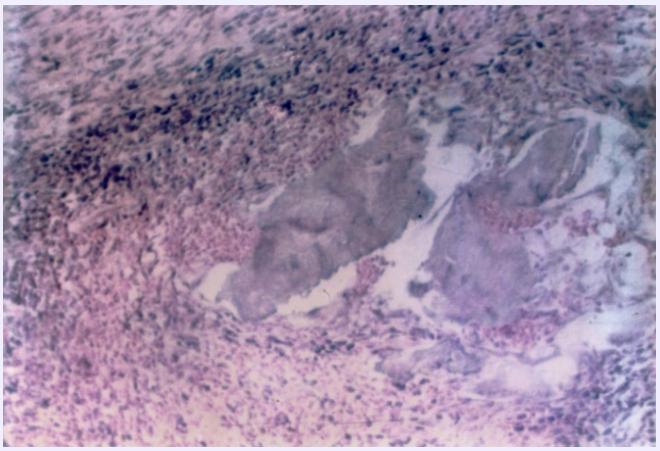

Fig.11: Chronic inflammation with pulpal calcification, after 30 days of treatment. With Hematoxylin – Eosin stain.

Discussion Of Results

As we stated in the beginning, the purpose of this study was the determination of anti-inflammatory and regenerative actions of royal jelly in inflamed pulpal tissue after coronary amputation. This was confirmed from the analysis of the ***first group***, where we implemented the composition of royal jelly only. It is well known in literature that the dentinal bridge stimulated by calcium hydroxide is incomplete, porous and does not provide long-term protection of pulp (Gomes., Brenda. 2002). Today, calcium hydroxide does not hold the position of glory as a single recovery pulp agent (Athanassiadis et al, 2007). Creating a suitable terrain in pulp through the curative material, which allows the healing, has opened the way for new alternative research efforts, where our modest work is also included.

Our histopathological results showed that we respected the conditions of radicular pulp to be healthy (Rroku C, Pavli E. 1979).

In various biological treatments, preparations based in antibiotics, sulfanilamide, glucocorticoids, antiseptic, proteolytic enzymes, and bio-substrates, etc. have been used

These preparations in a number of cases reduce inflammation, but do not stimulate the reparative dentin, and may even annihilate the dentine-genesis (such as glucocorticoids) (Gomes, Brenda. 2002).

Royal jelly is an albumin metabolic bio-stimulator and of cell regenerative processes (Rembold H. 1965**;** Xu Ming et al,. 1993). Decenoic acid is the main factor of antimicrobial action of royal jelly. It shows a high level of antimicrobial activity in relation to gram positive microorganisms, antiviral action was evident in water fractions of jelly, and among them, especially in albumin substance such as gamma globulin. So, the active factor of royal jelly, with antibacterial function, antiviral and antitoxic, are fatty acids, and in particular decenoic acid, but also the albumin substance (Hattori N. 2007).

Pastes with royal jelly positively influence on radicular pulpal tissue, haste the healing and stimulate the odontoblasts. It is important to note that, medicinal paste without the presence of calcium hydroxide stimulated the odontoblasts with relation to the presence of royal jelly, which is a biocatalyst of vital processes of the cell (Wu Cui-Wen., et al. 1993; Xu Ming et al 1993).

Conclusion

In conclusion, we managed to, that royal jelly composed in the pastes combined with propolis, possessed an expressed action of:

- ✓ analgesic,
- ✓ anti-inflammatory,
- ✓ odontoblasts stimulant,
- ✓ regenerative.

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SERUM TRACE ELEMENTS IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Abstract

INTRODUCTION: Many trace elements play important roles in activating or inhibiting enzymatic reactions, by competing with other elements and metal proteins for binding sites, by affecting the permeability of cell membranes and by other mechanisms. They play important roles in the oxidant/antioxidant balance. As such, trace elements are thought to be involved directly or indirectly in the pathogenesis of several diseases.

Free radicals have harmful effects on cells and tissues and are thought to be responsible for the pathogenesis of many diseases. Trace elements are required for the antioxidant enzymes and hence the optimal function of the immune system. Changes in the levels of these elements may lead to a reduction in antioxidant activities in various diseases.

The aim of this survey is to put in evidence possible alterations of copper, zinc and selenium status in patients suffering by oxidative stress caused by cardiovascular disease (CVD).

MATERIAL AND METHODS: We investigated serum levels of copper, zinc, and selenium in 83 patients hospitalized by acute myocardial infarction in some hospitals in Tirana and in 70 healthy persons. Serum copper and zinc measurements were carried out using flame atomic absorption spectrometry Shimadzu 7700 (AAS) and serum selenium measurements were carried out using atomic absorption spectrometry hydride method. Statistical processing of the data was carried out using Statistical Package for Social Science (SPSS 20).

RESULTS AND DISCUSSION: The results of this study have shown that levels of copper have increased in patients compared to control group, while the levels of zinc, and selenium decreased in patients compared to control group. Significantly low serum zinc ($p < 0.001$) and serum selenium ($p < 0.001$) were found in patients suffering by oxidative stress caused by CVDs compared to the group of healthy persons, whereas significant difference between the groups included in our survey was also found for serum copper ($p < 0.01$).

CONCLUSION: The results of this study indicate that there are alterations in serum concentrations of trace elements in cardiovascular patients, suggesting that they may play a role in the pathophysiology of these diseases by virtue of their role in oxidative stress.

KEYWORDS: CVD, serum zinc, serum selenium, serum copper, oxidative stress.

Introduction

Heart disease is one of the major health problems of developing countries of the world. Oxidative stress plays an important role in the progression of adverse complications in CVD, and many trace elements are involved in the oxidant-antioxidant balance. Essential trace elements copper, zinc and selenium are important parts of antioxidant enzymes as superoxide dismutase, glutathione peroxidase as well as of transport protein with antioxidant properties-ceruloplasmin. Mentioned trace elements may affect antioxidant defence system. Abnormalities associated with trace elements have not received much attention from clinicians in the past; however, in the past few years there has been a veritable explosion of knowledge about trace elements which are associated with abnormalities in experimental animals as well as in humans. The information explosion is rapidly reaching the stage where clinicians will be called upon more frequently to diagnose and treat trace element-related diseases (Linder and Hazeghazman, 1996, Kralik et al, 1996, Cordova and Alvarerez-Mon 1995).

Normal cardiovascular function is affected profoundly by a large number of processes at the molecular level. Many other etiological factors play a role. In the human body, trace elements function in a similar way; most of them are found at the active sites of enzymes or of physiologically active substances of the body.

Dietary deficiency causes a variety of clinical signs and symptoms through the decreased activity of these active substances. Slight or severe trace elements imbalances are considered risk factors for coronary heart disease (Metz 1982, Wada and Yanagisawa 1996).

Many elements exert a very strong influence on CVD risk factors such as disorders of blood lipids, blood pressure, coagulation, glucose intolerance and circulating insulin.

Detection and correction of trace elements imbalance in populations reduce the incidence of atherosclerotic heart disease by diminishing individual risk factors. Recent developments suggest that marginal deficiencies of microelements are common in human nutrition.

Copper, zinc and selenium are essential elements that have an important role in protection against oxidative stress, which has been implicated in the pathogenesis of over 100 human diseases (Halliwel and Cross 1994, Telisman 1995, Cerrutti, Ghosh, Oya, Amstad 1994). Stress can affect many vital processes and increase individual susceptibility to various diseases (Chrouspos and Gold 1992, Ader et al, 1995)

The aim of this survey is to put in evidence possible alterations of copper, zinc and selenium status in patients suffering by oxidative stress caused by CVD

Materials And Methods

We estimated serum copper, zinc and selenium levels of 83 patients hospitalized by acute myocardial infarction in some hospitals in Tirana, as well as 70 healthy persons as the control group. Blood samples were collected by vein puncture in plastic tubes. The samples were then centrifuged (2000 g at 4º C for 10 min) immediately. The sera were stored in - 20º C until subsequent analysis.

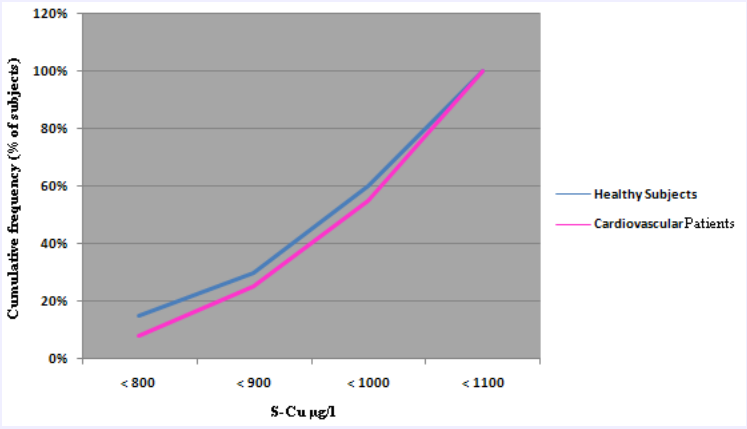
Serum copper and zinc measurements were carried out using Shimadzu AA 7000 flame atomic absorption spectrometry and serum selenium measurements were carried out using atomic absorption spectrometry hydride method. Statistical processing of the data was carried out using Statistical Package for Social Science (SPSS 20).

Results And Discussion

Data on levels of copper, zinc and selenium in sera are summarized in tables 1-3 and data on the cumulative frequency distribution are presented by the respective graphs.

Table 1. Levels of Cu in sera of cardiovascular patients and of control group (µg/L)

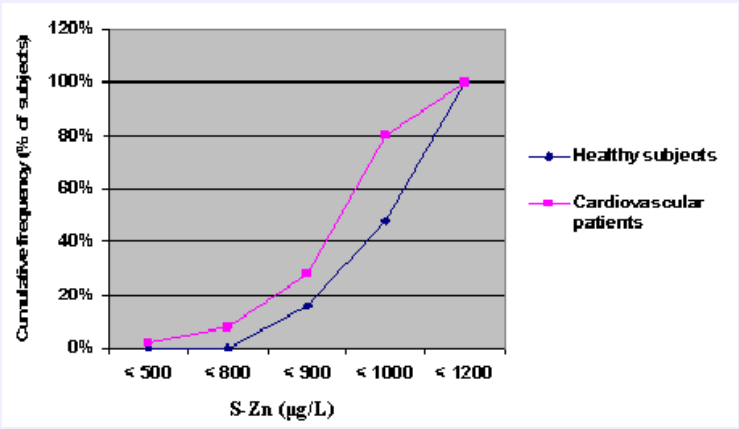
	Patients group	Control groups	Significance
Range	772 - 1105	698 - 1062	P < 0.01
Mean ± SD	981.85 ± 12.20	815.55±18.11	
Median	964.18	769.45	
CI 95%	925.82 – 985.21	788.41 – 824.57	
Number of samples	83	70	



Graphic 1. The cumulative frequency distribution of the copper serum (S-Cu) in healthy subjects and cardiovascular patients.

Table 2. Levels of Zn in sera of cardiovascular patients and group of control (µg/L)

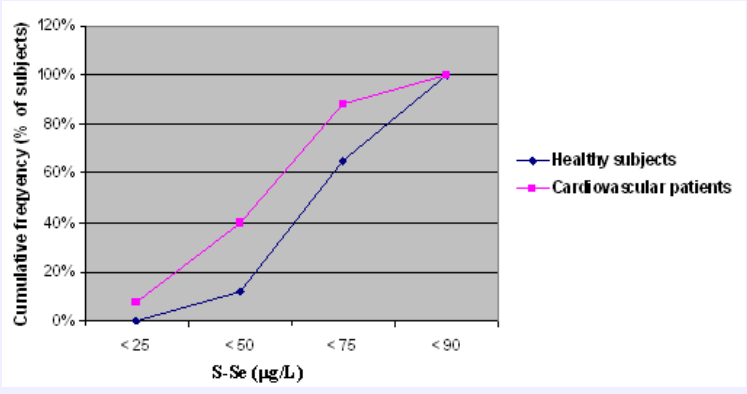
	Patients group	Control groups	Significance
Range	428-980	825-1170	P< 0.001
Mean ± SD	790±32.86	995.04±18.85	
Median	786.34	1005.00	
CI 95%	723.23 – 856.46	956.14 – 1033.94	
Number of samples	83	70	



Graphic 2. The cumulative frequency distribution of serum zinc (S-Zn) in healthy subjects and cardiovascular patients.

Table 3. Levels of Se in sera of cardiovascular patients and group of control (µg/L)

	Patients group	Control groups	Significance
Range	14.20-80.60	41-88	P< 0.001
Mean ± SD	46.98±3.44	65.84±2.063	
Median	45.90	65.60	
CI 95%	41.84 – 52.12	61.60 – 70.12	
Number of samples	83	70	



Graphic 3. The cumulative frequency distribution of the serum selenium (S-Se) in healthy subjects and cardiovascular patients.

As it is shown by tables and graphs, there is a difference on the concentration of microelements under survey between patients suffering by CVD and the control group. Obtained results shown a decrease of levels of serum zinc and serum selenium in patients compared to healthy control group, as well as an increase of levels of serum copper in patients versus healthy control group. Similar results have been obtained by other authors in their studies (Yahya et al, 2014).

The association between blood Zn and Cu concentration and prevalence of CVD has been described controversially (Cikim et al, 2003, Sinning et al, 2010). Previous reports demonstrating an association between low Zn and Cu concentrations and incidence of cardiovascular events in epidemiological studies but other recent studies revealed controversial data; for example in several prospective (Venardos et al, 2007, Venardos et al 2004, Rayman 2000) and retrospective (Blankenberg 2003) studies low serum Zn, Cu and selenium levels were associated with increased risk of coronary heart disease, however, in other studies this association could not be confirmed.(Navarro-Alarcon et al, 2000, Flores-Mateo et al, 2006, Bleys et al, 2006).

The controversies and complexities surrounding the oxidant/antioxidant role of copper zinc and selenium will be emphasised as there is now substantial evidence that some , but not all, of the potential role of changing of levels of these trace elements in cardiovascular mechanisms are related to oxidant stress and compromised antioxidant defence. Finally, the relationship between copper, zinc and selenium status and major biological mechanisms associated with CVD, especially atherosclerosis, arterial compliance and thrombosis will be evaluated using data from experiments in man and animals.

