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Review

MANAGEMENT OF UPPER AIRWAY OBSTRUCTIVE SYNDROME IN CHILDREN EVALUATED BY POLYSOMNOGRAPHY

РЕШАВАЊЕ НА ГОРНО ОПСТРУКТИВЕН СИНДРОМ КАЈ ДЕЦА ЕВАЛУИРАНИ СО ПОЛИСОМНОГРАФ

Vesna Petreska-Dukovska and Aco Dimov

Department of ENT head and neck surgery, Pediatric surgery - PGH Remedika, Skopje, Republic of Macedonia

Abstract

Introduction. Adenotonsillectomy and adenotonsillotomy are the most frequent procedures performed in managing upper airway obstructive syndrome in children both in recurrent tonsillitis and in pediatric obstructive sleep apnea (OSA).

Aim. Determination of the healing rate after tonsilloadenoidectomy or tonsillotomy in pediatric sleep apnea syndrome (OSAS).

Methods. This review paper summarizes the results and conclusions obtained in 25 clinical studies conducted in pediatric preschool children (2-6 years) with normal development, without any anomalies, but with obstructive sleep apnea evaluated with polysomnography and otolaryngological examination.

Results. The mean age of patients included in the studies was 4.8, and the mean tonsil size on a scale of 0-4 was 3.0. The measure was the percentage of children successfully treated with tonsilloadenoidectomy (T/A) based on preoperative and postoperative apnea hypopnea index (AHI), which mean value reduced from 18.6 to 4.8. Postoperatively the mean reduction of AHI in all patients was 13.6, which was a significant improvement of OSAS as a result of the surgical intervention.

Conclusion. A first line of management of OSA in children is reduction or removal of adenoids and tonsils with a significant percentage of success in these pediatric patients. In case of correct indication the success rate is very high, however in general, the healing percentage reaches over 60%.

Keywords: adenotonsillotomy, adenotonsillectomy, diode laser and laser vaporisation

Апстракт

Вовед. Аденотонзилектомијата и аденотонзилотоми-

јата се најчестите хирушки процедури употребувани во решавањето на горно опструктивниот синдром кај децата и тоа и кај рекурентните тонзилити и кај педијатриската опструктивна ноќна апнеа.

Цел. Утврдување на стапката на излекување, со примена на тонзилоаденоидектомија или тонзилотомија кај педијатрскиот сплеп апнеа синдром (ОСАС).

Методи. Во овој ревиски труд ќе се обидеме да ги сумираме резултатите и заклучоците добиени од 25 клинички студии, кај педијатрски претшколски деца (2-6 години) со нормална градба, без аномалии, со опструктивен сплеп апнеа синдром евалуирани со полисомнограф и орл егзаминација.

Резултати. Во овие студии средната возраст на пациентите изнесува 4,8, а средна големина на тонзиларното ткиво по скалата од (0-4) изнесува 3,0. Мерка беше процентот на деца успешно тертирани со тонзилоаденоидектомија (Т&А) базирани на предоперативниот и постоперативниот АХИ индекс чија средна вредност од 18,6 се намали 4.8. Средна вредност на намалувањето на АХИ индексот кај сите пациенти постоперативно е 13,6 што претставува сигнификантно подобрување на ОСАС со реализираната оперативна интервенција.

Заклучок. Прва линија на решавање на ОСА кај децата е редукација или отстранување на аденоидите и тонзилите со изразен висок процент на успешност кај овие педијатрски пациенти. При правилна индикација просекот на успешност е висок меѓутоа генерално процентот на излекување достигнува преку 60%.

Клучни зборови: аденотонзилотомија, аденотонзилектомија, диоден ласер, ласер вапоризација

Introduction

Obstructive sleep apnea (OSA) is a syndrome, which is characterized by recurrent apneas, hypopneas and snoring that disturb normal sleep and cause somnolence and fatigue.

Correspondence to: Vesna Petreska-Dukovska, Department of ENT head and neck surgery, Pediatric surgery - PGH Remedika, 1000 Skopje, R. Macedonia; E-mail: v_petreska@yahoo.com

Apnea—pause in breathing during sleep, is defined as an absence or a significant decrease (more than 70%) of airflow through the nose and mouth, which lasts 10 seconds at least.

Hypopnea—reduction in oronasal airflow or amplitude of thoracic abdominal movement for over 30% in a period longer than 10 seconds. There is also reduction in the oxygen saturation of 4% or over 4%. Majority of the respiratory episodes last 10-30 seconds, but sometimes they can last up to 1 minute or longer [1-7].

Snoring is a sound produced during sleep, resulting from vibration of soft tissues in the upper respiratory tract during breathing. Snoring is a condition where sound phenomenon plays a central role, but it is associated with significant restrictions of airflow and respiratory disorders during sleep.

There are many controversies about the correct diagnosis and adequate treatment of pediatric obstructive sleep apnea syndrome (OSAS). From the aspect of pediatric otorhinolaryngology snoring and OSAS in children have many common characteristics and are tightly connected between themselves. Today the American Academy of Pediatrics recommends clinical screening protocol for all children with OSAS [8-10]. Data about snoring in children during night, episodes of apnea and hypopnea, disturbed sleep, anxiety, problems in the behavior, are obtained by heteroanamnesis from the concerned parent along with the clinical examination made by an otolaryngologist including oropharyngoscopy (assessment of hypertrophy of adenoids and tonsils evaluated on a 0-4 scale), fiberoptic nasopharyngoscopy, rhinomanometry, craniofacial ratio CT or MRI and the gold standard in the OSA diagnostics—polysomnography [11]. The validity of data given by the parent together with the clinical examination and the mandatory polysomnography test are considered to be responsible for the clinical conclusion in management of upper airway obstructive syndrome in children. Based on the results obtained from the evaluation of each pediatric patient a conclusion has been made that adenotonsillectomy or adenotonsillotomy (partial reduction of palatine tonsils) are a method of choice in management of upper airway obstructive syndrome in children [12-18].

Pathophysiology

Anatomic and physiologic factors are combined in order to create pathognomic pharyngeal collapse, which is associated with obstructive sleep apnea. Imbalance between forces that cause dilatation of airways and those that cause collapse results in sleep apnea [19-23]. Dilatation of upper airways is caused by the following factors:

- Hypotonic pharyngeal muscles
- Larger dimension of airways and mandible
- Large lung volume

- Collapse of upper airways is caused by the following factors:
- Small dimension of upper airways
- Resistance in upper airways
- Negative inspiratory pressure
- Small mandible
- Inflammation of upper respiratory airways (non-specific and specific).

The resultant between the tonus and the pressure in the airways and the collapse of its contents cannot maintain the airways open and causes hypotonic or apneic episodes which finish in activating a reaction for releasing the airways. It is a condition of sleep with increased brain activity that does not lead to total awakening. This excitation of the body develops as a result of sympathetic stimulation, and the end purpose is a struggle for opening the airways. There is a considerable individuality in the sensitivity of chemoreceptors and mechanoreceptors, which influences on the duration of apneic episodes and the probability for the onset of significant oxygen desaturation, hypoxemias and hypercapnias.

