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Antimicrobial Activity of a Synthesized Fluoroquinolone with Potential Therapeutic Use

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Abstract

Antimicrobial resistance is one of the major public health threats of the 21st century. The need of developing new drugs is an emergency to avert a global crisis. In this context, the study aims to determine the antimicrobial activity of a new synthesized fluoroquinolone at the National Institute for Chemical Pharmaceutical R&D, Bucharest on some strains of *Staphylococcus, Streptococcus* and *Escherichia coli*. The study is sustained by the previous *in silico* molecular docking tests with promising results. The minimum inhibitory concentration was determined by two methods: the microdilution technique and agar disk diffusion method.

The results were compared with the breakpoint value of the antimicrobial activity recommended by EUCAST.

Keywords: antimicrobial resistance, quinolone

Introduction

Infectious diseases are one of the main causes of mortality worldwide. In spite of the prophylactic measurements and drug therapy available nowadays, a dramatic increased proportion of pathogens became resistant and generated severe diseases. Multi-drug-resistant bacteria are considered one of the major public health threats of the 21st century. The need of developing new drugs is an emergency to avert a global crisis [1-3].

Fluoroquinolone are potent antibiotics with a broad-spectrum against Gram-positive and Gram-negative pathogenic bacteria. Current prescribing guidelines recommend this antibiotic class as a second line agents when other narrow spectrum agents fail [4-6].

In this context, several fluoroquinolones with potential therapeutic effect were synthesized at the National Institute for Chemical Pharmaceutical R&D, Bucharest. The previously *in silico* molecular docking tests were promising for some molecular structures. One of these new fluoroquinolones, noted FPQ30 was selected for further studies to determine the therapeutic potential [6].

The study aims to evaluate the antimicrobial effect on some strains of *Staphylococcus, Streptococcus* and *Escherichia coli,* which are frequently involved in severe infections. The best method to evaluate the sensitivity of a micro-organism towards antimicrobials is determining the minimum inhibitory concentration (MIC) [7-9]. MIC was determined by two methods: the microdilution technique and agar disk diffusion method. The results were

compared with the breakpoint value of the antimicrobial activity recommended by EUCAST [8].

Materials and Methods

The antimicrobial substance tested was a fluoroquinolone noted FPQ30 synthesized at the National Institute for Chemical Pharmaceutical R&D, Bucharest.

Bacterial strains

For testing fluoroquinolone FPQ30, both reference and wild-type strains were used, the lasts being previously isolated from different patients. The reference strains were: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739.

The wild-type tested strains were the following: *Staphylococcus aureus* MRSA (1-16), *Streptococcus spp*. β-hemolytic (1-14), *Streptococcus spp*. γ-hemolytic (1-9) and *Escherichia coli* (1-14).

Multiple microdilution technique

The preparation of the FPQ30 stock solution was achieved by dissolving it in DMSO so that its concentration is 5000 mg/L. From the stock solution were obtained successive dilutions using the Mueller-Hinton broth. The dilutions used within the study were the following: 128 mg/L, 64 mg/L, 32 mg/L, 16 mg/L, 8 mg/L, 4 mg/L, 2 g/ml. To avoid and eliminate any errors, all dilutions were prepared using the stock solution and not by dilution of the immediate superior solution. [10, 11].

The preparation of the inoculum was carried out from cultures with previously checked purity. From each culture was homogenized in saline water the corresponding quantity so that the value 0.5 was obtained on the McFarland scale, which corresponds to a bacterial load of approx. $1\text{-}2x10^8$ CFU/ml. The suspension obtained was diluted in the Mueller-Hinton broth until obtaining the final inoculum of approx. $5x10^5$ CFU/ml [7, 12].

The method is in accordance with the standard recommended by CLSI [13, 14]. For this purpose, there were used plates adapted to microdilutions, with 96 wells. For each strain tested, 8 wells have been used, with the following distribution (below is presented the case of a stem in the first row of the wells): A1 – 128 mg/L, B1 – 64 mg/L, C1 – 32 mg/L, D1 – 16 mg/L, E1 – 8 mg/L, F1 – 4 mg/L, G1 – 2 mg/l, H1 – 0 mg/l (Negative control). In each well was added 50 µL inoculum and 50 µL FPQ30 in the concentrations mentioned above.

After the addition of the inoculum and quinolone in the wells, the plates were incubated in aerobic atmosphere at 37 °C for 24 hours [13].

Results

After the incubation was appreciated the degree of bacterial development in each pitting.

The increase in the micro-organism appears in the form of a deposit in the inferior part of the well [7, 8, 14] (Fig. 1). The minimum inhibition concentration is the lowest concentration of the antimicrobial agent capable of inhibiting the growth of the micro-organism in the broth, namely the first concentration in which bacterial development is not observed.

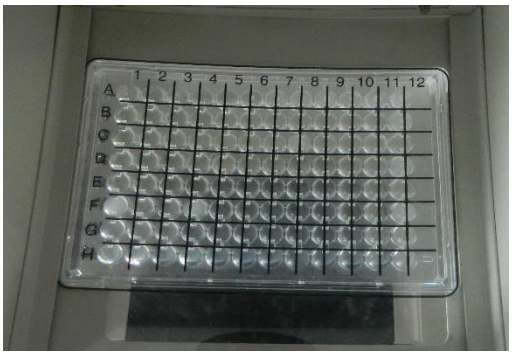

Fig. 1. Appreciation of the degree of bacterial development by analyzing the deposit in the well – the microdilutions method

Disk diffusion method (adaptation)

For this method, dilutions of FPQ30 were obtained from the stock solution using DMSO as shown in Table 1.

Table 1. Dilutions of the FPQ30 stock solution in the disk diffusion method

Stock solution FPQ30 (µl)	**DMSO (µl)**	**Concentration (mg/l)**	**FPQ30 (µg)**
100	50	3330	66,6
100	100	2500	50
100	150	2000	40
100	200	1660	33,2
100	250	1425	28,5
100	300	1250	25
100	400	1000	20
0 (negative control)	300	0	0

The bacterial suspension used was 0.5 McFarland turbidity standard, which corresponds to a bacterial load of approx. 1-2x10^8 CFU/ml. The inoculum has been distributed throughout the environment. After complete drying of the inoculum, the surface of the agar has been made of paper discs soaked with 20 µl FPQ30 and DMSO with specific concentrations. The plates were incubated in aerobic atmosphere at 37 °C for 24 hours.

Interpretation of the results. After incubation, the results were read by measuring the inhibition area occurring around the discs containing the antimicrobial substance [13, 14] (Fig. 2). The diffusion method is based on the diffusion of an antimicrobial agent, in a specific concentration, in the solid environment, in the presence of an inoculum located in pure culture.

The results obtained by this method can be influenced by the growth rate of the bacteria, the diffusion rate of the antibiotic, as well as the density of the agar used. The interpretation of the results is based on the determination of the inhibition zone, which is directly proportional to the sensitivity of the micro-organism to the tested antibacterial agent.

Fig. 2. Interpretation of the results by measuring the inhibition area around the discs containing the antimicrobial substance – the disc-diffusion method (*Escherichia coli* ATCC 8739)

Discussions

The centralized results obtained from the testing of wild-type strains by the microdilution method are presented in Table 2.

Table 2. The minimum inhibitory concentration of quinolone FPQ30 in the case of some reference and wild-type strains

Strain	No.	MIC (mg/l)															
		≤2		2		4		8		16		32		64		128	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Staphylococcus aureus MRSA	16	3	18.75	1	6.25	7	43.75	5	31.25	0	0	0	0	0	0	0	0
Streptococcus spp. β - hemolytic	14	0	0	0	0	0	0	2	14.29	2	14.29	0	0	5	35.71	5	35.71
Streptococcus spp. γ - hemolytic	9	0	0	0	0	2	22.22	3	33.33	1	11.11	0	0	2	22.22	1	11.11
Escherichia coli	14	0	0	0	0	2	14.29	1	7.14	0	0	4	28.57	4	28.57	3	21.43

The results obtained by disk diffusion method are presented in Table 3.

Table 3: Results obtained after testing quinolone FPQ30 through the disc-diffusion method, on various bacterial strains

Bacterial species	Strain	Minimum inhibitory concentration (MIC) FPQ30 (mg/l)							
		66,6	50	40	33,2	28,5	25	20	0
Staphylococcus aureus	ATCC 25923	26	24	24	23	23	21	21	6
	ATCC 6538	26	26	26	26	26	24	24	6
	Wild type MRSA – 1	24	24	24	24	24	24	24	6
	Wild type MRSA – 2	24	24	24	24	24	24	24	6
	Wild type MRSA – 3	24	24	24	24	24	24	24	6
	Wild type MRSA - 4	24	24	24	24	24	24	24	6
	Wild type MRSA - 5	24	24	24	24	24	24	24	6
	Wild type MRSA -6	24	24	24	24	24	24	24	6
	Wild type MRSA - 7	20	20	20	20	20	20	20	6
	Wild type MRSA - 8	24	24	24	24	24	24	24	6
	Wild type MRSA - 9	20	20	20	20	20	20	20	6
	Wild type MRSA - 10	20	20	20	20	20	20	20	6
	Wild type MRSA - 11	24	24	24	24	24	24	24	6
	Wild type MRSA - 12	20	20	20	20	20	20	20	6
	Wild type MRSA - 13	20	20	20	20	20	20	20	6
	Wild type MRSA - 14	24	24	24	24	24	24	24	6
	Wild type MRSA - 15	24	24	24	24	24	24	24	6
	Wild type MRSA - 16	24	24	24	24	24	24	24	6
Streptococcus spp.	Wild type β-hemolytic - 1	10	8	6	6	6	6	6	6
	Wild type β-hemolytic - 2	8	8	8	8	6	6	6	6
	Wild type β-hemolytic - 3	6	6	6	6	6	6	6	6
	Wild type β-hemolytic - 4	18	18	18	18	18	16	16	6
	Wild type β-hemolytic - 5	8	6	6	6	6	6	6	6
	Wild type β-hemolytic - 6	18	18	18	18	16	16	16	6
	Wild type β-hemolytic - 7	18	18	18	18	18	18	16	6
	Wild type β-hemolytic - 8	6	8	6	6	6	6	6	6
	Wild type β-hemolytic - 9	6	6	6	6	6	6	6	6
	Wild type β-hemolytic - 10	10	10	10	10	8	8	8	6
	Wild type β-hemolytic - 11	8	8	6	6	6	6	6	6
	Wild type β-hemolytic - 12	12	12	10	10	10	8	6	6
	Wild type β-hemolytic - 13	8	8	8	8	8	8	8	6
	Wild type β-hemolytic - 14	16	16	16	16	16	16	14	6

	Wild type γ-hemolytic - 1	18	18	18	18	18	18	18	6
	Wild type γ-hemolytic - 2	18	18	18	18	18	18	18	6
	Wild type γ-hemolytic - 3	6	6	6	6	6	6	6	6
	Wild type γ-hemolytic - 4	18	18	18	18	18	16	16	6
	Wild type γ-hemolytic - 5	10	10	10	6	6	6	6	6
	Wild type γ-hemolytic - 6	10	8	8	8	8	6	6	6
	Wild type γ-hemolytic - 7	18	18	18	18	16	16	16	6
	Wild type γ-hemolytic - 8	12	12	12	12	10	12	10	6
	Wild type γ-hemolytic - 9	18	18	18	16	16	16	16	6
Escherichia coli	ATCC 8739	8	8	8	8	8	8	8	6
	Wild type - 1	6	6	6	6	6	6	6	6
	Wild type - 2	6	6	6	6	6	6	6	6
	Wild type - 3	12	12	10	10	10	10	10	6
	Wild type – 4	10	10	10	10	10	10	10	6
	Wild type – 5	12	8	8	8	8	8	8	6
	Wild type – 6	16	16	16	16	16	8	8	6
	Wild type – 7	18	16	16	16	16	8	8	6
	Wild type – 8	10	10	10	10	10	10	10	6
	Wild type – 9	6	6	6	6	6	6	6	6
	Wild type – 10	16	16	16	16	16	8	6	6
	Wild type – 11	10	10	10	10	10	10	10	6
	Wild type – 12	10	10	10	10	10	10	10	6
	Wild type – 13	12	12	10	10	10	10	10	6
	Wild type – 14	12	12	10	10	10	10	10	6

FPQ30 has shown the strongest capacity to inhibit bacterial development of the *Staphylococcus aureus* MRSA. Thus, 18.75% of the tested strains have MIC value below 2 mg/l; considering that the breakpoint value for ciprofloxacin (the same class as the substance in the study) in the case of *Staphylococcus* is 2 mg/l, we may consider by extrapolation that these strains are susceptible to this fluoroquinolone. Following the testing of *Staphylococcus* strains through the disc-diffusion method, the inhibition area obtained was significantly increased, sustaining the ability of the substance to inhibit the development of these micro-organisms [8].

Regarding the activity of FPQ30 on the *Streptococcus* strains, the effect has been moderate, with an increased antimicrobial action on *Streptococcus spp*. γ-hemolytic compared to *Streptococcus spp*. β-hemolytic. According to EUCAST, the breakpoint for ciprofloxacin is 4 mg/l; however, this value is not applicable in case of other substances [8].

The most limited action of the FPQ30 proved to be against the strains of *Escherichia coli*, most of the strains could developed at high concentrations of antimicrobial substance. In the case of some strains, a moderate inhibition was noted using the disc-diffusion method.

These results sustain the antimicrobial capacity of FPQ30. Still, further studies are required because the tests were carried out *in vitro* on wild type strains, which, however, have undergone different passages in the laboratory, and their susceptibility to some antimicrobials may have been altered.

Conclusions

The new synthesized FPQ30 demonstrated an increased antimicrobial activity on *Staphylococcus aureus* MRSA and on *Streptococcus spp*. γ-hemolytic, a moderate effect on *Streptococcus spp*. β-hemolytic and a limited action on *Escherichia coli* strains. The promising results on some bacterial strains obtained *in vitro* correlated with the previous *in silico* molecular docking study sustain a potential therapeutic effect of the FPQ30 fluoroquinolone.

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In Vivo Preliminary Testing of Some Scars Treatment Ointment Formulations with Hyaluronic Acid

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Abstract

Worldwide, millions of people are affected by scars. Some ointments can help prevent scar tissue from developing excessively. Several modalities have been developed to assess scars in order to evaluate the efficacy of treatment with such ointments. Usual measured parameters are: pliability, firmness, degree of erythema and pigmentation of the skin, perfusion, thickness, and 3-dimensional topography of the scars. In this study, two new formulations of ointment (Formulation A and Formulation B) containing different strengths of hyaluronic acid as main active ingredient, developed in order to be used for the treatment of postsurgical scars, were preliminary assessed. The evaluation of the ointments effects on uninjured skin was made by evaluating the pliability, the erythema and melanin indices and the hydration, using non-invasive and easy-to-use devices. The clinical tolerance and acceptability of the formulations were also assessed. According to the obtained results, Formulation B was chosen for further studies and all the measured parameters proved to be valuable for the evaluation of the effectiveness of ointment during post-operative care for its soothing and repairing effects.

Keywords: scar, hyaluronic acid, pliability, erythema index, hydration index

Introduction

The skin is the soft outer tissue covering our body and is the largest organ of the body. The skin protects us from microbes and other elements, helps regulate body temperature, and permits the sensations of touch, heat, and cold. Any burn, injury, or other trauma, such as surgery, can cause a scar.

A scar is an area of fibrous tissue that replaces normal skin after an injury. Scars result from the biological process of wound repair in the skin, thus scarring is a natural part of the healing process. While every wound will create some bit of scar tissue, rich collagen production or inadequate wound care can result in thick, unsightly scar formation after injuries to the skin.

Worldwide, millions of people are affected by scars. A significant percentage of scars (38-70%, depending on the study) results in a pathological condition, i.e., causing pain, functional and psychological disorders, or cosmetic damage [1, 2].

Several modalities have been developed to assess scars in order to evaluate the treatments.

Scar-measuring devices should be non-invasive, accurate, reproducible, and easy-to-use to facilitate objective data collection and have clinical utility. Existing devices assess parameters

such as pliability, firmness, colour, perfusion, thickness, and 3-dimensional topography of the scars.

Some ointments can help prevent scarring from becoming excessive after burns, cuts, or acne outbreaks. Hyaluronic Acid (HA) represent an interesting and valuable option for the development of medical devices for epidermal and dermal wound treatment. HA is an important polysaccharide component of the extracellular matrix. It is among the most hygroscopic natural molecules; when absorbing water, HA molecules can swell in volume up to 1000 times, forming loose hydrated matrices [3, 4]. Also, HA is involved in the early stages of tissue repair and wound healing being, together with fibrin, a component of the matrix which forms to aid fibroblast and endothelial cell organization into the injury site [5-7].

Two new formulation of ointment (Formulation A and Formulation B) containing different quantities of hyaluronic acid as main active ingredient were developed in order to be used for the treatment of postsurgical scars. The final goal of our study is to evaluate the effectiveness of a new formulation ointment used during post-operative care for its soothing and repairing effects. In this preliminary *in vivo* study, the main objectives were the evaluation of the ointments effects on uninjured skin, and the evaluation of the clinical tolerance and acceptability of the formulations, while the selection of the formulation and the screening of parameters to be tested on injured skin and their corresponding ranges were secondary objectives. At this stage of the study, pliability, erythema and melanin indices and hydration of skin were assessed.

Materials and Methods

Volunteers

Measurements were performed on twelve healthy volunteers without scars. The study included male and female patients, from 28 to 59 years of age. The following volunteers were excluded: volunteers suffering from dermatosis, severe chronic or progressive diseases, known intolerance to any of the components of the product under study; patients undergoing corticotherapy or being treated with NSAIDs or anticoagulants and children, pregnant or nursing women. The Declaration of Helsinki Protocols was followed and before enrolment, all volunteers signed an informed consent form. The study protocol was approved by the coordinating ethics committee.

Product to be tested

Two new ointment formulations (Formulation A and Formulation B) containing different quantities of hyaluronic acid were tested. One registered product available on the Romanian market was used as comparator. A first application of a thick layer of products was performed on a selected skin area of the forearms of the volunteers enrolled in the study. After 30 minutes, the first measurements were made. In the evening of the measurements day and for twenty-one days thereafter, the volunteers continued to apply the ointments by means of a light massage until complete penetration. The measurements were performed also on an untreated skin area as control sample.

Effectiveness Evaluation Criteria

The evaluation criteria were: the pliability, the pigmentation and erythema indices, as well as the hydration of the skin. These parameters were determined at the following time-points: 30 minutes after the first ointment application ($t_{30\ min}$), after 7 days of treatment ($t_{7\ days}$) and after 21 days of treatment ($t_{21\ days}$).

The pliability

Pliability represents a set of biomechanical skin properties that provide its elasticity, firmness, extensibility, and tensile strength. Scarring could negatively affect the skin pliability, therefore this is an important means in the evaluation of scar characteristics, as it affects movement and correlates with the patient's overall satisfaction [8, 9]. The pliability was assessed by the measurement of skin elasticity. The Reviscometer RVM 600 (Courage and Khazaka Electronic, Cologne, Germany) measures the anisotropy of the skin by analyzing its vertical deformation in response to an acoustic shock wave. It measures the resonance running time (RRT) of the shock wave. The longer is the time the waves need to be propagated through the material, the higher is the measuring value is and the material is less elastic. Thus, a high RRT would correspond to scar tissue, which can be considered as less elastic compared with normal skin, and a low RRT would correspond to normal skin [10].

The degree of pigmentation

Skin scar pigmentation is determined by the vascularity and pigmentation of the tissue involved. After cutaneous injury, abnormal skin color is a common complaint by patients. The inflammatory process also induces an increase in vascularity and erythema and more active scars have a higher blood supply. Pigmentation can be usually quantified by determination of the intensity of light that is reflected from cutaneous tissue, acting with specific wavelengths and by patterns in melanin and hemoglobin light absorption that are known [11-13]. The pigmentation was assessed by the measurement of erythema and melanin index. The Mexameter MX 18 (Courage and Khazaka Electronic, Cologne, Germany) use spectrophotometric color analysis to calculate erythema and melanin index. Pigmentation is quantified by measuring the light intensity that is reflected from cutaneous tissue, for distinct wavelengths and by light absorption patterns which are known for melanin and hemoglobin.

The hydration

Scars shows increased transepidermal water loss compared to healthy skin [14, 15], thus the measurement of skin hydration was considered and the hydration index was determined.

Measurement of hydration of the upper skin layer (stratum corneum) was performed by the electrical capacitance method with the Corneometer CM 825 (Courage and Khazaka Electronic, Cologne, Germany). The Corneometer measures any change of the dielectric constant, due to skin surface hydration that changes the capacitance of a precision capacitor. An increase of Corneometer values shows a skin moisturizing effect [16, 17].

Tolerance Evaluation Criteria and Acceptability *Evaluation Criteria*

Tolerance was appraised every day by the patient on the basis of the appearance of side effects of allergic or irritating nature, such as itching and eczematisation. At the end of the study, the tolerance was assessed using a 4-step scale: bad, rather good, good, and very good.

Cosmetic acceptability of the product under study was evaluated by the patient at the end of the study according to ease of application and penetration, soothing effect, agreeable or disagreeable texture and odour [18].

Results and Discussions

The pliability

The pliability was assessed by the measurement of skin elasticity. The mean values of the resonance running time (RRT) are presented in a tabular form (Table 1) and variation of the skin elasticity is highlighted in Fig. 1.

Table 1. The mean value of the resonance running time

Sample	$t_{30\ min}$	$t_{7\ days}$	$t_{21\ days}$
Control	322.91	566.20	343.63
Formulation A	645.43	455.14	298.64
Formulation B	500.07	440.62	289.40
Comparator	493.31	508.98	281.94

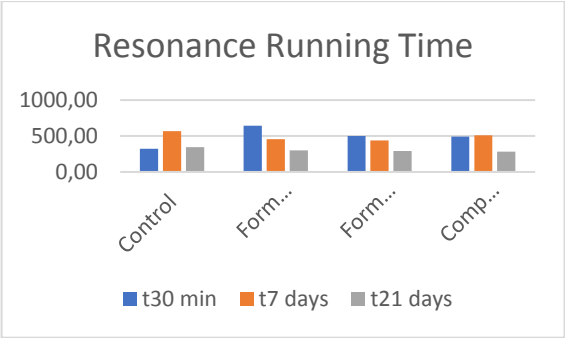

Fig. 1. The differences in the skin elasticity

It is obvious that the time the waves need to propagate is longer at 30 minutes after the application of the ointment for all formulations, including the comparator, compared to the untreated skin (control). Thus, the ointments are acting like a barrier until they are completely absorbed and the measurements are interfered. After 7 days of administration, the RRT mean values were lower for all the treated skin areas, compared to the untreated skin. This shows an improvement of the skin pliability, higher for the new formulations than for the comparator.

Better results are obtained for Formulation B. After 21 days of administration, the RRT mean values remained lower for all the skin areas on which the formulations were applied, compared to the untreated skin areas, but the differences were less noticeable. The higher effect of Formulation B was constant for 3 weeks.

The pigmentation

Coloration of the skin scar is determined by vascularity and/ or pigmentation of the tissue involved. Thus, for assessing the colour of th scar, both erythema and melanin index should be measured. In this preliminary study, only uninjured skin was considered for the ointment application, so the colour was assessed only by the measurement of erythema index in order to evaluate the potential side effect of the ointment. The mean values of the erythema index are presented in a tabular form (Table 2) and variation of the skin colour is highlighted in Fig. 2.

Table 2. The mean value of the resonance running time

Sample	$t30_{\ min}$	$t7_{\ days}$	$t21_{\ days}$
Control	234.13	199.40	202.24
Formulation A	223.03	242.00	204.16
Formulation B	187.27	184.12	184.44
Comparator	212.30	198.33	209.96

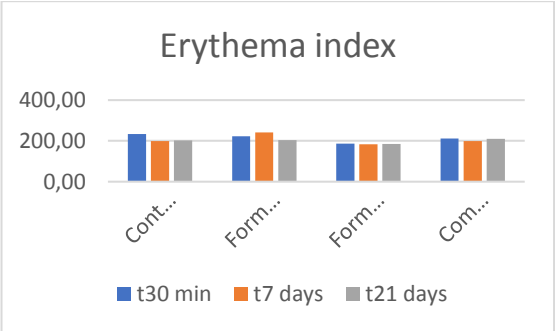

Fig. 2. The differences in the skin colour

It is observed that 30 minutes after the application of the ointments the erythema index values were lower for all formulations, including the comparator, versus the untreated skin (control).

Thus, no irritation effect was observed immediately after the administration. After 7 days of administration, the erythema index mean values were higher for Formulation A, meaning that a possible irritation effect can occurr. After 21 days, this effect is reduced. Better results were obtained for Formulation B, which showed no irritant effect, since the erythema index decreased compared to both the untreated skin and the comparator.

The hydration

The skin hydration was assessed by measuring the hydration index with a Corneometer, in order to evaluate the moisturizing effect of the formulations. The mean values of hydration index are presented in a tabular form (Table 3) and variation of the skin hydration is shown in Fig. 3.

Table 3. The mean value of the Hydration Index

Sample	*t30 min*	*t7 days*	*t21 days*
Control	39.31	43.28	23.61
Formulation A	65.55	43.40	32.72
Formulation B	61.26	43.29	34.06
Comparator	50.48	41.74	40.47

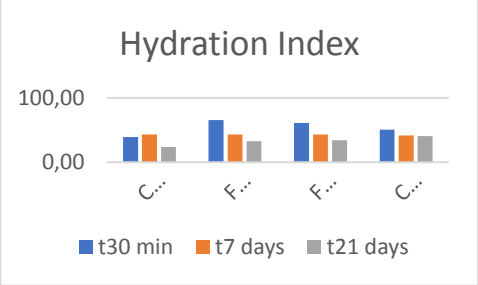

Fig. 3. The differences in the skin hydration

It is noticeable that hydration index average values were higher at 30 minutes after the application of the ointment for all formulations, including the comparator, versus the untreated skin (control). The effect is more evident for Formulation A. Both formulations were superior to the comparator, a possible consequence of film-forming properties. After 7 days of administration, the hydration mean values were similar for all skin areas. After 21 days of administration, the moisturising effect of the ointments was observed. Formulation B was superior to Formulation A, but not to the comparator.

Tolerance Evaluation Criteria and Acceptability Evaluation Criteria

No allergic or irritating side effects were reported neither for the test products nor for the control product. The findings on tolerance were very good.

All patients were satisfied or very satisfied with the ease of application and texture of the cream and found penetration easy and fast for both products. The odour of all products was found to be agreeable.

Conclusions

Two new formulations of ointment (Formulation A and Formulation B) with different strengths of hyaluronic acid as main active ingredient were developed, in order to be used for the treatment of postsurgical scars. In this preliminary *in vivo* study, evaluation of the ointments effects on uninjured skin using non-invasive and easy-to-use devices and evaluation of the clinical tolerance and acceptability were performed. Formulation B proved better results and less irritating potential than Formulation A. The clinical tolerance and acceptability reported by the volunteers were very good for both formulations. Further studies will focus on Formulation B and all the assessed parameters prove to be valuable for the evaluation of the effectiveness of ointment during post-operative care for its soothing and repairing effects.

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Debulking Surgery for Thoracic Metastases from Ovarian Cancer

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Abstract

Ovarian cancer is recognized for the high incidence of patients diagnosed in advanced stages of the disease, as well as for the poor prognostic if distant metastases develop. However, debulking surgery seems to be capable of providing a better long-term outcome of such cases.

The aim of this study is to demonstrate the feasibility and safety of thoracic resections for thoracic metastases from ovarian cancer. *Material and methods*. We present a case series of six patients in whom intrathoracic resections of ovarian cancer metastases were successfully performed. *Results*. The main associated resections consisted of pleurectomy in three cases, atypical pulmonary resections for oligometastatic disease (in two cases) while in the sixth case resection of the cardiophrenic lymph nodes was performed. Postoperatively no reoperation was needed. *Conclusions*. In selected cases presenting with oligometastatic intrathoracic lesions from ovarian cancer, cytoreductive surgery is feasible and effective, without increasing the perioperative morbidity.

Keywords: Thoracic metastases, ovarian cancer, debulking surgery

Introduction

Ovarian cancer remains one of the most commonly reported gynecologic malignancies; unfortunately, an important number of cases are diagnosed in advanced stages of the disease; it has been widely demonstrated that the overall survival rate significantly decreases once the International Federation of Obstetrics and Gynecology (FIGO) stage increases, actually the estimated survival for stage IV of disease being less than 20% at 5-year follow-up [1]. However, it seems that the most important factor in terms of survival remains the residual tumoral volume after debulking surgery, this factor being even more important than the stage at initial diagnosis [2-4]; due to the fact that most often patients with ovarian cancer or recurrent disease present multiple metastases, cytoreductive surgery is expected to involve various visceral resections in order to maximize the debulking effort [5-8]. A more particular situation remains the one of the patients diagnosed with extra-abdominal metastases from ovarian cancer. The extra-abdominal dissemination of ovarian cancer usually occurs via hematogenous route; therefore, the patient will often present disseminated lesions, not amenable to cytoreductive surgery with curative intent. When it comes to the thoracic involvement in ovarian cancer, it seems that it can occur via peritoneal, lymphatic or hematogenous route. The anatomical flow of the intraperitoneal

fluid in the abdominal cavity is the main responsive factor for the apparition of diaphragmatic metastatic lesions, while the pleuro-peritoneal diaphragmatic communication will provide the spread of the malignant cells in the pleural cavity, leading to the apparition of pleural metastases. In the meantime, the lymphatic outflow from the inter-aortico-caval adenopathies and the intra-thoracic lymph nodes play a central role in the intra-thoracic spread. Moreover, hematogenous dissemination will lead most frequently to the apparition of parenchimatous pulmonary metastases [9].

Material and Methods

Among the 32 patients diagnosed with thoracic metastases from ovarian cancer, 26 of them were excluded due to the presence of unresectable lesions; most commonly these lesions were represented by pleural carcinomatosis or disseminated hematogenous pulmonary lesions. The remaining six patients diagnosed with oligometastatic intra-thoracic disease were successfully submitted to debulking surgery to no optimal cytoreduction (defined as the absence of any residual tumoral volume).

Results

Between January 2015 and January 2017, 32 patients were diagnosed with ovarian cancer thoracic metastases; 25 of the 32 patients were diagnosed with thoracic involvement at the time of relapse, while in the other seven cases thoracic involvement was found at the time of the initial diagnosis. Among these 32 patients, 24 cases were diagnosed during the preoperative studies with extended lesions which qualified them only for performing palliative chemotherapy. In other two cases the preoperative studies did not reveal any unresectable disease; however, when performing a video assisted thoracoscopy, both patients proved to have extensive lesions of pleural carcinomatosis as source for their pleural effusion, so the attempt of cytoreductive surgery was abandoned. In all the remaining six cases resection of the thoracic metastases was performed. These patients were submitted to debulking surgery as part of primary cytoreduction in two cases, while the remaining four cases benefitted from thoracic surgery at the time of secondary cytoreduction. When it comes to the performed thoracic resections, they consisted of pleurectomy in three cases, atypical pulmonary resections in two cases and cardiophrenic lymph node resection in the sixth case. Among the patients who benefitted from atypical pulmonary resection, in one case a complex resection consisting of atypical resection of the inferior right pulmonary lobe en bloc with the underlining diaphragm, pleura and peritoneum was needed. It was the case of a 43-year-old patient diagnosed with a bulky metastasis invading the right diaphragm, the pleura and the inferior right pulmonary lobe at the time of the secondary cytoreduction. The reconstruction of the diaphragm did not necessitate the placement of any prosthetic material, separate stiches being used in order to close the diaphragmatic defect. The second case who necessitated atypical pulmonary resections for ovarian cancer lung metastases was the one of a 46-year-old patient who was initially submitted to surgery for stage IIIC ovarian cancer, at that time a total hysterectomy with bilateral adnexectomy, total omentectomy, pelvic and para-aortic lymph node dissection as well as pelvic peritonectomy being performed; at 18 months follow-up, the patient was diagnosed with bilateral ovarian metastases, so she was submitted to iterative pulmonary resection through thoracotomy at four weeks interval. Postoperatively no patient reported complications requiring re-operation. One patient necessitated temporary oxygen therapy postoperatively for the next two weeks, while another patient developed a febrile syndrome of unknown origin. The median hospital in stay was of six days (range 3-15 days).