Recent research has shown that free radicals, particularly, reactive oxygen species (ROS) play an important role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction (Bandyopadhay et al 2004) Oxidative stress describes the condition where an excessive production of ROS overwhelms endogenous anti-oxidant defence mechanisms. Te resultant elevation in ROS levels has a detrimental effect on cellular function, a consequence of ROS-induced damage to lipid membranes, enzymes and nucleic acids. Generation of ROS has been involved in various cardiovascular disorders, including ischaemia/reperfusion (I/R), atherosclerosis and cardiotoxicity induced by drugs (Scolletta et al, 2007) These ROS caused an injury to vascular cells and cardiac myocytes directly, and can initiate a series of local chemical reactions that result in an amplifcation of the initial ROS-mediated cardiomyocyte dysfunction (Blankenberg et al 2003).

In-vivo antioxidant nutrients which include vitamin C, trace elements such as Se, Zn and Cu play a crucial role in defending against oxidant damage (Thangadurai et al, 2012) . The results of published papers showed that the levels of trace elements (Zn, Se) of patients significantly decreased compared with control groups. The lowering in these values reflected the principal function of these elements as antioxidants (as free ion elements or bounding with enzyme) in biological systems (Paola et al , 2013) . The decrease in zinc level and its concomitant effect on copper level may affect the activity of some antioxidant enzymes that use these elements as cofactor within its structure. Hence those enzymes lose some of their activity and ability to remove free radicals (Beck et al, 1997).

Human body uses selenium to produce glutathione peroxidase, which works with vitamin E to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals produced by oxidative metabolism, Selenium is taking center stage as a potential anticancer agent by promoting formation of white blood cells which destroy the cancer cells and are an essential component of more than ten selenoproteins with multiple biochemical functions. Moreover, it boosts the immune system by increasing the activity and number of white blood cells and prevents premature aging, degenerative diseases, CVDs, inflammatory diseases, stroke, cataracts, and rheumatoid arthritis. It is also necessary for normal thyroid functions and protection

of heavy metal toxicity. Deficiency of the element can cause Keshan disease, characterized by an enlarged heart and poor heart function (Čuparigova et al,2011). Highly significant increase in copper level was observed in the sera of patients compared with control group. Among the cationic ligands, copper deserves particular consideration because it acts as a transition metal, and it is very potent to generate ROS after a reaction with oxygen. Free Cu (II) ion can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, copper is generally less susceptible to participate in the Fenton reaction **(**Marjolaine et al, 2008). In the reported literature the ranges of so-called "normal value" of these microelements are relatively large (Cornelis, Sabbioni, Var den Venne 1994, Alfthan and Neve 1996, Minoia et al. 1990). The concentration of microelements in sera of general population depended on the exposure to other metals and their interaction, as well as on different dietary habits, and the content of microelements in food (Telisman 1995, 1997, Golubkina and Alfthan, 1999).

We have not reported or published data about the levels of microelements in sera of population in our country. The scarce data that we have show that the concentration of these microelements is in general slightly lower for S-Zn and S-Cu and lower for S-Se compared with those reported in literature of other countries. The lower than normal value content of selenium in the serum of healthy persons perhaps depends on low concentration of selenium in soil or low bioavailability of the selenium for plants, and also on eating habits.

A study from Germany reported mean serum values of Cu (mg/l), Zn (mg/l) and Se (μg/l) in adults to be 1.048, 1.079 and 63.2, respectively (Rukgauer etal, 1997). Studies of Se levels in a healthy Spanish population showed a mean value of 80.7 μg/l (Torra et al, 1997) which is very high compared to that of our survey. On the other hand, higher mean serum Se values (122 μg/l) were reported in a population from Singapore (Huges et al, 1998)

Our results showed that the levels of S-Zn and S-Se in cardiovascular patients were significantly lower than levels of the control group, whereas significant difference was found and for S-Cu, but in this case S-Cu levels in patients were significantly higher than levels of control group. These significant differences may be due to the consequence of increased oxidative stress caused by the disease.

This survey has several strengths; it is the only published study of the trace elements in patients with CVD in Albania. Other strength is the significant difference levels of trace elements between patients with CVD versus control healthy group and a well-selected control group. We would like to mention the weakness of our survey, that was the limited number of patients with CVD and control group that were included in it.

Conclusion

CVD seems to be due to alteration of levels of trace elements in serum of patients, in this specificity, the significant increase of copper, and decrease in zinc and selenium

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QUALITY EVALUATION OF GASTRORESISTENT TABLETS OF ACETYLSALICYLIC ACID

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Abstract

INTRODUCTION: Acetylsalicylic acid (aspirin - ASA) is an important drug of nonsteroidal anti-inflammatory group with analgesic, antipyretic and anti-inflammatory properties. Delayed-release tablets are used to increase the bioavailability and reduce the risk of hospitalization of cardiac diseases. The efficacy of aspirin in preventing myocardial infarction is associated with preventing the formation of thrombi by slowing the aggregation of blood cells. Six types of 100 mg acetylsalicylic acid tablets are registered in the Albanian market and in the reimbursement list. The aim of this investigation was the quality evaluation of gastroresistent tablets of aspirin 100 mg and the estimation of dissolution profiles.

MATERIALS AND METHODS: The methodology used was based on the monograph of British Pharmacopeia 2007. Tablets control underwent organoleptic (eye inspection), physical and chemical. The in vitro dissolution studies were carried out in the dissolution apparatus, which was initially left for 2 hours in 0.1M HCl solution and then for 90 min at pH 6.8 phosphate buffer medium. Samples taken at various times were analyzed in UV-VIS spectrophotometer at, 276 nm using 0.1M hydrochloric acid as reference cell and at 265 nm for the phosphate buffer medium, respectively. Four brands of delayed release tablets 100 mg , used for analysis, were collected from Albanian market.

RESULTS AND CONCLUSIONS: There was no significant change in the measurement of these parameters for the four dosage forms with reference to British Pharmacopoeia (BP) values. From the dissolution profiles obtained, the dissolution of drug in medium pH 6.8 was achieved within the first minutes on most tablets.

Key-words: Acetylsalicylic acid, delayed release tablets, dissolution, dissolution apparatus

Introduction

Acetyl Salicylic Acid (ASA) or Aspirin (Figure 1) is one of the oldest and the most commonly used non steroidal anti-inflammatory drugs (NSAID). It is an effective analgesic, anti-inflammatory, anti-thrombotic and antipyretic agent that primarily acts by permanently inactivating the cyclooxygenase (COX) mediated activities of prostaglandins through irreversible binding unlike other NSAIDs, which are reversible inhibitors. Cyclooxygenase is required for prostaglandin and thromboxane synthesis. Aspirin acts as an acetylating agent where an acetyl group is

covalently attached to a serine residue in the active site of the COX enzyme [1,2,3].

Aspirin's efficacy in preventing Myocardial Infarction is related to preventing thrombus formation by decreasing platelet aggregation [4].

In addition to its effects on pain, fever, and inflammation, aspirin also has an important inhibitory effect on platelets in the blood. This antiplatelet effect is used to prevent blood clot formation inside arteries. Aspirin prevents blood from clotting by blocking the production by platelets of thromboxane A2, the chemical that causes platelets to clump. It has an antiplatelet effect by inhibiting the production of thromboxane [1].

Aspirin often causes acute gastric mucosal damage that can be seen endoscopically or assessed indirectly (for example, by measuring increased gastrointestinal blood loss). The occurrence of most adverse effects is apparently related to the dose administered. This dose-response effect, evident in both endoscopic and microbleeding studies done after acute or short-term aspirin administration, is also associated with the risk of developing chronic gastric ulcer [5].

In the Albanian market and in the reimbursement list are registered six types of tablets 100 mg of acetylsalicylic acid. The aim of this investigation was the quality evaluation of gastro-resistant tablets of aspirin 100 mg and the estimation of dissolution profiles.

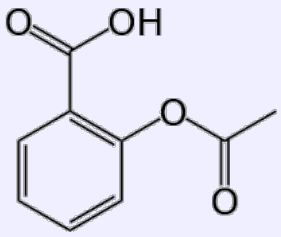

Figure 1. Structure of Acetyl Salicylic Acid (ASA).

Materials And Methods

Four different brands of gastroresistent ASA100 mg tablets were purchased from the retail pharmacy and were signed as samples M1, M2, M3 and M4. All the reagents used were purchased by Sigma Aldrich Company.

Equipments

Tablet Hardness- Guoming YD-2; Disintegration Test Apparatus - Guoming YD-2; Dissolution

Test Apparatus - Guoming YD-2; Spectrophotometer UV-VIS model UV765; quarts cuvettes, 1 cm.

Methods

For all the tablets selected, the tests of identity, uniformity of weight and diameter, crushing strength, disintegration were done, and also the assay for the content of active ingredients by iodometry and spectroscopy as described in the British Pharmacopoeia [9] and literature [6,7,8,10]. All the assessments were triplet and the results presented are the average of them.

Also all the tablets underwent in vitro dissolution study using USP apparatus type II.

Data analysis

Data for hardness, diameter, weight uniformity test, disintegration and content uniformity of the tablets were analyzed by determining the mean and standard deviation.

Hardness

Hardness of the tablet was measured using Tablet Hardness- Guoming YD-2tester. Seven tablets of each brand were randomly selected and the hardness of the tablets was determined (n=7).

Diameter

Random samples of 10 tablets were selected for each brand and their diameter was calculated in centimeters with the help of micrometer.

Uniformity of mass

The weights of ten tablets were determined individually using an analytical balance. The average tablet weight and standard deviation were calculated and compared with the permissible limits [7].

Identification

A quantity of powder equivalent to 0.3 g of Aspirin was boiled for 2 to 3 minutes with 10 ml of 5M sodium hydroxide, was cooled, and adding an excess of 1M sulphuric acid, a crystalline precipitate was produced. To a solution of the precipitate in water, iron (III) chloride solution [9] was added.

Disintegration

For this test, the disintegration apparatus with two baskets was used. The 900mL beakers were first filled with hydrochloric acid 0.1 M and were kept at 37±0.5°C for two hours. Six tablets of each brand were selected and placed in each of the cylindrical tubes of the basket without discs. After 120 minutes the medium was changed with phosphate buffer solution pH 6.8. Discs were used to avoid the floating of tablets while tube moved upwards and downwards in water,. The time taken to break each tablet into small particles and pass out through the mesh at the bottom of the tube was recorded [9].

Assay

20 tablets of aspirin were weighed and pulverized and an amount equivalent to 100 mg of Aspirin was transferred to a 250 ml Erlenmeyer flask, in which 2 ml ethanol 70 % and 5 mL of 0.5 M sodium hydroxide was added. The mixture obtained was gently mixed and was left to stand for 60 minutes. The base excess was titrated with 0.5 M hydrochloric acid, using phenolphthalein as indicator. The same procedure was performed to the blank test. Each ml of 0.5 M sodium hydroxide is equivalent to 45.040 mg of Aspirin [10,11].

Dissolution test

The dissolution was carried out using first as the medium 1000 ml of 0.1M hydrochloric acid and rotating the basket at 100 revolutions per minute. After 2 hours, a sample of the medium was withdrawn, was filtrated and the absorbance of the filtrate was measured at 276 nm using 0.1M hydrochloric acid in the reference cell. The absorbance of a suitable solution of standard aspirin in 0.1M hydrochloric acid was measured and the total content of aspirin in the medium was calculated, using the declared content of ASA in standard aspirin.

After this procedure the medium in the baskets was replaced with 900 ml of mixed phosphate buffer pH 6.8, previously held at 36.5° to 37.5°. After 45 minutes, a sample of the medium and filter was withdrawn. Immediately the absorbance of the filtrate was measured, at 265 nm using dissolution medium in the reference cell. Accordingly, the absorbance of a suitable solution of standard aspirin in the dissolution medium was measured, and the total content of aspirin [9, 12] was calculated.

Dissolution profile

The dissolution profile was obtained using the same methodology described for the dissolution test, but this test was performed for 45 minutes with aliquots collection at 10, 20, and 45 minutes. In each sample, a 10 ml aliquot of dissolution medium was removed, this volume being immediately replaced [10].

Results And Discussion

All the trial samples (M1, M2, M3 and M4) were evaluated using pharmacopoeial (they have undergone visual inspection, thickness, disintegration and hardness tests) and nonpharmacopeial tests (identification, assay and dissolution tests).

The results obtained for each sample after the hardness test, diameter and uniformity of mass measurements are given at the table 1.

Table 1: Parameters of ASA 100 mg tablets											
Products	Thickness/mm										
M1	85.2	82.5	82.7	82.9	82.4	85.2	85.2	88.2	82.7	82.9	
M2	75.4	75.3	75.4	75.3	85.5	75.5	75.3	75.4	75.4	75.3	
M3	75.8	79.1	75.8	71.6	72.4	71.4	71.2	79.3	71.9	72.5	
M4	87.6	88.4	87.4	88.3	88.4	87.6	87.9	87.5	88.2	87.9	
	Hardness/N										
M1	126.2	112.5	103.5	128.2	119.1	99.7	101.5	114.7	127.5	130.1	
M2	52.8	51.2	53.0	49.9	50.8	52.4	51.9	51.5	52.6	52.2	
M3	78.6	77.9	80.2	79.1	78.2	77.8	81.1	78.6	75.9	77.5	
M4	72.2	71.8	70.9	72.0	73.1	71.5	72.2	71.2	73.0	72.2	
	Weight variations										σ σ = aw ±7.5%
M1	0,2156	0,2156	0,2156	0,2156	0,2156	0,2156	0,2156	0,2156	0,2156	0,2156	]0.14086;0.29086[
M2	0.1305	0.1305	0.1305	0.1305	0.1305	0.1305	0.1305	0.1305	0.1305	0.1305	]1,2089;1,3589[
M3	0.1482	0.1482	0.1482	0.1482	0.1482	0.1482	0.1482	0.1482	0.1482	0.1482	]0,07292;0,22292[
M4	0.1369	0.1369	0.1369	0.1369	0.1369	0.1369	0.1369	0.1369	0.1369	0.1369	]0,06109;0,21109[

After the physical tests, the tablets were subjected to chemical tests that include identification, assay, disintegration and dissolution.