The activated sympathetic results in tachycardia, increase in the heart output and blood pressure. Hypoxemia is responsible for activation of neutrophils, which are adhered to endothelial cells and are responsible for releasing of oxygen-free radicals, endothelial dysfunction and increased vasoconstriction.

OSA is a cause of increased secretion of insulin and cortisol, which results in increased serum glucose and development of insulin resistance leading to diabetes. There is imbalance between leptin and ghrelin, hormones that regulate the hunger and satiety. Consequently, it leads to imbalance of lipogenesis and lipolysis with the end result, insufficient lipid breakthrough—hyperlipidemia and increase of body weight.

Consequences and sequels of OSA

From the analyzed 350 papers published over the last 10 years valid data have been obtained that point out to severe sequels due to untreated OSAS in children. The average IQ index in these studies was significantly lower in comparison with that in children who did not suffer from OSA [24-32]. Children with OSAS have reduced total lung capacity and significantly increased residual volume. In addition, all cognitive functions are reduced including remembering, memory, verbal communication, phonological skills, analytical thinking and judging, that is, all executive functions. Behavioral changes in cognitive attitude in these children have been verified, manifested with apathy, daily somnolence, depression, hyperexcitability and aggression.

Diagnosis of OSA

Polysomnography and clinical examination by a pediatric otorhinolaryngologist is a gold standard in establishing the diagnosis of OSA[33-36].

Apnea-hypopnea index (AHI) is the number of apnea and hypopnea episodes with different time duration and presence of desaturation in an hour.

- AHI < 5 – normal result in healthy individuals
- AHI 5 - 14 –mild apnea
- AHI >30 – severe apnea

Classification of the severity of night apnea:

1. Mild degree –AHI of respiratory disorder ranges between 5 and 14, that is, <20. Daily somnolence is minimally manifested. The episodes of apnea and hypopnea are not associated with significant hypoxemia.
2. Moderate degree-AHI ranges from 20 to 30. Daily somnolence does not disturb daily activities. Episodes of apnea and hypopnea cause considerable hypoxemia and clinical signs of heart arrhythmia. There is daily somnolence or excitation.
3. Severe degree–AHI is >30. It is characterized by constant daily somnolence and the normal activities of the subject are disturbed. Apnea and hypopnea episodes are associated with severe hypoxemia, threatening with disordered heart rhythm. There is an increased blood pressure during sleep. Chronic respiratory and heart insufficiency may be present.

There is no standardized approach to clinical verification based on tonsillar hypertrophy to establish a diagnosis of clinical OSAS.

We have searched the published papers to determine whether tonsil size is in clinical correlation with diagnosis of OSAS in comparison with the data obtained with the polysomnography test.

No clear answer related to this issue has been given; there are number of investigations that have shown conflict results. In 17 of 25 papers the results presented positive relevance and in 8 negative. In general, the degree of tonsillar hypertrophy is one of the subjective factors derived from the limitations and variations among the studies [36-42].

The difference varies significantly in relation to age, geographic location, ethnic affiliation and other factors. If the subjective aspect of the otolaryngology specialist is eliminated, than an attempt has been made for standardization of the degree of tonsillar hypertrophy graded on a 0-4 scale [43-49].

The degree of tonsillar hypertrophy can be divided into:

- tonsils hidden in the tonsillar fossa – grade 0;
- tonsils slightly enlarged of the fossa – grade 1;
- tonsils enlarged beyond the tonsillar fossa, but not close to midline - grade 2;
- tonsils enlarged almost up to midline -grade 3;
- tonsils enlarged and are in contact with each other - grade 4.

We cannot conclude that a child has OSA or that it is present or absent only based on the tonsil size, although this is a very common situation. All children with tonsillar hypertrophy prior to establishing a diagnosis of OSA have to undergo a polysomnography test. Pediatric patients with heteroanamnesis given by a parent, with a history of snoring, difficulty in breathing, sweating during sleep, gasping for air are candidates for having OSAS regardless of tonsil size and they deserve to be subjected to additional examinations such as polysomnography.

What is the correlation between the size of the tonsils and severity of OSAS?

Since tonsils (and adenoids) are larger than airways in children, they lead to different degree of OSAS that correlates with the tonsil size graded on a scale of 0-4. Firstly, the size determined on a scale is subjective and hence these findings are prone to prejudices and errors. However, each otolaryngology doctor who deals with this pathology knows that obtaining a clear visual assessment of the tonsils is only a scale for assessment of the size obtained by the ratio between the tonsils and pharyngeal walls.

Many studies have suggested that the real size of the tonsils, regardless of the relation with the pharyngeal walls, is in a positive correlation with OSAS. This is a challenge to develop a better way for clinical evaluation of the tonsil size.

How can the size of the tonsils be better clinically evaluated?

It can happen only if the doctors analyze the tonsils in three dimensions during the oropharyngeal examination. The combination of this approach with a scale that measures three dimensions (instead of one-dimensional approach that utilizes the traditional 0-4 scale) can significantly improve the accuracy of the clinical evaluation.

In addition to the simple physical examination, other approaches include ultrasound or endoscopy for better assessment of tonsil size.

The first line of choice and treatment of pediatric OSAS is removal of hypertrophic adenoid or tonsillar tissue. It can be done with adenoidectomy, tonsillectomy and tonsillotomy. There is a statistically significant AHI improvement in almost all patients who have undergone T/A for OSAS. In all 25 papers the results have shown a significant improvement of AHI on postoperative versus preoperative polysomnography, with a total average decline of AHI of 13.6.

We support T/A as an approach important in treatment of pediatric OSAS, but we also advocate the idea of recognizing the limitations of T/A.

There is a lack of published data with genuine results.

As a routine test physicians include polysomnography as one of the diagnostic examinations for pediatric sleep apnea. Prejudiced reporting is done by authors

who have obtained negative results that they do not publish with an aim to prove that T/A is not effective in treatment of pediatric OSAS. However, it is our opinion that in over 60% of cases tonsilloadenoidectomy is a positive and successful solution for OSA.

Many published studies have not examined children with comorbidities, including obese children. Although it seems logical to exclude rare comorbidities such as craniofacial abnormalities, chromosomal abnormalities, and neurological syndromes, exclusion criteria based on the weight or body mass index do not reflect the real picture. Parallel to the increased percentage of overweight children in the US, there is an increased percentage of sleep apnea. It is important to recognize that T/A cannot often yield desired results in this population.

Studies that have reported on the effectiveness of T/A in obese children or have compared the results between obese and normal-weight children have shown that OSA is less likely to resolve only with T/A.

Obstructive sleep apnea is distinctly associated with obesity. Obese children ($BMI > 24 \text{ kg/m}^2$) are at a higher risk of OSA in comparison to normal-weight children, that is, up to 75% of children with OSA are obese by measuring BMI. It is assumed that obese children have larger fatty deposits in their neck that cause collapse in the upper airways in lying position during sleep. Several studies have supported the hypothesis that AHI significantly reduces by losing weight in a larger number of children.