Discussion

The development of thoracic involvement in cases diagnosed with advanced stage ovarian cancer is considered as stage IV of disease, so the patient will be rather a candidate for neoadjuvant or palliative chemotherapy rather for debulking surgery; in certain cases, the reported results after neoadjuvant chemotherapy will transform cytoreductive surgery in a suitable therapeutic protocol [10, 11]. Moreover, it has been reported that in a significant number of cases the presence of pleural involvement (which has been misdiagnosed during the preoperative studies) is revealed only during surgery; in these cases, although the preoperative intent had been to obtain an optimal debulking surgery, the attempt of cytoreduction should be stopped due to the high risk of performing a suboptimal procedure [12, 13]. In our study, this situation was seen in two cases and leaded to the abandon of the attempt of cytoreduction. Both cases were preoperatively diagnosed at computed tomography with pleural effusion in the absence of visible solid pleural lesions, so thoracoscopy was performed; in both cases pleural carcinomatosis was seen so the idea of cytoreduction was abandoned. In order to avoid such situations certain studies, recommend association of positron emission tomography in order to diagnose the presence of solid malignant lesions of the pleura; for example, in the study conducted by Erasmus *et al*., and published in 2000 [14], as well as Orki's study published in 2009 [15], the accuracy of this positron emission computed tomography ranged between 92% and 97.5%. Moreover, other authors proposed to routinely perform video assisted thoracic surgery in order to improve the accuracy of staging and assess the feasibility of per primam cytoreduction. In the study conducted by Klar *et al*., on 17 patients, performing a video assisted thoracoscopy modified the therapeutic protocol in six cases (providing an upstage of the disease in three cases and respectively a downstage of the malignant process in other three cases) [16].

However, the management of these patients is not well standardized, due to the paucity of cases suitable for surgery. It seems that debulking surgery for oligometastatic disease or neoadjuvant chemotherapy followed by debulking surgery for stage IV of disease offer the best results in terms of survival [17, 18].

Due to the fact that in an important number of cases the criteria of unresectability is established by the presence of disseminated pleural lesions not amenable to cytoreduction, certain authors recently recommended performing hyperthermic intra-thoracic chemotherapy; in this way the principles of hypertermic intraperitoneal chemotherapy were successfully translated in thoracic surgery. A recent case has been reported by Jun *et al*., in a 46-year-old patient diagnosed with advanced stage ovarian cancer and intrathoracic metastases [19]; the patient was successfully submitted to abdominal debulking surgery consisting of total hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, lower anterior rectal resection, partial cystectomy and appendectomy, no visible residual disease being found; however, surgery was considered as suboptimal due to the presence of residual intra-thoracic disease. Postoperatively the patient was submitted to adjuvant chemotherapy consisting of carboplatin and paclitaxel in association with bevacizumab, the patient reported the stabilization of the thoracic lesions as well as no intra-abdominal progression of the disease, so she was submitted to hyperthermic intra-thoracic chemotherapy. At this moment, fulguration of the pleural metastases in association with pulmonary wedge resection was performed, the residual tumoral volume being lesser than 5 mm. Once the debulking process was ended, intra-thoracic hyperthermic chemotherapy was performed, the injected cytotoxic agent being cisplatin.

Postoperatively, the patient was submitted to additional eight cycles of adjuvant chemotherapy with good results; at 14 months from the moment of ending the treatment the patient was free of any sign of recurrence [19]. However, only few such cases have been reported so far for various primaries, a standard therapeutic protocol lacking for the moment [19]. When it comes to the diaphragmatic involvement in patients with ovarian cancer, the main surgical procedures which might be needed, in order to achieve complete cytoreduction, range

from superficial destruction of the lesions (proper for small, superficial lesions), diaphragmatic peritonectomy or stripping (for superficial disseminated lesions) and full thickness diaphragmatic resections (addressed for the deep lesions invading the entire diaphragmatic muscle as well as the underlining pleura and peritoneum; this last procedure is usually associated with higher morbidity rates consisting of pleural effusion or pneumothorax and might necessitate a more specific nursing therapy [20]. As for the pulmonary resections for ovarian cancer liver metastases, few case series have been published so far, so no clear guidelines and prognostic factors are known; however, it seems that the best candidate, in order to be submitted to pulmonary resection for ovarian cancer lung metastases, should have isolated pulmonary metastases in the absence of other distant lesions; moreover, a good pulmonary reserve is mandatory; another condition which seems to significantly influence the long term outcome is related to the disease free survival, an interval of at least 12 months being associated with a better outcome [21]. Interestingly, it seems that the right lung is more susceptible to develop metastases from ovarian cancer. As for the type of performed surgical procedure for pulmonary metastases from ovarian cancer, the most frequently reported resections include atypical lobectomy (or wedge resections), followed by anatomical pulmonary resections or laser excisions. In patients diagnosed with gynecological malignancies, thoracic metastases in whom wedge resection is feasible, it is recommended to have a disease-free margin of at least 2 cm for metastases smaller than 3 cm in diameter; in the meantime, lesions larger than 3 cm should be treated by lobectomy in order to diminish the risk of leaving in place any satellite microscopic disease [22]. In the study conducted by the Japanese surgeons and published in 2015, the authors included 23 patients diagnosed with lung metastases from gynecologic malignancies who were successfully submitted to surgery between 1985 and 2013 [22]; the reported results were compared to those of the patients who were submitted to systemic chemotherapy as a single therapeutic option. Among cases submitted to surgery, five patients had been initially diagnosed with ovarian cancer, 14 cases had been diagnosed with cervical cancer, while the remaining four patients had been previously diagnosed with endometrial cancer. When it comes to the way of performing pulmonary metastasectomy, it consisted of video assisted thoracoscopy in 13 cases and thoracotomy in the remaining 10 cases. Among all 23 cases submitted to pulmonary metastasectomy, the 5-year overall survival after thoracic surgery was 81.7%, no difference in regard with the primary site of the disease being reported; the only parameter which significantly influenced the overall survival was related to the histopathological subtype, patients with mucinous disease reporting a significantly poorer outcome. Moreover, six patients experienced pulmonary relapse, five of them being submitted to re-resection. In the meantime, the long-term outcomes of the patients who underwent pulmonary resections were compared to those of the patients submitted to chemotherapy-only group, the five-year overall survival rate being 81.7% among surgically treated patients and only 49.5% among the chemotherapy-only group [22].

In a recent study published by Nasser *et al*., in 2017 the authors reported nine studies dating from 2007 to present, which assessed the role of thoracic surgery in advanced stage or relapsed ovarian cancer, the number of operated patients ranging between 1 and 30, while the rates of complete cytoreduction ranged between 68 and 100%; in this way the authors demonstrated the feasibility and safety of debulking surgery for thoracic metastases from ovarian cancer [23].

Conclusions

Although an important number of patients diagnosed with ovarian cancer will develop at a certain point of their evolution intrathoracic disease, only very few of them will be amenable to surgery with curative intent due to the extent of intrathoracic and extrathoracic lesions.

However, whenever oligometastatic thoracic disease is suspected, surgery might be taken in consideration with curative intent irrespective of the site of the lesion (pleural, pulmonary or

lymphatic ones). Moreover, recent studies successfully reported the association of concomitant hyperthermic intrathoracic chemotherapy with promising results.

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Effect of Preoperative Treatment with Topic Mometasonefuroate in Nasal Permeabilization Surgery

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Abstract

Mometasone furoate is a 3rd class corticoid as potency, used in ENT pathologies for its anti-pruritic, anti-inflammatory and vasoconstrictive properties. The purpose of this paper was to evaluate whether it is relevant to propose the extension of the indications of topical corticosteroids in the preoperative preparation of patients undergoing functional endoscopic sinus surgery and turbinoplasty for chronic rhinosinusitis. Thus, a prospective, randomized, unblinded, single-centre study was conducted and took place during a 6 months period. The main outcomes were intraprocedural blood loss and total duration of the procedure. Patients receiving mometasone furoate had statistically significant lower intraprocedural blood loss (75.37±1.98 *vs.* 93.4±2.46 mL, $p<0.001$) and shorter duration of the procedure (46.27±1.33 *vs.* 58.83±2.49 minutes, $p<0.001$) than patients in the control group. Thus, it is proposed to broaden the indications for the use of topical corticosteroids, even if they are offtopic. We consider that the topical preoperative treatment with mometasone furoate is extremely useful in minimizing the intraoperative bleeding, thus shortening the time of the surgical procedure, increasing the intraoperative safety due to better visualization of the anatomical structures and decreasing the operating room costs.

Keywords: steroid, topical action, preoperative, turbinectomy, functional endoscopic sinus surgery

Introduction

Glucocorticoids have intense anti-inflammatory effects, regardless of the nature of the stimulus that provoked inflammation and are active in all phases of inflammation. They accumulate in the inflamed tissue, inhibit leukocyte migration and phagocytosis, stabilize capillaries and prevent their permeability, reduce local oedema formation, and maintain the reactivity of the vessels towards catecholamine action. In the late phase of inflammation, regeneration phenomena are reduced due to decreased number and proliferation of fibroblasts, disruption of collagen formation and limitation of capillary proliferation. Steroids have important anti-allergic effects irrespective of the stimulus that triggered the allergy being effective in certain allergic manifestations by the immunosuppressive effect and anti-inflammatory action. Thus, anaphylactic type reactions may be inhibited.

Diagnostic difficulties can be encountered when leukocytosis occurs as an effect of glucocorticoid action. It is worth mentioning that a prolongation of red blood cells lifetime, as well as an increase in their number and an increase of haemoglobin concentration, are observed [1, 2]. In the blood, the corticosteroid is largely fixed by proteins, initially binding to a specific globulin called Corticosteroid Binding Globulin (CBG), to which it has a high affinity, and then, when the hormone concentration increases, fixation is made by albumin to which the affinity is small, but the binding capacity is increased. The only active form of the glucocorticoid is when the hormone is free [1]. Therapeutically, glucocorticoids can be used as substitution medication in adrenal insufficiency and as pharmacological agents in many pathologies due to their anti-inflammatory and antiallergic properties.

Synthetic drugs with corticoid-like effects are widely used in a variety of pathologies ranging from allergy and respiratory medicine, dermatological, endocrinological, gastroenterological, haematological, ophthalmological and rheumatology/immunological conditions to multiple sclerosis, organ transplantation or traumatic brain injury. Although they are really useful in a variety of medical conditions, the use of corticosteroids comes with an impressive luggage of the most various adverse effects, some of which may be severe: steroid psychosis, anxiety, depression, steroid euphoria, fluid retention and hypertension, movement of body fat to the face and torso, insulin resistance, diabetes mellitus, colitis, peptic ulceration, cataract, retinopathy, candidiasis as well as low but important teratogenic effect [3-5].

The corticosteroids are frequently used for the screening of allergies to topical and systemic steroids. If a patient is allergic to one of the groups, the patient is actually allergic to all the steroids that define that group [6].

Considering the route of administration, steroids are defined as topical (skin, eye and mucous membranes use), inhaled (treatment of nasal mucosa, sinuses, bronchi and lungs), oral or systemic form (intravenous and parenteral injections).

Materials and Methods

The purpose of this paper was to evaluate whether it is relevant to propose the extension of the indications of topical corticosteroids in the preoperative preparation of patients undergoing functional endoscopic sinus surgery and turbinoplasty for chronic rhinosinusitis.

A prospective, randomized, unblinded, single-centre study was conducted and took place during a 6month period at a tertiary referral centre in Bucharest. 60 patients (30 female, mean age) were included in the study. All patients included were undergoing functional endoscopic sinus surgery and turbinoplasty for chronic rhinosinusitis. The main exclusion criteria were: coagulation dysfunction, antithrombotic drug use, the presence of benign or malignant rhinosinusal tumors. The first group of patients, the control group (30 patients), received intranasal saline solution, while the second group (30 patients) received intranasal mometasone furoate daily for a month before surgery. All patients were operated according to local protocols by a single experienced surgeon and the main outcomes were intraprocedural blood loss and total duration of the procedure.

Results

Univariate and multivariate analysis was performed. The variables taken into consideration for statistical analysis were age, sex, blood loss (ml) and surgery duration (min). The mean age of subjects enrolled in the study was 51 for the first group and 48.7 for the second group. Counts table for sex distribution shown that in the first group there were 17 women and 13 men, as for the second group there were 13 woman and 17 men, so there was a balanced number of women and men distributed by sex (Fig. 1). In addition, column percentage table and tests for row-

column independence (group by sex) were realised to create and re-evaluate the proper distribution by gender in the groups taken into the study.

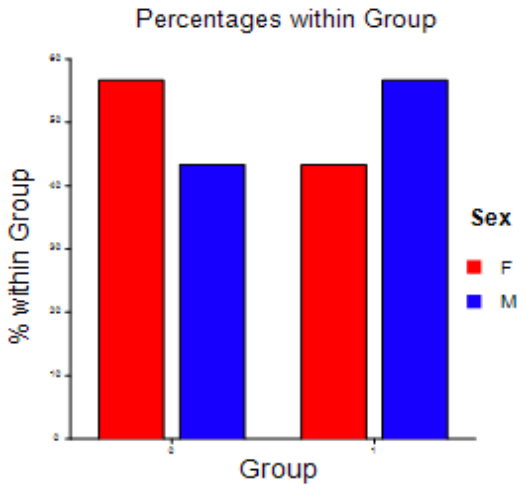

Fig. 1. Distribution by gender in the groups of study

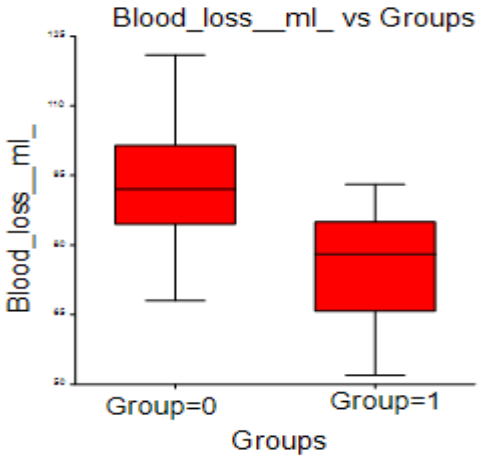

Fig. 2. Blood loss (ml) in each group of the study

Blood loss (ml) was evaluated by descriptive statistics, descriptive statistics for median, with analysis of the Two-Sided Confidence Interval for μ1 - μ2, with Mann-Whitney U or Wilcoxon Rank-Sum test for difference in location, Kolmogorov-Smirnov Test for comparing distribution and tests of assumptions. Mean and median, regarding blood loss in the selected groups of the study, calculated by descriptive statistics show almost identical values revealing a normal distribution. The first group lost intraoperative statistically significant more blood compared to the second group (93.4±13.4846 ml *vs.* 75.36667±10.877 ml, p<0.001 t-test). Thus, a schematic representation of blood loss in the two groups taken into the study could be achieved (Fig. 2).

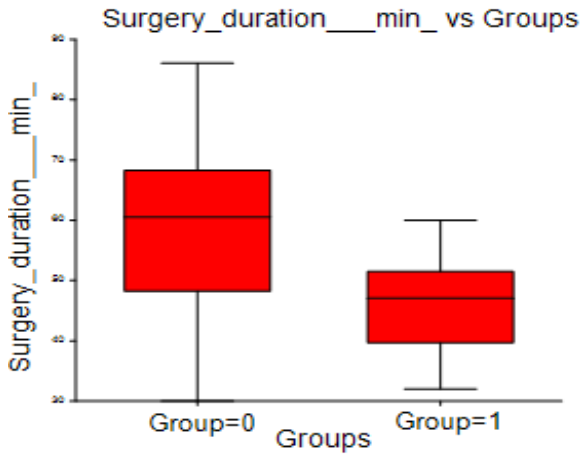

Fig. 3. Surgery duration (min) in each group of the study

As for duration of the surgery, the mean (46.26) and the median (47) calculated on the group that received mometasone furoate are almost identical, which reveals a statistically normal distribution as well.

Patients from the second group had a statistically significant shorter duration of surgery compared to the patients from the first group (46.26667±7.29635 *vs.* 58.8333±13.64092 min, p<0.001 t-test).

Similar to blood loss analysis, using the same statistical means, the diagram for the duration of surgical procedure was achieved, with the differences in the groups studied being evaluated depending on the variable in question. (Fig. 3).

Discussions

There are various classification systems all around the world utilised to define topical steroids according to their ability to constrict capillaries and provoke skin blanching. Thus, the World Health Organization Classification of topical corticosteroids utilizes 7 classes, the 1st class having an ultrahigh potency as the 6th and 7th classes have the lowest potency. Many countries admit only 4 classes, so as for the United Kingdom the 1st class is the strongest, while

in Continental Europe the 4th class is known as strongest. In Japan there are 5 classes, the 1st one being the strongest [6, 7].

Mometasone furoate is a salt form of mometasone, a man-made topical steroid used mostly as a suspension, inside a nasal spray, for its local anti-allergic and anti-inflammatory action in seasonal or permanent rhinitis in adults and children over 3 years of age, but it is also indicated for the treatment of nasal polyps in adults over 18 years of age (diminishes the volume of polyps, but does not remove the need for polypectomy or relapses). Basically, literature describes it as being a receptor agonist that has anti-pruritic, vasoconstrictive and anti-inflammatory abilities [8, 9].

This is a topical glucocorticosteroid that has local anti-inflammatory action at doses that are not systemically active. In each of the above classifications mometasonefuroate corresponds as potency to a 3rd class [10].

Systemic bioavailability after nasal administration is 0.1% in plasma. The digestive absorbed substance suffers an important first hepatic passage effect, with urinary and biliary elimination [9].

The product produces normal nasal mucosal architecture, diminishing the inflammatory response, this steroid's properties mainly rely on its ability to determine the inhibition of allergic reaction mediators' release, as leukotrienes [11].

The anti-inflammatory actions of third class corticoids recorded in the literature are: reduce vascular permeability and tissue oedema; enhances the function and number of beta adrenergic receptors; inhibits the eosinophil decreasing the release of cytokines and other inflammation mediators; reduces the synthesis and release of mast cell mediators by preventing the release of arachidonic acid from the cell membrane by inhibiting A2 phospholipase by lipocortins, thus blocking the formation of pro-inflammatory metabolites; reduce mucus secretion, possibly by inhibiting the metabolism of eicosanoids.

The nasal spray delivers by each comminution a metered dose of 50 micrograms of mometasonefuroate. In terms of posology, the loading dose is for adults (over 12 years of age) with 2 sprays in each nostril per day, and in the infant (3-11 years of age) a spray in each nostril per day. In case of allergic rhinitis, if the symptoms improve, the doses may be decreased. It was demonstrated that a clinically significant onset of action could be for some patients within 12 hours after the first dose, but full benefit of treatment may not be reached in the first 48 hours, thus this product should be used regularly. In the case of nasal polyps, if the symptoms are not adequately controlled at 5-6 weeks of use, the dose may be increased to 2 sprays twice daily in each nostril [10]. This product has a good safety profile and as possible side effects of using it, mentioned in literature, one can include epistaxis, nasal irritation, mucosal dryness, nasal and pharyngeal infections with candida albicans (discontinuation of treatment) and, rarely, septal perforations, ocular hypertonia or hypersensitivity reactions [12-14]. The situations in which the use of this product is contraindicated are represented by hypersensitivity to the active substance or any other of the excipients, epistaxis, oronasal or ophthalmic infections with herpes virus and, due to the inhibition that steroids have on wound healing, it is not recommended to use nasal corticosteroids in patients who have recently experienced nasal surgery or trauma until the healing process has ended. Special attention should be paid when proposed to administer the product in pregnancy, breast-feeding or when the patient wishes to have a baby because it is unknown if mometasone furoate is excreted in human milk and studies in animals have shown reproductive toxicity, but no effects on fertility [9]. Since its discovery and placing on market, mometasonfuroate has been the subject of numerous studies that have tried to demonstrate its efficacy and safety and also expand its use limits. Thus, nowadays, it is trying to be used to relief the nasal blockage in chronic or idiopathic rhinosinusitis, in the treatment of rhinitis medicamentosa and even trying to reduce volumetrically the adenoid hypertrophy [11, 13, 14].

The first group included in study had 17 woman and 13 men, as for the second group there were 13 women and 17 men, all mean age.

The control group received intranasal saline solution, while the second group received intranasal mometasone furoate, daily, for a month before surgery.

Patients receiving mometasone furoate had statistically significant lower intraprocedural blood loss (75.37±1.98 *vs.* 93.4±2.46 mL, $p<0.001$) and shorter duration of procedure (46.27±1.33 *vs.* 58.83±2.49 minutes, $p<0.001$) than patients in the control group.

Saline nasal solutions are a form of nasal irrigation that contain sodium chloride in different concentrations (hypertonic, isotonic or hypotonic) being used for their ability to alleviate nasal congestion, reduce thickness of nasal mucus and humidify the sinonasal cavities by maintaining secretions fluid and running.

On the other hand, mometasone furoate, by its β adrenergic receptor agonist effect and by inhibiting the metabolism of eicosanoids, therefore no pro-inflammatory metabolites in site, obtains an important intraoperative local vasoconstriction and reduced local postoperative oedema due to the length of the period being used preoperative which favors the marked deployment of its long-term effects. Due to this strong local vasoconstriction, the intraoperative blood loss, for those that used mometasone furoate, is significantly reduced. Thus, due to low intraoperative bleeding, the operator's time allocated to haemostasis is reduced and the overall duration of the surgical intervention is decreased in the case of the patient who previously received the steroid-based spray.

Conclusions

Taking into account the vasoconstrictive and anti-inflammatory properties of topical corticosteroids, in particular those of mometasone furoate and the results obtained by its preoperative administration in patients undergoing functional endoscopic sinus surgery and turbinoplasty for chronic rhinosinusitis, as well as the statistically significant results obtained from the aforementioned study, an assessment of widening the indications for the use of topical corticosteroids can be proposed, even if they are considered to be off-topic

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Quantitative Relationships of Methadone Plasma Levels in Patients Under Methadone Substitution Therapy

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Abstract

Despite its imperfections, methadone remains the commonly used substance in the treatment of opioid dependence, having a good acceptability. Several studies have shown insufficient methadone dose as a major cause of treatment failure. By using the plasma concentrations of methadone, this paper aims to develop mathematical models to predict the levels of methadone in biological samples, evaluating the combined effect of several variables (related to socio-demographic characteristics, addiction, co-morbidities).

The study sample consisted in 28 heroin addict patients, between 20-43 years old, who were voluntarily enrolled in the substitution treatment. Methadone plasma concentrations were determined by GC-MS method. The following parameters were assessed: plasma concentrations of methadone, methadone dose, age, duration of heroin use, heroin dose, the biochemical parameters relevant for the liver function (ex. AST, ALT, GGT) or for the general immune status (number of leukocytes) reflecting possible comorbidities. Quantitative relationships were performed using the software Statistica^{TM} StatSoft 6.0, the subroutine "piecewise linear regression with breakpoint" (linear regression through successive iterations).

Different quantitative relationships were obtained, such as methadone plasma concentration vs. methadone dose, age, duration of heroin use; vs. methadone dose, duration of heroin use, heroin dose; vs. methadone dose, age, heroin dose; vs. methadone dose, duration of heroin use, leukocyte number. The predictive power of the proposed mathematical models is high as the correlation coefficients obtained have values close to unity.

The mathematical models to predict the plasma methadone levels can provide an objective tool for the treatment monitoring, contributing to efficiency and individualization of therapy.

Keywords: methadone substitution therapy, methadone plasma levels, quantitative relationships

Introduction

Currently, the surveillance of methadone substitution therapy is still considered an ongoing challenge, due to need for an individualization and the increasing of the therapy efficiency.

Evidence from the literature argues that there is a positive correlation between plasma levels and methadone doses. Plasma concentrations of methadone, assessed after 24 hours after the daily dose, have been proposed as a measure of treatment efficacy [1]. Although the correlation between dose and plasma concentrations of methadone has been highlighted in numerous

studies, it is suggested that a linear correlation really significant is very difficult to obtain, since the plasma levels of methadone depends on many factors [2-4].

The correlation coefficients reported in different studies varied in a wide range, indicating weak to strong correlations: $r = 0.20$ ($p < 0.05$) [5]; $r = 0.55$ ($p < 0.01$) [6]; $r = 0.82$ ($p < 0.001$) [7]. Thus, genetic polymorphism of the cytochrome P-450 could cause slow, fast or ultra-fast metabolism. It was also suggested a higher correlation in patients with complete abstinence from illicit drugs [1]. In addition, there is research to suggest that the linear correlations between the dose of methadone and plasma concentrations, at lower doses (less than 80 mg) cannot be extrapolated to the higher doses (above 80 mg) [8]. Recent data indicate that infection with hepatitis C virus (HCV) affects the plasma concentrations of methadone, which is higher in HCV positive patients [9].

Since the results do not always highlight predictable correlations between the methadone plasma concentration and the analysed variables, we have predicted that the combined effect of these variables might reflect on plasma methadone levels.

In this regard, we have conducted a study to obtain several mathematical models for predicting the methadone plasma levels depending on various influencing parameters, based on a number of combinations of these factors, which could describe a certain level of plasma methadone concentration.

The influencing parameters considered were: methadone dose, age, duration of heroin use, heroin dose consumed, values of biochemical parameters relevant for hepatic function, e.g. aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) or for general immune status (leukocyte count), implicitly reflecting possible co-morbidities. Quantitative relationships were performed using the software Statistica™ StatSoft 6.0, the subroutine "piecewise linear regression with breakpoint".

Materials and Methods

Patients

The study sample consisted in 28 heroin addict patients, between 20-43 years old, who were voluntarily enrolled in the substitution treatment, all patients being monitored at Centre for Evaluation and Treatment of Addictions of Bucharest, Romania.

The individual data for substance use history, methadone therapy courses, clinical characteristics and socio-demographic elements were collected from the patient's medical records. The Declaration of Helsinki Protocols was followed and before enrolment, all volunteers signed an informed consent form. The study protocol was approved by the coordinating ethics committee.

Variables/parameters analyzed in the study

The following variable/parameters were evaluated in the study:

1. socio-demographic and addiction parameters: age, gender, age at the beginning of heroin consumption, time of heroin consumption, marital status, level of education, detention period (if applicable), the use of other drugs, associated comorbidities, urine tests for relapse evaluation;
2. methadone dose;
3. methadone plasma concentrations.

Quantification of methadone in plasma samples

To quantify methadone in plasma samples, several venous blood samples were taken, namely before administration of the daily methadone dose, at approximately 24 hours after the previous methadone dose intake, while the plasma has been separated.

A GC-MS method, which uses diphenylamine as an internal standard and a liquid-liquid extraction procedure (by hexane: i-propanol 97:3) were applied to quantify methadone in plasma samples. Data for quantification of methadone in plasma samples were previously published [10].

Statistics

Descriptive statistics and the frequencies distribution were performed using SPSS Statistics (ver. 21); information for different parameters were presented as the mean ± standard deviation/SD. Comparison between groups was achieved by applying Student t-test. The correlations between the investigated parameters have been evaluated using correlation coefficients (Pearson, Kendall and Sperman) [10]. All tests were considered statistically significant at threshold p_1=0.05 and p_2=0.01. Quantitative relationships between plasma methadone concentrations and different parameters analysed in the study were performed using the software Statistica™ StatSoft 6.0, the subroutine "piecewise linear regression with breakpoint" (linear regression through successive iterations) [11, 12]. To establish the quantitative relationships, the dependent and independent variables were defined. (Table 1)

Table 1. Defining the variables to establish the quantitative relationships

The dependent variable (Y)	Independent variables (X1-Xn)
Plasma methadone concentration (ng/mL) (CMTD)	Methadone dose (DMTD) Age (VS) Duration of heroin consumption (DCH) Heroin dose (DH) AST GGT Leucocyte count (LC)

Results and Discussions

The socio-demographic, history of substance use, and the clinical characteristics for the study sample

The study group was characterized in detail, considering demographic, comorbid and addiction characteristics, heroin use history, treatment history, clinical and paraclinical evaluation [10]. Table 2 describes the socio-demographic characteristics, substance use history, treatment characteristics and paraclinical evaluations of the study group.

Table 2. The socio-demographic characteristics, substance use history, treatment characteristics and paraclinical evaluations for the study group

Total cases	28
Gender distribution	20 men, 8 women
Men/women ratio	2.50
Mean age (± standard error)	32.89 (± 1.005) (range 20-43; median 33)
Frequent age groups	30-35 years (39%); 35-40 years (28%); 25-30 years (14%)
Duration of heroin use (years)	10.875 (±0.93) (range 1-9; median 13)
Other drug use	NPS (New Psychoactive Substances) 28.57% (8/28); Marijuana 10.71% (3/28); Benzodiazepines 35.71% (10/28); Cocaine 7.14% (2/28); Ketamine 7.14% (2/28)
Comorbidities – positive HVC (number of patients)	27 (96.43%)
Mean methadone dose (mg)	59.10 (range 30.00-85.00)
Mean methadone plasma levels (ng/mL)	303.35 (range 123.00-808.00)
AST (IU/L)	54.85 (range 11.00-225.90)

ALT (IU/L)	70.90 (range 4.70-262.30)
GGT (IU/L)	66.70 (range 4.00-370.00)
Leucocyte count	3320.01 (range 4600-6180)

All the patients in the group have been administered a substitute treatment with methadone.

The average dose of methadone was approximately of 59 mg, varying between 30-85 mg. The average methadone plasma level was of 303 ng/mL, and a large distribution concentration was shown, in the range of 123-808 ng/mL.

Quantitative relationships between the concentration of methadone in plasma and different parameters evaluated in the study

The correlation model is such as: Y_i (X_1, X_2) = [a_0 + a_1x_1 + a_2X_2]$_1$ breakpoint [b_0 + b_2X_2 + b_1X_1]$_2$ and consists in a discontinuous regression relation, applied when the nature of the relationship between independent variables and the dependent variable changes over the range of the independent variable. Thus, at some point there can be a discontinuity or a gap between the two types of variables. This discontinuity is quantified by the so-called point of discontinuity ("breakpoint" or "jump" of the regression line) [11, 12].