Identification test

After the above procedure, a deep violet color is produced in the solution indicating the presence of the aspirin for each sample.

Test of Disintegration

The ASA gastro – resistant tablets according to monographs BP 2007 specify the time of disintegration, 2 h in HCl 0.1 M at 370C, in the disintegration apparatus. There ought to be no cracking or coating break, and after that the tablets are put in phosphate buffer, pH 6.8 at the same temperature for 1 hour. Most of the tested tablets were disintegrated within ten minutes.

Assay

The drug content was found to be between the values 98% to 104%, values which are within the pharmacopeal limits. The amount of acetylsalicylic acid is shown for each sample in the table 2 below:

Table 2: The drug content	
Samples	The amount of ASA %
M1	102
M2	104
M3	98
M4	104

Dissolution test

Samples were taken from both media, and the total content of released aspirin in percentage has been calculated, relying on the obtained absorbance values, first n the HCl medium , λ = 276nm, and in buffer phosphate medium, λ = 265 nm in the end of 45th minute

The quantity of released aspirin in acidic environment does not surpass 5% of the declared (stated) quantity on the label of the drug container. The obtained values are summarily presented in the graphs of fig. 2

Table 3: The percentage of ASA release in each medium		
Samples	% release in HCl 0.1 M	% release in phosphate buffer pH 6.8
M1	3.02%	83.7%
M2	2.06%	82.3%
M3	1.86%	85.1%
M4	2.60%	83.2%

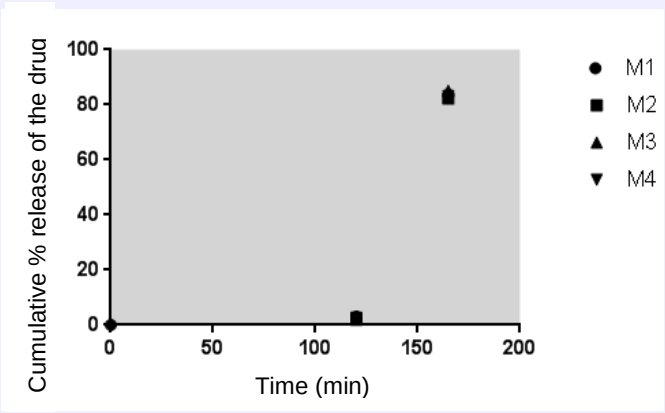

Figure 2: Graph of cumulative release of ASA in each medium HCl 0.1 M and buffer phosphate pH 6.8

From the graph we can conclude that each sample of the tablets has approximately the same cumulative percentage in the 45th minute, in buffer phosphate medium.

Dissolution profile

The obtained values of the cumulative quantity of the released drug after 10, 20 and 45 minutes are presented n the bottom graph (Fig.2). From this graph we may conclude that the larger quantity of released ASA occurred in the 20th minute for each of samples selected for analysis, and the higher percentage released was that of sample M2

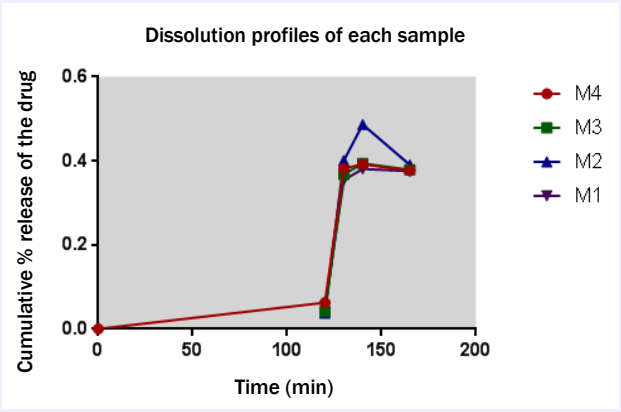

Figure 3: Dissolution profiles of ASA delayed release tablets in each medium HCl 0.1 M and buffer phosphate pH 6.8

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PRESCRIPTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN ALBANIAN COMMUNITY PHARMACIES

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Abstract

INTRODUCTION: Large number of medications is available to the public over the counter, obtained without a prescription. An example of these are over the counter analgesics, non-opioid analgesics with pain relieving effects. These agents include paracetamol and non-steroidal anti-inflammatory drugs such as acetylsalicylic acid, ibuprofen, and diclofenac, which can be consumed whenever required. Most of drugs are used for pain relief associated with many medical conditions including headaches, backaches, menstrual cramps, fever, and other pain related symptoms. These agents vary in their analgesic, antipyretic, and anti-inflammatory effects ranging from paracetamol, a good analgesic and antipyretic medicine with no anti-inflammatory effects to the non-steroidal anti-inflammatory drugs which possess powerful analgesic/antipyretic as well as exert anti-inflammatory actions.

The aim of the paper was to put in evidence the prescription of non-steroidal anti-inflammatory drugs in Albanian community pharmacies.

METHODS: A cross-sectional study was conducted for over 7 months, using a self-administered questionnaire. Pharmacists answered questions regarding prescription of non-steroidal anti-inflammatory drugs in Albanian community pharmacies.

RESULTS: The most frequently suggested over the counter analgesics were found to be Ibuprofen (57.8%) and Ketoprofen (47%). 86.2% of pharmacy professionals advised the maximum daily dosage, 19.6% advised taking over the counter non-steroidal analgesics after meals with plenty of water, 52.9% advised not to associate it with other medications. 26.4% of pharmacy professionals advised the preservation and 16.6% of them advised otherwise. About 80% of participants (pharmacy professionals) reported to have knowledge about the side effects, whereas 8% of them didn`t have any knowledge. 67% of respondents indicated that they had experienced at least one side effect from a non-steroidal anti-inflammatory drugs (75% of them were mild adverse drug reactions and 7% severe adverse drug reactions) whereas 33% of them had no such reports in their pharmacies. Most participants said having non-steroidal anti-inflammatory drugs in different types of dosage forms but injection dosage form had mostly adverse drug reactions. About 85.2% of adverse drug reactions were related to gastrointestinal tract. Non-steroidal anti-inflammatory drugs were prescribed more in females (75%) and in the age group between 30 and 40 years old.

CONCLUSION: There is a need that patients should take advice, so that they gain useful information regarding the possible reactions of drugs associated with food and other drugs intake, side effects on health and the risk factors.

KEYWORDS: Ibuprofen, non-steroidal anti-inflammatory drugs prescription, pharmaceutical dosage form, adverse drug reaction.

Introduction

Non steroidal anti-inflammatory drugs (NSAID) are medicaments with analgesic, antipyretic and anti-inflammatory effects (Hang et al. 2000). Certain NSAIDs including ibuprofen and aspirin have become accepted as relatively safe and available over the counter thus encouraging self-medication among the Albanian population for the relief the of pains, fever and inflammation. The outcome of self-medication and possible adverse drug reactions (ADR) are dependent on the quality of drug information given by the drug suppliers and their extent of use. The main ADR associated with NSAIDs relates to gastrointestinal (GI) and renal effects (Fillastre et al. 1997). These effects are dose dependent and in many cases pose serious risk of upper GI bleeding, ulcers, intestinal perforation and death. An estimated 10–20 NSAIDs associated upper GI adverse events resulting in 103,000 hospitalizations and 16,500 deaths per year in the United States have been reported (Feenstra et al. 2002). NSAIDs have also been reported to be associated with a relatively high incidence of renal ADR. The mechanism of these renal ADR is probably due to changes in renal haemodynamics through inhibition of prostaglandins. Analgesic nephropathy is less fashionable than it was 20 years ago since the dangers of analgesic mixtures became widely known and the sale of phenacetin, the most implicated drug, was banned in most countries (Matthew 1992). Moreover studies in a range of countries suggest that patients have little awareness of the risk of these medicines (Chroudhury et al.1997, Dde Broe 1998, Maxwell et al.2005).

Methods

We carried out a cross-sectional survey study in Albanian community pharmacies. The questionnaire was attached to a social network of professional pharmacists and completed in a period of 7 months (from 19 June 2016 until 25 January 2017). 247 professionals visited the link but only 102 of them were participants (they responded the entire questionnaire). 3% invited friends to participate and none of them has shared the survey in his own profile; 18% were desktop users and 82% mobile users. Average time spent completing the survey, was 1;29 seconds.

The questionnaire, described in more detail elsewhere, included questions, like: Which over the counter (OTC) drugs do you suggest to treat pain, what do you advise the patient when you prescribe OTC non-steroidal analgesics, do OTC non- steroidal analgesics have side effects and if they have been reported in your pharmacy, what type of adverse drug reactions are those, the form dosage of NSAIDs in your pharmacy and which of them have mostly adverse drug reactions, which system is more affected by adverse drug effects, NSAIDs are prescribed more in males or females, and in which age group NSAIDs are more prescribed.

Results

The most frequently suggested OTC analgesics were found to be *Ibuprofen* (57.8%) and *Ketoprofen* (47%), and the least one was *Migretil* (0.9%) (figure 1).

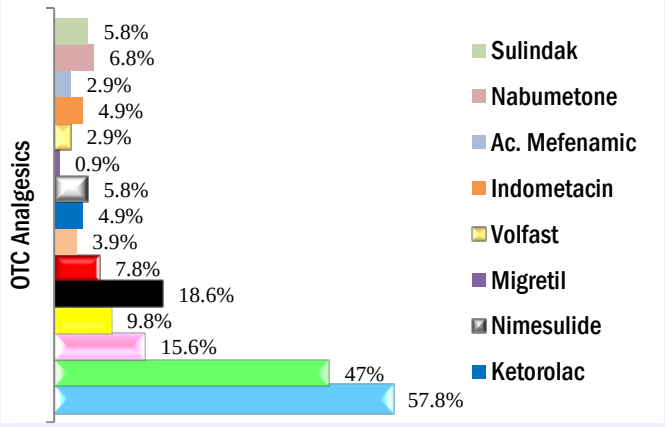

Figure 1. OTC analgesics suggested by pharmacy professionals.

86.2% of pharmacy professionals advised the maximum daily dosage, 19.6% advised taking OTC non-steroidal analgesics after meals with plenty of water, 52.9% advised not to take them with other medications (for example: ACE inhibitors, cyclosporine ore diuretics, because their use may increase the risk for nephrotoxicity). 26.4% of pharmacy professionals advised the preservation and 16.6% of them advised otherwise (figure 2).

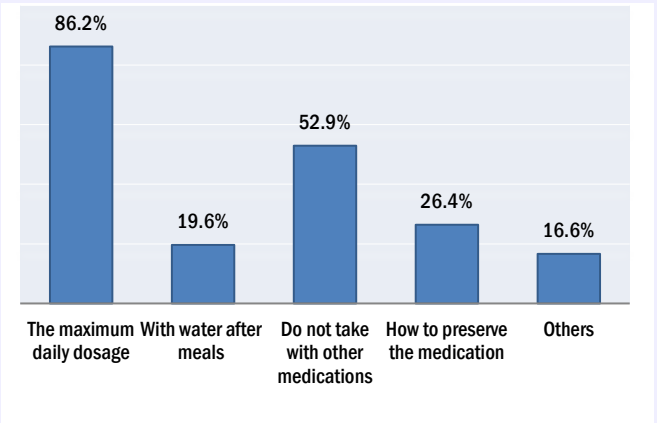

Figure 2. Advices in prescribing OTC non-steroidal analgesics.

About 80% of participants (pharmacy professionals) reported to have knowledge about the side effects, 12% did not, whereas 8% of them didn`t have any knowledge (figure 3). 67% of respondents indicated that they had experienced at least one side effect from a NSAID whereas 33% of them had no such reports in their pharmacies (figure 4).

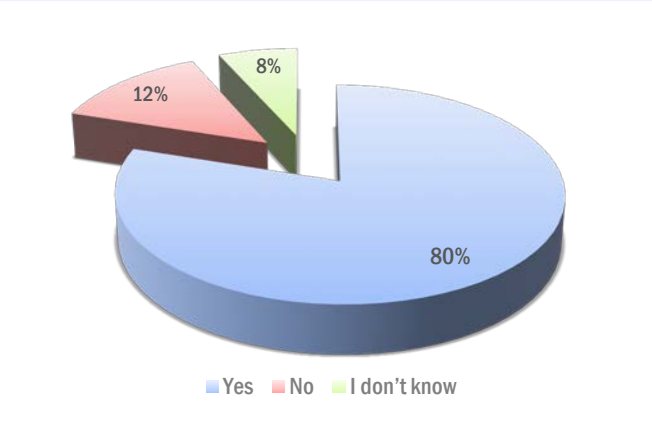

Figure 3. The knowledge about side effects.

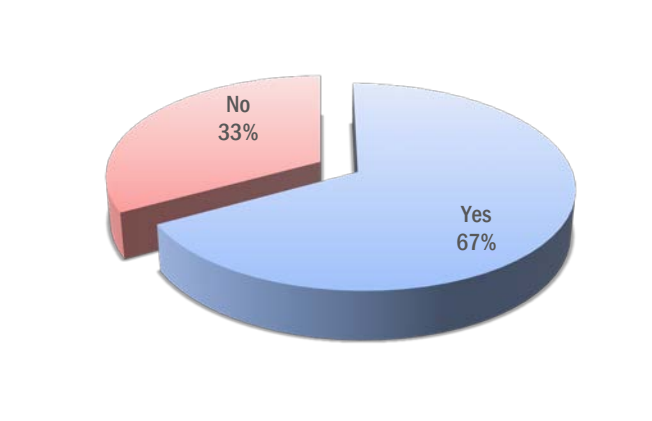

Figure 4. Percentage of respondents having/ not having experiences in their pharmacy.

Most of the respondents revealed having mild ADR in their pharmacies (75%), followed by moderate ADR (18%) and severe ADR (7%) (figure 5).

Most participants said having NSAIDs in different types of dosage forms: the tablets were mostly (96%), ointments (73.5%), injections (44.1%), pills (28.4%), pessaries (11.7%), as well as other pharmaceutical forms (28.4%) (figure 6).