In the 25 analyzed papers, 25% of the subjects had increased BMI in spite of the reduction of AHI after performed tonsilloadenoidectomy; their AHI reduced, but it was not normalized. It means that obesity generates OSA, and OSA by its pathophysiology creates conditions for further even higher increase in weight, thus suggesting the complexity of OSA treatment in obese children. Their treatment does not finish with tonsilloadenoidectomy; sometimes there is a need for additional examinations and interventions such as septoplasty, mucotome, orthodontics, reduction of lingual tonsil and above all, cure of the underlying disease – obesity.

Material and methods

This review paper summarizes in brief the results and conclusions obtained in a number of clinical studies, which have examined this huge problem in the pediatric otorhinolaryngology.

The focus of this review paper has been on studies that have presented results from examinations of diagnostics of upper airway obstructive syndrome in children obtained with mobile polysomnography, including studies that have analyzed the results of management of upper airway obstructive syndrome in preschool children aged 2-6 years.

We conducted a comprehensive literature search of the electronic databases (PubMed, MEDLINE, EMBASE, Current Contents, Science Citation Index and the Cochrane database) for the period from 01.01.2012 through 01.03.2017 of papers written in English. The search terms used included medical subject headings (MeSH) and keywords in the search strategy: apnea, obstructive sleep apnea, polysomnography, adenotonsillectomy, tonsillotomy.

This study was designed so as to see the results obtained by otorhinolaryngologists who deal with surgical treatment of OSAS (adenotonsillectomy and tonsillotomy) as an isolated procedure.

The aim of this study was to determine the success rate of T/A or tonsillotomy in elimination of pediatric OSAS. The following inclusion criteria were used in the examination as a protocol:

2. All children were younger than 6 years, that is, preschool age from 2 to 6 years.
3. Otorhinolaryngological examination was mandatory for assessment of adenotonsillar hypertrophy (on a 0-4 scale).
4. Mandatory polysomnography test prior to treatment and polysomnography test 6 weeks following the treatment.
5. Preoperative polysomnography test had to deviate from normal values of AHI which resulted with diagnosis of OSA.
6. All grades of OSA (mild, moderate and severe) were included.
7. The study was focused on children with normal constitution without anomalies in psychophysical development. Patients with craniofacial syndromes, chromosomal abnormalities or neuromuscular disorders were excluded.
8. Body mass index (BMI), that is, obesity was considered for “normal” finding and was not excluded as a criterion.

Results

Of identified 295 studies that analyzed this pathologic entity, we examined the results of a total of 25 studies that have entirely met the abovementioned criteria. 126 studies were excluded since they did not measure the AHI pre- and postoperatively. A number of these studies (102) were excluded because they got the indication for tonsilloadenoidectomy from the clinical picture and from the medical history, and made postoperative polysomnography tests to prove the positive effect and healing of OSAS after performed tonsilloadenoidectomy. Forty-two studies were excluded due to the presence of various syndromes, palatoschisis, craniofacial anomalies that they themselves lead to a high level of upper airway respiratory obstruction. All included studies analyzed the results of preoperative and postoperative polysomnography taking into

consideration as a measure the number of AHI, preoperative and postoperative otorhinolaryngological examination (the size of adenotonsillar tissue evaluated on a 0-4 scale), the influence of BMI on AHI before and after surgery, that is, all studies presented the number of successfully surgically treated patients either with adenotonsillectomy or tonsillotomy (partial reduction of tonsillar tissue) as an issue of efficacy of this method in obtaining normal polysomnography findings.

The mean age of patients in the analyzed studies was 4.8, and the mean tonsil size evaluated on the 0-4 scale was 3.0. In 17 studies tonsil size correlated with the AHI preoperatively (positive studies) contrary to the remaining 8 studies where no association between tonsillar mass and higher AHI was found (negative studies). Postoperative versus preoperative polysomnography showed improvement of AHI in all 25 studies. Preoperatively the mean AHI was 18.6 (6.9-69.3); AHI>5 and postoperative mean AHI summarized in all studies was 4.8 (0.39-14.2); AHI<5. Postoperatively the mean value of reduction of AHI in all patients was 13.6, which showed a significant improvement in OSAS after conducted surgical intervention.

As a separate entity we would like to emphasize BMI versus AHI preoperatively and postoperatively. In all 25 studies, written predominantly by American authors, children with increased body weight accounted for 25% of the total number of examined subjects. The mean value of preoperative AHI in these subjects (increased body weight) was 23.4 (10-69.3), whereas postoperative

AHI was 9.1 (7.9-14.2) where the overall decline of the mean index value was 14.3, which is a large decrease but it is never in normal ranges.

Discussion

Obstructive sleep apnea syndrome is one of the most common pathologic entities that appears in childhood, affecting even 1.2-2.8% of the whole children population at the age of 2-6 years[50-54]. According to many authors who deal with this problem nowadays, the probability of onset of OSA proportionally increases with body weight increase. The anatomy of upper airways along with their constituent structures is directly connected with the onset of OSA.

Nasopharynx is particularly important in children since adenoid hypertrophy is the most common obstruction and cause of OSA.

On the other hand, oropharynx is a constituent segment of palatine tonsils, soft tissue, both gingival arches, uvula and tongue, which ratio disorder has the principal and dominant role in the development of obstructive apnea.

Conclusions

Upper respiratory obstruction in preschool children is predominantly due to two factors: the size of tonsillar mass, anatomic structures and their correlation as well as sensory mechanisms that exist on the level of the

Table 1. Characteristics of analyzed studies

Papers	No. of patients	Mean age	Tonsil size	Mean AHI value pre-op	Mean AHI value post-op	BMI higher than 24	BMI higher than 24
Leach <i>et al.</i>	26	3.5	1	12.1	2	18%	74%
Niemenen <i>et al.</i>	13	3.7	3	24.4	7.9	42%	63%
Wang <i>et al.</i>	20	5.6	4	24.1	8.4	48%	61%
Brooks <i>et al.</i>	74	3.8	2	13.6	3.8	29%	73%
Niemenen <i>et al.</i>	81	5.6	3	15.7	4.6	20%	68%
Li <i>et al.</i>	56	4.2	3	15.1	9.9	26%	59%
Franco <i>et al.</i>	33	3.9	2	14.7	8.4	18%	62%
Wing <i>et al.</i>	18	5.7	1	12.5	3.6	16%	72%
Goodwin <i>et al.</i>	36	4.8	2	18.3	7.4	17%	61%
Bhattacharj <i>et al.</i>	58	4.6	4	21.8	10.3	28%	50%
Bhattacharjee <i>et al.</i>	42	5.4	4	23.7	10.2	29%	51%
Howard Brietzke <i>et al.</i>	84	4.9	3	16.9	6.6	15%	58%
Dayyat <i>et al.</i>	92	4.2	4	19.7	7.8	31%	55%
Bixler <i>et al.</i>	112	5.6	3	18.4	2.1	29%	70%
Stone <i>et al.</i>	68	5.8	1	12.9	2.2	24%	69%
Bar <i>et al.</i>	42	4.7	3	20.2	3.2	18%	65%
Shal <i>et al.</i>	18	4.4	4	24.2	4.1	32%	63%
Tal <i>et al.</i>	92	4.8	3	18.5	2.2	26%	73%
Kety <i>et al.</i>	114	5.9	3	17.3	2.3	24%	68%
Cook <i>et al.</i>	66	5.7	3	19.8	2.4	19%	71%
Knoxvile <i>et al.</i>	31	5.6	4	21.1	3.1	23%	67%
Witch <i>et al.</i>	94	5.9	3	17.8	2	18%	63%
Paul <i>et al.</i>	58	5.8	4	24.4	3.1	35%	74%
SpiegelLi <i>et al.</i>	34	5.9	4	24.5	3.8	40%	73%
Goodwen <i>et al.</i>	56	5.6	4	24.5	3.6	38%	71%