Different quantitative relationships were obtained, such as methadone plasma concentration (CMTD) *vs.* methadone dose (DMTD), age (AG), duration of heroin use DCH (DH); *vs.* methadone dose, duration of heroin use, heroin dose; *vs.* methadone dose, age, heroin dose; *vs.* methadone dose, duration of heroin use, leukocyte number (LC). The predictive power of the proposed mathematical models is high as the correlation coefficients (R) obtained have values close to unity. (Table 3)

Table 3. Mathematical equations on the correlations of methadone plasma concentration (C) with different influencing parameters; breakpoint at C=297.33 ng/mL

No	Quantitative relationships	Correlation coefficient (R)
1.	CMTD = f (DMTD, AG, DCH) = [250.376 + 1.696DMTD - 4.000AG + 1.464DCH]$_1$ *breakpoint* [– 384.544 + 7.869DMTD + 15.504AG – 20.364DCH]$_2$	0.9467
2.	CMTD = f (DMTD, AG, DH) = [244,.249 + 1.492DMTD – 2.852AG – 3.534DH]$_1$ *breakpoint* [-377.588 + 9.207DMTD + 12.777AG – 104.062DH]$_2$	0.9113
3.	CMTD = f (DMTD, DCH, DH) = [180.065 + 1.039DMTD + 0.244DCH – 7.848DH]$_1$ *breakpoint* [215,147 + 5,143DMTD -15,405DCH + 13,670DH]$_2$	0.8902
4.	CMTD = f (DMTD, DH) = [179.52 + 1.081DMTD – 7.618DH]$_1$ *breakpoint* [139.275 + 6.581DMTD – 71.791DH]$_2$	0.8689
5.	CMTD = f (DMTD, DCH, LC) = [171.237 + 1.684DMTD – 6.401LC]$_1$ *breakpoint* [602.303 + 4.775DMTD – 21.817DCH – 37.782 LC]	0.8987

A positive correlation between plasma concentrations of methadone and methadone dose for all proposed mathematical models has been observed. The correlation between plasma methadone concentration and the length of heroin use is generally positive before the breakpoint and negative after this point. The age has a positive correlation after the breakpoint, while comorbidities (as AST, ALT, GGT, LC values) have a variation usually negative after the breakpoint. These variations could be related to individual variability in the context of the presence of conditions that could affect the metabolism of methadone.

The results of the mathematical modeling based on nonlinear correlations with breakpoint have been completed by the implementation of the response surface methodology (RSM), an effective tool that can estimate the main effects and the interaction of the influencing parameters selected over the levels of methadone in plasma. The relationships between the dependent variable (the concentration of methadone in plasma) and the influencing factors (parameters) evaluated in the study illustrated by using the RSM allow to visualize the effects of these factors in the three-dimensional space.

There were graphically represented by RMS the following correlations:

- Plasma concentration of methadone *vs.* methadone dose and age. It is noticed a first peak of plasma concentrations of methadone at a high dose of methadone (80-90 mg) and young age (under 20 years) and a second peak (at a value of plasma concentration of methadone less than approx. 400 ng/mL) at a low dose of methadone (20-30 mg) and older age (approximately 40 years). (Fig. 1)
- Plasma concentration of methadone *vs.* heroin dose and the duration of heroin use. It is observed a positive correlation between the concentration of methadone in plasma and the duration of heroin use, while the correlation with the heroin dose reflects a "plateau" values, resulting in high concentrations of methadone plasma, regardless of the duration of consumption. (Fig. 2)
- Plasma concentration of methadone *vs.* methadone dose and GGT. The concentrations of methadone in plasma increase with dose of methadone, while GGT has a range (100-250) in which the methadone concentration reaches maximum values at the same time with the value of GGT. (Fig. 3)

The graphs indicating the simultaneous influence of the methadone dose and of a parameter possibly reflecting comorbidity (GGT, leukocytes) have similar shape (Fig. 3, Fig. 4), differing only one of the variables, suggesting the accuracy of the experimental data.

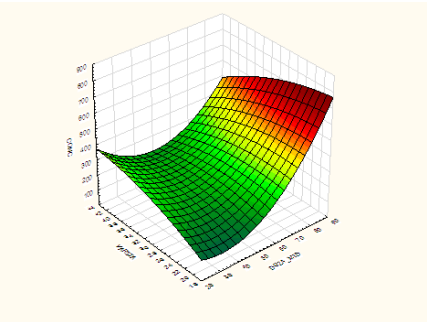

Fig. 1. Three-dimensional representation of the concentration of methadone in plasma *vs.* age and the methadone dose in patients under methadone maintenance treatment (MMT)

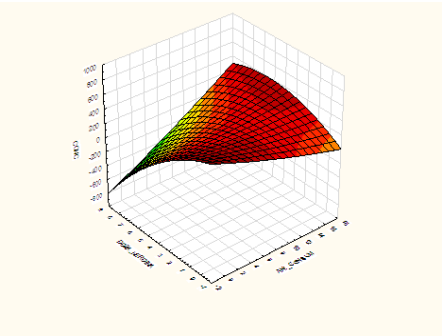

Fig. 2. Three-dimensional representation of the concentration of methadone in plasma *vs.* heroin dose and the duration of heroin use in patients under methadone maintenance treatment (MMT)

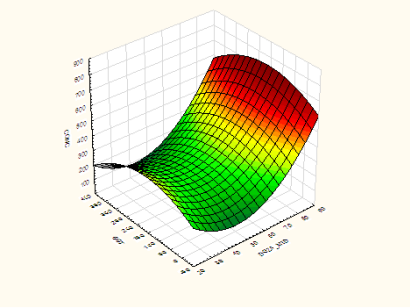

Fig. 3. Three-dimensional representation of the concentration of methadone in plasma *vs.* GGT values and the methadone dose in patients under methadone maintenance treatment (MMT)

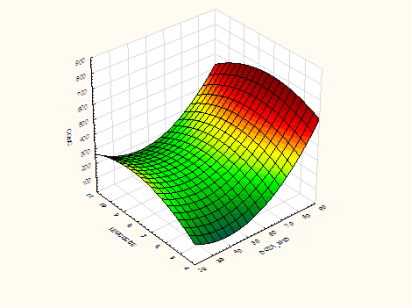

Fig. 4. Three-dimensional representation of the concentration of methadone in plasma *vs.* leukocyte count and the methadone dose in patients under methadone maintenance treatment (MMT)

Conclusions

Several mathematical models with a high predictive power (correlation coefficients with values close to unity) for methadone plasma levels depending on the influencing parameters (methadone dose, duration of heroin consumption, heroin dose, age, leukocyte count) have been established.

The correlation models obtained are constituted in discontinuous regression relations (breakpoint point correlations).

The mathematical models to predict the methadone plasma levels can provide an objective tool for the treatment monitoring, contributing to efficiency and individualization of therapy.

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The Effect of Acute a and B Hepatitis Viruses' Infections on Certain Salivary Constants in Chronic Alcohol Users

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Abstract

An observational study concerning the changes in salivary constants in alcohol users with hepatitis virus A (VHA) and hepatitis virus B (VHB) was made on a group of 45 patients. The obtained results show that acute VHA and VHB significantly modify saliva composition. The use of saliva for diagnostic tests has a series of advantages compared with serum tests, which is why it could become a useful method for the diagnosis and follow-up of patients with acute viral hepatitis.

Keywords: saliva, VHA, VHB, alcohol consumption

Introduction

The worldwide increase of alcohol consumption, as well as the increase and prevalence of viral hepatitis have been noticed over the past years. Acute virus A (VHA) hepatitis with manifest symptoms is still under clinicians' scrutiny and the association of virus B (VHB) hepatitis with liver cirrhosis and hepatocarcinoma account for the efforts undertaken for their early improved diagnosis. Both acute, and chronic alcohol consumption cause alterations in the oral cavity and salivary glands, and excessive chronic consumption triggers multiple systemic, particularly hepatic, afflictions, which have a negative impact on the patients' evolution [1-3].

Materials and Methods

A number of 45 patients were included in an observational study concerning the changes in salivary constants in alcohol users suffering from VHA and VHB infections. The inclusion

criteria for the subjects were: signing the informed participation consent form, stating the alcohol consumption and the results of serum tests performed for viral hepatitis infections. The alcohol consumption was 20 ml pure alcohol/day for women and 40 ml pure alcohol/day in men, not more often than 2 times/ week, for the past 6 months. The serum markers of hepatitis A, B, and C virus infections were determined in all subjects in order to diagnose VHA and VHB acute hepatitis. The patients with VHA infection were anti-VHA IgM-positive, AgHBs-negative, antiHBc IgM-negative, AgHBe negative, anti-VHC and ARN-VHC negative. The patients did not exhibit severe clinical signs. All cases had a self-limiting evolution. The patients with acute VHB viral infection were anti-VHA IgM-negative, anti-VHC and ARN-VHC negative, AgHBs-positive and anti-HBc IgM-positive. All patients with associated different types of neoplasm were excluded from the present study.

The subjects were divided into 3 groups comprising 15 subjects: the control group (12 men and 3 women), in which healthy subjects who drink moderate quantities of alcohol but are not infected with hepatitis viruses were included, a group including patients with acute VHA hepatitis (13 men and 2 women), and a group of patients suffering from acute VHB hepatitis (14 men and 1 woman). The saliva samples were taken in the morning, on an empty stomach, following an approximately 12-hour fast. The patients were only allowed to take the regular recommended medication, 24 h prior to taking the sample. 60 minutes before the saliva was taken, the patients brushed their teeth, as it is known that even minor lesions, such as those that may arise during brushing, may alter the saliva composition. The saliva tests performed were: flux, pH, total protein, IgA and IgG salivary immunoglobulines, biochemical tests (urea, creatinine, AST, ALT, chlorine, calcium and potassium).

The results were expressed as mean values ± standard deviation, for each group, and the differences among variables between groups were analyzed by the Student test (ttest) and ANOVA. The $p<0.05$ values show statistically significant differences, and $p<0.01$ shows highly statistically significant differences.

Results

A control group comprising 15 patients without hepatitis virus's infection and who stated they had a moderate chronic alcohol consumption. The mean values and the standard variations found are presented in Table 1.

Table 1. Salivary parameters in patients without hepatitis viruses' infection, with moderate chronic alcohol consumption

	Flux ml/min	pH	Urea mg/dl	Creatinine mg/dl	Proteins g/l	IgG mg/dl	IgA mg/dl	IgA/IgG
Mean value	0.64	6.70	45.49	1.07	1.32	2.25	11.86	4.45
Stand. dev.	0.20	0.73	15.65	0.44	0.27	0.17	3.58	2.34
	AST(TGO) U/l		ALT(TGP) U/l	AST/ALT	Chlorine mmol/l	Calcium mg/dl		Potassium mmol/l
Mean value	24.73		11.40	2.26	39.69	2.17		19.45
Stand. dev.	7.68		3.28	0.62	14.12	0.46		8.97

Table 2 presents the mean values of the saliva constants tested in patients with VHA hepatitis, and table 3 the p values which shows the statistical significance of the differences for non-hepatitis patients and the patients suffering from acute VHA hepatitis.

Table 2. Salivary parameters in patients with VHA hepatitis and moderate chronic alcohol consumption

	Flow ml/min	pH	Urea mg/dl	Creatinine mg/dl	Proteins g/l	IgG mg/dl	IgA mg/dl	IgA/IgG
Mean value	0.42	6.20	50.49	0.62	2.46	3.05	40.33	13.35
Stand. dev.	0.21	0.49	24.86	0.45	1.11	0.73	23.48	10.20
	AST(TGO) U/l		ALT(TGP) U/l	AST/ALT	Chlorine mmol/l	Calcium mg/dl		Potassium mmol/l
Mean value	117.67		112.40	1.28	63.73	1.73		14.12
Stand. dev.	25.59		62.06	0.59	8.74	0.44		5.34

Table 3. The statistical significance of the differences in the values found in the control group and the group of patients with VHA hepatitis

Flow	pH	Urea	Creatinine	Proteines	IgG	IgA	IgA/IgG	
0.004	0.01	0.26	0.005	0.0004	0.001	0.0001	0.009	
AST(TGO)		ALT(TGP)		AST/ALT	Chlorine		Calcium	Potassium
4.9826E-14		4.16419E-07		8.2546E-05	6.73028E-06		0.008	0.03

When compared to patients with a moderate alcohol consumption and without hepatitis, patients with viral VHA hepatitis exhibited significantly higher concentrations in total proteins, IgG, IgA, as well as the IgA/IgG, AST, ALT and salivary chlorine ratio. The saliva flux and the salivary pH, creatinine, calcium and potassium concentrations, along with the AST/ALT ratio, were significantly lower.

Table 4 presents the mean values of the salivary constants in patients with VHB hepatitis, and table 5 *p* values, which show the statistical significance of the differences found in patients without hepatitis compared to those with acute VHB hepatitis.

Table 4. Salivary parameters in patients with VHB hepatitis and moderate chronic alcohol consumption

	Flow ml/min	pH	Urea mg/dl	Creatinine mg/dl	Proteins g/l	IgG mg/dl	IgA mg/dl	IgA/IgG
Mean value	0.47	6.63	46.06	0.64	2.41	2.83	15.23	5.81
Stand. dev.	0.19	0.69	21.44	0.58	1.08	0.79	3.79	2.36
	AST (TGO) U/l	ALT(TGP) U/l		AST/ALT	Chlorine mmol/l	Calcium mg/dl	Potassium mmol/l	
Mean value	106.07	94.73		1.29	59.07	1.97	13.73	
Stand. dev.	52.28	37.95		0.78	9.83	0.40	3.71	

Table 5. The statistical significance of the differences in the values found in the control group and the group of patients with VHB hepatitis

Flow	pH	Urea	Creatinine	Proteins	IgG	IgA	IgA/IgG
0.01	0.40	0.46	0.01	0.0005	0.01	0.01	0.09
AST	ALT		AST/ALT	Chlorine		Calcium	Potassium
1.02871E-06	1.64147E-09		0.0004	0.0001		0.12	0.01

In patients with acute VHB hepatitis the saliva flux and creatinine were also significantly lower than in subjects with chronic moderate alcohol consumption and no hepatitis virus's infection. It was also found that the concentrations of total proteins, IgG, IgA, AST, ALT, the AST/ALT ratio, chlorine and potassium are significantly higher. The differences in the pH values, calcium and the IgA/IgG ratio are no longer significant, although the values are lower in VHB infected patients, just like in those with VHA infection, as compared to the patients in the control group. The salivary urea does not significantly change either in VHA or in the VHB infected patients, when compared to the subjects in the control group.

VHA infection showed the lowest salivary pH and the highest IgA concentrations. Table 6 emphasizes the fact that p indicated statistically significant differences in the pH, IgA and the IgA/IgG ratio, between patients with acute VHA hepatitis and the ones with acute VHB hepatitis.

Table 6. T tests-statistical significance in patients VHA patients and VHB patients

Flux	pH	Urea	Creatinine	Proteins	IgG	IgA	IgA/IgG
0.24	0.02	0.30	0.45	0.44	0.22	0.0001	0.004
AST (TGO)		ALT (TGP)	AST/ ALT	Chlorine	Calcium	Potassium	
0.22		0.17	0.47	0.09	0.06	0.40	

Discussions

It should be particularly noted that the statistically significant alteration of salivary IgA in VHA infected patients compared with subjects in the control group and in VHB infected patients. The local IgA synthesis may be stimulated and there is an increased extravasation of immunoglobulins in acute infections with hepatitis viruses, which would explain the increase in salivary IgG, being partly of plasmatic origin. Amarian GC and al. shows that hypergammaglobulinemia occurs in viral hepatitis, but also notes the local increase of immunoglobulins [4-6]. At the same time, there are studies that emphasize the connection between alcohol consumption and the salivary IgA, pinpointing its decrease and the subsequent decrease of the local immunological defence [7-9]. We noticed that the increase of IgA in chronic alcohol users may be interpreted as a change of the local immune response following VHA and VHB infection [10-12].

The use of saliva in diagnostic tests has a number of advantages over the serum tests. Saliva can be collected quickly, in a non-invasive manner and there is no possibility of contamination through a needle. It does not require the procedure to be performed in a hospital and no specialised medical staff is necessary to be present [13-15].

Conclusion

The results obtained in the present study show that acute VHA and VHB infections in patients who chronically drink moderate amounts of alcohol, significantly change many of the salivary parameters; observing these changes may be useful for the diagnosis and the development of the infections, as well as for the overall evaluation of the oral cavity. Further studies are necessary, which should consider also the likely alterations in glandular structures, periodontal tissues and dental structures in patients with acute hepatitis and chronic hepatitis.

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#6 (Jecan Radu), author #7 (Ionescu Dorin) and author #8 (Cristiana-Ileana Cosconel) contributed equally to the present work and thus are main authors.

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Spontaneous Bacterial Peritonitis – Risk Factors in Patients with Liver Cirrhosis – An Observational Retrospective Study Made in Constanta County Over 12 Months

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Abstract

Spontaneous bacterial peritonitis (SBP) is one of the lethal complications of liver cirrhosis (LC), no matter of etiology. The study was done to investigate the local main risk factors occurred during LC leading to the first episode of SBP. We retrospectively studied 192 patients diagnosed and admitted during 12 months in our clinic with LC of various etiologies from whom 45 patients (23.43%) developed SBP according to current EASL criteria of diagnosis.

Data were collected from the observation files. The univariate Cox regression analysis shown that Child-Pugh stage C, inadequate adherence to diuretic treatment, refractory ascites, the delay of paracentesis more than 24 hours from hospital admission, the electrolyte disturbances history, previous upper digestive bleeding, serum bilirubin >3.5mg/dl and albumin <2.5g/dl and low ascites fluid protein were more frequently encountered in patients with PBS than in other cirrhotic patients. The most common bacteria involved were *E. coli*, followed by *Streptococcus* group C and *Klebsiella pneumoniae*.

Keywords: liver cirrhosis, spontaneous bacterial peritonitis, risk factors

Introduction

The prevalence of PBS in patients with LC ranges between 1.5-3.5% in outpatients and in a percentage around 10% in hospital admitted patients [1-3]. The inflammatory reaction accompanying the peritoneum infection occurred is associated with an increasing number of neutrophils in the ascitic fluid. Peritoneal infection produces a resulting in an increased number of neutrophils in ascitic fluid. In many cases, the culture can remain sterile, but the diagnosis is positive when the PMN increases sufficiently, and symptoms are suggestive. SBP is diagnosed when the neutrophil count reaches 250/mm^3, but the highest specificity is attained when neutrophils count 500/mm^3[4-6]. Sometimes, the increased length of time spent for obtaining an ascitic fluid cell count makes the procedure useless, the reason for what there were introduced as rapid diagnosis procedures the reagent strips (RSs) [7]. The reagent strips method uses a colorimetric reaction that detects the esterase activity of leucocytes. Still, after a large meta-analysis, the technique proved the high risk of false negative results, and it is no longer used in large scale for rapid diagnosis of SBP [7]. Also, the culture, which detects

microorganisms from ascites fluid, remains negative in almost 40% of cases. In this situation, the positive diagnosis is fixed upon the PMN count >250/mmc determined by microscopy. The ascitic fluid culture is sometimes negative and is not compulsory for SBP positive diagnosis.

Still, it can help in choosing the best antibiotic, and for this reason, it should be done in all patients with suspicion of SBP. Besides, blood cultures should be performed in all patients with suspected SBP before starting antibiotic treatment [4, 8]. Less frequent but challenging is the case is known as bactericides, in whom patients have positive ascitic culture, associated with the neutrophil count less than 250/mm^3 [4, 9]. The clinical signs of inflammation or infection require antibiotic therapy. In these cases, the ascitic culture becomes positive at a second look.

If culture is negative at second look but the neutrophil count is >250/mm^3 should be treated with antibiotics, otherwise just followed [10]. The occurrence of SBP is often related to bacterial translocation through the enteric wall. Still, the prophylactic antibiotherapy should be recommended to patients at high risk of SBP preventing the imbalance of saprophyte flora and the equilibrium of host microbiota. The prophylaxis with antibiotics is now admitted by EASL guideline for patients with acute gastrointestinal bleeding, patients with low protein in ascitic fluid and no SBP in medical history (primary prevention) or for patients with antecedents of previous SBP (secondary prevention) [1, 11]. Having as background the complicate management of SPB and the high mortality related to it, we aimed to study the main local risk factors involved in the occurrence of SBP in patients with LC admitted in Gastroenterology Department of Emergency Hospital of Constanta, Romania, to detect those patients at higher risk better.

Materials and Methods

Medical files of 192 patients with LC of various etiologies admitted during 12 months in the above department were studied. The diagnosis of SPB was established according to EASL guideline: PMN count from ascites fluid (AF)>250/mmc with positive AF culture or blood culture; secondary peritonitis and PMN>500/mmc with negative culture. Were excluded other causes of ascites as carcinomatosis, pancreatitis, hemorrhages or tuberculosis using the lab and imagistic tests. Data collected from patient's files were obtained from the hospital archive. The medical history involving the etiology of LC, the length of medical history, the previous medical treatment, the treatment adherence and history of refractory ascites were noted. Blood and ascites fluid samples were investigated. Tests involving liver and kidney function were routinely done, and electrolytes and bacterial cultures were performed to fix the diagnosis according to the above criteria. As statistic analyze, we used the univariate Cox proportional hazards regression procedure to calculate the HR and CI 95% interval of confidence for each parameter studied as a risk factor involved in HCC occurrence.

Results

From the total of patients, 23% of patients (45 patients) were diagnosed with SBP during 1st January 2016 and 31st December 2016. Patient selection was performed based on inclusion and exclusion criteria. The data were collected from the observation sheets. Patients with decompensated liver cirrhosis were divided into 2 subgroups: the group of patients with liver cirrhosis and SBP (N=45), the group of patients with hepatic cirrhosis without SBP (N=147).

The demographic data analysis revealed that 112 were male (58%) and 80 females (42%).

As regards to the distribution of cases over decades of age, the group studied noted an increase in the incidence of cirrhosis after 40 years of age, the maximum number of cases being in the 50-59 age group. According to patient's provenience, we noted that 122 of cases came from urban areas (64%) and 70 examples from rural areas (36%). Depending on the etiology of LC, the cases were divided into four sub-classes: viral etiology B-31 cases (16%), viral etiology

C-56 cases (29%), and alcoholic etiology-85 cases (44%), mixed etiology (viral and alcoholic) 20 cases (10%). As regards evolution, 146 patients were discharged with a favorable evolution (76%), 3 patients with a steady progression (2%) and 43 cases of death (22%). Regarding the patients diagnosed with SBP, the etiology of LC revealed that 15 patients (33.33%) had viral etiology of the disease, 23 patients (51.11%) had alcoholic etiology and 7 patients (15.56%) had mixed etiology (Table 1).

Table 1. Demographic characteristics of patients with liver cirrhosis and spontaneous bacterial peritonitis

	N=192	**N=147**	**N=45**
Gender, n%			
Female	80(41.67)	66(44.90)	14(31.11)
Male	112(58.33)	81(55.10)	31(68.89)
Sex, mean	55.59(11.79)	52.09(10.76)	67.02(6.62)
Provenience, n%			
Urban	122(63.54)	90(61.22)	32(71.11)
Rural	70(36.46)	57(38.78)	13(28.89)
Etiology, n%			
Viral B	31(16.14)	26(17.69)	5(11.11)
Viral C	56(29.16)	46(31.29)	10(22.22)
Alcoholic	85(44.27)	62(42.18)	23(51.11)
Mixed	20(10.41)	13(8.84)	7(15.56)
Evolution, n%			
Favorable	146(76.04)	113(76.87)	33(73.33)
Stationary	3(1.56)	3(2.04)	0
Death	43(22.39)	31(21.09)	12(26.67)

The laboratory characteristics of patients on admission are demonstrated in Table 2.

Table 2. Laboratory characteristics of patients with liver cirrhosis and spontaneous bacterial peritonitis

	N=192	**N=147**	**N=45**
Hemoglobin(g/dl)	10.9± 2.16(6-13.9)	10.79± 1.96(6-13.9)	7.83± 0.85(6-9.3)
Platelets(mm^3)	133.68±74.21(32-378)	149.69±77.18(32-378)	81.04±17.72(44-101)
Total bilirubin(mg/dl)	2.31± 1.17(0.6-6.2)	2.05± 0.93(0.6-3.6)	3.17± 1.44(2-6.2)
Albumin (g/dl)	3.25± 1.03(1.2-5.4)	3.53± 0.98(2-5.4)	2.35 0.59(1.2-2.9)
Creatinine(mg/dl)	1.57 ±1.15(0.5-8.6)	1.17± 0.83(0.5-8.6)	2.89 ±1.07(1.6-5.2)
Sodium(mmol/l)	128.65±6.63(115-145)	129.78±6.21(121-145)	124.93±6.67(110-132)
Proteins from ascites(g/dl)	1.17± 0.76(0.4-2.4)	1.31± 0.81(0.4-2.4)	0.68± 0.14(0.5-0.9)
C reactive protein(mg/dl)	1.02± 0.91(0.2-3.9)	0.66± 0.52(0.1-2.3)	2.21± 0.91(1-3.9)

The univariate Cox regression analysis showed that Child-Pugh stage C, inadequate adherence to diuretic treatment, refractory ascites, the delay of paracentesis more than 24 hours from hospital admission, the electrolyte disturbances history, previous upper digestive bleeding, serum bilirubin >3.5mg/dl and albumin <2.5g/dl and low ascites fluid protein were more frequently encountered in patients with PBS than in other cirrhotic patients. Data are shown in Table 3, along with the p-values.

Table 3. Univariate Cox proportional hazards regression analyze estimating the main risk factors for HCC occurrence

Risk Factor	Parameter Estimate	The hazard ratio (95% CI for HR)
Child-Pugh C stage	0.11765	1.19(1.055-1.624)
Low adherence to diuretics	0.55711	1.61(1.009-1.988)
Refractory ascites	0.84447	1.99(1.034-2.087)
Paracentesis >24 h from hospital admission	0.12118	1.71(1.111-2.115)
History of serum electrolyte disturbances	0.06223	1.15(1.034-1.991)
Serum bilirubin >3g/dl	0.03451	1.01(0.999-1.755)
Serum albumin <2.5g/dl	0.22211	1.14(1.001-1.087)
Ascites fluid albumin <1,1g/dl	0.43272	1.43(1.231-1.879)
History of previous UDB	0.56811	1.76(1.173-1.954)

The most common bacteria involved in gut translocation to the peritoneum revealed that most commonly was detected *E. coli* (p=0.0232, 95% CI 27.22±4.55), followed by *Streptococcus* group C (p=0.0451, 95% CI 11.24±3.11) and *Klebsiellapneumoniae* (p=0.0499, 95% CI 9.24±2.01). Other bacteria involved were other types of streptococcus (*Staphylococcus aureus*, *Streptococcus pneumoniae*), *Pseudomonas fluorescens*, *Enterococcus faecium*, *Enterobacter*.

Discussions

Literature demonstrated that the best prediction factor is the Child-Pugh score and score parameters: serum bilirubin, serum albumin, prothrombin time, hepatic encephalopathy and ascites. The MELD score and gradient of hepatic venous pressure have been shown to predict mortality in over two-thirds of a significant number of studies. However, regarding alanine aminotransferase, the results of studies have shown that this indicator should no longer be incorporated into prognostic models because it is not a predictor of mortality [12]. The environment in which the infection is acquired does not appear to affect survival in the short or long term. In contrast, patients who developed a first episode of SBP pose an increased risk of developing further episodes of SBP in the future. Of the patients who survived the first episode, approximately 50-70% will develop SBP over the next year. Factors predisposing to disease recurrence are the degree of severity of liver disease (higher Child-Pugh score) and low protein concentration in ascites fluid [13]. Shi *et al*., used a regression and classification model to identify a predictive model based on biological results and stratify the risk of developing PBS in mild, medium or severe with an accuracy of 88%. The group with the high risk of developing PBS showed elevated creatinine, total bilirubin, prothrombin time and leukocytes, but no variable had demonstrated independent predictive value [14]. Kim *et al*., performed, in his study, a retrospective analysis of 239 patients with PBS and demonstrated that early-onset paracentesis in the first 12 hours was associated with a lower in-hospital mortality rate compared to paracentesis performed 12-72 hours after admission to hospital (13% *versus* 27%).

Each hour of delay, in this study, was associated with a 3.3% increase in hospital mortality [15]. In our study, the delay of paracentesis over 24 hours from admission was also associated with 1.71 times higher risk of SBP than in patients with rapid paracentesis. Angeli and colleagues found that cirrhotic patients with severe hyponatremia had an increased risk of developing complications such as SBP, hepatic encephalopathy and hepatorenal syndrome [16].

Kim *et al*., assessed 188 patients with cirrhosis and showed that, in comparison with patients with serum sodium ≥136 mmol/l, cirrhotic individuals with serum sodium concentration of ≤130 mmol/l presented a significantly higher risk of development of SBP (33.3% *versus* 16.3%;

p=0.037) [17]. In our study, most patients with SBP had in their history low serum sodium levels. In study by Nadagouda *et al*., out of 9 patients of SBP and its variants, 8 (88.89%) patients were in Child Pugh's Class C and only 1 (11.11%) case was in Child Pugh's Class B.

Also, the mean serum bilirubin level was 6.48±4.2 mg/dl, mean serum albumin was 2.41±0.39 mg/dl [18]. In our study, patients with a Child-Pugh C stage, a total bilirubin >3mg/dl and albumin <2.5 mg/dl, had an increased risk of developing PBS. Upper gastrointestinal bleeding (UGB) increases the risk of SBP and other infections during or immediately after the bleeding episode (first 5-7 days), with an incidence ranging between 16% (compensated cirrhosis) and 66% (advanced cirrhosis) [19]. Patients from our study who had previous UDB in their history had a higher risk of 1.76 to develop SBP. Research data demonstrated that refractory ascites has a poor prognosis and is always associated with severe electrolytic, hepatic or renal disturbances [20, 21]. Our study results revealed that the low adherence to diuretics and refractory ascites are among the SBP risk factors.

Conclusions

The management of SBP should clearly state the steps required for SBP avoidance to prevent one of the lethal complications of LC.

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The Role of Inguinal Lymph Node Dissection in Gynecologic Malignancies

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Abstract

Gynecologic malignancies, such as vulvar or vaginal cancer, are recognized for their propensity in developing inguinal lymph node metastases. When it comes to other pathologies such as endometrial, cervical or ovarian cancer inguinal metastases have been rarely reported.

Material and method

We present a case series of 28 cases of patients with gynecologic malignancies in which inguinal lymph node dissection was performed in order to provide an R0 resection.

Result

Among the 28 cases, the most commonly encountered malignancies consisted of vulvar cancer (in 22 cases), followed by vaginal cancer (in four cases), ovarian and endometrial cancer (each pathology presenting one such case). Postoperatively two cases submitted to surgery for vulvar cancer developed a perineal wound dehiscence and necessitated a prolonged conservative treatment. The histopathological studies confirmed the presence of inguinal lymph node metastases in 21 cases with vulvar cancer, in two cases with vaginal cancer as well as in the cases diagnosed with endometrial and ovarian cancer.

Conclusion

Inguinal lymph node metastases from gynecologic malignancies such as vaginal or vulvar cancer are commonly encountered, therefore inguinal lymph node dissection has been widely recommended. In other cases, such as ovarian or cervical cancer only few cases have been described so far; however, the routine investigation of inguinal lymph node stations should not be omitted, in rare cases inguinal metastases being reported.

Keywords: Gynecologic malignancies, inguinal lymph node metastases, dissection

Introduction

The most commonly reported origin of inguinal lymph node metastases from gynecologic malignancies are represented by vulvar and vaginal cancer. However, in rare cases other

gynecologic malignancies such as ovarian, cervical cancer or even endometrial cancer might induce the apparition of metastatic lesions at this level. Moreover, inguinal lymph node metastases form cervical, endometrial or ovarian cancer are considered as distant metastasis and are most often associated with disseminated lesions, transforming the patient into a candidate for palliative oncologic treatment. According to FIGO (International Federation of Obstetrics and Gynecology) classification, the presence of inguinal metastases in ovarian, cervical and endometrial cancer is considered as stage IVB of disease (as well as parenchimatous hepatic or splenic metastases) [1-2]. In rare cases, isolated inguinal lymph node metastases might develop, in this eventuality surgery with curative intent being taken in consideration [3-4].

Materials and Methods

28 patients with gynecologic malignancies have been submitted to surgery with curative intent between 2015 and 2017. Macroscopic R0 resection was defined by the absence of any visible residual disease while microscopic R0 resection was defined by the presence of positive resection margins.