Injection dosage forms were on the top of the list having adverse drug reactions (65.6%) , then tablets (43.1%), ointments (16.6%), pessaries (10.7%), pills (7.8%) and other pharmaceutical forms, non mentioned above, had less ADR (5.8%) (figure 7).

Most of the adverse drug reactions were related to gastrointestinal tract (GIT) 85.2%, to the cardiovascular system (CVS) 50%, to the urinary system (US) 36.2%, and to the other systems only 6.8% (figure 8).

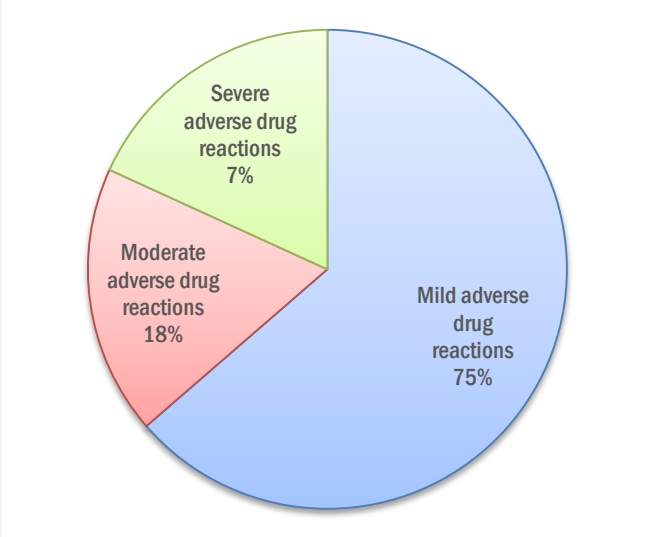

Figure 5. Type of adverse drug reactions forms of NSAIDs

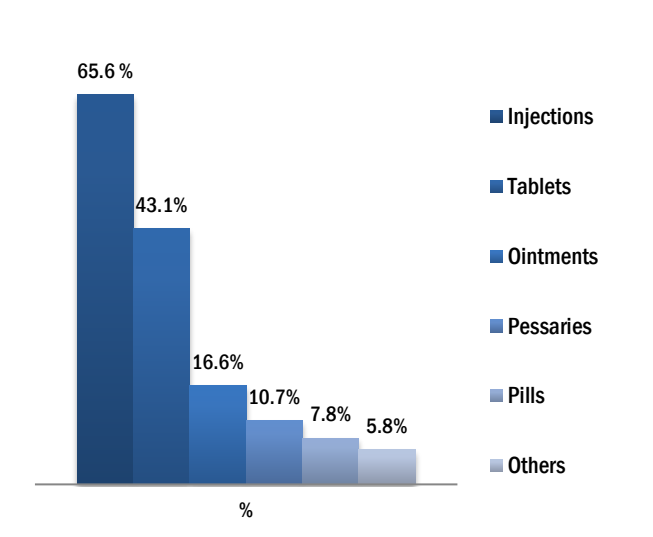

Figure 7. Pharmaceutical dosage forms and their ADR.

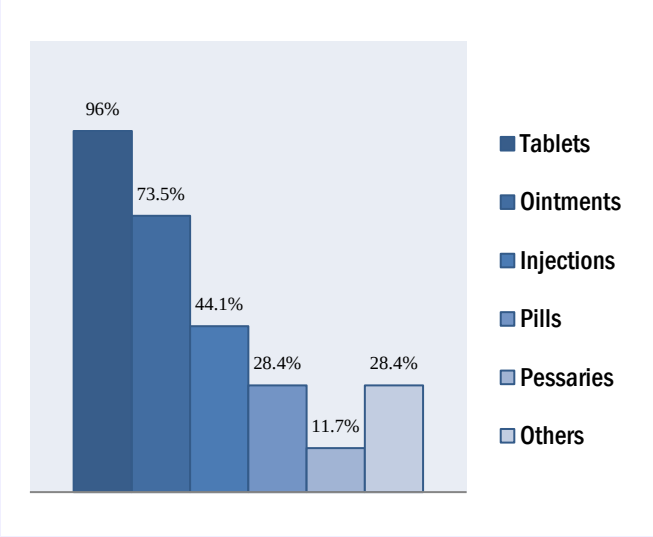

Figure 6. Pharmaceutical dosage.

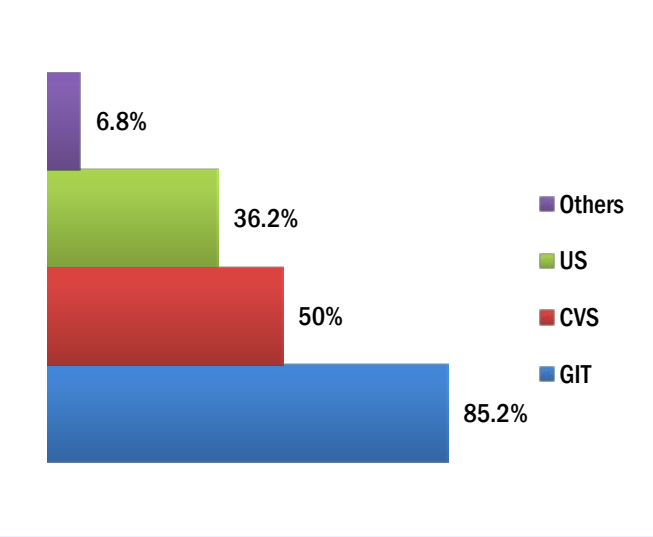

Figure 8. Evaluation of adverse drug reactions to different body systems.

NSAIDs were prescribed more in females, 75%, whereas in males , 25% (figure 9). NSAIDs were prescribed more in the age group between 30 and 40 years old, and none of those was prescribed in the age group 0-10 years old (figure 10).

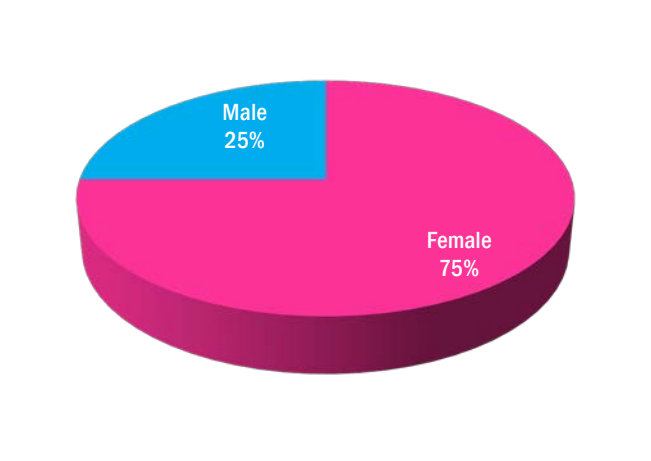

Figure 9. NSAIDs prescription in females and males.

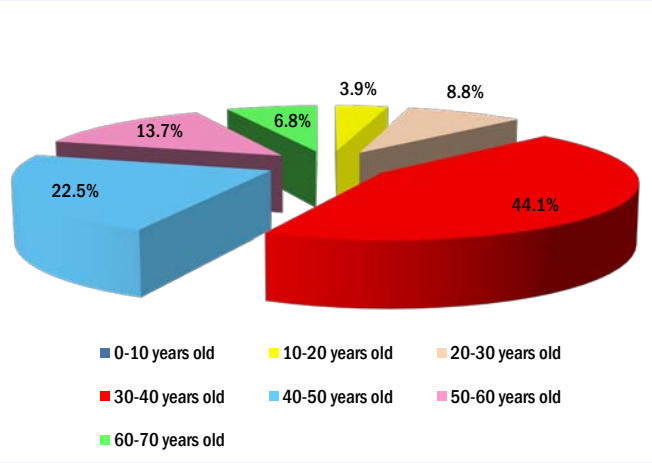

Figure 10. NSAIDs prescription according to the age groups.

Discussions

Non steroidal anti-inflammatory drugs (NSAIDs) are primarily used for their analgesic, anti-inflammatory, and antipyretic effects, but low-dose aspirin may also be used for cardiac prophylaxy (Hillis 2002). Pain relief is a prevalent and a growing need. While there are many conditions associated with pain necessitating treatment, including migraine and dysmenorrhea, musculoskeletal pain continues to be one of the most common. In 2011 alone, it is estimated that there were 116.5 million low-back and neck acute pain cases combined in the United States, and this number is projected to grow by 10% to 128.5 million by 2021 (Buurma. et al. 2012). The recent studies have accounted NSAIDs for more than 70 million prescriptions and 30 billion nonprescription purchases annually (Wehling 2014). Available nonprescription NSAIDs include aspirin, ibuprofen, and naproxen. Ketoprofen was previously available as a nonprescription agent; however, due to decreased demand all current formulations are only by prescription (http://clinicalpharmacology-ip.com.proxy.campbell.edu/2008) (www.fda.gov/cder/ob/default.htm.2008). This study has clearly demonstrated that OTC analgesics are consumed enormously nowadays, as it was reported the consumption of a large number of OTC analgesics by Albanian patients. It was found that Ibuprofen and Ketoprofen were consumed mostly, both as prescription and non prescription analgesics: the information from print/electronic media and relatives/friends as well as cost-effectiveness may be the reasons, as they have low cost with reasonable efficacy.

While purchasing over the counter OTC drugs, a high percentage of pharmacists, about 86.2% of them advised the maximum daily dosage, this is important to prevent side effects when over passing the daily dose. Usually elder people suffer with several diseases at one time; hence administering 5 ore 6 medicines at a time. If such people use ibuprofen (NSAIDs) for pain relief, it

can be harmful for them because their body undergoes some pathological changes, so in this way they are unaware of the severe adverse effects related to NSAIDs. Such patients can suffer from hemorrhage if they are taking corticosteroids, anticoagulants and SSRIs (selective serotonin reuptake inhibitors). Albanian pharmacists give their contribution in the awareness of the patients about the risk of drug interaction, as 52.9% of them advised not to take OTC non steroidal drugs with other medication.

The study revealed that almost 80% of pharmacy professionals had knowledge about the side effects of NSAIDs because most of them had experienced this in their pharmacies. Patients seem to be careful in reporting their side effect cases to the professionals, owing to the possibility of taking advice on continuation of the treatment.

About three quarters of ADR were mild and only 7% of them were severe, this indicates that patients are cautious in taking medicines and are always interested to receive information about their medications.

Several frustrations about the GIT events of NSAIDs (about 85.2% of them) were likely produced among Albanian patients; this due to the fact that patients were not advised to take their medication after meals with plenty of water, as well as they were not asked whether they suffered from any gastrointestinal diseases. Similar studies reported that between 1998 and 2001, the FDA's Adverse Event Reporting System identified 279 cases of gastrointestinal bleeding associated with the use of nonprescription NSAIDs. Of these cases, 197 were attributed to ibuprofen, ketoprofen, or naproxen use, and 82 were attributed to aspirin use. The following risk factors for gastrointestinal bleeding with NSAID use, either prescription or nonprescription, have been identified, as: use of concomitant medications, age >60 years, high dosage, previous history of gastrointestinal bleeding, concomitant use of alcohol and/or tobacco [www.fda.gov/bbs/topics/news/2008]. Current literature suggests that NSAIDs do not increase the risk of first-occurrence heart failure, but do substantially increase the risk of relapse of pre-existing heart failure, particularly in patients using concomitant diuretic therapy (Hillis 2002] (Rodriguez et al.2003). Approximately 1% to 5% of NSAID users may experience renal effects, though it is uncommon for nonprescription NSAIDs to cause acute renal failure (Hulisz et al. 2008). However, caution should be taken in individuals with decreased effective circulating volume, such as those with CHF, hepatic cirrhosis, chronic renal disease, or dehydration (Peterson 2005). In studies, the risk of serious GI bleeding, CV, and renal adverse events increases in a dose-dependent manner (Castellsague J. et al. 2012: McGettigan et al. 2011: Huerta C. et al. 2005). Investigators found a 2- to 3-fold reduction in serious GI adverse events like bleeding, perforation, and ulceration with use of low rather than high NSAID doses (Lewis. et al. 2002).

NSAIDs circulated in different dosage forms in Albanian community pharmacies, the tablets were mostly prescribed, thus due to the best compliance and the large marketing alternatives. Injections dosage forms of NSAIDs had greater adverse drug reactions because of their greater bioavailability compared to the other forms.

This study found that NSAIDs analgesics were the mainstay for pain relief in Albanian females, due to their awareness of health care needs. NSAID prescription revealed to be 44.1% in the group age between 30 to 40 years old, which is the average age of the Albanian population of higher work efficiency.

Conclusions

The questionnaire was attached for several months but only 41.2% of pharmacists were participants (participants were less than half of those who had visited it). Although pharmacists are usually the custodian of drugs and are charged with the responsibilities of delivering safe and efficacious medicines to the public, their interest to offer information about NSAIDs prescription was low. There are different non steroidal anti-inflammatory dosage forms circulating in Albanian pharmaceutical market, such as: tablets, injections, syrup, pills etc, being useful as prescription or non prescription analgesics. There is a need that patients should take advice, so that they obtain information regarding the possible reactions of drugs with food and other drugs, side effects on health and the risk factors. It should be strongly noted that the pharmacists' role is vital in patient-medication education issue, as many complained of the insufficient counseling, especially regarding the SEs, unclear handwriting and inadequate labeling.

Further studies evaluating knowledge of non steroidal anti inflammatory analgesics among Albanian patients should be conducted to further correlate the relationship between analgesics awareness and health care service provided by pharmacists.

Limitation

The limitation of this survey- based study was its small sample size. However, such a study can be carried out involving a larger sample size.

Acknowledgments

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We would like to thank also all pharmacist professionals for their support and great patience.