upper respiratory tract. The first line of management of OSA in children is reduction or removal of adenoids and tonsils, which results in a high percentage of success in these pediatric patients [55-60]. In case of correct indication the success rate can be high, but in general the percentage of healing reaches over 60%. In the remaining children with severe upper respiratory apnea, additional interventions in the upper respiratory tract are necessary such as septoplasty, mucotomy or orthodontic interventions [61].

Struggle against obesity has to be particularly emphasized as an additional factor for exacerbation of the upper respiratory obstruction in children. Polysomnography remains the principal method and gold standard in diagnosing OSA and in controlling the success of the conducted therapy.

Conflict of interest statement. None declared.

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Original article

NON-CYSTIC FIBROSIS BRONCHIECTASIS IN HOSPITALIZED PATIENTS

БРОНХИЕКТАЗИИ НЕАСОЦИРАНИ СО ЦИСТИЧНА ФИБРОЗА КАЈ ХОСПИТАЛИЗИРАНИ ПАЦИЕНТИ

Zoran Arsovski¹, Biserka Jovkovska-Kjaeva¹, Dejan Dokic¹, Gorica Breshkovska¹, Kiril Naumoski, Anita Arsovska², and Viktor Isjanovski³

¹University Clinic of Pulmonology and Allergy, ²University Clinic of Neurology, Faculty of Medicine, University "Ss Cyril and Methodius", Skopje, ³Psychiatric Hospital Skopje, Republic of Macedonia

Abstract

Introduction. Non-cystic fibrosis bronchiectasis (NCFB) is associated with certain immunodeficiency conditions, autoimmune or congenital diseases. The aim of this study was to assess the epidemiologic data in hospitalized patients with NCFB and to correlate age and sputum findings with length of hospitalization and the presence of comorbidities.

Methods. A retrospective study in patients with NCFB was performed during a period of 78 months. A total of 614 hospitalizations of 366 patients were analyzed with the computer program SPSS Statistics 20.

Results. Out of the total number of hospitalizations, 53.6% were male and 46.4% were female patients, with average age of 61.3 years. Bilateral NCFB was found in 74.9% of patients; 13.2% had right-sided, and 11.9% had left sided bronchiectasis. Only 4.6% had bleeding NCFB. No statistically significant correlation between bleeding bronchiectasis and smoking was registered. Most patients had only one comorbidity. Most frequent comorbidity was chronic obstructive pulmonary disease (61.2%). A statistically significant correlation was found between bleeding NCFB and the presence of comorbidities. 48.5% of patients were non-smokers, 25.9% were former smokers, while 25.6% were active smokers. Positive sputum culture was found in 125 patients and *Candida albicans* was the most common pathogen. Average duration of hospitalization was 10.3 days. Most of the patients, 265 (72.5%), were hospitalized once. Three percentages of all patients were hospitalized 6 or more times.

Conclusion. Our study could bring new insight into epidemiology of data concerning hospitalized patients with NCFB.

Keywords: non-cystic fibrosis bronchiectasis, chronic

obstructive pulmonary disease, sputum

Апстракт

Вовед. Бронхиектазиите неасоцирани со цистична фиброза (БНЦФ) се најчесто асоцирани со одредени состојби на имунодефициенција, автоимуни или вродени заболувања. Цел на оваа студија е да се одредат епидемиолошките податоци кај хоспитализираните пациенти со БНЦФ и да се корелираат возраста и наодите од спутум, со должината на хоспитализација и присуството на коморбидитети.

Методи. Направена беше ретроспективна студија на хоспитализирани пациенти со БНЦФ за период од 78 месеци. Вкупно беа анализирани 614 хоспитализации на 366 пациенти со компјутерскиот програм SPSS Statistics 20.

Резултати. Од вкупниот број хоспитализации, 53.6% беа мажи и 46.4% беа жени, со просечна возраст од 61.3 година. Билатерални БНЦФ беа најдени кај 74.9% од пациентите, 13.2% имаа деснострани, додека останатите 11.9% имаа левострани бронхиектазии. Само 4.6% од сите пациенти имаа БНЦФ со крварење. Не беше регистрирана статистички сигнификантна корелација меѓу бронхиектазиите со крварење и пушењето. Најголем дел од пациентите имаа само еден коморбидитет. Најчест коморбидитет беше хроничната опструктивна белодробна болест (61,2%). Статистички сигнификантна корелација беше најдена меѓу БНЦФ со крварење и присуството на коморбидитети, при што 48.5% од пациентите беа непушачи, 25.9% беа поранешни пушачи, додека 25.6% беа активни пушачи. Позитивна култура на спутум беше најдена кај 125 хоспитализирани пациенти, а *Candida albicans* беше најчест патоген. Просечно, пациентите со БНЦФ беа хоспитализирани 10.3 дена. Најголем дел од пациентите, 265(72.5%), беа хоспитализирани еднаш, додека 3% од сите пациенти беа хоспитализирани шест или повеќе пати.

Correspondence to: Zoran Arsovski, University Clinic of Pulmonology and Allergy, Faculty of Medicine, "Vodnjanska" 17, 1000 Skopje, R. Macedonia; E-mail: arso66@yahoo.com

Заклучок. Нашата студија придонесува за нов увид во епидемиолошките податоци на хоспитализираните пациенти со БНЦФ.

Клучни зборови: бронхиектазии неасоцирани со цистична фиброза, хронична опструктивна белодробна болест, спутум

Introduction

Bronchiectasis is defined as a pathological, permanent enlargement or dilatation of the distal, small and medium bronchi. The pathogenesis of the disease is widely known and accepted as a vicious cycle of microbial colonization, severe and/or chronic inflammation, destruction of the airways and mucus stasis. Etiologically, non-cystic fibrosis bronchiectasis (NCFB) most commonly appears after an infection (pneumonia, recurrent childhood infections, tuberculosis). NCFB are associated with certain immunodeficiency conditions (primary or secondary), autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, Crohn's disease), congenital diseases (α_1 antitrypsin deficiency, primary ciliary dyskinesia, tracheo-bronchomalacia). A separate group of NCFB is the idiopathic bronchiectasis. The symptoms and clinical manifestation may vary between having no symptoms or persistent cough and daily sputum production. Some patients (pts) can experience symptoms only at the time of an exacerbation. Other usual symptoms that occur during an exacerbation are: increased sputum production, increased cough, hemoptysis, dyspnea, wheezing, fever, fatigue, reduced pulmonary function etc. NCFB are characterized as cylindrical, cystic, or varicose in nature [1]. Chest X-ray and bronchography can be used in diagnostics, but with the appearance of computer

tomography, high-resolution computer tomography quickly became the gold standard in diagnosing NCFB [2,3,4]. With this method bronchiectasis in children with cystic fibrosis as young as 10 weeks old can be confirmed [5,6,7]. In some studies done in the USA, the prevalence of NCFB has risen from 2000 to 2007 by 8,74% [8]. The management of bronchiectasis is primarily focused on preventing the progression of the disease and improving the quality of life by decreasing the symptoms and exacerbations.