Results

Among the 28 cases, the most commonly encountered malignancies consisted of vulvar cancer (in 22 cases), followed by vaginal cancer (in four cases), ovarian and cervical cancer (each pathology presenting one such case). The preoperative diagnostic of malignancy was established through biopsy in all cases; in cases with cervical, vaginal and vulvar cancer the biopsy was taken directly from the primary tumor; the patient with ovarian cancer was initially investigated for the apparition of an inguinal tumoral mass which was biopsied, while the result demonstrated the gynecological origin of the metastatic inguinal nodes. Therefore, the patient was submitted to a computed tomography which revealed only the presence of disseminated adenopathies located in both the pelvic and para-aortic area, with no other suspect lesion; the patient was further investigated by performing a positron emission tomography which revealed a high area of capitation in the adnexal areas as well as in the lymphatic chains. The main histopathological subtypes of tumors included: squamous cell carcinomas in all cases diagnosed with vaginal cancer, in 21 cases of vulvar cancer as well as in the case diagnosed with cervical cancer; one patient with a vulvar lesion was diagnosed with vulvar leiomyosarcoma while the patient with ovarian cancer was diagnosed with the epithelial subtype.

Patients with vulvar cancer reported a median age of 67 years (range 62 to 80 years) and were submitted to hemivulvectomy in two cases, while all the remaining patients necessitated a total vulvectomy; moreover, anterior pelvic exenteration was associated in six cases, posterior pelvic exenteration in five cases and total exenteration in six cases; in all cases except the two patients who benefited from hemivulvectomy bilateral inguinal lymph node dissection was performed. Patients in whom pelvic exenteration was needed were also submitted to para-aortic and bilateral pelvic lymph node dissection. The median number of retrieved inguinal lymph nodes was 18 (range 8 to 21) while the median number of positive lymph nodes was 3 (range 0 to 10). The histopathological studies confirmed the presence of inguinal lymph node metastases in 21 cases, a single patient submitted to hemivulvectomy presenting negative inguinal lymph nodes. Macroscopic resection was R0 in all cases while microscopic R0 resection was confirmed in all but one patient; the last case was submitted to total pelvic exenteration; however, the histopathological studies revealed a positive resection margin on the anterior board of the specimen. The postoperative course was uneventful in all but two cases which experienced a perineal wound dehiscence and necessitated a prolonged time (of three weeks and five weeks respectively) to recover, conservative management of the wound being

performed. The patient in whom microscopic R1 resection was reported had the poorer outcome, the wound dehiscence being healed five weeks after surgery.

Patients diagnosed with vaginal cancer reported a median age of 59 years (range 54 to78 years); they presented lower vaginal lesions in two cases, middle vaginal lesions in one case and upper vaginal lesions in the last case; the patient diagnosed with middle vaginal lesion was initially submitted to radiation therapy; however she developed a fulminant hemorrhage so she was submitted to emergency surgery, a total hysterectomy with bilateral adnexectomy, pelvic, para-aortic and inguinal lymph node dissection being performed. The histopathological examination confirmed the presence of lymph node metastases at the level of the inguinal lymph nodes. The two patients diagnosed with lower vaginal lesions were initially submitted to radiation therapy; however, they experienced recurrences at 12 months and respectively 18 months follow-up so they were submitted to surgery consisting of total pelvic exenteration in the first case and posterior pelvic exenteration in the second case; in both cases pelvic, para-aortic and inguinal lymph node dissection was performed. The patient diagnosed with upper vaginal cancer had been previously submitted to surgery and adjuvant radiation therapy for a stage II cervical cancer three years previously; at the moment of diagnostic of the vaginal lesion the patient was submitted to per primam surgery, radiation therapy being no more indicated. In this case total exenteration with inguinal lymph node dissection was performed. The histopathological studies confirmed the presence of metastatic inguinal lymph nodes in the case diagnosed with recurrent vaginal cancer at 12 months follow-up as well as in the case diagnosed with upper third vaginal cancer. The median number of retrieved nodes was 15 (range 5 to 21) while the median number of positive lymph nodes was 2 (range 0 to 8). The postoperative course was uneventful in all cases.

The patient diagnosed with ovarian cancer and inguinal lymph node dissection was submitted to surgery as first intent therapy, total hysterectomy with bilateral adnexectomy, bilateral pelvic, inguinal lymph node dissection, para-aortic lymph node dissection as well as total omentectomy. The histopathological studies confirmed the presence of a moderately differentiated epithelial ovarian cancer, all the 18 retrieved inguinal lymph nodes presenting metastatic cells. The postoperative course was uneventful, three weeks after surgery adjuvant chemotherapy being instituted.

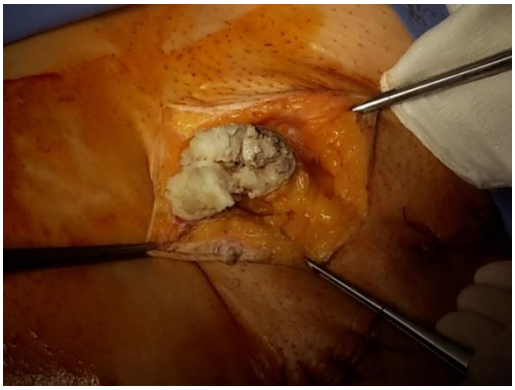

Fig. 1. Large necrotized adenopathic mass in the inguinal area

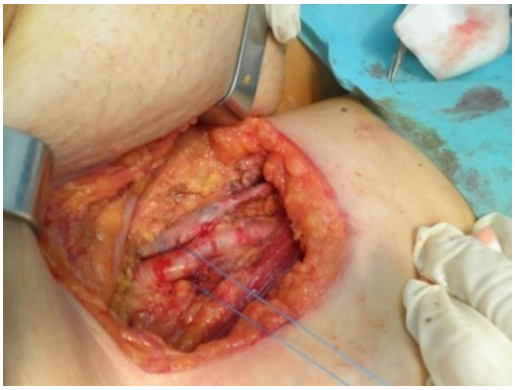

Fig. 2. The final aspect of inguinal lymph node dissection

The last case was the one of a 48-year-old patient diagnosed with a moderately differentiated endometroid endometrial cancer in association with pelvic and inguinal adenopathies. The patient was submitted to surgery, a radical total hysterectomy with bilateral adnexectomy, pelvic, para-aortic and inguinal lymph node dissection. The histopathological studies confirmed the persistent malignant cells in five out of the 16 inguinal lymph nodes. The postoperative course was uneventful. Intraoperative details are shown in Fig. 1-2.

Discussion

Lymph node metastases from vulvar cancer are expected when the primary tumor's depth exceeds 1 mm, the main pattern of spread consisting of the lymph channels draining to the mons pubis's area and, laterally to the superficial inguinal lymph nodes chains [5]. The chains originating from the superficial inguinal lymph nodes will perforate the cribriform fascia and will drain further in the deep inguinal lymph nodes. In the meantime, location of the lesion is responsible for the location of the lymph node metastases; in most cases, inguinal lymph node metastases develop on the ipsilateral part while central lesions usually drain in the both groins [6, 7]. When it comes to the most appropriate therapeutic protocol in patients diagnosed with advanced stage vulvar cancer and inguinal lymph node metastases, it seems that radical surgery of the primary tumor in association with debulking surgery of the bulky lymph nodes followed by radiation therapy provides the best results for long term [8].

Lymphatic spread of vaginal cancer is strongly influenced by the location of the lesion; while cases presenting upper third tumors will preponderantly lead to the apparition of pelvic lymph node metastases, lesions located in the middle and lower third will lead to the apparition of groin lymph node metastases. However, due to the complexity of the lymphatic anatomy of this organ, whenever vaginal cancer is diagnosed, both pelvic and inguinal lymph nodes should be carefully inspected [9]. Once inguinal lymph node metastases are diagnosed in patients with vaginal cancer, the overall prognostic is significantly decreased. For example, in the study conducted by Hockel *et al*., on 105 patients with advanced or recurrent cervical or vaginal cancer submitted to surgery with curative intent between 1999 and 2015 the presence of mesorectal or inguinal metastases was associated with a significantly poorer outcome, after a median follow-up period of 40 months none of these patients being alive anymore [10].

In patients with ovarian cancer is estimated that the incidence of inguinal lymph node metastases does not surpass 3% of cases, therefore inguinal lymph node dissection is not routinely recommended [11]. When it comes to the incriminated pattern of spread of ovarian cancer cells to the inguinal lymph node groups, it seems that the round ligaments play a central role [12]. Due to the fact that in ovarian cancer the best therapeutic strategy remains debulking surgery to no residual disease, in such cases inguinal lymph node dissection might be performed in order to maximize the cytoreductive effort [13, 14]; although inguinal lymph metastases are considered as stage IV of disease, debulking effort might improve the outcome. This fact has been also demonstrated in patients presenting other distant lesions such as pancreatic, hepatic or even splenic metastases [15-18].

The presence of inguinal lymph node metastases from endometrial cancer has been rarely reported so far, the main incriminated mechanism consisting of the tumoral migration via the round ligaments [19]. The first cases of endometrial cancer and inguinal lymph nodes metastases were reported by Paulusen *et al*., in 1978; surprisingly, in these cases the lymph node metastases were diagnosed six months and respectively two years before the diagnostic of endometrial cancer [19]. In a more recent case presented by Scholz *et al*., the patient presented for a tumoral inguinal mass which was resected and the results revealed the presence of a mucinous adenocarcinoma. The bioptic hysteroscopy confirmed the endometrial origin of the tumor so the patient was resubmitted to surgery, total hysterectomy with bilateral adnexectomy, omentectomy, pelvic and para-aortic lymph node dissection being performed [20].

When it comes to the lymphatic drainage of the uterine cervix, it has been considered that there are three levels: the first level is represented by the parametrial and obturator lymph nodes, the second levels consist of the internal and external iliac lymph nodes while the third level is represented by the inguinal and the common iliac lymph nodes [2, 6]. The classical patterns of lymphatic spread for cervical cancer begins from the serosal lymphatics of the uterine cervix which will drain the lymph in the parametrial lymph nodes; from this level, the next stations

will be represented by the obturator, external, internal, common iliac lymph nodes, and, finally para-aortic lymph nodes. However, multiple anatomic variations have been reported; for instance, in certain cases metastases in the para-aortic stations will develop even in the absence of pelvic metastases due to the presence of aberrant channels [2, 6]. As for the inguinal lymph node metastases, it is estimated that their incidence reaches 0.07% of cases diagnosed with cervical cancer [3]. Reported cases so far diagnosed with cervical cancer and inguinal lymph node metastases were treated by radio- and chemotherapy, a good response being reported initially [3].

Conclusions

Inguinal lymph node metastases in gynecological malignancies are considered in most cases as distant metastases and are usually associated with poor prognostic. However, in certain cases in which no other metastatic lesions are found, surgery should be taken in consideration in order to maximize the debulking effort.

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HPV and its Correlation to Oral Squamous Cell Carcinoma

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Abstract

Squamous cell carcinoma of the oral cavity is considered to be sixth to eight most common cancer in human pathology worldwide, this being under debate in most studies regarding the implication of oropharynx in the extension of the disease. All sites of the oral cavity can be involved in the tumor developing process so that the TNM classification is mandatory for staging the disease and for a proper oncological therapy. Epidemiological studies have shown that there is a great correlation between HPV infection and age and also with the sexual behavior of head and neck cancer patients. Alcohol and tobacco consumption along with the presence of HPV subtype 16 increase the risk of developing squamous cell carcinoma of the oral cavity from 1,2 to 9 folds. HPV infection in squamous cell carcinoma of the head and neck region changes the staging system that has been currently in use with the correlation of the tumor and lymph node implication. More fields of research regarding HPV infection have to identify the response of HPV positive head and neck tumors to external beam radiation therapy as well as the need for screening and vaccination.

Keywords: oral cancer, staging system, progression to malignancy

Introduction

Squamous cell carcinoma of the oral cavity is considered to be sixth to eight most common cancer in human pathology worldwide, this being under debate in most studies regarding the implication of oropharynx in the extension of the disease [1, 2]. All sites of the oral cavity can be involved in the tumor developing process so that the TNM classification is mandatory for staging the disease and for a proper oncological therapy. HPV has a great variety in subtypes having as much as 150 different forms. In the pathology of squamous cell carcinoma subtypes 16 and 18 are the most encountered in oral and cervical cancer [3].

HPV is classified into genera, species, types, subtypes and also according to its cutaneous or mucosal invasive characteristics. The mucosal types infect the structures at a celular level since the structure of the virus is capsid like with double-stranded DNA. The intracellular infection starts by invasive mechanism through microlesions of the epithelium or the mucosal outter layers and it is considered that the replication of the virus initiates in the basal cells of the squamous epithelium [4]. Depending on the invasiveness of the infection some viral proteins are being expressed. The persistent infection leads to the expression of E1 and E2 viral proteins

that along the progression of the infection are being integrated with the aid of E6 and E7 viral proteins. The integration process makes the transition from low grade dysplasia to high grade dysplasia to invasive cancer [5, 6].

Methodology

Up until 2012 some 20% of the oral squamous cell carcinomas were linked to the progression of the invasiveness of the HPV infection. From that moment on the International Agency of Research of Cancer (IARC) stated that according to the data gathered from a large number of studies the subtype 16 of HPV is consistently associated with oral cancers [7]. Epidemiological studies have shown that there is a great correlation between HPV infection and age and also with the sexual behavior of head and neck cancer patients [8]. Sexual preferences such as oral and vaginal sex without protection, high number of patients and sexual intercourse started at a young age are considered to be favorizing factors for HPV pathogenesis for oral squamous cell carcinoma [9, 10].

Oral lesions generated by the HPV associated infection have a large variety of presentation.

However, they usually present themselves as exophytic strawberry-like tumors that can be present at any site of the oral cavity. Despite the origin in the oral cavity some precancer lesions may originate in the oropharynx. This type of lesions progress to invasive cancer in a time dependent manner, thus the need for clinical observation through regular biopsies. Targeted biopsies for premalignant lesions are the optimal way of appreciating the progression of the lesion to invasive cancer. The clinician needs to be aware of the sexual behavior of each individual and has to address the patient with endoscopic examination and NBI enhanced imaging to search for microlesions of the mucosa. HPV infection can also be prevalent when having to deal with contaminated materials since HPV is very resistant to heat and most disinfectants.

The transmission of the infection can also be vertical so screening from infected mothers need to be done. The malignant progression is produced after a long period of latency and is cytokine mediated. This can confer the aspect of healed patients. According to an overview of epidemiology and common risk factors for oral squamous cell carcinoma presented by the otolaryngologic clinics of North America there is a relative risk that is higher for patients that are exposed to several risk factors, both intrinsic and extrinsic. Alcohol and tobacco consumption along with the presence of HPV subtype 16 increase the risk of developing squamous cell carcinoma of the oral cavity from 1,2 to 9 folds [11-13]. Moreover, the quantification of packs of cigarettes consumed by the patient every day correlates to a relative risk from low to high. These studies have been the key point in developing a validation protocol for an HPV related cancer staging system. HPV infection in squamous cell carcinoma of the head and neck region changes the staging system that has been currently in use with the correlation of the tumor and lymph node implication [14]. The new staging system considers genotype transformation due to three phenotypic changes, immortality, lack of control for the cellular proliferation and marinization. Further fields of research need to answer some questions regarding the correct appreciation of HPV infection in squamous cell carcinoma such as: does it influence overall survival rates; does it determine metastasis and does it determine resistance to therapy through genetic mechanism. Therefore, HPV related squamous cell carcinoma of the head and neck needs to be fought by a multidisciplinary team which includes head and neck surgeon, oncologist, radiotherapist, pathologist, infections and of course HPV specialists [15].

Discussions

Although the pathology and the invasive mechanism of HPV infection are known there is a possibility that the morphology of the sites of the oral cavity to be of interest when talking about

invasion. As with the genital tract there are areas in the oral cavity that are being completely keratinized such as the tongue, infrastructure of the maxilla and the hard plate and in which the invasion of the HPV occurs harder and less often. Considering this to homosexual behavior in men we need to take into consideration the fact that vaccination in this case could reduce the risk of developing HPV related oral cancer. Along with the correlation between HPV and oral cancer risk factors such as tobacco and alcohol have been known to add the risk of developing oral cancer to that of HPV infection. Chewed tobacco in contrast to regular smoking produces abrasion of the outlining of the mucosa creating means of invasion for HPV infection.

Accordingly, the World Health Organization has rated the Karachi South region in Pakistan as having the second highest risk for developing oral cancer [16, 17]. Further clinical attention needs to be addressed to preventing behavior particularities that can increase the risk of developing HPV related oral cancer.

Conclusions

HPV infection has a higher incidence in squamous cell carcinoma than two decades ago and it correlates with alcohol and tobacco consumption. These identifiable risk factors are basis for a new staging system which aims to better treat patients with squamous cell carcinoma of the oral cavity and oropharynx. Vaccination is a means of decreasing the incidence of HPV infection and its progression to invasive cancer. The subtype 16 of HPV is currently associated with the squamous cell carcinoma of the head and neck cancer and it correlates with the number of cigarettes consumed daily by the patients. According to Fakhry C., Gillison M.L. tumors positive for HPV infection have a better prognosis but this has to be confirmed by more cohort studies and metanalysis. More fields of research regarding HPV infection have to identify the response of HPV positive head and neck tumors to external beam radiation therapy as well as the need for screening and vaccination [18-19].

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Multidisciplinary Approach in a Patient with Stevens-Johnson Syndrome

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Abstract

Cutaneous drug reactions that involve the skin and the mucous membranes are the most frequent type of adverse drug reaction. These reactions, varying from simple pruritic eruptions to potentially lethal events, are an important cause of iatrogenic morbidity and mortality. The Stevens-Johnson syndrome (SJS) is a severe and potentially fatal cutaneous drug reaction. In SJS mouth, eyes, skin, genitalia, the intestinal and the respiratory tract can be affected. The aim of this paper is to present the therapeutic management of a patient with SJS.

We report a case of a 64-year-old female presented to the Department of Plastic Surgery of "Prof. Dr. Agrippa Ionescu" Emergency Clinical Hospital with erythematous and livid macules of the face, trunk, palms and soles, with erosions of the oral mucosa, with conjunctivitis and eyelid edema and erythema. The patient had a sever discomfort upon swallowing due to the glottic edema, but she did not have fever.

We highlight this case due to the fact that SJS is a rare syndrome that affects approximately 1 or 2/1000000 annually. Due to the high rate of mortality, the management of treatment of the patients with SJS is vital. A rapid diagnosis, identification of the causative drug and supportive care in an intensive care unit are essential.

Keywords: Stevens-Johnson syndrome, drug reaction, hypersensibility reaction

Introduction

The Stevens-Johnson syndrome (SJS) has the aspect of a partial-thickness burn that can determine a 100% loss of epidermis. In general, it is required the same specialized supportive care as a severe burn [1]. It was first reported in 1922, as an acute mucocutaneous reaction in two young boys. The syndrome was described by purpuric macules, acute and severe stomatitis

with necrotic lesions of the mucosa and conjunctivitis [2, 3]. It is considered to be potentially life-threatening and, in most cases, there is a culprit drug that produces the reaction.

The exact cause of SJS is unknown. Most often, it's a hypersensibility reaction to drugs [4].

Allopurinol is considered to be one of the most common drugs that cause SJS [2]. Other drugs that are at increased risk of determine SJS are: antibacterials (sulfonamides), anticonvulsants (phenytoin, phenobarbital or carbamazepine), nonsteroidal anti-inflammatory drugs of the oxicam-type [4]. Even though the majority of cases are drug-induced, there are some other factors that can determine SJS such as Mycoplasma pneumoniae infections, Herpes simplex virus, Lupus erythematodes and HIV [2].

Case report

A 64-year-old woman patient was referred to the Department of Internal Medicine with a cutaneous eruption on the trunk and with facial and glottic edema that appeared a few hours prior to presentation. The patient was a non-smoker, had diabetes mellitus type 2, class II obesity, hypertension and was allergic to insects' bites. After two days, painful ulceration of the lips and oral cavity lead to discomfort in mouth opening and eating. She presented burning sensation all over the body, with small blisters on the chest, arm, legs and thighs showing a painful distress. The vital signs were within normal limits, blood pressure was 140/70 mmHg, heart rate 75 beats/min, temperature 36,9 °C and oxygen saturation 98%. The blood tests revealed hyperglycaemia and normal renal function. Corticosteroid therapy was established, with a precise control of blood glucose. The glottic edema was reduced, but the cutaneous reaction remained.

After 4 days, the blisters increased in size being filled with a clear liquid. The dermatologist recommended specialized supportive care in an intensive care unit, antibiotherapy, immunotherapy and local treatment of the cutaneous lesions with methylene blue. After another 2 days, under corticotherapy the general status of the patient was critical, but stable. The transfer in the Plastic Surgery Department was decided due to the access to specialised intensive care unit.

At admission in our intensive care unit, the patient had large blisters on the thorax with clear liquid, purpuric eruption all over the body and macules on the abdomen with tendency to coalescence. The lips and the oral cavity presented ulcerated lesions with impossibility of eating and the eyes were erythematous, tearing with acute conjunctivitis. There were no signs of cervical lymphadenopathy. The cutaneous lesions were treated with methylene blue solution (Fig. 1). Blood tests, at presentation, revealed lower levels of albumin, normal renal function and high level of glucose.

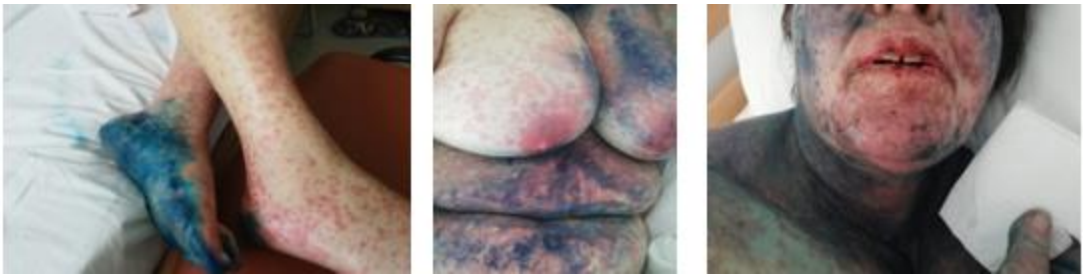

Fig. 1. Initial presentation of the case in the Intensive Care Unit – day 7

The patient was treated like a burned patient with superficial second-degree burns involving 27% of total body surface. Hidroelectrolytic resuscitation was realised and albumin was administered starting with day 4. The patient was washed with antiseptic solutions, including Polihexanidine and physiological serum, the blisters were evacuated and the lesions were

treated with oxytetracycline [5, 6]. A new plan of treatment was decided without immunotherapy.

In the next two days general bath was done in aseptic conditions, the new formed blisters were evacuated and oxytetracycine was sprayed all over the body. The face was treated with a solution with vitamin A. Ocular lesions were treated with ophthalmic solutions. Deep lesions of the thorax were treated in the first days with a dressing that absorbed the exudates and reduced the pain and after that with silver impregnated dressing [7]. She was haemodynamically stable with oxygen, and no intubation (Fig. 2, Fig. 3).

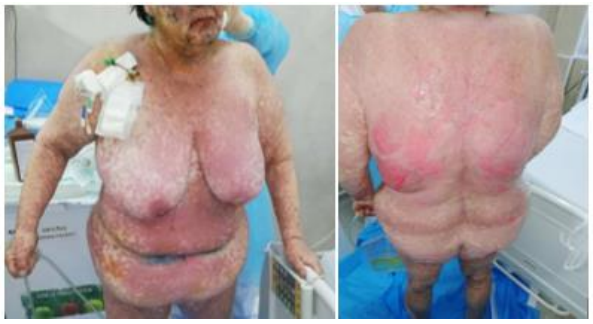

Fig. 2. Third day in the intensive care unit

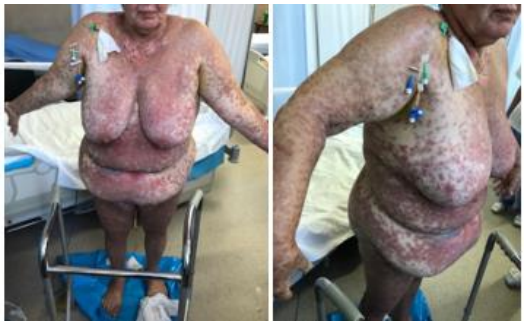

Fig. 3. Sixth day in the intensive care unit

After nine days in the intensive care unit she was transferred in our department. After the general bath, moisturizing cream was applied all over the body and dressings with silver nitrate were applied on the deep lesions. The evolution was favourable. The patient was fully recovered after 21 days of hospitalization and left the plastic surgery department in a good clinical condition (Fig. 4.).

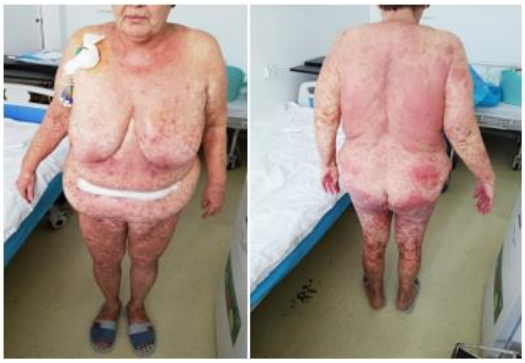

Fig. 4. Last day of hospitalization

Discussions

The pathological mechanism of SJS is only partially understood, but it is considered to be immune-mediated through CD 8 T-cells, cytolytic molecules FasL and granulysin [4, 8]. The management of treatment of SJS starts with a precise diagnosis in an early stage, withdrawal the causative drug(s), supportive care and specific therapy [7, 9]. There are recommended systemic steroids, intravenous immunoglobulin (IVIg) and plasmapheresis as the 3 first-line treatments of choice [10, 11]. Our patient received systemic steroids, but IVIg were not administered because the causative drug was a protein used to control diabetes without knowing the interfering effects of the two substances. It is not fully understood the effects of concomitant administration of IVIg and systemic steroids [4]. The patients with SJS should be treated like patients with partial-or full-thickness burns using the Parkland's formula for resuscitation [1].

Our patient had placed a central intravenous line in an area with normal skin, fluids and electrolytes were monitored with strict control of central venous pressure and urine output.

Parenteral nutrition was realised in the first 3 days in the intensive care unit, associated with prophylaxis with antibiotics, controlled analgesia and anticoagulant therapy.

The major differential diagnosis of SJS is other severe bullous skin diseases such as paraneoplastic pemphigus or Staphylococcal scalded skin syndrome [1]. Toxic epidermal necrolysis (TEN) is considered to be the sever form of SJS, differing only by their extent of skin detachment, more than 30% in case of TEN [12]. The average mortality in case of SJS is almost 5%, being higher in elderly patients [2, 13].

Conclusions

Stevens-Johnson syndrome is one of the most severe forms of cutaneous adverse drug reactions. It is a life-threatening condition and a public health issue. The patients must be treated in an intensive care unit as patients with partial-or full-thickness burns. Rapid diagnosis of the syndrome and cure of the patient by competent burn centre staff are the most important steps in the management of treatment of these patients. Corticosteroids are a choice of therapy for SJS in most of the cases. Further research in the pathogenesis of SJS and TEN is needed.

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Orbital Transposition. Do we Need it?

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Abstract

The management of frontal sinus pathology represents a challenge for ENT surgeons. In the past, the external approach was considered the gold standard for the pathologies involving the frontal sinus, but with the development of endoscopic surgery new, minimally invasive procedures appeared. To endoscopicaly reach lesions that involve the lateral orbital roof, exposure of the frontal sinus should be increased. Orbital transposition is a new and innovative technique that aims to expand the frontal sinus.

In this paper, we will present the steps of the orbital transposition technique, and we will evaluate its utility and safety.

Keywords: frontal sinus, endoscopic approach, orbital transposition

Introduction

The management of frontal sinus pathology represents a challenge for ENT surgeons. In the past, the external approach was considered gold standard for the pathologies involving the frontal sinus but with the development of endoscopic surgery new, minimally invasive procedures appeared [1].

There are three endoscopic endonasal approaches of the frontal sinus Draf I, Draf II a/b and Draf III [2]. The advantages of the endoscopic sinus surgery are faster recovery, no incision that leads to no scars and fewer side effects [2, 3].

Although, in recent years, the endoscopic technique is preferred by surgeons for frontal sinus lesions, there are some limitations and contraindications for this type of approach. When choosing an endoscopic approach, for the frontal sinus, the surgeon must take into consideration the anteroposterior diameter of the frontal sinus, the origin site of the lesion, if the location of the tumor is lateral or medial from the virtual sagittal line that passes through the lamina papyracea, the size of the tumor and erosions of the posterior sinus wall. Depending on these parameters, the surgeon decides between the endoscopic, external or combined approach [4, 5].

However, in cases with lesions localized lateral to the mid- pupillary line, with the development of new multi angled instruments and increased surgical endoscopic experience exclusive endoscopic endonasal approach of the frontal sinus is possible [6].

To reach lesions that involve the lateral orbital roof, exposure of the frontal sinus should be increased. Orbital transposition is a new and innovative technique that aims to expand the frontal sinus [6].

In this paper, we will present the steps of the orbital transposition technique, and we will evaluate its utility and safety.

Materials and Methods

By using the orbital transposition technique, the orbital content is transposed laterally after the superomedial wall of the orbit is drilled [7].

After performing Draf IIb or Draf III surgical procedures, the first step is identifying the anterior ethmoidal artery. The artery needs to be coagulated and carefully transected to prevent complications like massive hemorrhage or retrobulbar hematoma [7, 8].

The second step is the complete exposure of the *lamina papyracea*. Afterwards, the upper portion of the lamina is fractured and removed. During this maneuver, it is very important to preserve the periorbital layer intact as well as preserving the integrity of the trochlea of the superior oblique muscle to ensure normal eye mobility [9].

The orbit is displaced laterally, with the help of a malleable retractor, to expose the floor of the supraorbital recess. The bony floor is drilled with a diamond burr and removed until the coronal plane is reached. The risks of this procedure are dural exposure and CSF leaks [10].

By drilling the bone of the supraorbital recess, enough space is created for the surgeon to reach the lesions developed in the later aspect of the frontal sinus. Special surgical instruments like curved drills or shavers are needed to be able to reach the lateral area [11].

At the end of the surgical procedure, after the removal of the retractor, the orbit expands to its normal position [10].

Discussions

Traditionally, frontal sinus lesions developed in the lateral part of the frontal sinus were not eligible for endoscopic surgery, but for the external approach. The external procedures are the Howarth- Lynch procedure, The Lothrop procedure or the osteoplastic flap [11].

Lately, the indication for endoscopic surgery expanded with the advances made in instrumentation and with the increase of endoscopic surgical experience [11-13].

However, the endoscopic approach is still not commonly used in case of lesions developed laterally from the mid- pupillary line. Surgeons must take into consideration certain aspects of the frontal sinus before choosing this approach. All these aspects concentrate on the anatomy of the frontal sinus [11].

The pneumatization of the frontal sinus can be an obstacle for endoscopic surgery. If the sinus is well pneumatized chances of reaching lesions located on the lateral side of the sinus are small [11, 13].

The convexity of the orbital roof limits the access to the lateral aspect of the sinus as well [12]. For the passage of instruments, a minimum anteroposterior diameter of the frontal sinus is required [13].

Orbital transposition is a new technique that increases the space of the frontal recess and allows access to the lateral part of the frontal sinus. This technique is challenging and requires training, experience and skill. Also, special curved instruments are needed to be able to perform the surgery [14].

In choosing the correct approach and in establishing the surgical plan, radiological examination plays a very important role. Nevertheless, the assessment of the relationship between the frontal sinus and the lesion can, sometimes, only be done intraoperatively. This is one of the reasons why every surgeon must be trained to perform both endoscopic and external approaches. Another reason for this is that during any endoscopic surgery complications may arise, and the approach must be switched into an external one [15-18].