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PHYSIOTHERAPY EFFECTS ON PAIN WHILE WALKING IN PATIENTS WITH KNEE OSTEOARTHRITIS

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Abstract

Knee osteoarthritis is a chronic degenerative disease. The main symptom of patients with knee osteoarthritis is a degenerative and mechanical type of pain. Pain is very noticeable while walking in rugged terrain, during ascent and descent of stairs, when changing from sitting in standing position as well as staying in the sitting position for a long time. Many studies have shown that the strength of the quadriceps femoris muscle can affect gait, by improving or weakening it. Kinesio Tape is a physiotherapeutic technique, which reduces pain and increases muscular strength by irritating the skin receptors. The aim of this article was to verify if Kinesio Tape reduces pain while walking, in patients with knee osteoarthritis. 74 patients with primary knee osteoarthritis, aged 50-73 years, participated in this study. We observed the change of pain, while walking for 10 meters at normal speed for each patient, before, a day after the application and three days after the application of Kinesio Tape on quadriceps femoris muscle, with the help of numerical pain rating scale - NRS. Our results indicated that there was a significant decrease of the pain while walking for 10 meters.

KEY WORDS: knee osteoarthritis, gait, Kinesio Tape, rehabilitation, numerical pain rating scale - NRS, 10 meter walking test.

Introduction

Osteoarthritis is a widespread, slowly developing disease, with a high prevalence increasing with age. The most common large joints involved in the disease are the knees, where the disease is particularly disabling because of difficulty in rising from chair, climbing stairs, kneeling, standing and walking. These limitations are partly due to muscle weakness, especially quadriceps muscle (Van Baar et al, 1998, Fransen et al, 2002, Steultjens et al, 2001, Slemenda et al, 1997). It has been suggested that functional ability is also affected by poor proprioception (Sharma et al, 2003, Sharma 2003, Sharma et al, 1997, Bennell et al, 2003, Hurley et al, 1997, Pai et al, 1997, Marks 1994).

The primary complaints of patients suffering from osteoarthritis are pain, stiffness, instability and loss of function. In addition to this impaired muscle function is frequently observed in patients with osteoarthritis of the knee. It was found that 80% of patients with knee osteoarthritis reported problems related to muscle function, strength, endurance and coordination (Rogind et al, 1988).

Hassan et al. 2001 observed that knee joint pain and quadriceps strength were significant predictors of increased postural sway. Women who reported widespread pain had a 60% greater risk of falling compared to women with no or mild pain (Mandeville et al, 2008).

Additional factors associated with disability in persons with knee osteoarthritis include increasing age, obesity, female gender, co morbidity and quadriceps muscle weakness (McAlindon et al,1993, Dekker et al, 1993). Pain and muscle strength may particularly influence postural sway (Marks 1994). Impairments in knee joint proprioception have been mentioned by multiple authors (Marks 1994, Hurley et al, 1997, Tarigan et al, 2009). These deficits, in combination with the ageing process, may culminate in greater impairments of balance in this patient population, compared with their age-matched and healthy counterparts (Mandeville et al,2008).

Kinesio Tape is the original and authentic elastic therapeutic tape. According to Kenzo Kase (Kase 2003, Kase 2008), Kinesio Tape is a definitive rehabilitative taping technique that is designed to facilitate the body's natural healing process while providing support and stability to muscles and joints without restricting the body's range of motion as well as providing extended soft tissue manipulation to prolong the benefits of manual therapy administered within the clinical setting. These proposed mechanisms may include: 1. correcting muscle function by strengthening weakened muscles, 2. improving circulation of blood and lymph by eliminating tissue fluid or bleeding beneath the skin by moving the muscle, 3.decreasing pain through neurological suppression, and 4. repositioning subluxed joints by relieving abnormal muscle tension, helping to return the function of fascia and muscle. Chen et al. 2008 mentioned in their studies that Kinesio Tape can lift the skin to increase space between skin and muscle, reducing and localized pressure, promoting circulation and lymphatic drainage. This theoretically reduces pain, swelling, and muscle spasm. Chronic pain can be improved via the sensory stimulation of other types of nerve fibers. In these circumstances, Kinesio Tape may be effective for pain that persists after an injury has healed or for pain that is above and beyond the injury severity

To our knowledge, no study has directly investigated the effect of Kinesio Tape on pain intensity during walking in patients with knee osteoarthritis. The aim of this study was to verify if the application of Kinesio Tape on quadriceps muscle reduces pain while walking for 10 meters at normal speed, in patients with knee osteoarthritis before the application of Kinesio Tape, a day after the application of Kinesio Tape and three days after the application of Kinesio Tape on quadriceps femoris muscle.

Patients And Methods

The subjects (n=74), aged 50-73years (mean age 61.5), were consecutive out-patients with a clinical diagnosis of primary knee osteoarthritis made by a rheumatologist. The main criterion for the selection of the subjects in this study was the diagnosis of knee osteoarthritis by X-ray. Criteria for excluding subjects in the study were other musculoskeletal diseases, total knee replacement, significant hip or spinal arthritis, neurological diseases and diseases that affect balance and coordination. All of the subjects signed a written consent to participate in the study voluntarily.

Kinesio Tape was applied with a tonus regulation technique also called muscle technique on quadriceps femoris muscle. We measured the tape length in maximal stretched position of the tissue. The application was done with the patient in this maximal stretched position. The tape was applied without stretch following the course of the muscle borders from one insertion to the opposite one.

Figure 1. Apply of Kinesio Tape on quadriceps femoris muscle.

We observed the change of pain, while walking for 10 meters at normal speed for each patient, before, a day after the application and three days after the application of Kinesio Tape on quadriceps femoris muscle, with the help of numerical pain rating scale - NRS. The worse knee, as selected by the patient was the "index" knee. Pain was assessed by numerical pain rating scale (NRS), by instructing the patient to choose a number from 0 to 10 that best describes their current pain. 0 would mean "no pain" and 10 would mean "worst possible pain" (McCaffery et al,1989).

In the current study the alternative hypothesis that Kinesio Tape is effective to decrease the pain while walking on a distance of 10 meters in patients with knee osteoarthritis was tested.

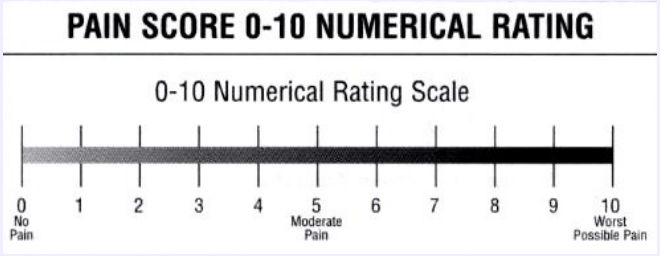

Figure 2. Numerical pain rating scale used in this study.

Results

Seventy four consecutive out-patients with a clinical diagnosis of primary knee osteoarthritis participated in this study, mean age of the participants was 61.5 (range 50-73 years), 67% of whom were female. The worse knee, as selected by the patient was the "index" knee.

We observed the change of pain, while walking for 10 meters at normal speed for each patient before the application of Kinesio Tape, a day after the application of Kinesio Tape and three days after the application of Kinesio Tape on quadriceps femoris muscle, with the help of numerical pain rating scale (NRS), where 0 would mean "no pain" and 10 would mean "worst possible pain" (19).

In Table 1 is shown the number of patients who chose the pain score while walking for 10 meters on normal speed before applying the Kinesio Tape. It is shown that 21 of 74 patients (28.4%) chose score 5, 15 of 74 patients (20.3%) chose score 6, 17 of 74 patients (22.9%) chose score 7 and 21 of 74 patients (28.4%) chose score 8 on the numerical pain rating scale.

Table 1. Numerical pain rating scale (NRS) scores before applying Kinesio Tape (KT)

NRS	Number of patients before KT	%
0		
1		
2		
3		
4		
5	21	28,4
6	15	20,3
7	17	22,9
8	21	28,4
9		
10		

In Table 2 is shown the number of patients who chose the pain score while walking for 10meters on normal speed one day after applying the Kinesio Tape. It is shown that 10 of 74 patients (13.5%) chose score 4, 26 of 74 patients (35.1%) chose score 5, 29 of 74 patients (39.3%) chose score 6, 5 of 74 patients (6.7%) chose score 7 and 4 of 74 patients (5.4%) chose score 8 on the numerical pain rating scale.

In Table 3 is shown the number of patients who chose the pain score while walking for 10meters on normal speed three days after applying the Kinesio Tape. It is shown that 15 of 74 patients (20.3%) chose score 2, 26 of 74 patients (35.1%) chose score 3, and 33 of 74 patients (44.6%) chose score 4 on the numerical pain rating scale.

Table 2. Numerical pain rating scale (NRS) scores scores one day after applying Kinesio Tape (KT)

NRS	Number of patients one day after KT	%
0		
1		
2		
3		
4	10	13,5
5	26	35,1
6	29	39,3
7	5	6,7
8	4	5,4
9		
10		

Table 3. Numerical pain rating scale (NRS) scores three days after applying Kinesio Tape (KT)

NRS	Number of patients three days after KT	%
0		
1		
2	15	20,3
3	26	35,1
4	33	44,6
5		
6		
7		
8		
9		
10		

Table 4. Numerical pain rating scale (NRS) scores before applying Kinesio Tape (KT), one day and three days after applying KT.

NRS	Number of patients before KT	%	Number of patients one day after KT	%	Number of patients three days after KT	%
0						
1						
2					15	20,3
3					26	35,1
4			10	13,5	33	44,6
5	21	28,4	26	35,1		
6	15	20,3	29	39,3		
7	17	22,9	5	6,7		
8	21	28,4	4	5,4		
9						
10						

Discussion

Lack of information about the impact of elastic therapeutic tape in pain relief in this diagnosis led us to carry out this research. Our objective was to determine whether the application of Kinesio Tape on quadriceps muscle in patients with knee osteoarthritis will lead to a pain relief while walking a 10 meter distance on a normal speed.

The results of this study showed no significant difference in pain intensity during walking one day after applying Kinesio Tape on quadriceps femoris muscle. However a significant decrease in pain intensity was shown during walking three days after applying the Kinesio Tape. Similar findings have been reported elsewhere. Kaya et al. 2011 studied 55 patients with shoulder impingement syndrome treated by Kinesio Tape or local modalities and found that although immediate effect of Kinesio Tape is greater than the local modalities, Kinesio Tape was similarly effective at the second week of the treatment.

The results of the study conducted by Miller and Osmotherly 2009 provided evidence for a short - term role for taping as an adjunct to routine physiotherapy program in different treatments. They found that Kinesio Tape has main effect on the early stage of treatment and that there was not a significant Kinesio Tape effect after several weeks. The immediate results and improvements following the Kinesio Tape are also reflected in the work of researchers who found significant improvements immediately following Kinesio Tape compared with placebo taping in patients with other musculoskeletal disorders such as patellofemoral pain syndrome (Crossley et al, 2009, Lan et al, 2010) or whiplash - associated disorders (Gonzalez et al, 2009, Nederhand et al, 2002).

Kase et al. 2003 and Kase 2008, Thelen et al. 2008 however, recommend at least three daily action of elastic therapeutic tape. Kase et al. mentions that three days after the application of Kinesio Tape can occur soft tissue changes, improvement of muscle function, increase of blood circulation and lymphatic drainage. Thelen et al. found that after three days of Kinesio Tape application, a significant decrease of the functional shoulder joint pain was reported, and an increase of movement.

Limitations in this study was the sample size, for with a greater sample size we could obtain more relevant results. In this study the effect of Kinesio Tape in pain relief in knee osteoarthritis was not assessed. Further studies are needed to investigate the effect of Kinesio Tape in pain relief of knee osteoarthritis.

Conclusion

There seems to be a significant decrease of pain three days after applying Kinesio Tape on quadriceps femoris muscle. However, no significant improvement was noted a day after the application. Kinesio Tape can be used in patients with knee osteoarthritis, especially when pain relief is a short term goal of the treatment. More clinical research is needed to investigate the effect of Kinesio Tape in pain relief of knee osteoarthritis.

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REVIEW ARTICLES

ISOTHIAZOLINONES AND CONTACT ALLERGY

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Abstract

BACKGROUND: Isothiazolinones appears to be a frequent cause of dermatitis in European countries.

OBJECTIVES: The aim of this review was to investigate the prevalence of contact allergy from methylisothiazolinone and methylchlorothiazolinone/methylisothiazolinone in different countries.

METHODS: Information on isothiazolinone allergy published during 2013-2017 was reviewed.

RESULTS AND DISCUSSION: The percentage of the positive reactions to the methylisothiazolinone in different countries were as follows: 3.7% in Denmark, 6.02% in Germany, 5.6 % in France, 12.94 % in Canada, 6.9 % in Italy, 5% Ireland, 7.2 % in Belgium and 6.8 % in United States. In most cases contact allergy was caused by use of cosmetics, cleaning agents, paints and baby wipes. The prevalence of the mixture MCI/MI was as follows: 9.4 % in United Kingdom, 9.41% in Canada, 9.04 % Italy, 6.3 % Ireland, 4.5% Belgium, 4.4% Germany, 8.3% British Isles and 10.3% Portugal. Dermatitis from isothiazolinones most frequently affected the hands, face, arms, legs, eyelids, anal-genital area, but also widespread areas on the body. Most cases have been caused by leave on cosmetic products.

CONCLUSIONS: An increase in prevalence of contact allergy to methylizothiasolinone and methylchlorothisolinone/methylisothiazoline was observed in European countries, Canada and US. The concentration of these preservatives in cosmetic products should be reduced to safer levels.

KEYWORDS: Isothiazolinones, methylisothiazolinone, Kanthon CG, contact dermatitis.

Introduction

The EU regulation 1223/2009 provides a list of allowed preservatives in cosmetic products with maximum concentration in ready for use preparation (Annex V). At the moment the list contains 57 chemical substances; however, only few of them are strongly represented on the market: formaldehyde, parabens, formaldehyde-releasers, methylchoroisothiazolinone (MCI) /methylisothiazolinone (MI). Preservatives are known as one of the two most relevant allergens found in cosmetic products (Timm-Knudson et al., 2006; Maier et al., 2009) and there is a significant increase in the level of reactivity (Schnuch et al., 2011).