The aim of this study was to assess the epidemiologic data (gender, age, smoking status, length of hospitalization, distribution of bronchiectasis, sputum findings, comorbidities) in hospitalized pts with NCFB and to correlate the presence of comorbidities, sputum microbiology findings and length of hospitalization.

Materials and methods

A retrospective study in pts with NCFB hospitalized at the University Clinic of Pulmonology and Allergy - Skopje, was performed during a period of 78 months, from July 2010 to December 2016. A total of 614 hospitalizations of 366 patients were analyzed.

Gender, age, type of bronchiectasis, duration of hospitalization, distribution, co-morbidities, smoking, sputum for microbiology and rehospitalizations were analyzed. In pts with NCFB, including those without a comorbidity, the diagnosis of cystic fibrosis was excluded. For the analysis of the gathered data a computer program SPSS Statistics 20 was used. The analysis was done with standard descriptive and analytical bivariate and multivariate methods.

Attributive statistical series were analyzed by determining the coefficient of relations, proportions, rates and determining the statistical significance between the found differences. The numerical series were

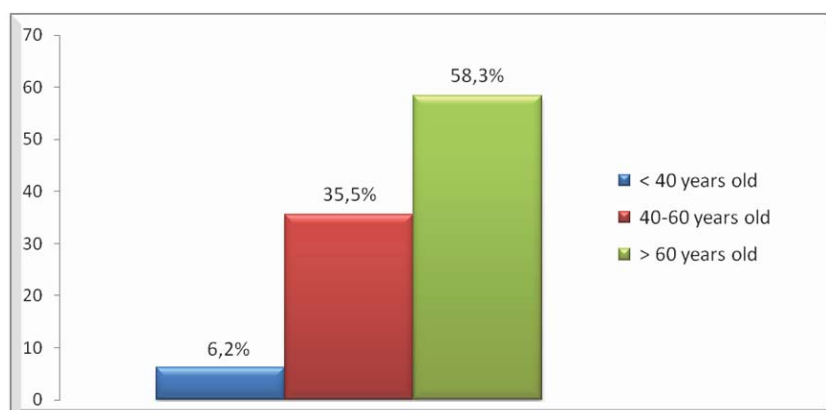


Fig. 1. Age groups of hospitalized pts

analyzed by measures of central tendencies and means of dispersion of data. Confidence interval was 95% ($p < 0.05$). The results are shown in tables and figures.

Results

Male gender was predominant in our group of patients (193 out of 366). Of all the hospitalizations in the

study (614), 329(53.6%) were male, while 285(46.4%) were female patients. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.016$, difference test). The average age of pts with NCFB was 61.3 ± 13.193 years, with a minimum of 16, and a maximum of 89 years. The average age within 329 male pts was 60.4 ± 13.42475 years, and within 285 female pts 62.4 ± 12.86719 years. This difference was statistically insignificant for $p > 0.05$ ($p = 0.072$).

The most dominant age group in the analyzed pts was the above 60 years group with 358(58.3%) (Figure 3). Only 38(6.2%) of the pts were under 40 years. The remaining 218(35.5%) were between 40 and 60 years old. The percentage difference was statistically signifi-

cant between the representation of the “above 60” group, compared to the other two modalities for $p < 0.05$ ($p = 0.0000$, difference test) (Figure 1).

Bilateral NCFB was found in 460(74.9%) of pts; 81(13.2%) had right-sided, while the remaining 73(11.9%) had left-sided bronchiectasis (Figure 2). The percentage difference was statistically significant between the representation of the bilateral bronchiectasis in respect to the other two modalities for location for $p < 0.05$ ($p = 0.000000$, difference test). Only 28(4.6%) of all pts had bleeding NCFB. No statistically significant correlation between bleeding bronchiectasis and smoking was registered (Pearson Chi-square: 2.91441, $p = 0.232887$).

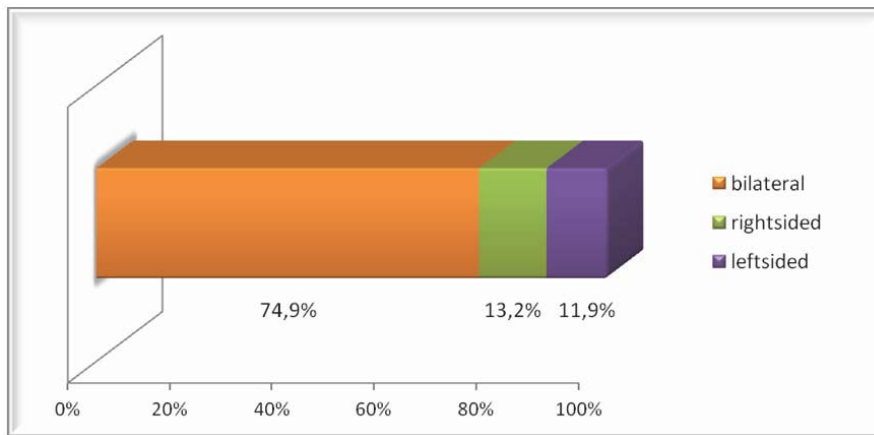


Fig. 2. Distribution of NCFB

In most of the patients with NCFB-202(32.9%), there was only one comorbidity. 144 patients or 23.4% had two comorbidities, three were found in 169(27.5%), four in 74(12.1%), five in 23(3.7%). Only two patients had six comorbidities. Of all patients who had a comorbidity, 376(61.2%) had chronic obstructive pulmonary disease (COPD). Other comorbidities by frequency were: chronic respiratory insufficiency (CRI) in 320(52.1%) pts, arterial hypertension (ATH) in 254(41.4%) pts,

cardiomyopathy (CMP) in 196(31.9%) pts, diabetes mellitus (DM) in 99(16.1%) pts, paranasal sinusitis in 44(7.2%), pulmonary fibrosis in 40(6.5%) and hemoptoe/hemoptysis in 21(3.4%) pts. No comorbidities were found in only 60(9.8%) patients (Figure 3). But, a statistically significant correlation was found between bleeding NCFB and the presence of comorbidities (Pearson Chi-square: 614.000, $p = 0.00000$).