Conclusions

The endoscopic approach of the frontal sinus with orbital transposition is an alternative to traditional open surgery for lesions that develop in the lateral side of the mid- pupillary line [19, 20].

Orbital transposition is a useful, yet challenging technique surgeons use in order to expand the frontal sinus and have better visibility of the lateral aspect. This type of surgery should be well planned ahead and only applied in selected cases where the anatomy of the frontal sinus allows the removal of the lesions with this technique. Surgeons need experience and special double curved instruments to perform this surgery and to reach the far later side of the sinus.

However, even experienced surgeons face situations when the lesion developed in the frontal sinus cannot be resected endoscopically. In those cases, the endoscopic approach must be switched into an external one.

Our conclusion is that even if the endoscopic approach is preferred by many surgeons, the traditional external approach is very important and sometimes the best option for optimal surgical results.

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Mechanisms of Lead Toxicity: Case Reports of Occupational Lead Poisoning

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Abstract

Exposure to heavy metals at workplace has a great impact on population health. In particular, exposure to lead is considered one of the oldest occupational exposures. Chronic lead poisoning in workers with long history of exposure to heavy metals result in different organ dysfunctions: nervous system, kidneys, liver or gastrointestinal system, accompanied by increased lead levels in the blood and the urine, and secondary anemia induced by the presence of the lead in the organism. $CaNa_2EDTA$ chelation therapy represents at the same time a diagnostic method, but also an effective measure of treatment.

Keywords: heavy metals, lead, delta-aminolevulinic acid, occupational exposure, chelation therapy, Copşa Mică

Introduction

Environmental pollution with heavy metals is as old as human ability to melt and process ores. Exposure to heavy metals has been known for thousands of years. In particular, exposure to lead is considered one of the oldest occupational exposures. Lead and its compounds play an important role in modern industry, being still the most used non-ferrous metal. Along with lead, other heavy metals encountered in professional exposure are cadmium, zinc, copper, mercury and manganese.

High concentrations of metals in the environment and workplace may lead to the accumulation of these toxic substances in the body, thus increasing the risk of chronic diseases such cancer, neurological degenerative disturbances or different organs and systems diseases [1]. The effects of lead are characterized by a cumulative effect; general disorders are accentuated as lead accumulates in the body. Initially, manifestations are not characteristic: weakness, asthenia, joint pain, digestive disorders [2]. In chronic lead poisoning several syndromes are described: asteno-vegetative, nervous, anemic, digestive, cardiovascular, and renal syndrome. The harmful action of lead in the human body include: blocking the synthesis of hemoglobin and the red blood cell membrane, a process leading to anemia; toxic action on

the nervous system producing motor neuritis and encephalopathy; morphological and functional lesions in the kidney that can lead to nephritis; endarteritis and vascular spasm [1].

The hypertension induced by the presence of lead in the body has been suggested to result from the reduction of nitrogen monoxide (NO) activity in the walls of the blood vessels. Studies conducted by Vaziri *et al*., (2001) showed that lead increases the amount of NO in occupationally exposed persons by increasing nitric oxide synthase (NOS), a mechanism of NOS overexpression being also suggested [3]. In severe stages, lead intoxication is manifested by seizures, paralysis of the limbs, coma and ultimately death.

Materials and Methods

Our research was conducted in Copşa Mică, Romania, a region intensely polluted with heavy metals (Pb, Cd, Cu, Zn) due to nonferrous ores extraction and metallurgical processing.

This paper present four case reports of workers in Copsa Mica metallurgical smelter and hospitalized in a Labor medicine clinic in Sibiu in the period 2010-2015. All patients had a long history of workplace exposure to heavy metals and they were diagnosed with occupational lead poisoning as primary diagnostic. Clinical presentation, occupational exposure data, laboratory findings and treatment during hospitalization are provided.

Description of the study area

Copşa Mică is a small town located in the north-west of Sibiu County (geographical coordinates 46°6′45″N and 24°13′5″E). This region was considered "one of the five most polluted industrial sites of the communist world" as reported in the Atlas of Our Changing Environment [4]. Copşa Mică metallurgical plant was established in 1939-1940 and included equipments for Zn and Pb extraction, recovery of Cd and other metals such as bismuth, gold, silver and antimony. In 1998 the factory has entered into a phase of ecological rehabilitation, and presently its activity was reduced to the recovery of heavy metals from the equipment [5].

The long operation of this industrial unit induced an extensive environmental contamination (air, water and soil pollution) in the region. Previous research found that lead and cadmium are the dominant pollutants accumulated in soils and vegetables harvested from these contaminated soils [6].

Case reports of occupational lead poisoning

Clinical presentation, occupational exposure data and laboratory findings of patients are summarized in Table 1 and Table 2.

Case report 1

A 52 years old male, employee of the metallurgical plant of Copsa Mica (occupational exposure to heavy metals 28 years) with dizziness, nausea, vomiting and vision disturbances.

Computer tomography shows images of cerebellar and paracerebellar infarction.

Biochemical analyses revealed a blood lead level of 51 µg/dL and urinary lead levels 206 µg/dL. Patient treatment consisted in forced diuresis, chelating therapy with calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) and supportive therapy with B complex vitamins.

Case report 2

A male worker, aged 37 years and 14 years history of occupational exposure to heavy metals, presented with complaints of diffuse abdominal pain, loss of appetite and fatigue. Clinical examination revealed gingival pigmentation (Burton's line) which is a specific sign of chronic exposure to lead [6]. The patient was diagnosed before with chronic gastroduodenitis.

Biochemical analyses revealed hypochromic hemolytic anemia, elevated liver enzymes and high blood lead level and urinary lead levels after chelating therapy - 78,1 µg/dL, and respectively 575 µg/dL. Treatment included $CaNa_2EDTA$ chelating therapy, complex B vitamins, and symptomatic therapy (pain relievers, antispasmodics).

Case report 3

Male patient, 40 years old, worker in the same metallurgical plant (occupational exposure of 18 years) presented at hospital with headache, asthenia, epigastralgia (pyrosis), and abdominal pain. Laboratory analyses revealed elevated liver enzymes and high blood lead level and urinary lead levels after chelating therapy - 72,8 µg/dL, and respectively 520 µg/dL. Examination by esophagogastroduodenoscopy confirms the diagnosis of chronic gastritis. Forced diuresis was initiated and therapy with vitamins, hepatoprotectors and prokinetics.

Case report 4

The patient is a 45 years old male, with long history of exposure to heavy metals in workplace (21 years) presented asthenia, fatigue, and symptoms of renal dysfunction (pollakiuria, disuria). Laboratory findings revealed hypochromic hemolytic anemia, elevated serum creatinine, urea nitrogen and uric acid in the blood. Also, 47,4 µg/dL of lead was found in the patient blood and 106,2 µg/dL of lead in the urine. The echographic examination shows hepatic steatosis and chronic nephropathy. Patient treatment included chelation therapy, hepatoprotectors and vitamins.

Table 1. Summary of occupational lead poisoning case reports

Patient ID	**Age (years)**	**Occupational exposure (years)**	**Main diagnostic**	**Associated dysfunctions**	**Symptomatology**
Patient 1	52	28	Occupational lead poisoning	Cerebellar infarction; paracerebellar syndrome	dizziness, nausea, vomiting, vision disturbances
Patient 2	37	14	Occupational lead poisoning	Chronic gastroduodenitis; Secondary anemia	diffuse abdominal pain, loss of appetite, fatigue, gingival pigmentation (Burton's line)
Patient 3	40	18	Occupational lead poisoning	Chronic gastritis; Secondary anemia	headache, epigastralgia (pyrosis), asthenia, abdominal pain
Patient 4	45	21	Occupational lead poisoning	Chronic nephropathy; Hepatic steatosis; Chronic gastroduodenitis; Secondary anemia	asthenia, fatigue, pollakiuria, dysuria

Table 2. Biochemical parameters and lead exposure indicators

Patient ID	Hb (g/dL)	Ht (%)	Blood lead levels (µg/dL)	Urine lead levels (µg/dL)	Delta-aminole vulinic acid (mg/L)	Siderem ia (µg/dL)	TGO (u/L)	TGP (u/L)	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Patient 1	12,3	38,1	51	206	18,1	82	20	20	1,06	44	5,6
Patient 2	9,8	29,3	78,1	575	19,25	134	85	145	1,17	48	5,5
Patient 3	10,3	32,2	72,8	520	30,25	161	108	106	1,13	53	6,6
Patient 4	11,8	35,7	47,4	106,2	14,3	69	38	45	1,6	54	7,6

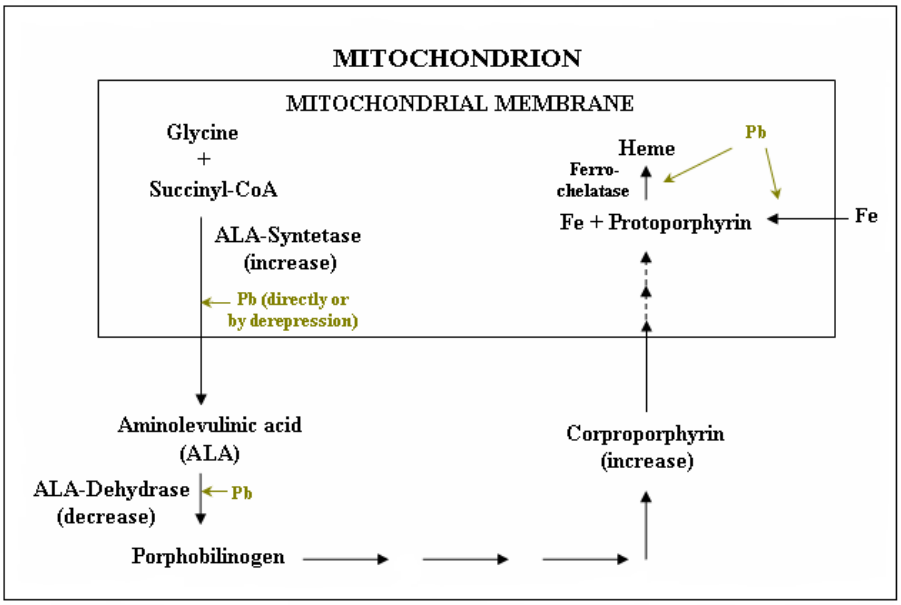
Discussions

All patients had a long history of occupational exposure to heavy metals (14-28 years) and elevated blood and urine lead levels. The reference blood lead level (BLL) for adults was designated 5 µg/dL [7]. The U.S. Occupational Safety and Health Administration (OSHA) established standards for occupationally exposed workers and recommend that workers to be removed from lead exposure when blood lead levels are equal or higher than 50 µg/dL (construction industry) or 60 µg/dL (general industry) and allow workers to return to work when the BLL is below 40 µg/dL [7].

One of the primary effects of lead is inhibition of heme synthesis, with subsequently anemia.

The secondary anemia was present in three of the four presented patients. The hematotoxicity mechanisms are based on the inhibition of several enzymes in the heme biosynthesis pathway: delta-aminolevulinic acid dehydratase (ALA-D), delta-aminolevulinic acid syntetase (ALA-S), coporphyrinogen oxidase and ferrochelatase (Fig. 1). ALA-D is strongly inhibited by lead in human organism. The degree of ALA-D inhibition has been used clinically to quantify the degree of lead poisoning [8]. Inhibition of this enzyme by lead has been demonstrated in circulating erythrocytes, but also in the kidneys, bone marrow, brain, liver and heart. Inhibition of the enzyme begins at blood lead levels of 10-20 µg/dL and is quasi-complete at levels of 70-90 µg lead/dL [9]. Decrease of ALA-D activity leads to high levels of delta-aminolevulinic acid in blood, one of the biomarkers of early lead biochemical adverse effect in population exposed in contaminated workplaces, but also in low levels exposure situations as seen in the general population [10]. The accumulation of δ-aminolevulinic acid (δ ALA) also contributes to oxidative stress of cells through auto-oxidation (Fig. 1). Inhibition of ferrochelatase, another enzyme that occurs in the synthesis of the heme, leads to the accumulation of iron in the blood. Two patients associated severe anemia with increased sideremia, a common condition in lead poisoning.

Fig. 1. Mechanism of lead action on heme synthesis



Gastroduodenal tract damage may also be related to lead exposure; gastritis or gastroduodenitis was present in three patients. Symptoms such abdominal pain, nausea, diarhea or constipation commonly occur when blood lead levels exceed 40 µg/dL in adults [11].

Lead induces cellular toxicity through multiple mechanisms: calcium substitution (without replacing its functions) in a series of receptor-dependent or enzyme mediated transport

processes; production of oxidative stress through initiation of membrane lipid peroxidation by stimulating ferrous ions; inhibition of nitric oxide synthases or alteration of gene expression with over-expression of pro-inflammatory cytokines and enzymes that mediates neuroinflammation [12, 13].

Also, the exposure to lead results in depletion of gluthatione reduced (GSH) reserves and accumulation of lipid peroxidation products. Subacute exposure to lead modifies the activity of the glutathione-related enzymes, such as GR, GST, and G6PD [14]. Many factors, such as history of lead exposure, the duration of such exposure, and age are influencing the response of an exposed individual to the toxic action of lead.

In brain the effects are based on lead's ability to pass through the blood brain barrier by substituting calcium ions and the influences on neurotransmitter system (glutamatergic, dopaminergic and cholinergic), mitochondria, cerebrovascular endothelial cells, astroglia and oligodendroglia [15]. Lead accumulates in astroglial cells, the glial cells being vulnerable to lead due to their deficiency in lead detoxifying protein [16]. Chronic lead exposure can alter NMDAR subunit gene expression in the brain, with impairment of learning and memory processes [17].

Lead interferes with glutamate, the most common neurotransmitter in the brain, and thus interferes with neuronal development. Biochemical changes consist of decreased cholinesterase activity, increased activity of dopamine and alteration of gamma-aminobutyric acid (GABA) activity [18].

Some authors have experimentally observed a hypervascularization in the brain, proportional to the duration of lead uptake, and cerebral haemorrhage. The exposed workers were characterized by diffuse alteration of the cerebral artery and an increase in the tonus of the blood vessels, especially in the arterioles, which may be the cause of the headache described in patient no. 2 [19].

In the kidney, lead has direct action on proximal countable tubes (Fanconi-like syndrome) and indirect action on the renal artery, with vasoconstriction and ischemia [20]. Chronic lead exposure, with blood lead levels persistently above 70-80 μg/dl, represent a risk factor for chronic kidney disease [21]. Chronic exposure to lead results in over expression of angiotensin II and intranuclear p65 NF-kβ in tubular and infiltrating cells [22].

Initiation of chelation therapy led to the elimination of lead in large quantities in the urine and the decrease of the blood lead level in all patients.

Conclusions

Workers with a long history of exposure, diagnosed with occupational lead poisoning, presented elevated blood and urinary lead levels. Chronic lead poisoning result in different organ dysfunctions: nervous system, kidneys, liver or gastroduodenal system, the secondary anemia joining the increased lead levels in the blood and urine of occupationally exposed patients. The treatment included forced diuresis, symptomatic and chelating therapy with $CaNa_2EDTA$, but the most important recommendation is limitation or cessation of occupational exposure.

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Splenopancreatectomy for Isolated, Late Recurrence after Surgically Treated Poor Differentiated Ovarian Cancer

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Abstract

Tumoral lesions of the pancreas are most often primary pancreatic neoplasms, metastases at this level being rarely reported. We present the case of a 56-year-old patient who was diagnosed with an isolated metastasis involving the spleen and the pancreatic tail two years after debulking surgery for stage IIIC ovarian cancer followed by adjuvant chemotherapy. The patient was successfully submitted to a distal pancreatic resection en bloc with splenectomy. The histopathological studies confirmed the metastatic origin of the respective tumour. The postoperative course was uneventful, and the patient was discharged afterwards in the sixth postoperative day.

Keywords: Oligometastasis, ovarian cancer, distal pancreatectomy, splenectomy

Introduction

Tumoral lesions involving the pancreas are in the vast majority primary pancreatic neoplasms, metastatic lesions being unusual; however, the most commonly incriminated malignant primaries which might lead to the apparition of pancreatic metastases are represented by renal, pulmonary, gastrointestinal malignancies or melanomas [1]. The largest series studying the role of pancreatic resections for metastatic disease have been reported among patients with renal cell carcinomas; the encouraging results reported so far lead to the successful association of pancreatic resections for other origins of the metastatic lesions [2-4]. As for ovarian cancer, it can lead to the apparition of pancreatic metastases via multiple routes (including the peritoneal, hematogenous and lymphatic route); unfortunately, ovarian cancer pancreatic metastases usually occur as part of systemic, unresectable relapse and are therefore rarely amenable to cytoreductive surgery. However, in rare cases, when debulking surgery to no residual disease is feasible or if pancreatic lesions develop as oligometastatic disease, surgery might be taken in consideration with acceptable results in terms of perioperative complications; in such cases an improved long-term survival is expected [5-7].

Case report

A 54-year-old patient with no significant medical history presented for diffuse abdominal pain and asthenia; the abdominal ultrasound demonstrated the presence of a moderate quantity of ascites in association with tumoral transformation of the ovaries, while the tumoral markers revealed an increased level of CA 125 (of 1450U/ml). The patient was submitted to a computed tomography which confirmed the suspicion of ovarian cancer; in the meantime, a diffuse peritoneal and omental thickening as well as enlarged pelvic lymph nodes were also seen, so the patient was submitted to surgery: a total hysterectomy en bloc with bilateral adnexectomy, parietal and pelvic peritonectomy, pelvic and para-aortic lymph node dissection as well as total omentectomy. At that moment the postoperative course was simple, the patient being discharged in the fifth postoperative day. The histopathological examination of the specimen confirmed presence of a poorly differentiated serous ovarian neoplasm/adenocarcinoma, two out of the 18 pelvic lymph nodes being involved; however, none of the resected para-aortic lymph nodes presented metastatic involvement. Three weeks after surgery the patient was referred to the oncology clinic where she was submitted to six cycles of carboplatin and paclitaxel. At the end of the oncological treatment the levels of CA 125 were normal (23U/ml), so the patient was further submitted to an active protocol of follow-up. At the eighteen months follow-up, the patient presented a slightly increased level of CA 125 levels (measuring 65U/ml); however, the imagistic studies did not reveal any modification. At the two-year follow-up, the serum levels of CA 125 reached 223U/ml while the computed tomography revealed the presence of a solitary tumoral lesion involving the pancreatic tail and the spleen. The patient was resubmitted to surgery, a distal pancreatectomy en bloc with splenectomy being performed (Figures 1-3). The postoperative course was favourable, the patient being discharged in the sixth postoperative day while the histopathological studies confirmed the metastatic origin of the tumoral lesion.

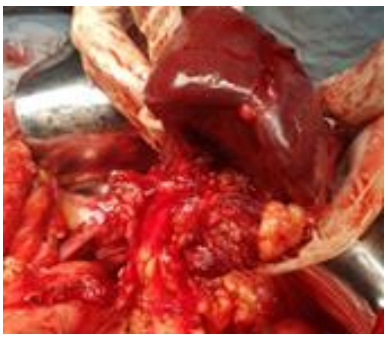

Fig. 1. Intraoperative aspect after mobilization of the pancreatic tail en bloc with the spleen.

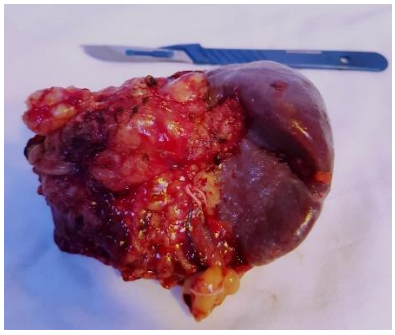

Fig. 2. The specimen – distal pancreatectomy en bloc with splenectomy.

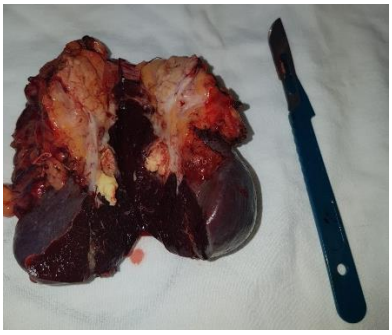

Fig. 3. The specimen-pancreatic metastasis developed at the level of the pancreatic tail invading the splenic capsule.

Discussion

Ovarian cancer remains one of the most common gynaecological malignancies with poor long-term outcomes due to the fact that most patients are diagnosed when disseminated lesions are already present. The main patterns of spread in ovarian cancer patients include the peritoneal, hematogenous as well as the lymphatic route, leading to the apparition of distant metastases involving both the lower and upper abdomen [8, 9]. When it comes to the rate of the distant metastases, is estimated that up to 8% of cases present with distant metastases at the time of diagnostic, while 22% of cases will develop distant metastases at a certain point of the evolution of the disease [5, 10].

Pancreatic metastases from ovarian cancer remain rare eventualities, only few cases being reported so far. In such cases an essential step is the differential diagnostic with a primary pancreatic neoplasm, which is most often established after performing the biopsy of the pancreatic lesion; however, in cases presenting a previous history of another malignancy, the metastatic origin of the tumor should be also taken in consideration [4, 5 ,11]. As for the pancreatic metastases from ovarian cancer, although only rare cases have been reported so far, in autopsy studies the reported incidence reaches 21% [12].

When it comes to the most appropriate therapeutic approach of patients diagnosed with pancreatic metastases from ovarian cancer, the decision will be taken accordingly to the extent of the disease. In cases presenting disseminated lesions in which a radical cytoreductive surgical procedure cannot be expected, the most efficient management consists of palliative chemotherapy. Whenever debulking to no or minimal residual disease is expected, surgery might be taken in consideration in order to obtain a good control of the disease. However, initially it has been considered that the presence of extended tumoral lesions in the upper abdomen represented a formal contraindication for debulking surgery. For example, in the study conducted by Eisenhauer *et al.*, the authors included 262 patients submitted to debulking surgery between 1998 and 2003 [13]; however, upper abdominal resections (including pancreatic resections) were performed after the year of 2000. Therefore, the authors divided the patients included in the study into three groups: the first group included patients submitted to debulking surgery including the upper abdomen, the second group included cases in which lesions were limited to the inferior abdomen while the third group included patients presenting disseminated lesions submitted to incomplete debulking surgery; the long term outcomes demonstrated similar outcomes for the first two groups, which reported a significantly better outcome when compared to the third group; in this way the authors demonstrated the feasibility of upper abdominal resections as part of debulking surgery [13]. The largest studies which included pancreatic resections are summarized in Table 1.

Table 1. Studies reporting pancreatic resections as part of debulking surgery

Name, year	Period of the study	No of patients	Median age (years)	No of cases submitted to pancreatic resections (%)	Type of surgery: primary/secondary cytoreduction	Postop. morbidity	Pancreatic resections postoperative morbidity
Beneditii Panici 2015[14]	2006-2014	126	55	48 (39,6%)	Primary	28,5%	4,7% (13% of patients who were submitted to pancreatic resections developed postoperative complications)
Burton, 2011 [15]	1995-2009	20	59	1 (5%)	Secondary	25%	0
Fanfani, 2010 [16]	2005-2008	87	55	3 (3,4%)	Primary and secondary	42,5%	0
Hoffman, 2007 [17]	2002-2004	6	NR, age raging between 43-76	2 (33%)	Primary	50%	0
Tate, 2017 [18]	2008-2012	106	60	21 (20%)	Primary	20%	7 cases of pancreatic leakage – conservative management
Rodriguez, 2013 [19]	2001-2004	482	57	12 (0,5%)	Primary	NR	NR
Eisenhauer, 2006 [13] (pancreatic resections were performed only after the year of 2000)	1998-2003	57	59	6 (11%)	Primary	12%	NR

Later on, pancreatic resections have been reported as part of debulking surgery in the upper abdomen and were included in the papers which studied the efficacy of cytoreductive surgery at this level. However, it should not be omitted the fact that performing a pancreatic resection as part of cytoreductive surgery might increase the rate of the postoperative morbidity, the most fearful complications being related to the risk of developing pancreatic leaks.

In a more recent study conducted on the theme of pancreatic resections as part of debulking surgery for advanced stage or relapsed ovarian cancer, the authors included six cases which necessitated distal pancreatectomy at the time of primary cytoreduction (one case), secondary cytoreduction (four cases) and tertiary cytoreduction respectively (one case) [7]. Among these six cases two patients developed pancreatic leaks, one of them being treated in a conservative manner while the second one necessitated reoperation. However, the overall mortality was null while the long-term outcomes reported a survival of 54 months for the case submitted to pancreatic resection as part of primary cytoreduction, a median survival of 36 months for patients submitted to pancreatic resections as part of secondary cytoreduction, and 10 months for the patient submitted to distal pancreatectomy at the moment of tertiary cytoreduction [7].

These data come to demonstrate that distal pancreatectomy can be safely performed as part of cytoreductive surgery for advanced stage or relapsed ovarian cancer, a significant benefit of survival being expected.

As for the role of splenic resections as part of cytoreductive surgery, it seems that splenectomy can be also safely associated in order to maximize the debulking effort for both advanced stage or relapsed ovarian cancer; however, it seems that the pattern of splenic involvement might induce a difference in terms of survival, cases presenting capsular lesions being associated with a better outcome when compared to cases presenting hematogenous splenic metastases [20-22].

Conclusions

Although are rare situations, pancreatic metastases from ovarian cancer can be encountered in both advanced stage and relapsed ovarian carcinomas. Whenever debulking surgery to no residual disease is expected, distal pancreatectomy (sometimes en bloc with splenectomy) should be performed in order to maximize the debulking effort. However, it should not be omitted the fact that pancreatic resections might associate a higher risk of postoperative complications such as pancreatic leaks. Therefore, it should be performed only in specialized centres.

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Male Sexual Dysfunction in Diabetes Patients. Diagnostic and Therapeutic Management Options

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Abstract

Diabetes mellitus (DM) is the cause of multiple medical and psychological complications.

The increase in DM prevalence, both Type 1 and Type 2, and its complications, also entails an increase in the incidence of sexual dysfunction in these patients, this being considered one of the early complications in this category of patients. Male Sexual Dysfunction (DSM) is much better investigated and documented today, and medical treatment is complemented by lifestyle optimisation, psychological counselling and, last but not least, good DM control.

Keywords: diabetes mellitus, male sexual dysfunction, erectile dysfunction

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases and is a real public health problem. The number of people suffering from DM is on the increase due to urbanisation, increasing the prevalence of obesity and reducing physical activity on the other hand, due to the rise in life expectancy, the emergence of modern and sophisticated investigations as well as new therapies in the field.DM is the cause of multiple complications, both medical and psychological. The increase in DM prevalence and its complications also entails an increase in the incidence of sexual dysfunction in these patients. Numerous studies have shown that DM patients are at increased risk of developing sexual dysfunction earlier than 6-10 years compared to non-diabetic patients. This is due to poor control of DM, its age, but also to the presence of its complications [1]. Sexual dysfunction may be the first sign of deterioration in vascular status in patients with DM [2, 3].

Materials and Methods

We conducted a review of the literature by looking into the MEDLINE database of the most representative articles published so far. More than 20 articles were selected from the research, using the search by keywords: diabetes; diabetic erectile dysfunction; diabetic neuropathy, endothelial dysfunction; phosphodiesterase inhibitors and intracavernous injection.

Results and Discussions

Diabetes mellitus (DM) is responsible for lesions of the somatic and autonomic peripheral nervous system, urinary tract injuries as well as psychological changes. Vegetative diabetic neuropathy affects almost all organs, especially: heart, kidney, urinary bladder, gastrointestinal apparatus, pupil but also the reproductive system. Sexual deficiencies are common problems for diabetic patients and are primarily related to the degree of glycemic control and the duration of diabetes. Sexual and urological complications are the cause of the damage to both nerves and small blood vessels.

It is estimated that 30 million male Americans develop along the evolution of DM erectile dysfunction (ED). Male impotence can develop at any age, but usually the most affected are senior men. A recent study of 1300 patients showed that 52% of the patients in the suds had different degrees of erectile dysfunction and their age was between 40 and 70 years. Over 50% of the 10 million men diagnosed with type 2 diabetes have various degrees of sexual impairment. In patients with well-controlled DM, the impotence rate is lower, while patients with poorly controlled DM between 50-70% of men with sexual dysfunction [4, 5].

Nowadays, the prevalence in patients with DM is 28%, probably much underestimated, and is present in subjects aged 40-70 years, compared with the incidence in non-diabetic subjects, where the values are about 9.6%. The age at which ED is established in patients with DM is lower than in non-diabetic patients, with a prevalence of 1-10% in 30-year-old men reaching 20-40% in those aged 60-69 years. Of ED patients, nearly 40% are diabetics, of whom more than a quarter were not diagnosed with DM, in other words, the ED diagnosis was almost concomitant with the DM diagnosis [6]. A recent study showed that at least 11.5% of patients with ED had undiagnosed diabetes [7]. The prevalence of ED in patients with Type 1 DM and Type 2 DM is equal. ED is more common in diabetic patients with neuropathy [5-7].

DSM is much easier to diagnose and is the most well-studied pathology of sexual dysfunction.

DSM is also referred to as erectile dysfunction (ED) and represents the inability to obtain and maintain a penile erection sufficient for satisfactory sexual intercourse [8, 9].

From an etiopathogenic point of view, ED is a disease with multiple trigger factors. Not always cause-effect association is well established and documented. ED can be various, from psychogenic erectile dysfunction, neurological disorders (parasympathetic vegetative system) endocrine, veno-occlusive incompetence, iatrogenic causes, drugs to cause infectious causes (balanitis, prostatitis).

In patients with ED and DM, the factors involved in ED are more specific as age, duration of DM, glycemic control level by evaluation of glycosylated hemoglobin (HbA1C%), presence of micro- and macrovascular complications, especially neuropathy and vascular damage, dyslipidemia, smoking, alcohol consumption, treatment followed for both DM and its complications, disorders of androgen secretion, as well as alteration of endothelial function and contractility of the cavernous muscle. Several recent studies have also shown a direct link between vitamin 25 (OH) D and ED deficiency.

On the other hand, in this category of patients, the psycho-emotional disorders have an important role in triggering the pathology [10, 11].

The ED classification, according to the International Index of Erectile Function (IIEF-5) Questionnaire, is divided into 4 stages, depending on the scores that this questionnaire records: score 22-25 points = no ED, 17-21 points = ED mild, 12-16 points = moderate ED, 8-11 = Moderately severe ED, 5-7 points = severe. The chest is validated and is a very lasting tool in ED evaluation along with clinical and laboratory investigations [12, 13].

From a pathophysiological point of view, penile erection depends on the increase in blood flow in the cavernous body, which in turn depends on the infusion pressure, the relaxation of the arterioles that irrigate these bodies and the relaxation of the cavernous muscle. The

atheromatous bone is frequently associated with DM by 40% more than non-diabetics. The atheromatous attenuation of the internal shingles and the internal iliac artery may explain the poor erection. In diabetic patients nocturnal penile tumescence (TPN) is much reduced [14] and the explanation may be related to neuropathic affection but also The primary area of dysfunction is at the level of NO synthesis and release, both from the neural and endothelial sources, but not from the intracellular level of the cavernous muscle [15].

Endothelial cells form a permissive layer that regulates the flow of nutrients and the action of bioactive circulating molecules in the blood on the underlying tissue, mainly vascular smooth muscle. This is achieved by a series of receptors attached to the membrane and junctional proteins. The primary marker of dysfunction endothelial cells in diabetics is the presence of microalbuminuria, thus indicating the presence of renal microangiopathy. Therefore, the low activity of NOSe, the inactivity of NO barely released and the growth of advanced glycation end products (AGES) are the mechanisms involved in ED. The three phases of erection (excluding detumescent phase) are characterised by different clinically established parameters (Table 1).