Isothiazolinones are a group of broad spectrum preservatives with good antimicrobial activity against

gram-positive, gram-negative, bacteria, yeast and fungi used in different products to inhibit the growth of microorganisms. The most widely used isothiazolinones in cosmetics, household and industrial products are: methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), methylisothiazolinone/ methylchloroisothiazolinone (MI/MCI), benzisothiazolinone (BIT), octylisothiazolinone (OIT) and methyltrimethyleneisothiazolinone. MI and BIT are used as preservatives in cleaning agents and MI in cosmetics and body care products. BIT and OIT are used in industrial products (Friis et al., 2014). They are all classified as skin sensitizers.

Methylisothiazolinone and methylchloroisothiazolinone (MI/MCI 3:1) are also used together but the mixture is highly allergenic (Smith et al., 2016). In the early 1980s MI/MCI was introduced into personal care products at levels of up to 50 ppm. Kathon CG was a preservative system contaning as active ingredients MCI and MI, widely used in the early 1980s. Rapid increase in contact allergy to Kathon CG was observed in different European countries during 1985-1989. The prevalence of allergy was: 4.6 % in Finland (Hannuksela 1986), 4.4% in Sweden (Björkner et al. 1986), 3.43% in Germany (Frosch and Schulze-Dirks, 1987), 0.8% in Denmark (Hjorth and Petersen, 1986), 3.6 % in USA (Fransway 1986), 3.5 % in Spain (Hasson et al. 1990) and 5.5 % in Switzerland (Pasche and Hunziker 1989).

Atopic dermatitis on hand, face and lower leg was the most common of skin disorders. Prevalences of allergy symptoms are shown in Table I. Cosmetics were found to be the major cause of sensitization. MCI/MI has been limited at a concentration of 15 ppm. From July 2015 MCI/MI mixture is not allowed in leave-on cosmetics.

MI was used alone in cosmetics since 2005 at concentrations up to 100 ppm. Methyisothiazolinone is an important cause of contact dermatitis in European countries and USA. Also other preservatives have shown to be relevant sensitizers. Perrenoud et al. (1994) studied the frequency of sensitization of 13 common preservatives on 2295 Swiss patients.

Table 1. Kathon CG allergy in different countries.

Country	Prevalence of allergy from Kathon CG		Symptoms	Reference
Finland	Kathon CG 100 ppm	0.7% 1985 to 4.6% 1986	Atopic dermatitis, chronic hand dermatitis, lower leg dermatitis.	Hannuksela 1986
Sweden	Kathon CG 300 ppm	4.4 % 1986	Contact dermatitis	Björkner et al. 1986
Germany	Kathon CG 100 ppm	3.43% 1986	Contact dermatitis on the face and hands.	Frosch and Schulze-Dirks
Denmark	Kathon CG 100 ppm	0.8% 1986	Contact dermatitis	Hjorth and Petersen 1986
USA	Kathon CG	3.6% 1986	Allergic contact dermatitis	Fransway 1986
Spain	Kathon CG	3.5 % 1988-1989	Contact dermatitis	Hasson et al. 1990
Denmark	Kathon CG	1.3 % 1988	Contact dermatitis	Menné and Hjorth 1988
Switzerland	Kathon CG 100 ppm	5.5 % 1986-1987	Contact dermatitis	Pasche and Hunziker 1989

The percentages of positive reaction to the preservatives studied were as follows: formaldehyde 5.7%, benzalkonium chloride 5.5%, Kathon CG 5.5%, thiomersal 4.2%, chlorhexidine digluconate 2.0%, DMDM hydantoin 1.7%, paraben mix 1.7%, chloroacetamide 1.5%, bronopol 1.2%, imidazolidinyl urea 1.0%, quaternuim 15 1.0%, triclosan 0.8%, 2,4 – dichlrobenzyl alcohol 0.4 %. According to Mose et al. (2013) and Aerts et al. (2016) octylisothiazolinone is also a relevant sensitizer. Cases of occupational allergic contact dermatitis caused by benzisothiazolinone are also reported (Meysman and Groossens 2017).

Benzisothiazolinone is widely used in paint and varnishes, but also in household cleaning products but is not allowed in cosmetic products. Schwensen et al. (2015) have reported high concentration of BIT in paints (0.1 to 462.5 ppm). Friis et al. (2014) reported that the most widely isothiazolinones (in Danish markets) are benziisothiazolinone, methylisothiazolinone and methylchloroisothiazolinone and may occur in high concentrations.

Materials And Methods

Information on isothiazolinone allergy published in the period 2013-2017 was reviewed. Keywords used were: isothiazolinone allergy, methylisothiazolinone allergy and methylchloroisothiazolinone allergy.

Results And Discussion

Few years ago, a higher prevalence of contact allergy was registered in different countries. In Europe, USA and Canada several groups have documented the frequency of allergy to methylisothiazolinone and to the mixture methylisothiazolinone/ methylchlorothiazolinone. In the EU, MI was approved as a preservative in cosmetics and household products in 2005 at a concentration of 100 ppm. Since then, several cases of MI contact allergy have been reported. The prevalence of contact allergy has reached the levels: 3.7% in Denmark (Lundov et al. 2013), 6.02% in Germany (Uter et al. 2013), 6.0% in some European countries (Schwensen et al. 2016), 5.6 % in France (Hosteing et al. 2014), 12.94 % in Canada (Wilford et al. 2017), 6.9 % in Italy (Gallo et al. 2016), 5% Ireland (Murad and Marren 2015), 7.2 % in Belgium (Aerts et al. 2014) and 6.8 % in United States (Yu et al. 2015). More detailed data are presented in the Table II.

MI is also an emerging allergen in the pediatric population (Madsen et al., 2014). Wet wipes with MI were frequently the cause of atopic contact dermatitis (Chang et al., 2014). Frequently these are misdiagnosed as eczema, impetigo, or psoriasis.

The prevalence of the mixture MCI/MI was as follows: 9.4 % in United Kingdom (Ali et al. 2014), 9.41% in Canada (Wilford et al. 2017), 9.04 % Italy (Gallo et al. 2016), 6.3 % Ireland (Murad and Marren 2015), 6.3 % (Madsen and Andersen 2014), 4.5% Belgium (Aerts et al. 2014), 4.4% Germany (Molin et al. 2014), 8.3% British Isles (Johnston 2014), 10.3% Portugal (Gameiro et al. 2014).

As we can see from the Table II, there is an increase in the prevalence in the period from 2010/2011 to 2013. Studied conducted in Canada (Wilford et al. 2017) showed that prevalence of contact allergy from MCI/MI has had very high values in 2015 (12.94%). However we should take in consideration the fact that different methods, number of patients and allergen concentration are used by different groups.

Table 2. Methylisothiazolinone and methylchloroisothiazolinone prevalence of contact allergy in different countries.

Country	Prevalence of contact allergy		Symptoms	Publication
Denmark	MI MCI/MI	2.0% in 2010 to 3.7% in 2012 1.0% in 2010 to 2.4 % in 2012	Hand and face dermatitis	Lundov et al. 2013
Germany	MI	1.94% in 2009 to 6.02% in 2012	Face and ano-genital dermatitis	Uter et al. 2013
European countries	MI	6% in 2015	Hand, face, arms, eyelids dermatitis	Schwensen et al. 2016
France	MI 200 ppm	1.5 % (2010) to 5.6 % (2012)	Contact dermatitis	Hosteing et al. 2014
Sweden	MI	0.55 to 6.5%	Contact allergy	Isaksson et al. 2015
Europe	MCI/MI 100 ppm MCI/MI 200 ppm	1.2% 2.1%	Contact allergy	Bruze et al. 2014
Canada	MCI/MI MI 2000 ppm	9.41% (2015) 6.6% (2013) to 12.94 % (2015)	Contact allergy	Wilford et al. 2017
Italy	MI 2000 ppm MCI/MI	2.3 % (2012) to 6.9% (2013) 6.76% (2012) to 9.04% (2013)	Hand and face dermatitis	Gallo et al. 2016
Ireland	MCI/MI 200 ppm MI 2000 ppm	6.3 % (2012-2014) 5.0 % (2012- 2014)	Facial dermatitis	Murad and Marren 2015
Denmark	MI MCI/MI 0.02% MI 0.02%	4.8% (2011) to 6.5% (2013) 5.1% (2011) to 6.3% (2013) 3.1 % (2011) to 3.8% (2013)	Contact allergies	Madsen and Andersen 2014
Belgium	MCI/MI MI	4.5 % (2012) 6.0% (2012) to 7.2% (2013)	Hand and facial dermatitis.	Aerts et al. 2014
France-Belgium	MI	6.0% (2012) to 7.0% (2013)	Facial and hand dermatitis	Aerts et al. 2015
Germany	MCI/MI	4.4 % (2013)	Skin allergy	Molin et al. 2014
British Isles	MCI/MI MI	4.3 % (2010) to 8.3 % (2013) 1.7% (2010) to 11.1 % (2013)	Contact allergy	Johnston 2014
United Kingdom	MCI/MI	9.4 % (2011-2013)	Skin allergy	Ali et al. 2014
United States	MI	2.5 % 2012 to 6.8 % 2014	Hand and face allergy	Yu et al. 2015
Finland	MI	10.3% 2012 to 13.2% 2014	Contact allergy	Lammintaustra et al.2014
Portugal	MI (500 ppm) and MCI/MI (100 ppm)	5.15% 2012 to 10.9 % 2013	Contact allergy	Gameiro et al. 2014
United Kingdom	MI (500 ppm)	0.5 % 2010 to 5.7 % 2012	Contact allergy	Mc.Fadden et al.2013

Dermatitis most frequently affected the hands, face, arms, legs, eyelids, anal-genital area, but also widespread areas on the body. In most cases, patients were aged >40 years, both males and females. Cosmetics were the most common products causing allergy. According to Madsen and Andersen (2014) sources of exposure from methylisothiazolinone or methylchloroisothiazolinone were different consumer products such as: cosmetics (43%), cleaning agents (7%), paints (14%) and baby wipes (1%). Other authors suggest that primary source of exposure are cleaning agents and paints. Leave-on cosmetic products were the most allergic. Allergic reaction from wet wipes (baby wipes) were frequently reported with hand dermatitis in parents, chronic perianal and facial dermatitis in babies.

Different authors suggest that high prevalence is related with increasing use of MI and MCI/MI in cosmetic products. Other authors suggest that higher concentrations of MI are used in cosmetic products. Alvarez-Rivera et al. (2012) revealed higher isothiazolinone content for some rinse off products (baby care products). Methylisothiazolinone (0.025%-0.36%), benzalkonium chloride (1%) and triclosan (0.4%) were found in cosmetic products sold in European markets in concentrations higher than the limit allowed by European Regulation 1223/2009 Banned preservatives such as methyldibromo glutaronitrile were also found (Neza and Centini, 2016).

Conclusions

This review shows the increasing prevalence of methylisothiazolinone and methylchloroiso-thiazolinone/methylisothiazolinone contact allergy in different European countries , Canada and US. The concentration of these preservatives in cosmetic products should be reduced to safer levels.

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DUAL ANTIBIOTIC SYSTEMIC THERAPY IN THE TREATMENT OF PERIODONTAL DISEASES AND THE ROLE OF METRONIDAZOLE

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Abstract

INTRODUCTION: Periodontitis is an inflammatory disease of bacterial origin, which affects the supporting tissues of the tooth. There are a number of procedures and protocols aimed at the prevention of the progression of the lesion and maintaining current status of periodontal tissues. To support such procedures, in addition to monotherapy with antibiotics, dentists frequently use a combination of antibiotics, known as the dual antibiotic therapy. Among the pharmacological therapeutic options, with metronidazole and its combinations with other antibiotics, positive results are obtained in managing the disease.

OBJECTIVE: This study aims to assess the role and advantages of dual systemic antibiotic therapy, one of the antibiotics being Metronidazole, in the treatment of periodontal disease, compared with other treatment options.

MATERIALS AND METHODS: A retrieval of on-line scientific literature up to 31 January 2017 was conducted in the US National Library of Medicine (PubMed) on clinical studies for periodontitis. Inclusion criteria for the selection of studies were: published in English, controlled clinical trials in humans, and cohort studies of > 1 month duration with a comparison group; subjects with aggressive or chronic periodontitis who received two antibiotics. The final selection was independently completed by the two reviewers reading the selected articles, and their results were compared.

RESULTS: After an initial selection, 79 papers were identified by the electronic search; 18 papers met the inclusion criteria. Total study population was 1261; 1086 with ChP and 175 with AgP. The most common parameter evaluated was pocket depth (PD) assessed in 13 studies, followed by bleeding on probing in 9 studies and clinical attachment level (CAL) in 8 studies. In almost all of the studies, systemically administered antibiotics exhibited a more positive attachment level change than the control group in the study. The shortest therapy regimen was determined 3 days and the longest regimen was 4 weeks.

CONCLUSIONS: Analyzed data showed that systemically administered adjunctive antibiotics with and without SRP and/or surgery appeared to provide a greater clinical improvement in attachment levels than therapies not employing these agents. The use of metronidazole and amoxicillin in patients with aggressive periodontitis showed statistically significantly higher PD reduction and lower number of pockets ≥7 mm compared to only SRP (Scaling and Root Planning). Administration immediately after initial SRP provides more PD reduction and CAL "gain" in initially deep sites than late administration of SPT (Supportive Periodontal Therapy) with reinstrumentation after 3 months. Literature suggests that metronidazole can also be used in combination with ciprofloxacin. This is a very powerful combination against mixed and resistant infections.

KEYWORDS: metronidazole, periodontitis, dual therapy.