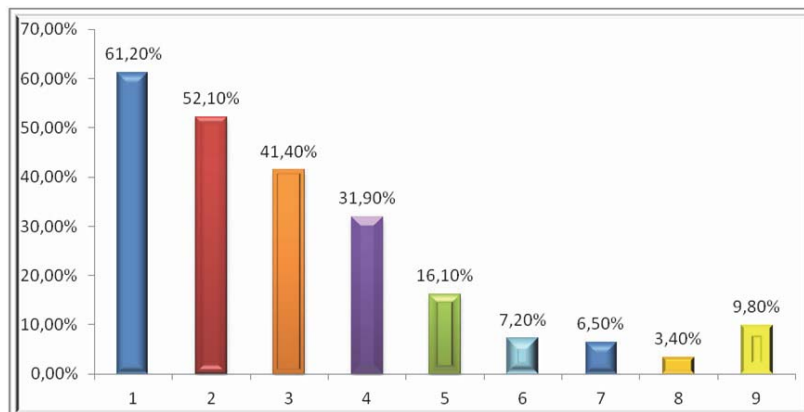


Fig. 3. Comorbidities in patients with NCFB

Legend: 1 - Chronic obstructive pulmonary disease (COPD), 2 - Chronic respiratory insufficiency (CRI), 3 - Arterial hypertension (ATH), 4 - Cardiomyopathy (CMP), 5 - Diabetes mellitus (DM), 6 - Paranasal sinusitis, 7 - Pulmonary fibrosis, 8 - Hemoptysis/hemoptoe, 9 - None

298(48.5%) of the analyzed patients were non-smokers, 159(25.9%) were former smokers, while 157(25.6%) were active smokers. The percentage difference was statistically significant between the representation of the non-smokers and the other two modalities for $p < 0.05$ ($p = 0.0000$).

Sputum for microbiology was not taken from the majority of patients, 388 or 63.2%. The reason for this was that pts had already been treated with antibiotics in the primary and/or secondary care, or had started empiric antibiotic treatment for the exacerbation due to

their primary diagnosis for hospitalization, or they had already had a proven *Pseudomonas* infection. In some pts the admission diagnosis was not bronchiectasis; however, after initial appropriate and antibiotic treatment and further investigations bronchiectasis was established. In 101 or 16.4% of patients, no pathogens were found. Of all patients that had sputum taken for analysis, a positive sputum culture was found in 125. *Candida albicans* was the most common, found in 62 pts. *Pseudomonas aeruginosa* was the second most frequent pathogen, found in 43 pts (Table 1).

Table 1. Sputum findings in patients with NCFB

Sputum	No.	%
Not taken	388	63.2
No pathogens	101	16.4
<i>Candida albicans</i>	52	8.5
<i>Pseudomonas aeruginosa</i>	33	5.4
Other	9	1.5
<i>Acinetobacter</i> species	5	0.8
<i>Pseudomonas aeruginosa</i> + <i>Candida albicans</i>	5	0.8
<i>Streptococcus pneumoniae</i>	4	0.7
<i>Streptococcus pyogenes</i> gr A	4	0.6
<i>Haemophilus influenzae</i>	2	0.3
<i>Pseudomonas aeruginosa</i> + <i>Streptococcus pyogenes</i> gr A	2	0.3
<i>Candida albicans</i> + <i>Haemophilus influenzae</i>	2	0.3
<i>Pseudomonas aeruginosa</i> + other	1	0.2
<i>Pseudomonas aeruginosa</i> + <i>Streptococcus pneumoniae</i>	1	0.2
<i>Candida albicans</i> + <i>Streptococcus pyogenes</i> gr A	1	0.2
<i>Candida albicans</i> + other	1	0.2
<i>Acinetobacter</i> species + other	1	0.2
<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter</i> species + others	1	0.2
<i>Candida albicans</i> , <i>Streptococcus pneumoniae</i> + others	1	0.2

On average, patients with NCFB were hospitalized for 10.3 days (10.3 ± 4.1), with a minimal stay of 1, and a maximal of 29 days of hospital stay.

Regarding the correlation between age and length of hospitalization, there was a statistically significant ($p = 0.030$), weak negative correlation ($r = -0.0873$) (Figure 4).

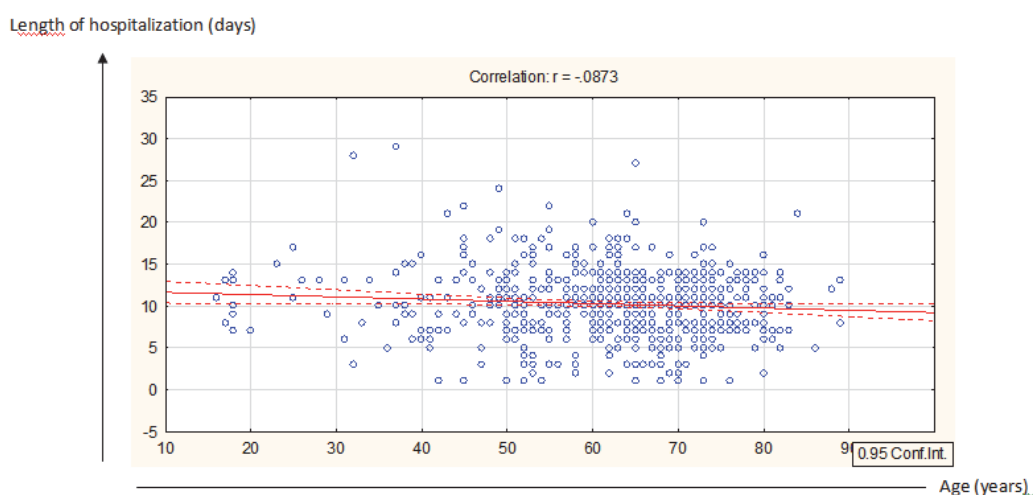


Fig. 4. Correlation between age and length of hospitalization

Within the patients with NCFB that had sputum taken for analysis, on average 101 pts with a negative sputum culture were hospitalized for 11.0 ± 4.222453 days, while 125 pts with a positive sputum culture were

hospitalized for 11.6 ± 4.563643 days. The difference was statistically insignificant for $p > 0.05$ ($p = 0.356007$); t -test = -0.924912 .

In the study, most of the patients, 265 or 72.5%, were hospitalized once. From the rehospitalized patients (27.5%), 56 or 15.3%, had only one rehospitalization. Three percentages of all patients were hospitalized 6 or more times. One patient was hospitalized 21 times.

Discussion

In literature, NCFB is most commonly found in females [9]. On the other hand, in our study the predominant gender was male (52.73%). Furthermore, males (53.6%) were also predominant in the number of hospitalizations, with statistically significant difference for $p < 0.05$. The average age was 61.3 years old. Our data corresponds with the global data where the average age is from 60 to 62 years [10,11]. Some studies that take into consideration children younger than 16 years found that hospitalizations related to bronchiectasis have two peaks, one under 10 years of age, the other above 65 [9]. In our study, the oldest age group, above 60 years, was the most commonly affected group and the difference between the representation of the "above 60" group in respect to the other two modalities (under 40 and between 40 and 60 years old) was statistically significant for $p < 0.05$. These results also correspond with the rest of the world, where the prevalence rises with age [8].