Table 1. Methods of clinical evaluation of ED

1. Intracavernosal pressure (PIC) creates penile rigidity.
2. The arterial discharge at the level of penile arteries by Doppler examination determines the peno-brachial index.
3. Cavity-venous flow determines induction rate and erection flow rate.
4. The penile tumescence test (TPN) is the standard of diagnosis and is especially useful in differential diagnosis (ED due to mental impairment is not characterised by low TPN).
5. The papaverine test induces a pharmacological erection and excludes the vascular cause.
6. The trial for alprostadil (prostaglandin PGE1) injectable is among the first objective investigations, but it has an orientational value, distinguishing between vascular DE and non-vasculogenic DE.
7. Sonographic evaluation and colour Doppler provide information on the presence of blood flow, its meaning and its speed.
8. Cavernography provides information on cavernous filling and the existence of penile fibrosis.
9. Internal arterial arteriography is the "golden standard" for the diagnosis of arteriogenic impotence.
10. Electrocardiogram of cavernous bodies gives information about the degree of inertia of the corpus cavernosum (EMG-CC).

The clinical diagnosis of ED is not the first easy to translate. A detailed history, including a personal account, the duration of diabetes, its degree of control (HbA1c%), the presence of hypoglycemia, the presence of complications, the pharmacological treatment of DM/complications, the compliance to treatment and diet, the family/comorbidities, alcohol/alcohol status, surgery history, sleep disturbances can often be present and can mask an ED. In this context, the ED history should be detailed and include information on the history of sexual function, reproductive history, the age of dysfunction, the way of installation (sudden/slow), aspects of intimate life: the couple's relationship, traumas, abuses. The general and specific abstracts must most often be doubled by the physical and biochemical examination, general and specialist investigations (urology -andrology, diabetes) as well as a psychological evaluation (see also Table 1).

Once the ED has been established, and its degree can be made a treatment plan. Currently, therapeutical options are multiple and include, on the one hand, DM treatment and on the other hand ED treatment and action on risk factors: cessation of smoking, limiting alcohol consumption and regular exercise. The choice of treatment will be made by a multidisciplinary team including the urologist, diabetologist, psychologist/psychiatrist, sexologist and andrologist [16]. Most of the time, the improved chronically imbalanced glycemic imbalance does not lead (HbA1c), and future complications have also found improvement in DE.

Antidiabetic medication can be criminalised for the onset or deterioration of ED only if, within two weeks of therapy administration, there is a sudden decline in ED. Elimination of risk

factors, psychological/sexual counselling, testosterone therapy - initiated only in the context of hypogonadism clinically proven by hormone dosing (testosterone, SHBG) along with oral and injectable preparations recommended in ED complete the therapeutic management (Tables 2, 3, 4).

Table 2. Oral preparations used in ED treatment

Oral therapy	**Action**	**Common side effects**
The Class of Inhibitors of Phosphodiesterase-5 (PDE5I)	Relaxation of smooth vascular muscles	Vomiting Rush facial Dyspepsia Headache
1.Sildenafil (Viagra)	It acts primarily on PDE 5 and, to a lesser extent, on PDE-6	It is not associated with nitrates -> cardiovascular risk Problems of discolouration of the blue/green colours due to the action on PDE-6
2.Tadalafil (Cialis^{r})	It acts primarily on PDE 5 and, to a lesser extent, on PDE-6	It can produce hypotension in the context of chronic alcoholism Retinal complications
3.Vardenafil (Levitra^{r})	It primarily focuses on PDE 5 and, to a lesser extent, on PDE-11	It should not be given after a meal rich in lipids.
4.Avanafil (Stendra)	It acts primarily on PDE 5 and has the fastest action in its class, entering in action in 15 minutes	Can cause ejaculatory disorders, visual disturbances, low blood pressure, priapism

Table 3. Intracavernosal injection

Injection treatment	**General action**	**General side effects**
Phenoxybenzamine	It produces a full erection and is maintained during sexual intercourse	Rush
Papaverine	Relaxation of smooth muscles	Hypotension
Prostaglandin E1	Vasodilatation, Relaxation of smooth muscles	The lack of response to the administration of the preparation, the short duration of action is rapidly metabolised
Phentolamine	It has a powerful vasodilator effect through a direct impact on vascular smooth muscle and alpha-adrenolytic outcome (more less). Inhibits 5-HT (hydroxytryptaminergic) receptors.	Priapism, a risk of injection site fibrosis

Table 4. Other therapeutic alternatives in the treatment of ED

Therapy	**Action**	**Adverse effects**
Endoureteral preparations: Alprostadil gel (Muse)	Promising but weaker results than injectables, causing erection	Less than injectable preparations
External Devices: Vacuum Devices	Prolonged erection	They have not been described
The arterial revascularisation of the deep penile artery	In clinically documented atheromatosis, the results are good, improving local blood flow and enhancing erection.	Priapism, surgical defects
Penile prosthesis with semirigid/inflatable prosthesis/reservoir through a surgical approach	Penile erection is weaker than previous methods.	Local infection Extrusion The irreversible destruction of cavernous tissue Pain Mechanical denture defect

Recent results have shown that administration of L-arginine or L--citrulline improves erectile function. These amino acids have the potential to increase nitric oxide and indirectly increase penile blood flow. This aspect is even more important in people with DM where the level of nitric oxide is low [17]. And the administration of vitamin D has a beneficial effect in patients with ED, diabetes and 25 (OH) vitamin D deficiency [18, 19].

Conclusions

ED is today better diagnosed, investigated and treated. The most important step is to correctly diagnose the diagnosis, more precisely if the ED is psychogenic or organic [20]. It is a real psychological problem when installing the first Symptoms of ED, but the collaboration between specialists of diabetes, urologists, gynaecologists and psychologists makes these pathologies much better today, increasing the quality of life of patients with DM. The treatment must be phased out, as proposed above, and interventional cardiology procedures, as well as revascularisation procedures, can bring real benefits to ED patients.

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Gastroprotective Effects of the Fatty Acid Esters and Ethanol-Amides Synthesised from Extra-Virgin *Oleum Olivae* – On Pharmacological Induced Ulcers and Gastric Secretion on Rat

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Abstract

Bioactive foods are gaining increasing attention due to the scientific evidences related to the correlations between diet and health status. The mediterranean diet is associated with reduced risk of developing chronic degenerative disorders such as cardiovascular diseases, type 2 diabetes and cancer, it can improve diseases symptoms in inflammatory degenerative diseases, acting both at local and systemic levels. The role of the extra virgin olive oil (EVOO) is essential, many studies showed the importance of a rich in olive oil diet. The role of polyfenols and free fatty acids in the EVOO is extensive investigated, corelating the action of the fatty acids and the endogenous endocannabinoid system. In this purpose, we measured the protective effect of the esthers and ethanolamides from the extra-virgin Oleum Olivae on experimental gastric ulcers and on the gastric secretion on rat. The therapy with the ethanolamides of the fatty acids in the presence of indometacin reduced the number of ulcers with 89.96% compared with indometacin and methylic esthers, reduced with 53.61%. The gastric acidity of the animals treated with indometacin and ethanolamides of the fatty acids decreased with 60.66% compared with the animals treated with indometacin only, and with 38.11% for the ethylic esters and indometacin; the ethanolamides of the fatty acids from the extra-virgin Olive oil presented a protective effect in the ulcers induced by indometacin.

Keywords: faty acids methylic esters, oleamides, anandamide, cannanbinoid, fatty acids ethanol amides, extra-virgin olive oil, gastric ulcers

Introduction

The extra-virgin olive oil (EVOO) is a bioactive food that contains polyphenols and mainly fatty acids, glicerids of the linoleic acid and arahidonic acid, trioleine, tripalmitine, sterols (beta-sitosterol) delta 7-stigmasterols, delta 5-avanasterol, insaponificable substances that are represented by iridoids, lignanic compounds and triterpens [1]. Several studies proved the importance of a rich diet in olive oil [2-4], the extra virgin olive oil containing polyphenols with biological action in therapy of arterial hypertension and also prevention of cardio-vascular diseases, improving the endothelial markers involved in blood pressure control in hypertensive women [5-7]. According to the current research data, olive oil polyphenols revert endothelial dysfunction induced by high glucose/free fatty acids. The regular consumption of extra virgin olive oil (EVOO) inputs high content of polyphenols with a recognised role in modulating

several molecular pathways and protects the plasmatic lipids from the oxidative stress; to the phenolic compounds but also due to the monounsaturated fats content, that have antioxidant, anti-inflammatory and immunomodulatory properties [8]. Polyphenols comprise thousands of compounds of plant secondary metabolites including flavonoids, isoflavonoids, phenolic acids, proantho cyanidins and other tannins, and lignans with different biological activities. The major polyphenols in olive oil are phenolic acids (hydroxytyrosol, tyrosol), secoiridoids (e.g. oleuropein) and lignans (e.g. pinoresinol) [9]. Polyphenols have been found to decrease heart disease risk factors by lowering blood pressure and cholesterol, reducing blood clotting and improving the health of artery linings. In the EU EFSA Regulation 432/2012 [9] the health claim regarding "the protection of blood lipids from oxidative stress" that can be attributed to the EVOO relates to the level of olive phenolic compounds, respectively "may be used only for olive oil which contains at least 5 mg of hydroxyl-tyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 mg of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20mg of olive oil" [10].

The olive oil comprises mainly of the monounsaturated fatty acid oleic acid (C18:1); a EFSA approved health claim on the unsaturated fatty acids (Commission Regulation (EU) 432/2012) reccomends that replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels.

Considering the fact that in the olive oil are present mono- and poly-unsatturated fatty acids, principaly in the form of triglycerides (Table 1) we elaborated a method of transformation of the extra virgin olive oil fatty acids in the corresponding methyl esters and ethanol amides at the National Institute of Chemical and Pharmaceutical Research and Development.

Several studies showed that ethanol amides of the fatty acids would represent a new class of the biologically active compounds "endocannabinoidome" [11] with potential anti-inflammatory and immune modulatory action [12] CNS function regulation of food intake and sleep [13], cicatrizing action [14]. According to the protective effects of the endocannabinoid system against the gastric lesions and its regulatory role in feeding behavior and inflammatory bowel disease [15-17], this ubiquitous signaling system is considered as an emerging target for the therapeutic interventions in the gastrointestinal (GI) disorders [18].

We approached two synthesis strategies based on previous experience in oleamide synthesis [19] to obtain the fatty acid ethanol amides starting form the *Olivae oleum virginale:* a) the aminolysis of the glycerides b) the transformation of the glycerides in the corresponding methyl esters.

Synthesis of amides like anandamide (N-arachidonoyl-ethanolamine – AEA – lipid mediator that acts as an endogenous ligand of CB1 receptors, exerts an overall modulatory effect on the brain reward circuitry [20]), prostamides, fatty acids amides, macamides, could be realized by several methods: direct estherification of acids with amines or with carbonyl-di-imidazole, acid chloride and amines, esters with amines catalyzed by bases like MeONa, we synthesized the tested products according with [11, 19, 21].

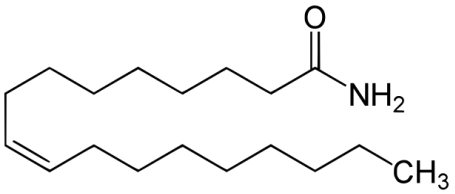

Fig. 1. The structure of oleamide – (Z)-Octadec-9-enamide (C18H35NO) – amide of oleic acid

The final product is a mixture of the corresponding ethanol amides in which predominates according to the procentual composition the ethanolamide of the oleic acid, the ethanolamide of the linoleic acid and a small quantity of the ethanolamide of the stearic acid [19]. The

structure of the methylic esters and of the 1-ethanolamides obtained was confirmed by the 1H-RMN spectres and IR. The present synthesis is subject of a patent.

In the present paper we tested the cicatrising action of the fatty acids esters and ethanol amides from the extra-virgin olive oil on the experimental ulcers provoked by indometacin and on the gastric secretion on rat.

Materials and Methods

The *Olivae oleum virginale* (olive oil) used in the study was manufactured in Greece, obtained by cold pressing and centrifugation of the mature fruits of the species *Olea europea L.* with quality specifications according to the European Pharmacopoeia [22], the monography *Olivae oleum virginale.* We transformed the olive oil fatty acids in the corresponding methylic esters and ethanol amides. We took into study pharmaceutical grade raw materials processed and controlled in our laboratory. We took into study batches of white, male Wistar rats, weighing 200+/-10 g, kept into mobile cages, in special shelters, in standard laboratory conditions. The animals were purchased from the Animal Biobase of the University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania.

With 24 hours before the study the animals were restricted from food during 24 hours and water "*ad libitum*". All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002. The animals were distributed in six batches of eight animals each.

The first batch was the control, with 24 our food restriction before being sacrificed. The second batch was orally treated with indometacin 10 mg/body weigh (b.w), orally, during 4 days, the third batch received indometacin 10 mg/b.w. orally and methylic esters of the fatty acids in dose of 250 mg/b.w. orally during a 4 days period; the fourth batch received indometacin 10 mg/b.w. orally and the ethanol amides of the fatty acids from the olive oil in dose of 250 mg/b.w. orally during a 4 days period and the batch 5 was treated with indometacin 10 mg/b.w. orally and 5 ml/b.w. orally extra-virgin *Oleum olivae*. The batch 6 received ranitidine 10 mg/b.w. orally as a reference substance because inhibits the basal secretion and stimulated and it has a long acting activity.

The measurement of the gastric secretion was done by the Shay method of ligature of the pillory. After two hours the animals were sacrificed under chloroform anesthesia. The stomach was excised and dissected on the large curve margin, the gastric secretion was collected and the stomachs fixed on the contention plates, the mucous was examined with the magnifying lens, marking the ulcers appeared and their dimension.

After the measurement of the gastric secretion volume, the gastric content was centrifuged and the gastric acidity was measured by the Topfer Linossier method. The percentage index of protection (IPP) is calculated with the formula:

$$IPP=M/Tx100 \quad [8]$$

M=gastric acidity for the control animals; T=gastric acidity for the treated animals

The statistical evaluation of the results was made by the "t" student test. Data were analyzed using t tests (for single between-group comparisons). Data were expressed as mean ± standard error. A p value of 0.05±SD was considered statistically significant.

Results and Discussions

The macroscopic analysis

After the sacrification of the animals the stomach was excised, cut on the large curve margin, washed for eliminating the eventual impurities and fixed on contention plates. The anatomopathological preparates are examined with the magnifying lens and measured the

lenght of the lesions for each stomach. The indometacine treated batches presented multiple ulcers of variate dimensions (**Fig. 2-7**).

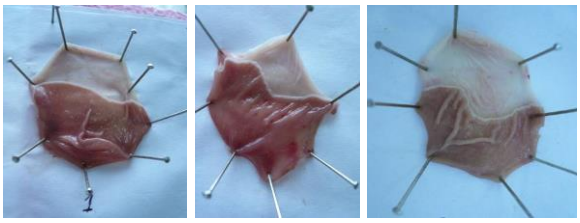

Fig. 1. Anatomo-pathological aspect of the gastric mucosa in the control batch

Fig. 2. Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine

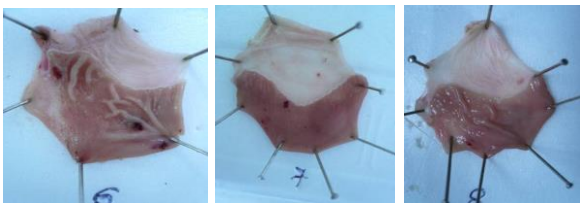

Fig. 4. Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine and olive oil

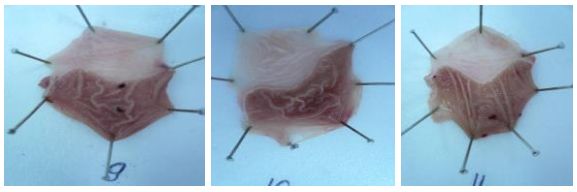

Fig. 5. Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine and amides

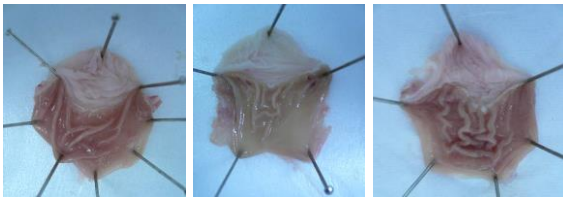

Fig. 6. Anatomo-pathological aspect of the gastric mucosa after administration of indomethacin and famotidine

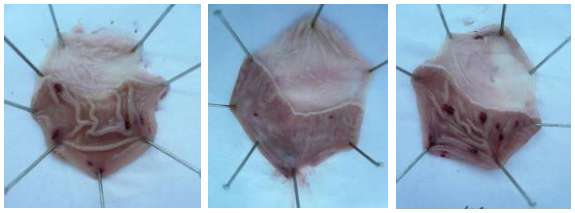

Fig. 7. Anatomo-pathological aspect of the gastric mucosa after administration of indomethacin and esters

The association of the indometacin with the ethanolamides of the fatty acids from the olive oil had a protection of 89,96% compared with the treated batch only with indometacine and a protection of 53,61% for the batch treated with the mehylic esthers. Also the extra-virgin *Olivae oleum* presented a protection of 66,26% compared with the indometacine (**Table 1-2**).

Table 1. The influence of the ethanolamides and of the esters of the fatty acids from extra-virgin *Oleum Olivae* on the experimental ulcers produced by indometacin

Tested product	**Ulcers no. x+/-e.s.**				**media**	**Effect%**
	<0,5 mm	**2 mm**	**4 mm**	**6 mm**		
Untreated controls	2,3±0,40	1,6±0,50	1±0,6	0	1,22	-
Indometacine	5,2±0,70	6±0,80	3,2±0,4	2,2±0,3	4,15	-
Indmethacine + methilic esthers	4,4±0,30	3,3±0,4	0	0	1,92	-53,61
Indometacine +ethanolamides	1,75±0,20	0	0	0	0,43	-89,96
Indometacine + *Oleum Olivae*	2,6±0,20	3±0,25	0	0	1,40	-66,26
Ranitidin	1±0,09	0	0	0	0,25	-93,97

x+/-e.s.=media +/- standard error, p<0,05

Determination of gastric secretion acidity

The animals treated with the methylic esters of the fatty acids from *Oleum olivae* presented a decrease of the gastric acidity with 38,11% compared with indometacine, and those treated with ethanolamides presented a decrease of 60,66%. Also the extra-virgin *Olivae oleum* reduced the gastric acidity with 32,84% compared with the animals treated with indometacine.

Table 2. The influence of the ethanolamides and of the esters of the fatty acids from extra-virgin *Oleum Olivae* on the gastric secretion produced by indometacin

Batch	**Gastric volume content** (mL)		**Total acidity** (mEq/L)		
	xe.s.	*Efect%*	*xe.s.*	*Effect %*	*IPP*
Untreated controls	2,75±0,3	-	14,67±1,3	-	-
Indometacine	4,10±0,45	49,09	27,10±2,90	84,73	-
Indmethacine + methilic esthers	3,28±0,40	19,27	16,77±1,40	-38,11	87,47
Indometacine + ethanolamides	2,36±0,32	-14,18	10,66±0,95	-60,00	137,61
Indometacine + *Oleum Olivae*	3,10±0,35	12,72	18,2±1,60	-32,84	80,60
Ranitidin	1,3±0,22	-52,72	6,85±0,70	-74,72	214,16

x+/-e.s.=media +/- standard error, p<0,05, IPP = percentage protection index

Conclusions

The ethanolamides of the fatty acids from extra-virgin *Olivae oleum* presented the most pronounced effect in the ulcers provoked by indometacine and of decrease of the gastric acidity on rat.

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The Release of Simvastatin from Coated Tablets Containing Inclusion Complexes Between Simvastatin and Methyl-Beta-Cyclodextrin

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Abstract

The aim of the present work was the study of in vitro release of simvastatin from coated tablets containing inclusion complexes between simvastatin and methyl-beta-cyclodextrin and the comparison of dissolution profiles obtained for the tested product and the reference product (Zocor®, Merck Sharp & Dohme Corp.), showing the influence of methyl-beta-cyclodextrin on this.

The dissolution studies were made according to the USP 34 methodology, by using the paddle apparatus Erweka DT 800. The official method in USP 34 is a derivate spectrometry method, used to clear the influence of the excipients, being a precise, high sensitivity and selectivity method. A spectrometer UV-VIS Nicolet Evolution 100 was used.

In comparison with Zocor® 10 mg coated tablets which released after 45 minutes only 80% of the contained quantity, the coated tablets containing simvastatin – methyl-beta-cyclodextrin inclusion complex showed an excellent drug release, with significant differences, releasing in the first 30 minutes almost all quantity of simvastatin contained. This is due to the fact that simvastatin forms with methyl-beta-cyclodextrin aqueous soluble complexes.

Keywords: simvastatin, methyl-beta-cyclodextrin, inclusion complex, coated tablets, dissolution rate

Introduction

Simvastatin (SV) is a drug that decreases cholesterol level, being structurally derived through fermentation of an Aspergillus terreus product, being largely used to treat hypercholesterolemia. It is a potent inhibitor of 3-hydroxyl-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, that catalyzes conversion of HMG-CoA to the mevalonate. This conversion is rate-limiting and an early step in cholesterol biosynthesis. SV is a white crystalline powder and nonhygroscopic, insoluble in water and to a lesser extent absorbed from the gastrointestinal tract. Moreover, SV is easily degraded in an oxidation process that is accelerated by humidity, high temperatures, light, and oxidizing agents. Complexation of SV with methyl-β-cyclodextrin (Me-β-CD) offers the possibility to enhance the aqueous solubility and dissolution rate of drug from its oral dosage forms, and also to increase the stability of SV [1-3].

Me-β-CD has a high aqueous solubility (over 20%) and a good ability to stabilize the drug molecule [4].

In order to ensure the stability of SV in tablets, we prepared coated tablets containing inclusion complexes between simvastatin and methyl-beta-cyclodextrin. The aim of the present work was the study of in vitro release of simvastatin and the comparison of dissolution profiles obtained for the tested product and the reference product (Zocor®, Merck Sharp & Dohme Corp.), showing the influence of methyl-beta-cyclodextrin on this.

Materials and Methods

SV was obtained from Biocon Limited Biopharmaceuticals, India, and HP-β-CD was purchased from Sigma-Aldrich Chemie GmbH, Germany. The core-tablets were prepared by direct compression method, by thus avoiding heat and humidity factors during the manufacture of the tablets, using a Korsch EK-O tabulating machine adjusted to obtain core-tablets with an average weight of 200 mg, this corresponding to a contain of 10 mg simvastatin/tablet [5]. The coating of the core-tablets was made with an 8 % aqueous dispersion of Opadray II yellow (produces by Colorcon Ltd, England), which forms an aqueous soluble film, with immediate disintegration for fast, active release. The coating was performed using the fluid-bed technique, and the Caleva Mini Coater/Drier 2, produced by Caleva Process Solutions Ltd, Great Britain.

The resulting coated tablets were subjected to the dissolution test, according to the USP 34 methodology [6], by using the paddle apparatus Erweka DT 800.

The official method in USP 34 is a derivate spectrometry method, used to clear the influence of the excipients, being a precise, high sensitivity and selectivity method [7].

The dissolution test was performed using a dissolution medium consisting of 900 ml of pH 7 buffer solution containing 0.5% sodium dodecyl sulfate (for its tensioactive effect) in 0.01 M sodium phosphate, the apparatus being settled at 50 rpm.

We determined the amount of simvastatin that is dissolved from difference existing between UV absorbances that at the wavelengths of maximum and minimum absorbance, which takes place at about 247 nm and 257 nm, respectively, on portions that are filtered on simvastatin test solution, in comparison with a standard solution. A spectrometer UV-VIS Nicolet Evolution 100 was used. According to USP 34, not less than 75% of the labeled amount of simvastatin should be dissolved in 30 minutes [8].

The extracted concentrations of the samples at different periods of time are using to calculate the amount of active differential released (in the spell between two analyses).

The formulas used to calculate the quantity of simvastatin released are:

After 15 minutes:

$$\% \text{ simvastatin released} = \frac{A_p}{A_e} \times \frac{m_e}{D_e} \times \frac{D_p}{10} \times 100$$

where:

A_p=the absorbance of the test solution
A_e=the absorbance of the standard solution
m_e=weight of the standard simvastatin (mg)
D_e=the dilution factor of the standard solution (ml)
D_p=the dilution factor of the test solution (ml)
10=the labeled quantity of simvastatin in each tablet (mg)

After 20 minutes – 45 minutes:

$$\% \text{ simvastatin released} = \frac{1}{A_e} \times \frac{m_e}{D_e} \times \left[(D_p \times A_p) + (20 \times A_{p\,15(20,30,45)}) \right] \times \frac{100}{10}$$

where:
A_p=the absorbance of the test solution
A_e=the absorbance of the standard solution
m_e weight of the standard simvastatin (mg)
D_e=the dilution factor of the standard solution (ml)
D_p=the dilution factor of the test solution (ml)
$A_{p\ 15\ (20,\ 30,\ 45)}$ = the absorbencies of test solutions at 15, 20, 30, 45 minutes
10=the labeled quantity of simvastatin in each tablet (mg)

Simultaneously, to compare the obtained results for the studied tablets, we performed the dissolution test also, for the reference product Zocor® 10 mg, coated tablets.

Results and Discussions

We tested 6 coated tablets of each simvastatin-Me-β-cyclodextrin complex and Zocor series, all calculations being made with a double precision as defined by ANSI/IEEE STD 754 – 1985, but they were rounded by display reasons [9].
Figure 1 shows the simvastatin release from the reference product, Zocor coated tablets.

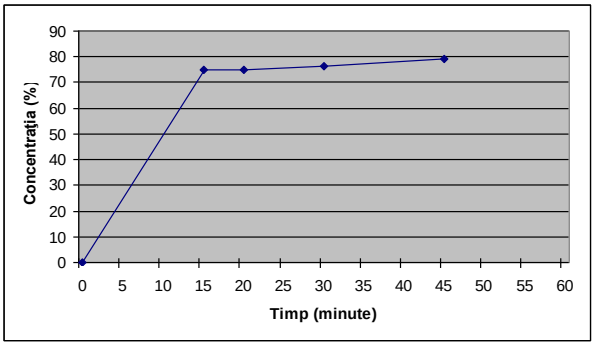
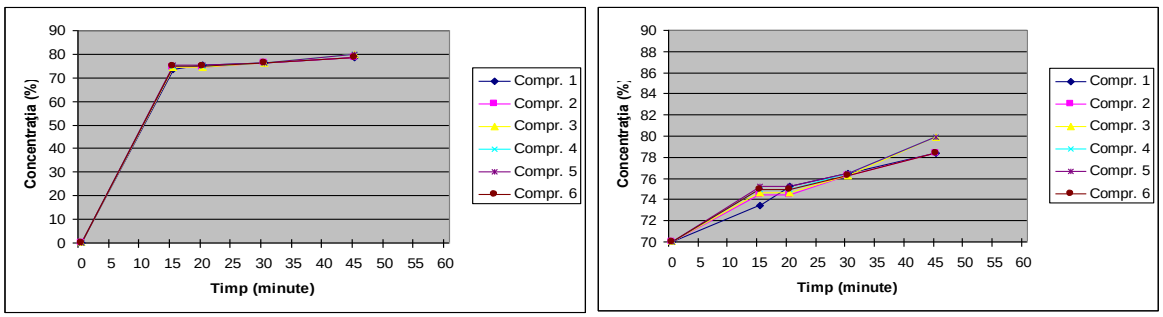


Fig. 1. The variation of medium concentration of simvastatin released in time by Zocor 10 mg coated tablets

Fig. 2 (a and b) presents the variation of simvastatin concentration released in 45 minutes for the 6 tested coated tablets of Zocor 10 mg.



2a 2b

Fig. 2. The variation of simvastatin concentration released in 45 minutes for the 6 tested coated tablets of Zocor 10 mg

We can notice the fact that simvastatin is released from the Zocor coated tablets in a large quantity after 15 minutes, in the interval 20-45 minutes the increase of the concentration occurs, but not being significant. It is important to mention the fact that not even after 45 minutes the entire quantity of simvastatin contained by the tablets and revealed in the assay studies was released, but only 80% of this. Anyway, this is a predictable behavior for a lipophilic and practically insoluble in water, substance as simvastatin.

The tables no. 1, 2, 3, and 4, and figure 3 show the simvastatin release from the coated tablets containing SV-Me-β-CD inclusion complex.

Table 1. The values of specific absorbances at 247 nm and 257 nm and of the concentration of the 6 coated tablets containing SV-Me-β-CD complex, at 15 minutes

No.	*Standard*			*Sample (coated tablets containing SV – Me – β – CD complex)*			*Concentration %*
	Maximum of absorbance	*Start moment*	*The difference*	*Absorbance at 247 nm*	*Absorbance at 257 nm*	*The difference*	
1.	0,744	0,023	0,721	0,570	0,169	0,401	95,2
2.				0,571	0,170	0,401	95,2
3.				0,567	0,168	0,399	94,9
4.				0,571	0,171	0,400	95,1
5.				0,571	0,170	0,401	95,2
6.				0,570	0,171	0,399	94,9
Media							95,08

Table 2. The values of specific absorbances at 247 nm and 257 nm and of the concentration of the 6 coated tablets containing SV-Me-β-CD complex, at 20 minutes

No.	*Standard*			*Sample (coated tablets containing SV – Me – β – CD complex)*			*Concentration %*
	Maximum of absorbance	*Start moment*	*The difference*	*Absorbance at 247 nm*	*Absorbance at 257 nm*	*The difference*	
1.	0,744	0,023	0,721	0,573	0,170	0,403	95,7
2.				0,574	0,171	0,403	95,7
3.				0,572	0,170	0,402	95,5
4.				0,572	0,169	0,403	95,7
5.				0,572	0,168	0,404	95,9
6.				0,571	0,169	0,402	95,5
Media							95,67

Table 3. The values of specific absorbances at 247 nm and 257 nm and of the concentration of the 6 coated tablets containing SV-Me-β-CD complex, at 30 minutes

No.	*Standard*			*Sample (coated tablets containing SV – Me – β – CD complex)*			*Concentration %*
	Maximum of absorbance	*Start moment*	*The difference*	*Absorbance at 247 nm*	*Absorbance at 257 nm*	*The difference*	
1.	0,744	0,023	0,721	0,573	0,169	0,404	95,9
2.				0,572	0,169	0,403	95,7
3.				0,573	0,169	0,404	95,9
4.				0,572	0,169	0,403	95,7
5.				0,574	0,170	0,404	95,9
6.				0,571	0,168	0,403	95,7
Media							95,80

Table 4. The values of specific absorbances at 247 nm and 257 nm and of the concentration of the 6 coated tablets containing SV-Me-β-CD complex, at 45 minutes

No.	*Standard*			*Sample (coated tablets containing SV – Me – β – CD complex)*			*Concentration %*
	Maximum of absorbance	*Start moment*	*The difference*	*Absorbance at 247 nm*	*Absorbance at 257 nm*	*The difference*	
1.	0,744	0,023	0,721	0,574	0,170	0,404	95,9
2.				0,574	0,170	0, 404	95,9
3.				0,575	0,171	0, 404	95,9
4.				0,573	0,169	0, 404	95,9
5.				0,575	0,171	0,404	95,9
6.				0,573	0,170	0, 403	95,7
Media							95,87

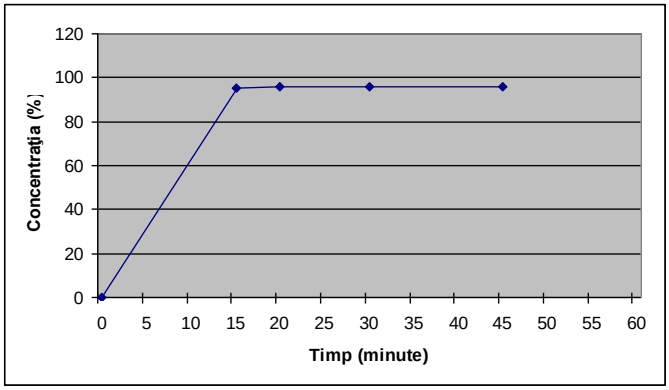
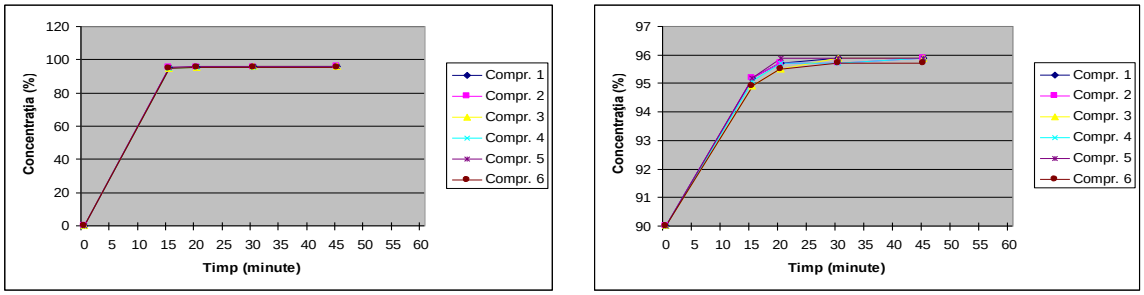


Fig. 3. The variation af medium concentration of simvastatin released in time by the coated tablets containing SV-Me-β-CD complex

Fig. 4 (a and b) presents the variation of simvastatin concentration released in 45 minutes by the 6 tested coated tablets containing SV-Me-β-CD complex.