Introduction

Periodontitis is an inflammatory disease of bacterial origin, which affects the supporting tissues of the tooth. It constitutes one of the most frequent bacterial infections in adults. There are hundreds of bacterial species associated with this disease, and this fact makes it more complicated the achievement of a successful specific therapy for periodontitis (Paster et al, 2001). Among these, the most relevant are Aggregate bacter Actinomycetem comitans (*A.a.*), Porphyromonas gingivalis, Treponema Denticola, Fusobacterium Nucleatum, PrevotellaIntermedia, Campylo bacterium Rectus and Eikenella Corodens (Nishihara et al, 2004 , Feng et al, 2006). P.Gingivalis is considered as the main cause of chronic periodontitis, though no less important is the A.a., which is recognized as the leading cause of aggressive periodontitis(Nishihara et al,2004, Slots et al, 1999). The difficulties faced by periodontists lie in the fact that the restoration of normality for the periodontal tissues becomes difficult with time, and if left untreated, it can progress into an irreversible situation (Lindhe 2008). There are a number of procedures and protocols aimed to prevent the progression of the lesion and maintaining current status of periodontal tissues. To succeed in these procedures, in addition to manual curettage, the systemic antibiotic therapy plays an important role. There is evidence that manual mechanical removal of supra and subgingival plaque, without the use of antibiotic therapy, is incapable to eliminate pathogenic bacterial species and thus to maintain gingival levels of adhesion (Seiler, AAP 1966). To support such procedures, except monotherapy with antibiotics, which consists in the use of only one type of antibiotic, dentists frequently use a combination of antibiotics know as a combined therapy or dual antibiotic therapy (Herrera et al, 2008, Ciancio 2002). Among the pharmacological therapeutic options, metronidazole, and its combination with other antibiotics, has had positive results in managing the disease.

Materials And Methods

The purpose of this systematic review is to determine whether systemically dual therapy with antibiotics improves primary clinical outcomes and the role of Metronidazole in the combined therapy regimen. A computerized search of the literature of clinical studies for Periodontitis was conducted independently by two reviewers up to 31 January 2017 in the US National Library of Medicine (PubMed), using the search terms and combinations presented in Table 1. Inclusion criteria for the studies selection were: published in English, controlled clinical trials in humans, and cohort studies of > 1 month duration with a comparison group; subjects with aggressive or chronic periodontitis who received two antibiotics, when one of them was Metronidazole and that compared the effectiveness of the therapy either with placebo, or with another pharmacologic therapy, or another intervention (surgical or non-surgical intervention).Studies involving systemic reviews in vitro experiments, combinations of locally plus systemic antibiotics, were excluded. Initially (phase 1), the search encompassed published abstracts with the following combination of keywords: (‘*periodontitis, metronidazole, two, anti-bacterial agents*). Eligibility of potential studies was determined by reading the title and abstracts of each article identified by the search engine. All the articles that appeared to meet the inclusion criteria on the basis of their abstracts were selected and collected. Secondly, the full-text articles were obtained for manuscripts with missing abstracts or those in which insufficient relevant information was included in the published abstract. The final selection was independently completed by the two reviewers reading the complete articles and their results were compared. Disagreements were resolved by discussion between the two review authors. Excel worksheets

were designed to list the selected articles and the specific parameters for each case. These parameters included the authors and year of publication, type of study, therapy used in the two groups, parameters evaluated for measuring clinical outcomes and mail conclusion of the studies.

	Term used	Number of studies
	periodontitis OR periodontal infection OR chronic periodontitis OR aggressive periodontitis OR periodontal disease	59,354
And	("periodontitis"[MeSH Terms] OR "periodontitis"[All Fields]) AND ("metronidazole"[MeSH Terms] OR "metronidazole"[All Fields]) AND two[All Fields] AND ("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR "anti-bacterial agents"[All Fields] OR "antibiotic"[All Fields]) AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields])	79
**MESH: Medical Subject Heading*		

Figure 1. Search strategy stages in PubMed.

Results

After an initial selection, 79 papers were identified by the electronic searches; 18 papers met the inclusion criteria. Thirteen for chronic periodontitis and 5 for aggressive periodontitis. Total study population, for both control and test groups, was 1261;1086 with ChP and 175 with AgP.The most common parameter evaluated was pocket depth (PD) assessed in 13 studies, followed by bleeding on probing in 9 studies and clinical attachment level (CAL) in 8 studies. Seven out of 18 studies involved comparison of placebo with the combined dual antibacterial therapies, from which 6 conducted on patients with ChP and 1 on patients with AgP. In almost all of the studies, systemically administered antibiotics exhibited a more positive attachment level change than the control group in the study. The shortest therapy regimen was determined 3 days and the longest regimen was 4 weeks. Overall, scaling and root planning (SRP) plus systemic antimicrobial groups demonstrated better results in CAL and PD change than SRP alone, or in placebo groups.

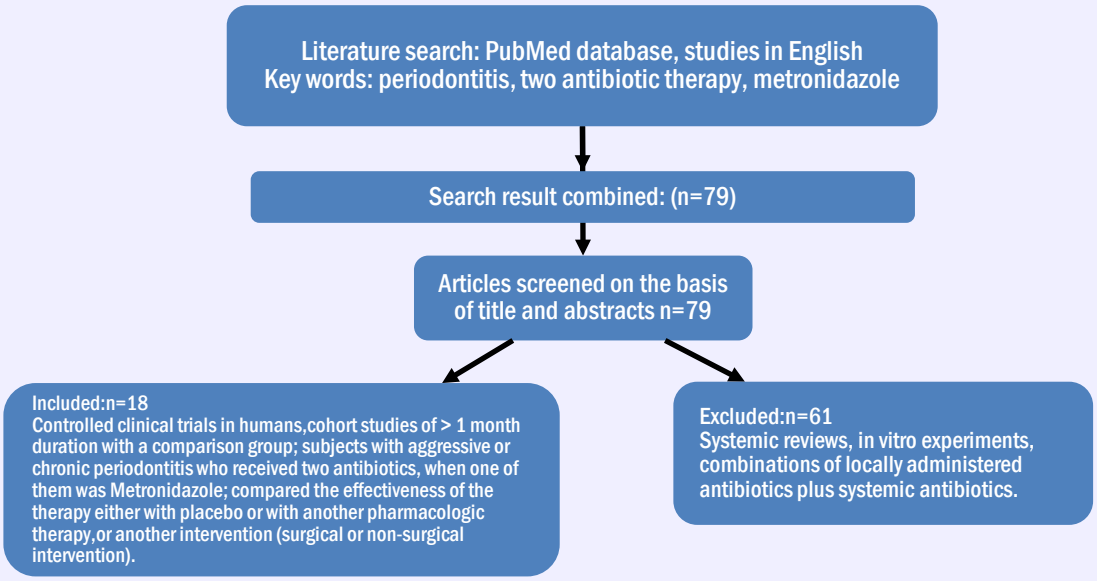

Figure 2. Scheme of search strategy.

Table 1. Information relating to 18 papers selected from the search.

Author	Type of study	Nr. of patients	Type	Therapy	Parameters evaluated	Conclusions
Cosgarea et al (2016)	placebo-controlled, randomized clinical study	102	ChP	SRP + placebo or SRP + AMX + MET (both 500 mg × 3 times daily) for 3 days or SRP + AMX + MET (both 500 mg × 3 times daily) for 7 days	PD, CAL, BOP, FMPS, GBI prior to treatment and at 3, 6 months post-treatment.	3 or 7 days' systemic administration of AMX + MET may lead to significantly greater clinical improvements compared to non-surgical therapy alone
Mombelli et al (2016)	group control, randomized clinical trial	80	ChP	375 mg AMX and 500 mg MTZ three times per day for 7 days during the non-surgical treatment phase/ during surgical phase.	Resistance of VGS to penicillin and erythromycin	Amoxicillin plus metronidazole did not significantly affect the resistance pattern of the VGS to penicillin or erythromycin.
Ercan et al (2015)	group control, retrospective record study	45	AgP	SRP only; SRP plus azithromycin (AZT group); and SRP plus MTZ and AMX (M+A group)	PD, CAL, GI, PI, BOP recorded at baseline and 3-month post therapy.	Nonsurgical therapy reduces PD, CAL and clinical inflammation findings. The scores were decreased more in the AZT and M+A groups than the controls, but this difference did not reach significance.
Yang et al (2015)	group control, randomized clinical trial	138	ChP	Minocycline hydrochloride; MTZ sustained-release film with minocycline hydrochloride, 4 weeks	therapeutic effect, adverse reaction and relapse situation of patients	Metronidazole sustained-release film combined minocycline hydrochloride can evidently improve patients' periodontal status, enhance drug therapeutic effect. It has less adverse reaction and low relapse rate, thus is worthy of clinical promotion.
Mombelli et al (2015)	single-center, randomized placebo-controlled crossover clinical trial	80	ChP	Group A, 500 mg MTZ plus 375 mg AMX three times per day for 7 days during the first, non-surgical phase of periodontal therapy (T1) and placebo during the second, surgical phase (T2); and group B, placebo during T1 and antibiotics during T2	PD, BOP	Giving the antibiotics during T1 or T2 yielded similar long-term outcomes, but antibiotics in T1 resolved the disease quicker and thus reduced the need for additional surgical intervention.
Arweiler et al (2014)	group control, randomized clinical trial	36	AgP	SRP and either systemic administration of AMX+MTZ or 7 days or with two episodes of PDT.	PI, BOP, PD, GR, CAL	Both treatments resulted in statistically significant clinical improvements, AB showed statistically significantly higher PD reduction and lower number of pockets ≥7 mm compared to PDT.
Almaghlouth et al (2014)	placebo-controlled, randomized clinical study	40	ChP	Full-mouth SRP within 48 h with either adjunctive systemic AMX and MTZ or placebo	Serum cytokines, acute-phase proteins	Subjects with untreated periodontitis may show high peaks for several inflammatory markers in serum simultaneously.Nonsurgical periodontal treatment with or without antibiotics reduced most of these peak levels.
Arweiler et al (2013)	randomized, group controlclinical trial	36	AgP	Full-mouth SRP, then randomly divided into two groups: Group AB received AMX and MTZ 3 times a day for 7 days. Group PDT received two applications of PDT on the day of SRP as well as at follow-up after 7 days	PI, BOP, PD, GR, CAL	Both treatments led to statistically significant clinical improvements. The systemic administration of antibiotics, however, resulted in significantly higher reduction of PD and a lower number of deep pockets compared to PDT.
Faveri et al (2014)	group control, clinical trial	64	ChP	SRP combined with MTZ (400 mg 3 times daily) + AMX (500 mg 3 times daily for 14 days applied in 32 smokers and 32 non-smokers	PD	Smokers with CP benefit less than non-smokers from treatment by the combination of SRP, MTZ, and AMX.
Feres et al (2012)	group control, clinical trial	118	ChP	SRP only or with MTZ 400 mg/3 a day or MTZ+AMX (500 mg 3 times day) for 14 days. Half of the subjects in each group rinsed	PD	Treatment of generalized ChP is significantly improved by the adjunctive use of MTZ+AMX and MTZ.

Table 1. Information relating to 18 papers selected from the search.

Author	Type of study	Nr. of patients	Type	Therapy	Parameters evaluated	Conclusions
				with 0.12% chlorhexidine twice a day (BID) for 2 months		
Goodson et al (2012)	group control, randomized clinical trial	187	ChP	SRP plus none, SRP + Systemic amoxicillin + metronidazole (SMA), local tetracycline delivery (LTC) and periodontal surgery (SURG)	CAL, PD	Patients receiving adjunctive therapies generally exhibited improved CAL gain and/or PPD reduction when compared with the outcome of SRP alone. Only additive, not synergistic effects of the various adjunctive therapies were observed
Mendonça et al (2012)	group control, randomized clinical trial	21	ChP	MTZ + AMX for 10 days with SD; MTZ + AMX for 10 days with NSD;	INF-γ, IL-17, IL-23 and IL-4	SD and NSD associated with systemic antimicrobials did not differ in terms of clinical benefits for RP in diabetics up to 6 months post-therapies. RP treated by SD presented increased levels of cytokines.
Casarin et al (2012)	randomized placebo controlled clinical trial	24	AgP	FMUD plus placebo, FMUD plus 375 mg AMX plus 250 mg MTZ for 7 days	PI, BOP, PD, GMP, CAL	amoxicillin/metronidazole improves clinical and microbiologic results of FMUD in AgP treatment.
López et al (2012)	parallel-arm, double-blind, randomized clinical trial	165	ChP	plaque control and root planning plus AMX and MTZ; plaque control instructions, supra-gingival scaling, and two placebos	Risk factors for cardiovascular disease, serum lipoprotein cholesterol, glucose, BMI, CRP, fibrinogen concentrations, PD	Reduction of periodontal inflammation either with root planning and systemic antibiotics or with plaque control and subgingival scaling significantly reduces CRP levels after 9 months in patients with MetS.
Dannewitz et al (2007)	post-operative follow up group control trial	53	ChP	amoxicillin/metronidazole or ciprofloxacin/metronidazole	A.a. test, PD	No differences were found between the subjects that were tested positive and negative for A.a in the postoperative period
Kaner et al (2007)	group control, clinical trial	34	AgP	SRP+AMX/MTZ, SRP +AMX/MTZ after 3 months	PD, CAL, BOP	administrationof amoxicillin/metronidazole immediately after initial SRP provides more PD reduction and RAL "gain" in initially deep sites than late administration at SPT with reinstrumentation after 3 months.
López et al (2006)	randomized, placebo controlled clinical trial	22	ChP	M+A for 7 days; (SRP) and two placebos	BOP, PD, CAL	Changes in clinical and microbiological parameters were similar after receiving systemically administered M+A as the sole therapy or after receiving SRP only
Giannopoulou et al (2006)	randomized, placebo-controlled, clinical trial	16	ChP	Half of the subjects received 250 mg MTZ and 375 mg AMX three times a day for 7 days; the other half received a placebo	GCF	Improved healing of the soft tissues has been noted clinically in non-surgically treated sites in subjects treated with antibiotics

**Abbreviations*

ChP- chronic periodontitis	GBI-gingival bleeding index (GBI)	CRP - C-reactive protein	AMX -Amoxicillin
AgP -aggressive periodontitis	AdvP -Advanced Periodontitis	GCF - gingival crevicular fluid	MTZ - Metronidazole
SRP- scaling and root planning	SD - surgical debridement	GR - gingival recession	BOP-bleeding on probing
GMP - gingival margin position	NSD - non-surgical debridement	CAL- clinical attachment level	PD- Pocked depth
FMPS- full-mouth plaque scores	FMUD - full mouth ultrasonic debridement	PDT- photodynamic therapy	

Discussions

Analyzed data showed that systemically administered adjunctive antibiotics with and without SRP and/or surgery appeared to provide a greater clinical improvement in attachment levels than therapies not employing these agents. Selection for an individual patient has to be made on other clinical factors.Systemically administered metronidazole, and especially the combination of metronidazole, amoxicillin and SRP leads to a beneficial change in the composition of the subgingival microbiota by reducing pathogens and allowing the growth of host-compatible species. In addition, the combination of systemic antibiotics and a strict control of supragingival plaque during the active phase of therapy has shown promising results in the treatment of chronic periodontitis. Furthermore, the use of metronidazole and amoxicillin in patients with aggressive periodontitis showed statistically significantly higher PD reduction and lower number of pockets ≥7 mm compared to only SRP. Administration immediately after initial SRP provides more PD reduction and CAL "gain" in initially deep sites than late administration at SPT with reinstrumentation after 3 months. Overall, systemic antimicrobials in conjunction with SRP, can offer an additional benefit over SRP alone in the treatment of periodontitis, in terms of CAL and PD change, and reduced risk of additional CAL loss. However, it is difficult to provide guidance to the more effective ones, since studies presented insufficient sample size for many of the antibiotics tested and very few of them were cross-over studies. Although, literature shows that metronidazole has a prominent effect on periodontitis, but alone it's not the drug of choice for treating A.a. infections. Instead, its combination with other antibiotics shows to be effective against these bacteria (Rams 1992). Also, it is effective against anaerobes such as P.Gingivalis and P.Intermedia (Jorgensen 2000).