It should be emphasized that in elderly people with symptoms similar or the same as those associated with bronchiectasis, we should take NCFB into consideration and do the necessary examinations.

Bilateral bronchiectasis was the most common modality with 74.9% of all hospitalizations, and the statistical difference was significant for $p < 0.05$. COPD was the most recurring comorbidity with 61.2% of all hospitalizations. Some studies have shown subgroups that initially fulfill the criteria for asthma or COPD, which later, with the help of HR-CT, were diagnosed with NCFB [12,13]. Also, some studies emphasize that we should take into consideration the possibility of NCFB in non-smoking patients who have been diagnosed with COPD [14]. In our studied group, only 9.8% did not have any kind of comorbidity. The majority were non-smokers (48,5%). Active smokers were represented with under 30% (25.6%).

These percentages almost completely coincide with the numbers from other studies [15,16] and provide clear insight that a positive smoking status weakly correlates with the appearance of NCFB. In patients with positive sputum findings, *Candida albicans* and *Pseudomonas aeruginosa* were the most commonly isolated in 62 and 43 sputums, respectively. In 5 cases these two microorganisms were found simultaneously. In a number of studies, *Pseudomonas* has been indicated as the most common and a bacteria that

closely correlates with the appearance of a greater decline in the pulmonary function, more frequent exacerbations and a lower quality of life [16-19]. A study conducted by Chalmers related to vitamin D, found that vitamin D deficient patients were more susceptible to *Pseudomonas* colonization and had more frequent pulmonary exacerbations [20]. Less information can be found about the fungal colonization and prevalence in patients with NCFB [21-23]. However, studies of extreme importance show that prolonged usage of antibiotics against the most common bacteria such as *Pseudomonas* can give way to fungal colonization with fungi that originate from the oral microflora such as *Candida albicans* [21,24]. In one patient with comorbidities (polyneuropathia, alcoholism, hemoptoe) *Aspergillus niger* was isolated in bronchoalveolar lavage. In another patient, with other comorbidities (emphysema, respiratory insufficiency, pulmonary fibrosis and status post-pulmonary tuberculosis) *Aspergillus fumigatus* was isolated in bronchial aspirate. Other detected microorganisms were: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli*, *Stenotrophomonas maltophilia*.

Regardless whether they had sputum taken for analysis or not, patients with NCFB on average stayed in the hospital for 10.1 days. Only 27.5% of all hospitalized patients were rehospitalized, but 15.3% have only one rehospitalization. In one study in New Zealand [9], from all 1172 patients hospitalized in a year, 227 or 19% were rehospitalized in the first 90 days of their release. In the full course of the study which lasted for 5 years, 2364 of 5494 patients or 43%, were rehospitalized in the first year of their release.

Conclusion

NCFB was more frequent in male pts, with average age of 61.3 years. The average hospital stay was 10.1 days and 27.5% of all hospitalized pts were rehospitalized. The most common finding was bilateral bronchiectasis, while COPD was the most recurring comorbidity. Positive smoking status had weak correlation with the appearance of NCFB. The most commonly isolated were *Candida albicans* and *Pseudomonas*.

This is the first comprehensive study on NCFB in the Republic of Macedonia. Most of our results correlate with the findings from the literature. Recently NCFB has been a disease of renewed interest due to an increase in their prevalence and their association with different comorbidities and because of their influence on increasing the treatment costs of hospitalized pts. The available data on NCFB come from relatively small trials. Our study could bring new insight into epidemiology data concerning hospitalized pts with NCFB.

Conflict of interest statement. None declared.

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Original article

THE PROGNOSTIC ROLE OF BETA-CATENIN IN PATIENTS WITH ADVANCED STAGE SEROUS OVARIAN CANCER

В-КАТЕНИНОТ И НЕГОВАТА ПРОГНОСТИЧКА УЛОГА КАЈ ПАЦИЕНТКИ СО НАПРЕДНАТ СТАДИУМ НА СЕРОЗЕН ОВАРИЈАЛЕН КАРЦИНОМ

Igor Aluloski¹, Gordana Petrushevska², Rubens Jovanovic² and Mile Tanturovski¹

¹Department of Gynecologic Oncology, University Clinic of Gynecology and Obstetrics, ²Institute of Pathology, Medical Faculty, University "Ss. Cyril and Methodius" Skopje, Republic of Macedonia

Abstract

Introduction. Serous ovarian cancer is the most common sub-type of epithelial ovarian cancer and is the leading cause of cancer-related death among gynecologic cancer patients. Beta-catenin plays a vital role in the genesis of certain types of cancers. Its implications in the survival and prognosis of patients with serous ovarian cancer is not yet fully understood.

The aim of the study was to analyze the association between beta-catenin expression, as well as certain other clinical and pathohistological characteristics of serous ovarian cancers, with the overall patient survival in advanced stage cases.

Methods. We conducted immunohistochemical analysis in tumor specimens from 40 patients to determine the expression of beta-catenin. We analyzed the relationship between beta-catenin expression and the FIGO disease stage and the tumor grade. We used Kaplan-Meier statistics to analyze the prognosis.

Results. We detected increased expression of beta-catenin in patients with FIGO Stage III or IV ($p=0.0003$). We did not detect a statistically significant association between beta-catenin expression and tumor grade ($p=0.817$). The positive expression of beta-catenin was associated with shorter average survival ($p=0.034$). There was no statistically significant relationship between beta-catenin expression and other pathohistological tumor features.

Conclusion. Beta-catenin expression is associated with poorer prognosis in patients with serous ovarian cancer.

Keywords: ovarian cancer, beta-catenin

Апстракт

Вовед. Оваријалниот серозен крцином претставува

најчест поттип од епителните оваријални карциноми и претставува водечка причина за смртен исход во групата на гинеколошки карциноми. Се наметнува неопходна потреба за развивање дијагностички и прогностички маркери за ова заболување. β -катенинот игра централна улога во туморогенезата на одредени типови карциноми. Нецелосно е разјаснета улогата на β -катенинот во дијагнозата и во прогнозата на оваријалниот серозен карцином.

Цел. Целта на оваа студија е да ја анализира корелацијата меѓу експресијата на β -катенинот и клиничките и патохистолошките карактеристики, кај серозен оваријален карцином во напреднат стадиум и неговата корелација, во однос на севкупното преживување.

Методи. Во оваа студија, експресијата на β -катенинот беше испитана кај 40 пациентки со оваријален серозен карцином преку спроведени имунохистохемиски анализи. Анализирани беа соодносот меѓу експресијата на β -катенинот и FIGO стадиумот на болеста и патолошкиот градус. Со помош на Kaplan-Meier методата е направена анализа на прогнозата.

Резултати. Зголемената експресија на β -катенинот беше детектирана кај пациентки со FIGO стадиум III и IV ($p=0.003$). Не е утврден сигнификантен сооднос меѓу експресијата на β -катенинот и патолошкиот градус ($p=0.817$). Позитивната експресија на β -катенинот е поврзана со пониска стапка на преживување ($p=0.034$). Не е утврдена статистичка сигнификантност меѓу експресијата на β -катенинот и другите патолошки параметри.