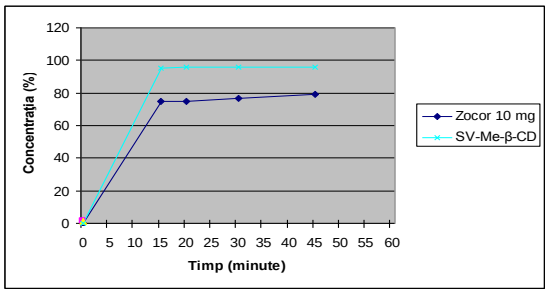


4a 4b

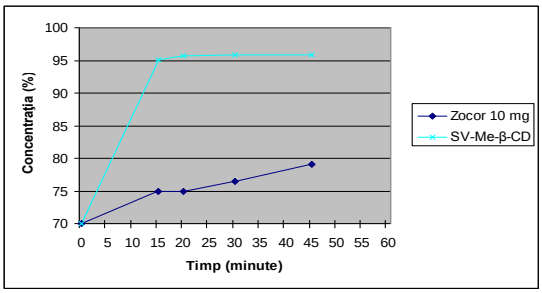
Fig. 4. The variation of simvastatin concentration released in 45 minutes by the 6 tested coated tablets containing SV-Me-β-CD complex

For the coated tablets containing SV-Me-β-CD complex, we can observe the fact that in the first 15 minutes almost entire quantity of contained simvastatin, as the assay studies showed, is released. We also notice the uniformity of the results obtained between the coated tablets.

The fig. 5 (a and b) presents the compared release of simvastatin, during the 45 minutes, from the coated tablets containing SV-Me-β-CD complex and the coated tablets of Zocor.



5a



5b

Fig. 5. The variation of the medium concentration released in time by the coated tablets containing SV-Me-β-CD complex and the coated tablets of Zocor

Unlike the coated tablets of Zocor which registered higher concentrations on the assay studies, but after 45 minutes they released only 80% of the contained simvastatin, the coated tablets containing SV-Me-β-CD inclusion complex presented an excellent release of the active, with significant differences, releasing after 15 minutes almost entire quantity of simvastatin contained.

In the Zocor tablets the simvastatin is processed and compressed after wet granulation, while in the tablets containing the inclusion complexes with Me-β-cyclodextrin this is direct compressed. This should not have a pronounced influence on the simvastatin release, more influence having the fact that it forms with β-cyclodextrins water soluble complexes.

Conclusions

In conclusion, we can say that the studied coated tablets containing SV-Me-β-CD inclusion complex present an optimal release of simvastatin, within the admissible limits provided by USP 34, being at least 75% after 30 minutes. We can notice that for the coated tablets containing SV-Me-β-CD inclusion complex the release of the active was fast, but released the quantity is proportional to complex solubility in aqueous solution at the enteric pH.

The release of the simvastatin from the Zocor coated tablets is actually a slow release, the value obtained after 45 minutes not being a saturation value, meantime at the SV-Me-β-CD coated tablets, after 45 minutes the saturation is reached. More, we can observe, that after 15 minutes, it is almost saturation. The analytical variability between the both types of coated tablets of the same series is insignificant, being of 2% for the SV-Me-β-CD tablets, and of 4% for the Zocor tablets. In these terms, the variability between series is more significant than the intraseries one, this being of 15%.

The simvastatin release ratio is great and could be distinguished also due to the used derivate spectrometry method, but mostly to the use of the sodium dodecyl sulfate with its tensioactiv effect. Also, in the fast release of simvastatin from the studied SV-Me-β-CD coated tablets, very important roles had also the used method of direct compression, with all its advantages, the direct compression excipient Flowlac, and the coated agent Opadry II.

By all these, we can conclude the fact that Me-β-CD by enhancing the aqueous solubility of simvastatin, much influences its release from the tablets, in the way of raising the available pharmaceutical quantity of the active.

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The Challenge of Portal Vein Thrombosis in Non-Cirrhosis Patients

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Abstract

The portal thrombosis is a severe and frequent complication of hepatic disease and general conditions, like congenital or acquired thrombophilias. The G20210A mutation in the Prothrombin gene is, after V Leiden factor, the second common congenital thrombophillia and is associated with a moderate risk of thrombosis in the venous system; the article presents two cases of portal and superior mesenteric thrombosis due to this anomaly, underlines the attenuate clinical picture and the necessity for seeking for a contributing factor and discuss the treatment options.

Keywords: Prothrombin Related Thrombophilia, G20210A factor II mutation, portal vein thrombosis

Introduction

The portal vein thrombosis is a common complication of liver cirrhosis and liver neoplasms, but it can appear also in other procoagulant circumstances, like acquired or inherited thrombophilia. Between the latter, the most known is the presence of the Leiden factor, but with a great prevalence is also the mutation G20210A in the fibrinogen gene, that induces an abnormal II Factor, more resistant to clearance. The article proposes a discussion about Factor II mutation 20210A and its clinical consequences, by presenting two cases of patients with portal vein thrombosis due to the above-mentioned mutation. Both patients were extensively investigated before to establish the diagnosis, and both were successfully treated with an antagonist of K vitamin. Beyond the practical issues, some problems remain to be solved: the residual risk, the treatment duration and, perhaps new therapeutic approaches.

Portal Vein Thrombosis

The portal vein thrombosis (PVT) implies occlusion with a thrombus of the portal vein, one of its intra- or extrahepatic branches, and sometimes is accompanied by other visceral thrombosis- most commonly in the superior mesenteric vein. It is a usual finding in hepatic cancer and in class C Child cirrhosis, where it aggravates a preexistent portal hypertension.

Other etiologies include abdominal sepsis, surgery or trauma, inherited or secondary thrombophilia, endoscopic sclerotherapy, transjugular intrahepatic portosystemic shunt, abdominal inflammation and mechanical compression (e.g. abdominal tumors, retroperitoneal fibrosis) [1].

Due to acute obstruction of the portal vein and/or its branches, the thrombus may extend into the mesenteric or splenic vein, complete or partial, with a quick elevation into the entire portal system; that can be followed, in the absence of a proper anticoagulant therapy, by the most severe complication- intestinal infarction.

Despite of its important clinical significance, the clinical manifestation can be mild or not so severe as is expected, with the main symptoms being abdominal pain; nonspecific complains, like dyspeptic and even fever, are also common.

There are no specific signs at physical examination and the diagnosis is established by abdominal imaging: ultrasonography and Doppler examination, CT- scan and MRI, usually with contrast. For an acute or subacute onset of PVT, suggestive is the presence of a lacunar image in the porta; a lack of radiological signs of cavernous portal transformation or of hyperechoic intravascular image are suggestive for an old, organized thrombus.

In the absence of C Child class cirrhosis (which is per se the most common etiology of PVT) or hepatic cancer, the next level in diagnosis is the identification of predisposing conditions, in order to obtain valuable clues for an eventually specific treatment, beside anticoagulation.

The anticoagulant therapy (standard, initially with heparin and after loading – dose, with antiK vitamin compounds, for an INR 2-3) is the actual standard [2], and its duration in patients with a good response (clinically and imagistic, with re-canalization or cavernomatous transformation of the culprit vessel) it is between three and six months. If the patient was diagnosed with a prothrombotic status, or the mesenteric vein thrombosis is present, the long-term therapy remains, in spite of the lack of clear guidelines, the most common approach.

The next two cases illustrate portal and superior mesenteric vein thrombosis, in two patients with a congenital prothrombotic status- the G20210A mutation in Factor II gene.

Case 1

A 53 years patient, male, non-smoker and without any known pathology, presented to our hospital for an abdominal pain episode, that occurred about 2 weeks before admission; it was preceded for 7 days of loss of appetite. He was hospitalized in a regional hospital, were he was diagnosed with portal vein thrombosis. He was referred to our hospital for extensive investigation regarding etiology.

There was no history of a recent travel, nor for environmental exposures or for abdominal trauma, surgery. He was non-smoker and with a moderate alcohol intake, about 3-5 units/week.

The clinical examination revealed a normoponderal patient, with normal general, respiratory and cardiac examination, with a slight sensibility during deep palpation in the upper abdominal quadrants, but normal liver and spleen findins. Noticeable, there were no jaundice, nor ascites or collateral circulation on the abdomen.

The common laboratory studies were unrevealing (Table 1), negative markers for hepatitis B or C, no abnormal tumoral markers; chest Rx-normal; ECG-normal sinus rhythm, the echocardiography was normal except a slight increased peak systolic arterial pulmonary pressure- 30 mmHg.

The abdominal ultrasound showed a dilated porta- 15 mm and a possible residual clot in it, no ascites and, supplementary, multiple renal cysts. CT images confirm the first diagnosis of portal vein thrombosis and superior mesenteric vein thrombosis.

The superior digestive endoscopy, colonoscopy and prostate examination were negative for cancer; bone marrow aspirate was normal and allow us to eliminate a procoagulant paraneoplasic status.

A panel for procoagulant status was performed: lupus anticoagulant, APCR (for factor V Leiden), antithrombin mutation – were normal, but a heterozygote mutation in the in the prothrombin gene II Factor was identified- mutation G20210A.

Therapy: acenocoumarol (for INR 2-2,5) and, after one year from the onset, the evolution was very good- no symptoms, no new thrombosis and the portal vein suffered a cavernomatous transformation. Three years after the diagnosis- the patient is asymptomatic, on antivitamine K treatment, no recurrence of any kind of thrombosis.

Table 1.

	June 2013	Julv 2013	December 2018
WBC (cells/ µl)	4950	5700	N/A
Hb (g/dl)	13,1	12.6	N/A
PLT (per µl)	237000	221000	N/A
Creatinine (mg/dl)	1.35	1.36	N/A
AST (U/l)	26	28	N/A
ALT (U/l)	40	30	N/A
ALKP (U/l)	55	64	N/A
GGT (U/l)	37	28	N/A
TP (serum)	7,4	7.2	N/A
Alb (g/dl)	4,2	4.1	N/A
T-Bil (mg/dl)	0.7	1.1	N/A
LDH (U/l)	437	428	N/A
D- Dimers qualit	positive	Negative	N/A
PT (sec)	12.8	33.3	N/A
Fibrinogen(mg/dl)	432	330	N/A
Lypase (U/l)	280	191	N/A

Case 2

The second case is also a man, 44-year- old, nonsmoker, admitted to hospital with a 3-week history of sudden onset of a mesogastric abdominal pain and constipation. He had no abdominal trauma or surgery in the past.

The patient's past medical history was not significant in the present context and only included an old (more than five years before) episode of gastroenteritis and a recently diagnosed grade 2 arterial hypertension (maximum systolic blood pressure=160 mmHg), dyslipidemia.

The physical examination was unrevealing, except a pain elicited by palpation the abdomen, in the periumbilical and epigastric regions; no ascites or collateral abdominal circulation.

The initial evaluation found a slight hepatic cytolysis, cholestasis (Table 2); no signs of hepatocellular failure, pancreatitis, the markers for hepatitis B or C were negative, the common tumoral markers (CEA, CA 19-9, AFP)- in the normal range.

The barium enema, gastroduodenoscopy and colonoscopy, PSA were all normal; bone marrow aspiration didn't reveal signs of a hematologic malignancy. All those allowed us, with a great probability, to rule out a cancer.

The seeking for an inherited thrombophilia found a normal APCR (for Factor V Leiden), normal C protein and S protein, negative for lupus anticoagulant, but molecular genetic tests identified a heterozygous mutation of factor II, mutation G20210A which we hypothesize that is the cause of the patient's prothrombotic state. He was started on anticoagulation therapy with acenocoumarol.

After 3 months of anticoagulant therapy, the symptoms ceased, the biochemical panel returns to normal (Table 2) and a second CT scan showed an increased portal vein diameter, portal cavernomatous transformation of the right portal branch, thrombosis of proximal part of the

superior mesenteric vein and collateral porto-systemic circulation at the level of the gall bladder bed, perigastric area and minimally in the splenic hilum.

The anticoagulant therapy was continued; the patient is asymptomatic after five years, without recurrence of thrombosis in the portal system or elsewhere.

Table 2.

	January 2015	April 2015	June 2018
WBC (cells/µl)	7800	6400	7200
Hb (g/dl)	13.3	14.4	14. 2
PLT (per µl)	448000	278000	395000
Creatinine (mg/dl)	0.9	0.86	0.87
AST (U/l)	57	32	35
ALT (U/l)	108	68	58
ALKP (U/l)	151	77	68
GGT (U/l)	348	81	80
TP (serum)	7.79	7.12	7.20
Alb (g/dl)	4.49	4.35	4.4
TBil (mg/dl)	0.9	0.9	0.91
LDH (U/l)	437	428	430
D-Dimers qualit	positive	Negative	Negative
INR	1.1	2.5	2.4
Fibrinogen (mg/dl)	432	275	310
Lypase (U/l)	293	N/A	N/A

Discussions

Prothrombin Related Thrombophilia (PTM, Prothrombin 20210G>A Thrombophilia, Factor II G20210A variant, *F2*-Related Thrombophilia, Factor II-Related Thrombophilia, Prothrombin G20210A Thrombophilia Prothrombin Thrombophilia).

Prothrombin related thrombophilia is one of the most common etiologies of inherited thrombophilia (approximatively 2% in general population, more frequent in the caucasians) [3].

It is due to a point mutation in the 3'untranslated region of the prothrombin gene (the substitution of A for G at position 20120). It is characterized by an enhanced clot formation and by venous thrombembolism (VTE) [4], but the clinical expression is variable; so, many individuals heterozygous or homozygous for the 20210G>A allele in F2 never develop thrombosis, but some adults experience recurrent episodes of thrombosis, with a relative risk of two- fivefold increase [5].

The clinical presentation has no specific features, the main clinical syndrome is venous thromboembolism; the arterial thrombosis (myocardial infarction, stroke, acute peripheral ischemia) appear to be as in the general population [6]. Many individuals never develop a thrombotic episode; from the symptomatic subjects, most of them have the first episode in adulthood, sometimes with recurrent VTE before the age of 30. Apart of common deep venous thrombosis in the legs and pulmonary embolism, individuals carrying this mutation have also

an increased risk for upper extremity thrombosis, for cerebral vein thrombosis (a six to tenfold increase risk, especially in women taking oral contraceptives), for unusual locations like retinal vein thrombosis or superficial venous clot formation. Among them there is an increased risk of portal vein involvement, both idiopathic or associated with cirrhosis [7].

As regard the recurrence after a first episode of VTE, there are data that support the idea of an increased risk and other that sustain the opposite [8]. In 2011, the EGAPP Working Group concluded that the heterozygosity is not a predictive factor for another thrombotic event in the adult people [9]. In the homozygous status for 20210G>A or multiple prothrombotic associations- the risk is more important, the same is higher for children and pregnant women (especially if the previous episode was related with the contraceptive uses).

Factors that predispose to thrombosis:

1. The number of 20210G>A alleles- the homozygotes have an increased risk versus heterozygotes;
2. Other coexistent inherited thrombophilic disorders:
    a) Factor V Leiden- a sevenfold increase in risk, if a subject is heterozygote for both mutations; these patients are prone to develop thrombosis in unusual locations (portal, mesenteric, cerebral sites);
    b) deficiency in anticoagulants proteins S or C;
    c) other genetic factors, like the (normal) variants of the F8 gene (coding factor VIII)- F8 Val34Leu or SERPINE 1 (coding Plasminogen Activator Inhibitor 1- PAI 1), SERPINE 1 4G polymorphic allele;
    d) the familial history of thrombosis, even for heterozygosity, in a first degree relative; the risk is higher with the number of relatives affected and with a younger age of the first episode.
3. Coexistence of acquired thrombophilic disorders: hyperhomocysteinemia, antiphospholipid antibodies, malignancy;
4. Circumstantial risk factors - are mainly related to estrogen exposure or pregnancy - excluded, because we investigated two man, without any kind of hormonotherapy; other situations- like organ transplant, central venous catheter, surgery, injuries or travel- were also not applicable to our subjects [10].

Our patients were not carrying an additional mutation for the factor V Leiden, but, of course, we cannot exclude other rarer mutations. Because of association with cancer, seeking for a malignancy was very important in these patients. Even the homozygotes subjects are at a higher risk for developing thrombosis than the heterozygotes, there are no differences in the severity of clinical manifestations or a special resistance to treatment between the two entities.

The treatment for the first episode starts with heparin (usually low molecular weight heparin) or fondaparinux and anti K vitamin drug (after a proper loading), for an INR~2,5 (2-3); the direct factor X inhibitor rivaroxaban is also approved [1]. The duration of therapy is under debate and depends of factors like individual risk of recurrence versus the risk of bleeding. The risk to develop a new thrombosis in the next five years after a first episode of VTE is approximately 30%, but there are no data available for recurrence after splanchnic thrombosis.

The risk of recurrence depends on the clinical circumstances of the first event, individual risk factors, as described earlier and the adequacy of initial treatment. Generally- anticoagulation for 3 to 6 months is indicated. Long term anticoagulation is administered to subjects with unprovoked thrombosis, with a good anticoagulation monitoring, in the absence of the bleeding risk factors; it is also reasonable in some homozygous for 20120G>A allele, or if another thrombophilia is present [11]. For long term therapy are used LMWH, acenocoumarol or warfarin; non-anti K vitamin anticoagulants: argatroban, dabigatran, lepirudine, apixaban and rivaroxaban are approved for use, too [9].

Considering that our two patients had a first episode of unusual site thrombosis (portal and superior mesenteric vein), unprovoked by a special circumstance and with a low risk of bleeding- we choose to prescribe long term anticoagulant therapy- in both patients acenocoumarol [12]. Prothrombin related thrombophilia is inherited as an autosomal dominant trait. The genetic testing is indicated in adult first degree relatives of probands, especially in those with a strong family history of VTE at a young age and female family members who intend to become pregnant or to begin a hormonal therapy with estrogens (like oral contraceptives) [9]. One of our patients has a daughter, but she was not tested until now.

Conclusions

The emerging data on thrombophilias explain some splanchnic thrombosis that appear "unprovoked"; it has a very unspecific clinical presentation. Between these kinds of procoagulant states, the factor II related thrombophilia appears to be sufficient common to justify an early screening in this clinical circumstance.

In this moment we don't have enough data regarding the duration of anticoagulant therapy, nor if the different classes are equal as efficacy and safety, but the current opinion is that is reasonable to do it indefinitely.

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Treatment Algoritm of Lip Cancer

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Abstract

More than 20% of all oral cavity carcinomas involve the lips and the first step of treatment requires surgical excision with oncological safety margins. Reconstruction of the lips remains a challenging procedure. A precise analysis of the defect and a preoperative planning is essential. There are a variety of reconstruction procedures depending on the size and the location of the defect and also on tissue elasticity.

For defects that involve less that 30% of the lip, primary suture is recommended in comparison with larger defects where local flaps that use tissue from the opposite lip or adjacent cheek are used. Total lip defects reconstruction is difficult to be realised with local flaps, so distant flaps or free flaps are usually utilised. In order to achieve the best functional and aesthetic results, surgeons should choose the most suitable reconstruction procedure. In this paper we review some of the techniques used for lip reconstruction in our department after surgical excision of cutaneous tumors.

Keywords: Lip tumour, lip reconstruction, local flap

Introduction

Reconstruction of lip defects represents a great challenge for plastic surgeons owing to the fact that lips are the focal point of the lower third of the face being responsible by the verbal and nonverbal communication. Lips have an essential role in daily living, being involved in speech, nutrition and facial expression [1]. These have also an important sensory and aesthetic role providing individualized beauty [2].

Perioral defects may be a consequence of trauma, oncological resections, burn or congenital deformities. The most frequent cause of lip defects is the excision of malignant lesions, squamous cell carcinoma being responsible for the majority of the cases (96%) and Merkel Cell carcinoma for the most aggressive ones [3], especially of the lower lip, in comparison with upper lip where basal cell carcinoma is most common and has a slow development [4, 5].

The management of lip defects needs a complex preoperative planning in order to preserve or to restore the function, the form and the aesthetics [6]. The preservation of the intraoral mucosal lining and the surface area of the oral aperture are the main two functional objectives, but also the orbicularis muscle sphincter must be viable, due to its role in the functional recovery. Moreover, the vermilion-cutaneous junction and lip aesthetic units must be maintained during the reconstruction [2]. The lips have a complex structure, consisting of skin and subcutaneous tissue, muscle, mucosal lining and vermilion. The lip vermilion is composed of keratinized glabrous epithelium with sebaceous glands. The vascularization is ensured by facial artery with its branches that form the superior and the inferior labial arteries. Motor innervation is provided by the buccal and the mandibular branches of the facial nerve [2]. The aim of this paper is to present our surgical experience in choosing the right surgery treatment method for reconstructing lip defects and possible complications.

Material and Methods

We realized a non-comparative retrospective case series study of twenty patients treated in the Plastic Surgery Department of Emergency Clinical Hospital "Prof. Dr. Agrippa Ionescu" for lip defects. Considering the zone of the defect, the dimension, the age and general health status of the patient different methods of reconstruction were approached. Based on defect size the reconstruction methods were grouped for small defects (less than one third of the lip), large defects (more than one third of the lip) and total lip defect (more than 80%).

Results and Discussions

Partial thickness defects

Partial thickness defects involve the cutaneous tissue and depending on their size are closed by primary suture or local flaps, the skin graft having a poor aesthetic outcome.

Nasolabial flap is one of the oldest techniques of reconstruction orofacial defect, being used in India in 600 BC [7]. This flap is based on the perforating branches of the angular artery [4].

Nasolabial flap has been used for the upper lip reconstruction as a rotation flap, for the philtrum as a tunnelled flap, for the commissure and for the lower lip as a transposition flap. In addition, in the majority of cases, it is used as thick as possible, full-thickness nasolabial flap being recommended in through-and-through lip defects [7]. In general, the flap is raised in a plane above the orbicularis muscle and it is sutured into the defect [6]. In our department, nasolabial flap was used in two cases for reconstruction of the upper lip defects (Fig. 1).

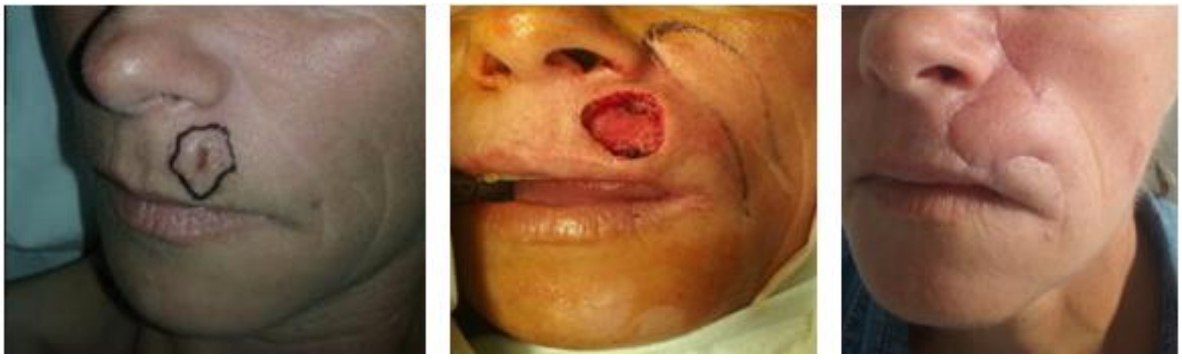

Fig. 1. Preoperative and postoperative views of the reconstruction of the upper lip using nasolabial flap

Small full thickness defects

Small full-thickness defects less than 1/3 of the lip may be treated by primary closure. The defects are usually smaller than 2 cm [8]. Local tissue is recommended whenever possible due

to the minimal donor-site morbidity, but also by keeping the sphincter function and by giving the natural colour and texture. Frequently, the excision is done in the shape of a V and the closure is realised in three layers (mucosa, muscle and skin) [6]. In our department six patients underwent this type of reconstruction.

Large full thickness defects

Regarding large full thickness defects, the reconstruction methods were grouped considering the zone of the defect (central or lateral).

The Karapandzic flap is a one-stage reconstruction procedure for central lower lip defects used in four cases [6]. It is considered an advancement-rotation flap, fully innervated that maintains lip mobility and sensation and can be used to reconstruct large defects with similar tissue [9]. The most important step of this procedure is the planning of the incisions [6]. A curvilinear incision parallel to the lip margin and towards the alar base is performed for creating the flap. The width of the flap is equal to the vertical height of the lip defect [8]. The mucosa is used just in case of intraoral defects. The neurovascular structure, the labial arteries and the buccal motor nerve branches, are identified and preserved, the muscle fibers being mobilized by fine dissection. The flaps are rotated and advanced to form the new lower lip (Fig. 2) [8]. It is highly important to preserve the flap thickness [6]. A side effect is the reduction in size of the lip circumference that can determine microstomia. However, the Karapandzic flap leads to an intact oral opening, with good function, sensation and mobility.

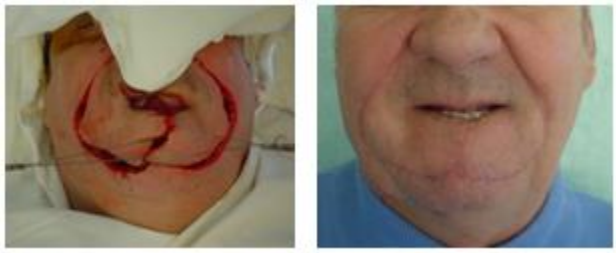

Fig. 2. Intraoperative and postoperative views of Karapandzic flap technique

The McGregor flap is a rectangular flap used to reconstruct full thickness lip defects, involving more than 50% of the lower lip (Fig. 3). It is designed by making a horizontal incision from the edge of the defect, equal to the vertical height of the lip defect. The flap length is double in size. Mc Gregor flap was utilised in three of our cases being rotated around the oral commissure without causing microstomia and without changing the position of the mouth corner [10].

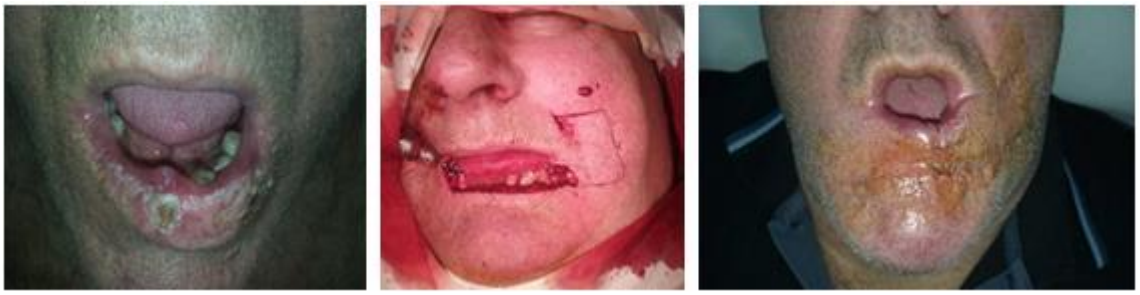

Fig. 3. Preoperative, intraoperative and postoperative photos of the reconstruction of the lower lip with McGregor flap

The Gilles fan flap was used for lower lip defects in another three cases. The incision is done from the inferior edge of the defect and it is a full-thickness one. The incision continues laterally around the commissure. The superior part is performed into the melolabial fold. Another

incision is created in the direction of the superior vermilion, paying attention at the superior labial artery. After these steps, the flap is advanced and rotated into the defect. Z-plasty can be used to rotate better the pedicle around the commissure [6] (Fig. 4).

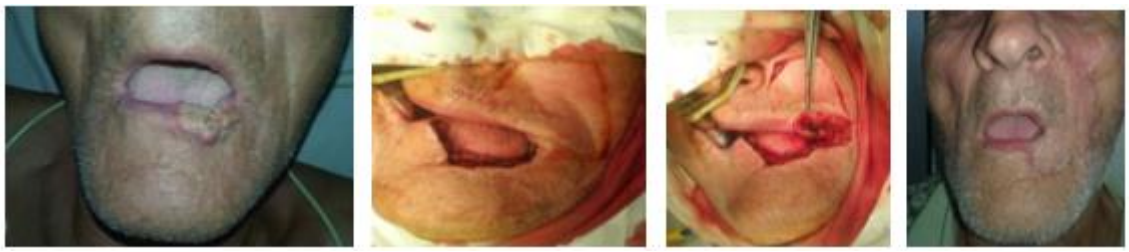

Fig. 4. Preoperative and intraoperative views of the Gillies flap

Total lip defects

Total lip defects are difficult to reconstruct with local flaps due to the poor functional outcome characterised by microstomia and opposite lip protrusion [10-12]. In this case distant pedicled flaps or free flaps can be used.

The deltopectoral flap is a pedicled axial fasciocutaneous flap based on the internal mammary artery perforator [13, 14]. It was used in one case for the reconstruction of a defect that includes the lower lip, the left commissure and a third of the upper lip (Fig. 5). The dissection is done from the anterior axillary line to almost 3 cm from the sternum, usually in the first 5 intercostal spaces. The flap is cut after 4 weeks, allowing the reconstruction of an extended area. The cosmetic results can be further improved.

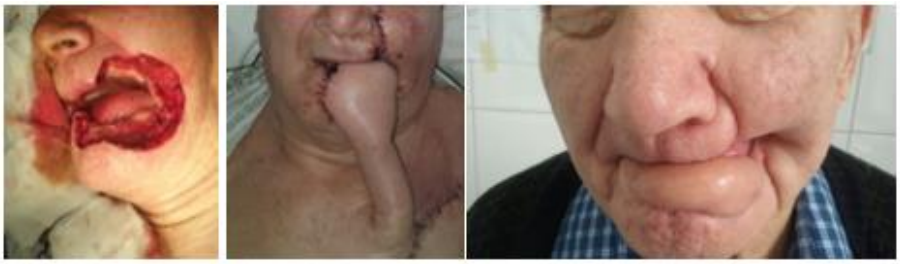

Fig. 5. Reconstruction of the lower lip using a deltopectoral flap

When distant flaps do not provide sufficient tissue for total lip reconstruction, free flaps can also be used with excellent results even if the donor site tissue has a different texture from the lip and facial skin. The disadvantages include restricted oral function and average aesthetic results [15]. Usually fasciocutaneous flaps are recommended being realised in a single-stage procedure [16]. One of the most common used flaps is the radial forearm flap with good results (Fig. 6). The radial forearm is transferred with the palmaris longus tendon for lip support [3].