Metronidazole combined with Amoxicillin may have a great impact in the management of patients with aggressive periodontitis (Abinaya et al, 2012). Amoxicillin is found to be useful in the management of patients with aggressive periodontitis, in both localized and generalized forms (Weinstein 1975). Literature suggests that metronidazole can be used in combination with ciprofloxacin. Actually, ciprofloxacin is the only antibiotic in periodontal therapy to which all strains of A.a. are susceptible. Metronidazole targets obligate anaerobes, and Ciprofloxacin targets facultative anaerobes. This is a very powerful combination against mixed infections. This combination provides a therapeutic benefit by reducing or eliminating pathogenic microorganisms and offers a prophylactic benefit by giving rise to predominantly streptococcal microflora (Rams et al, 1992). Periodontal infections contain a wide diversity of bacteria; hence, no single antibiotic can be effective against all putative pathogens (Walker et al, 1993). This "mixed infection" can include a variety of aerobic, microaerophilic, and anaerobic bacteria, both gram negative and gram positive. This scenario makes it mandatory to use more than one antibiotic, either serially or in combination (Jorgensen et al, 2000).

In addition to issues that arise from the inappropriate choice of the antibiotic prescribed, the duration of the treatment shows to be a problem itself. A short-term therapy may pose a risk in terms of antibiotic resistance rather than a treatment for the disease. This is true especially in cases of a chronic periodontitis where the presence of periodontal pathogens, specifically A.a., is known to endure in tissues after therapy and re-infect the pocket. Thus, the use of systemic antibiotics was thought to be necessary to eliminate pathogenic bacteria from the tissues. It is suggested that therapy in these cases should be at least 8 days or more for most of the antibiotics, which if greater than the data we found in our study (Christersson et al, 1987).

In practice, antibiotics are often used empirically without microbial testing. Studies conducted to

evaluate the effectiveness of microbial testing concluded that the usefulness of microbial testing may be limited and that empirical use of antibiotics, such as a combination of amoxicillin and metronidazole, may be more clinically and cost effective than bacterial identification and antibiotic-sensitivity testing. The practice of such measures can still be considered whenever a case of aggressive periodontitis is not responding or if the destruction continues despite good therapeutic efforts (Abinaya et al, 2012).

While the use of antibiotics in periodontal treatment will probably always be controversial, reports from both the American Academy of Periodontology and the European Federation of Periodontology contain valuable guidance for their use (Rams et al, 1992). Both these reports, following exhaustive literature searches, determined that patients with aggressive periodontitis appear to benefit from the adjunctive use of systemic antibiotics during treatment. The mechanical curettage without the addition of systemic antibiotics would probably be a failure considering the rapid bacterial colonization of periodontal pockets (Ciancio 2002). Systemic antibiotic therapy helps the manual curettage and improves immune response to eliminate subgingival bacteria, which are not affected by manual therapy (7???, Ciancio 2002). Based on WHO reports, bacterial resistance to antibiotics poses a "major global threat" to public health. Additionally, in some countries such as Norway, there is a national policy for the use of antibiotics with narrow spectrum in dental clinics to limit antibiotic-resistance (Mohammed et al 2007). Consequently, we must limit their use and prescribe the right dosage and duration of therapy to prevent further resistance.

Conclusions

From all the antibiotics available to dentists for periodontal diseases, metronidazole has a limited practice and it's not a first choice drug. A combined therapy with metronidazole and amoxicillin can be of great benefit for the patient because periodontal infections contain a wide diversity of bacteria; hence, no single antibiotic can be effective against all putative pathogens. For this purpose, we would suggest the combination of metronidazole and ciprofloxacin.

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CASE REPORT

THE FIRST REPORT OF AN ABCD1 GENE MUTATION IN AN ALBANIAN FAMILY

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X-linked adrenoleukodystrophy (X-ALD), a progressive neurodegenerative disease, is characterized by an abnormal function of the peroxisomes, which leads to an accumulation of the Very Long Chain Fatty Acids (VLCFA) in plasma and tissues, especially in the white matter of the central nervous system and the cortex of the adrenal glands (Kallabi 2016, Wiesinger 2015). X-linked adrenoleukodystrophy is a monogenic disease caused by mutations in the ABCD1 gene located on Xq28 (Wiesinger 2015). Mutations in the ABCD1 gene affect the function of the encoded protein ALDP, an ATP-binding cassette transporter located in the peroxisomal membrane protein (Kallabi 2016, Wiesinger 2015). Being an X-linked disease it affects mostly males, although some women who are carriers can have milder forms of the disease (Santosh 2013). The phenotypic expression and prognosis of an affected male is unpredicta variable (Kallabi 2016). Clinically, X-ALD can present with a wide range of phenotypic manifestations (Wiesinger 2015, Engelen 2012, Kemp 2016). Three main phenotypes are seen in affected males: 1) Childhood cerebral form – appearing in mid-childhood, 2) Adrenomyelopathy – occurring in men in their 20s or later and 3) Impaired adrenal gland function (called Addison disease or Addison-like phenotype) – adrenal gland does not produce enough steroid hormones. (Santosh 2013, Kemp 2016)

The present paper reports the clinical, biochemical, MRI imaging and molecular investigation in an Albanian family with an affected male with childhood cerebral adrenoleukodystrophy. His mother and sister carry the same mutation too. We believe that this is the first publication of ABCD1 gene mutation in an Albanian family.

Case Report

The patient was 8 years old when he was hospitalized due to severe headache, vomiting, confusion and intermittent *loss of consciousness (syncope)*. For months before the hospitalization, the patient had shown inattention, deterioration in handwriting skills and diminishing school performance, but his parents did not pay too much attention on them. The boy had febrile seizures when he was 7 and 22 months old. On admission to hospital, Magnetic Resonance Imaging (MRI) suggested adrenoleukodistrophy. MRI revealed the following findings: bilateral high signal lesion on T2 and FLAIR in bilateral peri-atrial regions, which show peripheral enhancement after administration of i/v contrast and

extention to splenium of corpus callosum and bilateral lemniscal tract. Biochemical reports also showed elevated levels of very long chain fatty acids in plasma.

After 3 weeks of treatment in the hospital the clinical status and neurological examination of the patient was almost normal. Later the patient showed difficulty in understanding speech, difficulty in reading, spatial orientation, and comprehension of written material; hearing difficulties, a decline in visual acuity and a hyperactivity.

Discussion

Leukodystrophies comprise a broad group of progressive, inherited disorders affecting mainly myelin (Santosh 2013, Kemp 2016). X-linked adrenoleukodystrophy is a neurodegenerative recessive disorder caused by mutations in the ABCD1 gene located on Xq28 (Kallabi 2016, Wiesinger 2015, Kemp 2016). The ABCD1 gene codes for the peroxisomal transporter ATP-binding cassette subfamily D member 1 (ABCD1, formerly ALDP) contain ten exons and spans 20 kb of genomic DNA. The 3616-bp transcript has 2,235 bp of coding sequence (Wiesinger 2015, Kemp 2016). ABCD1 gene mediates the import of very long-chain fatty acid (VLCFA) CoA esters across the peroxisomal membrane and its dysfunction results in impaired degradation of VLCFAs in peroxisomes and consequently leads to their accumulation in various lipid species in tissues and body fluids. (Kallabi 2016, Wiesinger 2015, Klouwer 2016, Wiesinger 2013,8). X-ALD manifests clinically as dysfunctions of the central nervous system (CNS), adrenal glands, and testicles (Kallabi F 2016, Wiesinger Ch 2015, Kemp S 2016).

The diagnosis of ALD is primarily based on clinical, MRI imaging, biochemical and genetic studies. (Santosh 2013, Wiesinger 2015, Kemp 2016)

The childhood cerebral form of three main phenotypes which are observed in affected males manifest themselves most commonly between ages four and eight years. Boys with symptoms of attention deficit disorder (ADD) show signs of progressive behavioral disturbance, vision loss, difficulty in understanding spoken language, worsening handwriting, incoordination or other neurologic disturbances and motor function ones, dementia follows the initial symptoms and often leads to total disability within two years. Other variants observed in approximately 5%-10% of affected males incclude headache, increased intracranial pressure, hemiparesis or visual field defect, aphasia or other signs of localized brain disease with the onset usually between age four and ten years (Kemp 2016, Wiesinger 2015).

Our patient had almost a normal development until he reached 8 years old when he was hospitalized due to severe headache, vomiting, confusion and intermittent loss of consciousness. His clinical data before hospitalization (inattention, deterioration in handwriting skills, diminishing school performance) and after hospitalization (difficulties in reading, in understanding speech, in spatial orientation, in hearing etc), are typically found in the childhood cerebral form of X-linked adrenoleukodystrophy. Our patient has reached a total disability within one year and benefits from the general supportive care of parents, as well as of the hospital.

Besides, MRI images of the patient were compatible and consistent with active demyelination as usually observed in childhood cerebral X-ALD (Santosh 2013). MRI is always abnormal in boys with cerebral disease and often provides the first diagnostic lead. In approximately 85% of affected individuals, MRI shows a characteristic pattern of symmetric enhanced T2 signal in the parieto-occipital region with contrast enhancement at the advancing margin (Santosh 2013, Kemp 2016).

Typically, when a diagnosis of X-ALD is suspected based on clinical presentation or magnetic resonance imaging abnormalities, biochemical testing for elevated plasma VLCFA levels is performed. Biochemical reports show elevated levels of very long chain fatty acids in plasma (Wiesinger 2015, Santosh

2013, Klouwer 2016, Wiesinger 2013). Plasma concentration of very long chain fatty acids (VLCFA) is abnormal in 99% of males with X-ADL regardless of age, disease duration, metabolic status, or clinical symptoms (Wiesinger 2015, Kemp 2016). Three parameters usually are analyzed: Concentration of C26:0 Ratio of C24:0 to C22:0 Ratio of C26:0 to C22:0. All three parameters are elevated in the majority of males, though some variation is observed (Kemp 2016). Thus, an elevated level of VLCFAs, as in our case, represents the standard biomarker for diagnosis of X-ALD, but does not predict the phenotype or progression of disease (Wiesinger 2015).

In our case report, magnetic resonance imaging (MRI) as well as the high levels of VLCFAs prompted the diagnosis the X-ALD. Molecular analysis of ABCD1 gene has shown a pathogenic mutation. Sequence analysis of all ABCD1 gene was performed and identified the following hemizygous mutation: c1553G>A (pArg518 Gln). No additional pathogenic mutations were identified in the ABCD1 gene. The found mutation is a missense one. A tremendous number of different disease-causing mutations have been described in X-ALD (Wiesinger 2015, Schackmann 2016, Karkar A 2015). Missense variants have been found in all parts of the gene but are most common in the membrane domain or the ATP-binding domain, emphasizing the importance of these two domains for the function of ALDP (Kemp 2016). A comprehensive overview of all described mutations can be found in the X-ALD database (http://www.x-ald.nl) (Wiesinger 2015). This mutation has been previously reported (http://www.x-ald.nl).

The phenotype cannot be predicted by VLCFA plasma concentration or by the nature of the ABCD1 pathogenic variant as the same pathogenic variant can be associated with each of the known phenotypes (Kemp 2016, Wiesinger 2015). No relevant genotype–phenotype correlation exists in X-ALD. Some current, ongoing SNP association studies suggest that multiple loci, rather than a single modifier gene, likely contribute to the phenotype (Kemp 2016, Brose 2012, Semmler 2009).

While the majority of patients typically inherit the defective ABCD1 allele from one parent, between 4.1% and 19% of X-ALD cases have been reported to carry mutations acquired de novo (Kemp 2016). The ABCD1 gene sequencing indicated the same missense mutation c1553G>A (pArg518 Gln) in the exon 6 of the ABCD1 gene in the patient, his mother and his sister too.

Approximately 20% of females who are carriers develop neurologic manifestations like mild to moderate spastic paraparesis that resemble AMN but have later onset (age ≥35 years) and milder disease than do affected males (Kemp 2016). His mother clinically has a mild form of the disease. Finding the same mutation at his sister is helpful to clarify the carrier status and for the discussion of the availability of prenatal testing before her future pregnancy (Kemp 2016).

As the conclusion, familiarity by the pediatricians, neuropediatricians with the clinical-pathologic manifestations and progressive MR imaging features of childhood cerebral X-linked ALD is helpful in evaluating affected patients (Santosh 2013, Wiesinger 2015, Klouwer 2016).

To establish the extent of disease and requirements in an individual diagnosed with X-linked adrenoleukodystrophy (X-ALD), the following evaluations are recommended: neurologic examination, brain MRI, adrenal function tests and medical genetics consultation. Evaluation of at-risk family members, often implemented insufficiently, is important for the management and genetic counseling (Kemp 2016, Wiesinger 2013).

References

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