Complications are possible and include: hypertrophic scarring, loss of sensation and microstomia [16].

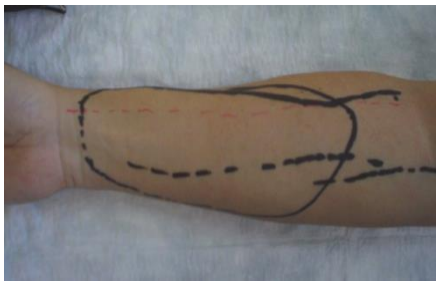

Fig. 6. Preoperative photo, the marking of the flap and postoperative view of lower lip reconstruction with radial forearm flap

After reconstruction surgery, patients were referred to the oncologist and radiotherapy was recommended in 8 out of 20 cases. One-year follow-up revealed good functional and esthetical outcomes with no local recurrences.

Conclusions

Lip reconstruction is challenging because the lips are the dynamic centre of the face.

Planning and choosing the most appropriate procedure for each patient is highly important.

Some patients may refuse different kind of reconstructive techniques due to aesthetic result and that is why the plastic surgeons should involve their opinion in decision making process.

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Surgical Management of Advanced Malignant Lymphomas of the Head and Neck Region

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Abstract

The differential diagnosis of the head and neck tumors includes malignant tumors that may be primary tumors or the expression of head and neck organs. Along with this there is a possibility of distant site metastasis in the head and neck region. Systemic disease is a constant question when setting the diagnosis. This is the case of lymphatic system malignant pathology.

All sites and organs of the head and neck region can be involved in the appearance of nodal or extranodal expression of lymphomas. Malignant lymphomas are treated in the hematology department with the aid of surgery departments for establishing the diagnosis. However, there are cases in which surgery is mandatory for therapy management, for cases that do not respond to chemotherapy or biological drugs. Surgery is reserved for advanced cases in which palliation is the means of therapy for ensuring the patients with management of life threatening situations or for a better quality of life. Patients that are non-responsive to therapy and have oral cavity, pharynx or larynx determinations of the disease benefit from the surgical therapy management for ensuring breathing via tracheostomy, for stopping bleeding from the tumor and for ensuring enteral feeding via nasogastric tube placement.

Keywords: malignant lymphoma, palliation, tracheostomy, surgical hemostasis, nutrition management

Introduction

Dealing with tumors of the head and neck region is a constant provocation for the head and neck surgeon this being the reason for approaching this pathology in a multimodal team. The tailored multi-specialty therapy is the current standard for diagnosis and therapy management.

The differential diagnosis of the head and neck tumors includes malignant tumors that may be primary tumors or the expression of head and neck organs. Along with this there is a possibility of distant site metastasis in the head and neck region. Systemic disease is a constant question when setting the diagnosis. This is the case of lymphatic system malignant pathology.

All sites and organs of the head and neck region can be involved in the appearance of nodal or extranodal expression of lymphomas. There are some head and neck sites predisposed to be affected by lymphomas such as the rhinopharynx, tonsils, base of the tongue, larynx, nasal cavity and paranasal sinuses. These tumor masses can mimic infection such as dental abscesses, different types of tumors, drugs consumption or other types of disease [1-3].

Malignant lymphomas represent as much as 5% of the total malignant neoplasias of the head and neck region [4]. According to the presence or absence of the Reed-Sternberg cells can be classified into Hodgkin or non-Hodgkin's lymphomas. The current used classification of malignant lymphomas is the one proposed by The World Health Organization (WHO) and it is the answer of all the questions asked and answered by the Hematology associations worldwide by combining morphology, immunohistochemistry, genetic data as well as signs and symptoms of the specific disease [5].

Malignant lymphomas are treated in the hematology department with the aid of surgery departments for establishing the diagnosis. However, there are cases in which surgery is mandatory for therapy management, for cases that do not respond to chemotherapy or biological drugs. Surgery is reserved for advanced cases in which palliation is the means of therapy for ensuring the patients with management of life threatening situations or for a better quality of life. Surgery in the head and neck area for non-responsive patients includes performing a tracheostomy, surgical hemostasis or nutritional management for large tumors that impede oral nutrition.

The incidence of non-Hodgkin lymphomas (NHL) is continuously rising worldwide for the past two decades especially in industrial countries reaching as much as 35%. Despite this increase in incidence the disease-free evolution and overall survival rates have improved in the past 7 years to over 50% [6]. There are categories of patients such as HIV patients, organ or stem-cell transplantation patients as well as patients with inherited or acquired immunodeficiencies or other autoimmune disease that have an increased risk of developing NHL [7]. An addition to the risk factors for developing this type of malignant lymphomas is the increased exposure to UV radiation [8]. There are also biological organisms that are linked to the development of NHL such as Epstein-Barr virus which is may cause the appearance of Burkitt's, nasal NK-cell or T-cell lymphoma, Helicobacter pylori which can generate lymphoma expressed in the mucosa-associated lymphoid tissue and HHV-8, HTLV-1, HCV, and SV40 [6, 9].

Methodology

Malignant lymphoma patients are being diagnosed according to a specific diagnostic protocol. Staging the disease into a particular subtype requires the evaluation of different parameters included into the Ann Arbor classification [6]. The Ann Arbor staging system is currently in use and it comprises 4 clinical stages based on symptoms and the location of the disease. Therefore, lymphoma can present itself as nodal or extranodal and accompanied or not by systemic manifestations. The B symptoms include fever, at least 38 °C, weight loss and night sweats. The weight loss has to exceed 10% of the total body weight on a time interval of 6 months prior to the diagnosis. Patients with lymphoma are being examined both clinically, general examination and endoscopic examination of the head and neck region, by using laboratory studies, such as CBC, liver and kidney function and by imaging studies. Imaging studies complete the diagnosis and the staging process according to the manifestations of the disease.

There area set of investigations that are essential for the diagnosis and staging of malignant lymphomas, as follows:

1. bone marrow aspiration and biopsy
2. fine-needle aspiration cytology
3. immunohistochemical analysis of the tumor
4. chest radiography
5. nasopharyngeal laryngoscopy.

Along with the core set of investigations to make the proper diagnosis and correct staging of the disease there are a number of investigations that will complete the general knowledge

regarding one's disease. Imaging studies will be performed depending on the presence of nodal or extranodal of specific site involvement. If adenopathy of the mediastinum, abdomen or pelvis are present CT of the chest, abdomen and pelvis are to be performed. If a tumor of in the head and neck region is present than CT scans need to comprise the area. However, PET-CT is limited to particular cases [10, 11].

High fidelity investigations such as cytogenetic analysis, polymerase chain reaction analysis or fluorescent in-situ hybridization may be useful in selected cases. Surgery is indicated for diagnosis in the case of the need of an excisional lymph-node biopsy which is also essential in HL and NHL, in the case of staging laparotomy or laparoscopy for HL.

Head and neck surgery are needed when lymphoma is considered to be present in one of the organs in this area and incudes endoscopic guided biopsies from tumor mases of the nose, paranasal sinuses, pharynx, oral cavity or larynx. One invasive procedure is still under debate that is unilateral or bilateral tonsillectomy. This is yet to be determined, however, at least unilateral tonsillectomy needs to be performed when suspecting this site of evolution for malignant lymphomas.

The most important contribution that surgery can make to the therapy of malignant lymphoma patients with head and neck determinations is to address life-threatening situations like bleeding from the tumor and acute respiratory insufficiency of the upper respiratory tract.

More than this, there is the possibility that the tumor size is large enough so that the patient can not eat properly. In the later situation a nasogastric feeding tube needs to be placed or if this is not possible a gastrostomy or a jejunostomy needs to be placed. In most cases the ligation of the external carotid artery as a means to stop bleeding from the head and neck organ region and the performance of the tracheostomy are emergency procedures since both bleeding and respiratory insufficiency tend to occur abruptly. Acute respiratory insufficiency may be prevented and a tracheostomy can be performed is a timely manner. It's been known that surgery is required in advanced cases in patients that are non-responsive to therapy and the prognosis is linked to the Ann Arbor stage, age, the presence of B symptoms, the presence of Ki-67 protein. More non-Hodgkin lymphoma patients tend to receive surgery for complications of the disease [12-14].

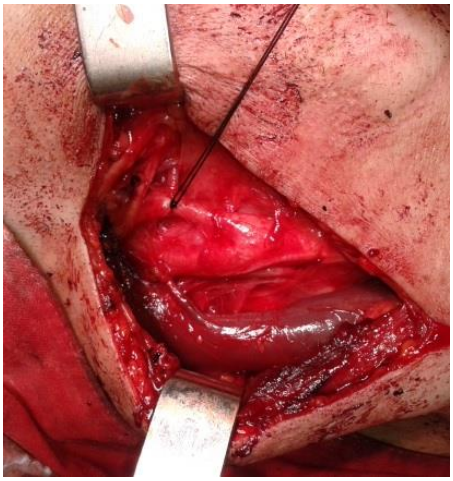

Fig. 1. Right external catorid artery ligation for oral bleeding from a tumor of the right hipopharynx diagnosed as extranodal NHL

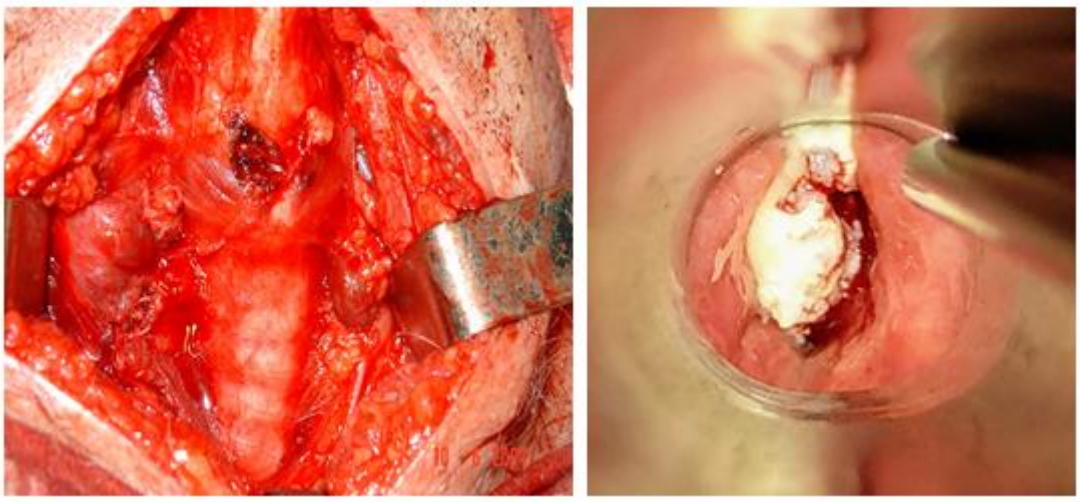

Fig. 2. Anterior wall of the trachea exposed for transistmic tracheostomy for NHL with larynx determination and acute respiratory insufficiency of the upper aerial tract

Discussions

The incidence of malignant lymphomas is rising and despite this attribute the disease-free periods and the overall survival rates have improved. The new diagnosis protocols and the better means of laboratory investigations leaded to the earlier detection of the disease. The advanced cases of malignant lymphomas need to be addressed with clinical, morphology and cytogenic evaluations to establish the grade of the lymphoma and the prognosis [15, 16]. The prognosis is dependent on different tumor biology factors such as the presence of the Ki-67 protein, the expression of Bcl-2 and Bcl-6 genes as well as the level of lactate, the production of β2-microglobulin. The B symptoms are a good predictor of the evolution and prognosis of the disease [17, 18]. The multimodal approach and the collaboration of the hematology, head and neck surgery department, general surgery department, imaging department and pathology department is a must when dealing with malignant lymphomas. Surgery is mandatory when facing life-threatening situations and in the case of non-responsive patients who need their quality of life improved. Surgical interventions for ensuring breathing and stopping bleeding from the tumor site are emergency procedures and are performed palliatively. Nutrition is one of the issues at hand when there are oral cavity or pharynx determinations. If oral feeding is not possible a nazo-gastric feeding tube should be placed. However, in most cases a percutaneous endoscopic gastrostomy is not possible due to the tumor volume and the possibility of bleeding.

In these cases, a classic gastrostomy or a jejunostomy are required [19, 20].

Conclusions

Patients that are non-responsive to therapy and have oral cavity, pharynx or larynx determinations of the disease benefit from the surgical therapy management for ensuring breathing via tracheostomy, for stopping bleeding from the tumor and for ensuring enteral feeding via nasogastric tube placement. The nasogastric tube is usually placed under endoscopic control so that the tumor is not injured and thus the risk of bleeding is decreased. In most cases the tracheostomy in permanent due to the fact that the progression of the disease is uncontrollable by targeted therapy, so these patients need to be educated in tracheostomy management, proper breathing and speech. It usually is a major disability for the patients and they require psychological support. Post surgery clinical evaluation of the tumor needs to be performed so that there might be a possibility of tracheostomy malfunction. Palliative therapy is a last resort surgical management of an otherwise non-surgical disease.

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The Influence of Oral Administration of Coenzyme E1 on Fatigue Laboratory Markers in High Performance Athletes

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Abstract

High intensity sustained training in high performance athletes is associated with increased mucle fatigue, muscle damage and increase of the muscle damage markers as lactate dehydrogenase (LDH) and creatine kinase (CK) and Aspartate transaminase (AST)/serum glutamic oxaloacetic transaminase (SGOT). Muscle fatigue is defined as a decrease in maximal force or power production in response to contractile activity. The increased physical performance, delayed muscular fatigue, reduced recovery time and improved endurance is the ultimate aim of mostly all athletes. Despite the lack of (semi-) official recommendations, there is a growing amount of data regarding potential benefic nonspecific treatments that could improve the exercise induced muscular fatigue syndrome. The nicotinamide adenine dinucleotide (NADH – coenzyme E1) is considered one of the strongest antioxidants in the body having a key role in restoring energy levels. To evaluate the benefits of NADH orally (25 mg/day combined with Vitamin C 50 mg) supplementation on fatigue and biochemical parameters in 16 high level sportswomen during an intense training period, we compared the biological markers of neuromuscular fatigue and metabolic recovery – ASAT, LDH, CK in two study groups, control and test. All the sportswomen were facing at the beginning of the observation, a period of intensification of training, so we were expecting the followed values to rise in both groups. This was confirmed by our determination, but the test group had the values less high than the witness group. This is especially evident for CK, a reduction of 50% was reported in treated group *versus* placebo that had the values at half as the test group, but also for the other parameters. These observations lead to the hypothesis that the oral NADH supplementation could confer potential benefits on training muscle fatigue and biochemical parameters in sportsmen, improve the recovery and sustain the physical performance. Larger sample trials are warranted to confirm these findings.

Keywords: muscle fatigue, hogh performance athletes, NADH, creatine kinase, ASAT, LDH, physical training, mitochondria

Introduction

High intensity sustained training in high performance athletes is associated with increased mucle fatigue, muscle damage and increase of the muscle damage markers as lactate dehydrogenase (LDH) and creatine kinase (CK) and Aspartate transaminase (AST)/serum glutamic oxaloacetic transaminase (SGOT) [1]. Muscle fatigue is defined as a decrease in

maximal force or power production in response to contractile activity [2]. Prolonged exercise, long time combat, military training and some related diseases (i.e. cancer, stroke) can cause muscle fatigue, that limits athletic performance combat ability and disease recovery. Muscle force production involves a sequence of events, extending from cortical excitation to motor unit activation to excitation-contraction coupling, and ultimately leading to muscle activation.

Muscle contractions activate ATPases and promote glycolysis, thus leading to an increase in intracellular metabolites, such as H+ generating tisular acidosis, lactate, piruvate and reactive oxigen species (ROS). In addition to acidosis, anaerobic metabolism in skeletal muscle also involves hydrolysis of creatine phosphate to creatine and piruvate; Creatine has little effect on contractile function, whereas piruvate, appears to be the most important cause of fatigue during high-intensity exercise more than acidosis [3]. A growing body of evidence proposes mitochondrial dysfunction as a central factor of the cronic fatigue syndrome due to activation of immune-inflammatory pathways that can impair the mitochondria [4].

ATP generation. The mitocondria

Maintaining skeletal muscle mitochondrial content and function is important for sustained health throughout the lifespan [5]. Mitochondria are the cellular "power houses", generating energy in a form that the cell can use using oxygen and water. ATP is a critical energy source for the majority of cellular functions-including muscle contraction and it's generated from ADP. The process consumes energy, generated by the conversion process that moves protons (H+) across the inner membrane of the mitochondria, creating an electrical "gradient", than drives protons back-through a molecular rotor [6].

The mitochondria imports citosol lactic acid, then metabolize it, being a carrier system for NADH oxidation [7]. The mitochondrial activity impacts the cell energy metabolism, calcium buffering, rate of ROS generation (good or bad), and apoptosis regulation [8].

Mitochondrial respiration produces ATP and consumes O_2, a process that generates ROS.

As the work intensity increases, ROS production increases, being implied in the muscle fatigue-pretreatment of intact muscle with a ROS scavenger significantly attenuates the developmentof fatigue [9]. Other metabolites with probable roles in fatigue include ATP, ADP, PCr and Mg [10].

This practice of prolonged activity/exercise with high intensity, as seen for example in triathlon training, can cause several physiological imbalances which could result in muscle fatigue and damage, changes in systemic inflammatory response, and finally reducing thus the athletes' physical performance [11]. Under normal circumstances, the total adenine nucleotide pool (ATP+ADP+AMP) remains constant. When the ATP supply is lowe than the consumption of ATP during exercise, fatigue occurs. In the case of inadequate oxygen supply, the oxidative phosphorylation is insufficient, ADP do not generate ATP, the ATP production shifts from aerobic processes (catabolism of glucose/glycogen, lipids, amino acids) to anaerobic glycolysis or glycogenolysis [12] resulting in lactate accumulation.

Muscle fatigue markers

According to the mechanism and metabolic changes during muscle fatigue were pointed three muscle fatigue markers: (1) ATP metabolism biomarkers-lactate, ammonia and hypoxanthine; (2) Oxidative stress biomarkers (ROS), – lipid peroxidation, protein peroxidation, and antioxidative capacity; and (3) Inflammatory biomarkers, – TNF-α, leukocytes, and interleukins [13].

The increased physical performance, delayed muscular fatigue, reduced recovery time and improved endurance is the ultimate aim of mostly all athletes. Despite the lack of (semi-) official recommendations, there is a growing amount of data regarding potential benefic nonspecific treatments that could improve the exercise induced muscular fatigue syndrome:

CNS-exciting drugs (amphetamine, caffeine), food supplements (i.e. American ginseng and *Rhodiola rosea*, vitamins, minerals, creatine) used clinically or experimentally [14-16].

Nutritional supplementation is regarded as legal by the International Olympic Committee (IOC) gaining popularity as a way to achieve performance enhancement.

Inflammation, oxidative stress, mitochondrial dysfunction, and CoQ_{10} deficiency have been well documented in pathological muscle fatigue as in the cronique fatigue syndrome (CFS) [17, 18].

Several studies show that nicotinamid adenine dinucleotide (NADH) could be safe and beneficial in the fatigue syndrome in performance athletes in association with CoQ10 [19].

Nicotinamide adenine dinucleotide (NAD^+)/β-nicotinamide adenine dinucleotide reduced form (NADH) is an essential electron transporter in mitochondrial respiration and oxidative phosphorylation. Based on its'crucial role in the mithocondrial biochemistry, NADH has been shown to improve cognitive functioning in patients with Parkinson's disease, depression, AD, decrease of the oxidative status associated with aging, improve the fatigue and biochemical parameters in chronic fatigue syndrome [18]. There are few studies on high performance athletes supplemented with NADH in our country; the present study investigates the influence of NADH 25 mg per day administered orally for 4 weeks – as a food supplement formula – on the muscle fatigue markers in high performance athletes during the intense preparation period compared with placebo [20].

Materials and Methods

We conducted a four week, randomized, blind placebo-controlled trial to evaluate the benefits of nicotinamide adenine dinucleotide NADH orally (25 mg/day combined with Vitamin C 50 mg) supplementation on fatigue and biochemical parameters in 16 high level sportswomen during an intense training period, practicing rowing at high level, of some biological parameters markers of neuromuscular fatigue and metabolic recovery.

A stabilized, sublingual reduced form of NADH 12,5 mg oral tablets was tested for quality, contaminants and adulterants, anti-doping tested and certified previously to the study. NADH was administered administrated orally, sublingual, *a jeun*. The test group (n=8) received 25 mg NADH daily (two tablets) combined in a formula with 50 mg Vitamin C and the control group (n=8) received vitamin C 50 mg daily for next 30 days of training. All other conditions for training (intensity, other medication to sustain effort, recovery) were identical for both groups.

The study protocol was previously approved by the Institute of Sport Medicine Medical Board.

All the physical performance parameters were monitorised during the training period; clinical checkups and laboratory determinations were done according to the routine training medical surveillance protocol. In order to analyse the effects of the NADH, the markers of muscle damage lactate dehydrogenase (LDH), ASAT and creatine kinase (CK) were supplementary determined together with the general biochemical parameters – during the study period. All the sportswomen had a progressive increase of the training workload from the beginning of the observation; the increase of the fatigue markers – LDH, CK, ASAT was expected as known from previous studies.

A value of 41 UI/L was set as the standard upper limit value by the laboratory - for ASAT, 26-140 UI/L for CK and 207-414 U/L for LDH.

The data was statistically evaluated using the "t" student test. Data was analyzed using t tests (for single between-group comparisons). Data is expressed as mean ± standard error. A p value of 0.05±SD was considered statistically significant.

Results and Discussions

Inflammation markers

At the beginning of the study the mean value for ASAT was of 41 UI/L±23,25 for the control group and of 43,62±9,48 in the test group; the mean values of ASAT after 15 days of supplementation were of 39±1,90 UI/L respectively 34±2,10UI/L and of 73,44±37,01 for the control batch compared with 64±30,39 UI/L for the test group.

The mean values for CK were of 297,25±98,08 UI/L for the control group and 311,5±141,38 UI/l for the test group; after 15 days the values decreased slowly (Fig. 3) and increased after 30 days to 1152±127,88 UI/L for the control group compared with 653,57±47,53 UI/L for the test group.

The LDH values increased less during the training, with mean values of 324,87±40,96 UI/L for the control group and 273±57,65 UI/L for test in the first day of the study, of 295±12,3 UI/L and 271±1,4 UI/L in day 15 or the study and grew at 439,22±97,60 UI/L for the control batch versus 378,28±46,58 UI/L for the tested group.

All the other biological parameters tested showed no significant changes during the 30 days training (study) period (Table 1)

Table 3. Average biochemical values in the tested groups on day 1 and at the end of the study (MEAN± STDEV)

	Test group				**Control group**			
	AVG day 1	**STDEV**	**AVG day 30**	**STDEV**	**AVG day 1**	***STDEV***	**AVG day 30**	***STDEV***
Proteins (g/L)	7,35	±0,33	7,38	±0,38	7,20	*±0,22*	7,05	±0,42
BUN (mg/dL)	37,85	±6,77	32,85	±5,63	38,75	*±5,28*	37,88	±7,75
GOT (UI/L)	43,62	±9,48	64	±30,39	41,00	*±23,25*	73,44	±37,01
CK(UI/L)	311,5	±141,3	653,57	±475,33	297,25	*±98,08*	1152	±127,88
LDH (UI/L)	273	±57,65	378,28	±46,58	324,87	*±40,96*	439,22	±97,6
Ca total (mg/dL)	10,23	±0,53	9,92	±0,23	9,85	*±0,30*	10,03	±0,22
Ca ionic (g/L)	43,87	±1,64	42,28	±2,13	42,87	*±2,10*	44	±2,15
Mg (mg/dL)	2,18	±0,28	2,21	±0,069	2,1	*±0,075*	2,11	±0,11
Iron (mg/dL)	115	±31,14	107,14	±48,82	122	*±41,31*	106,22	±31,1
Ferritin (ug/dL)	160,87	±17,31	93,42	±12,12	87,37	*±21,80*	92	±43
Hb (g/dL)	14,57	±0,48	12,57	±0,68	14,56	*±0,68*	12,58	±0,57
Hematocrit (%)	44,21	±1,52	38,3	±1,95	43,26	*±2,13*	38,08	±1,46

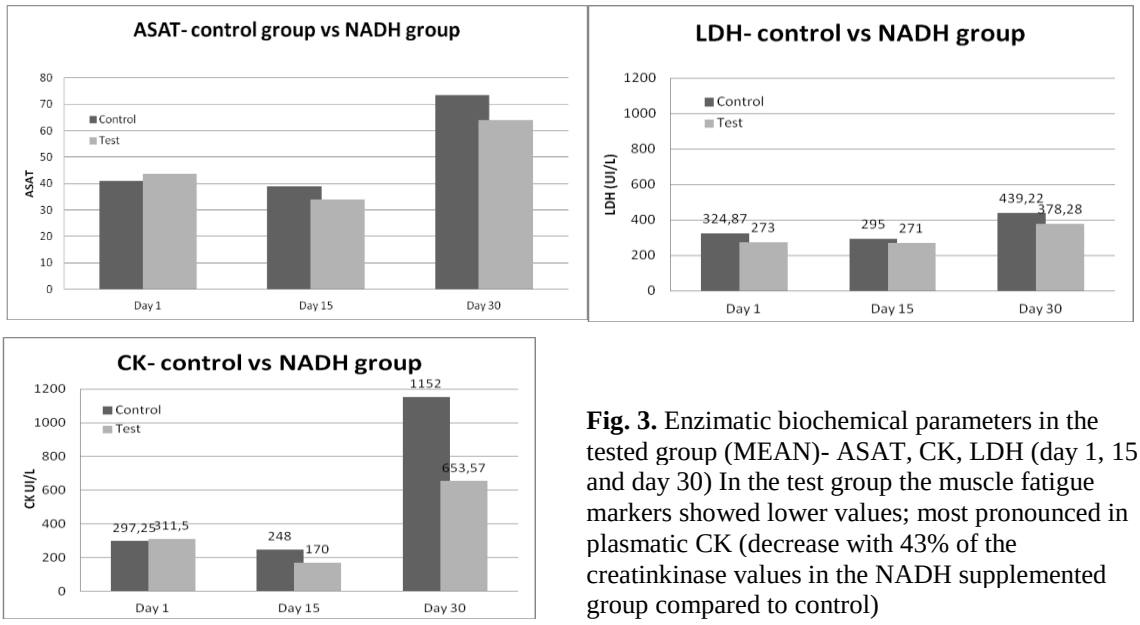

Fig. 3. Enzimatic biochemical parameters in the tested group (MEAN)- ASAT, CK, LDH (day 1, 15 and day 30) In the test group the muscle fatigue markers showed lower values; most pronounced in plasmatic CK (decrease with 43% of the creatinkinase values in the NADH supplemented group compared to control)

Conclusions

The supplementation of oral NADH for 30 days during intense physical training determined an increase of 109,81 or the plasmatic CK values compared with an increase of 287,55% of the circulating CK plasmatic levels in the control, untreated group. Oral NADH supplementation could have potential benefits on exertion muscle fatigue and biochemical parameters in sportsmen during intense training, could improve the recovery and sustain the physical performance. During the present study no other physical performance parameters were recorded. Larger sample trials are warranted to confirm these findings, evaluation of the correlated performance parameter can measure the real improvement of the physical performance and recovery time associated with the NADH use in performance sportsmen.

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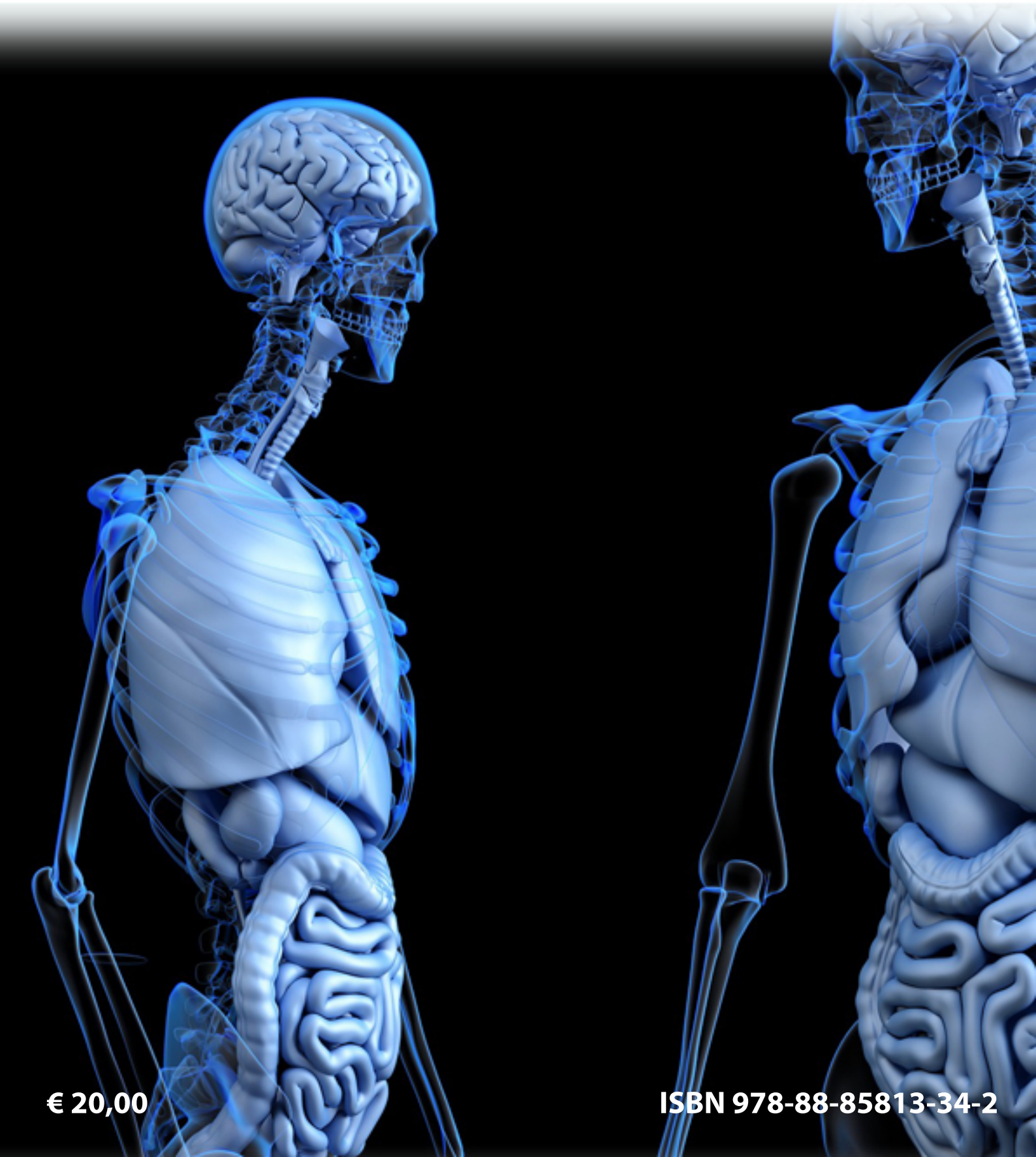

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