Заклучок. Експресијата на β -катенинот може да користи како предиктивен маркер за лоша прогноза кај пациентки со напреднат стадиум на серозен оваријален карцином.

Клучни зборови: оваријален карцином, бета-катенин

Correspondence to: Igor Aluloski, Department of Gynecologic Oncology, University Clinic of Gynecology and Obstetrics, Adress: ul. Anastas Mitrev 29/10, 1000 Skopje, R. Macedonia; E-mail: i_aluloski@hotmail.com

Introduction

Ovarian cancer is the fifth most common gynecologic

malignancy in the developed world and the leading cause of cancer-related death in women with gynecologic malignancies. It accounts for 1.3% of all cancers in the USA. The National Cancer Institute approximates that in 2017, 22440 women in the US would be diagnosed with ovarian cancer, and 14080 of patients would die as a result of the disease [1]. The high incidence and mortality could be partially explained by the fact that most patients are diagnosed in advanced stage because of the insidious nature of the disease and the vague early symptoms. There have been advances in the past couple of decades in the treatment of the disease, including surgical technique advances, as well as the development of new generations of chemotherapy, but in spite of that, the overall five-year survival rates of advanced stage patients have remained relatively constant at around 28.8% [1]. Most of these patients experience recurrences and chemo-resistance. The most important tumor-associated prognostic factors for survival are disease stage, residual tumor size, histologic subtype of the tumor and the tumor grade. It is therefore prudent to investigate specific prognostic markers for ovarian cancer survival. The unraveling of the molecular mechanisms behind the pathogenesis of ovarian cancer could lead to improvement in the treatment modalities which in turn could improve survival.

Beta-catenin is a multi-functional cytoplasmic protein. Its gene is located on the 3p21 chromosome. Beta-catenin, together with E-cadherin plays a vital role in the forming of the cyto-skeleton. Downregulation of beta-catenin expression in the cell membrane has been noted in advanced stages of serous and clear cell ovarian cancers, which is associated with poor tumor differentiation and the presence of metastasis [2]. Not only does beta-catenin serve as the “inter-cellular glue” in complex with the E-cadherin, but also plays a pivotal role in the tumor genesis [3]. The Wnt protein, which acts on the cell membrane via frizzled and lipoprotein receptor-related proteins, inhibits the phosphorylation and the degradation of beta-catenin [4].

It is noteworthy that there is only limited amount of data that regards the prognostic value of the immune expression of beta-catenin in serous ovarian cancer and the association of beta-catenin expression with the clinical and pathological characteristics of the tumor and the overall survival rate. In this study we will detect beta-catenin expression in a set of samples from serous ovarian cancer.

Materials and methods

Patients

We analyzed data obtained from the medical records of all patients hospitalized for surgical treatment of advanced stage serous ovarian cancer at the University

Clinic of Gynecology and Obstetrics, University of “Ss. Cyril and Methodius”, Skopje, Macedonia from 01/01/2010 to 31/12/2012. All patients were followed up at least 36 months. All histological analyses were done at the Institute of Pathology, University of “Ss. Cyril and Methodius”, Skopje, Macedonia. We included data from 40 patients with advanced stage serous ovarian cancer (FIGO stages III and IV).

We recorded and analyzed the following parameters: age, disease stage (according to FIGO), tumor grade (classified as low or high based on the two-tier grading system recommended by Shimazu and Silverberg [5]).

Immunohistochemistry

The immunohistochemical analysis of beta-catenin expression was done using a monoclonal antibody-Human β -catenin, clone 17C2 (Novocastro), diluted to 1:100.

The results from the immunohistochemical staining were analyzed on a NIKON 80 light microscope and were phot-documented. All specimens were analyzed by three independent observers, blinded for the clinical outcome.

The beta-catenin staining of the cell membrane, cytoplasm and nucleus was evaluated according to the description published by C.M. Lee *et al.* [6]. The staining of the cytoplasm and nucleus was considered positive. The immunohistochemical staining of cancer cells was done in a semi-quantitative fashion depending on the percentage of positive cells. The percentage of stained cells in each section was coded as follows: “0” - <5% cells stained, “1” 5-50% of the cells stained and “2” if over 50% of the cells were stained [6].

Statistical analysis

All statistical calculations were performed in MedCalcver 12.1.4.0 2011 statistical software (Broekstraat at 52, 9030 Mariakerke, Belgium). The statistical significance of the differences was analyzed using the Chi-square test. The univariate survival analysis was done using the Kaplan-Meier method. Overall survival was defined as the time between the date of the initial surgical treatment and the date of the last follow-up and/or the date of death (if the death was cancer-related). Values for $p < 0.05$ were considered statistically significant.

Results

The average age of the patients was 52 years (range 25-79 years). Thirty-six patients (90%) were in FIGO stage III, while 4 patients (10%) were FIGO stage IV. During the follow-up period, of the 36 patients included in the study, 25 patients died as a result of the disease. The average follow-up duration was 24 months (range 12-36 months), while the average survival was 15 months (range 7-23 months).

Seventeen patients (42.5%) had low-grade tumors, while 23 (57.5%) patients had high-grade tumors (Table 1). Beta-catenin proteins were located on the cell membrane, cytoplasm and nucleus of the ovarian cancer cells.

Predominantly positive immuno-staining was observed on the cell membrane, as well as the cytoplasm (Figure 2 and 3). We did not observe isolated membrane beta-catenin positivity in the analyzed ovarian cancer cells.

Table 1. Association of beta-catenin, FIGO disease stage and tumor grade in serous ovarian cancer

Parameter	No. of patients (%)			Total	P
	Beta-catenin				
	-	+	++		
<i>Stage</i>					
III	6 (16.6)	16 (44.4)	14 (38.8)	36 (90)	0,003
IV	0	1 (25)	3 (75)	4 (10)	
<i>Tumor grade</i>					
Low	3 (17,6)	5 (29.4)	9 (52.9)	17 (42.5)	0,817
High	3 (13)	9 (39.1)	11 (47.8)	23 (57.5)	

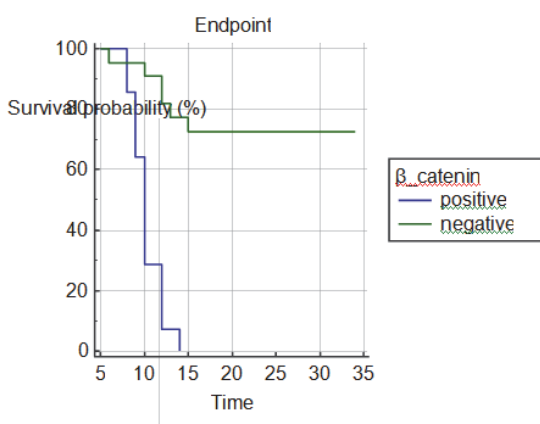


Fig. 1. Overall survival and beta-catenin expression in 40 patients with advanced stage serous ovarian cancer (Kaplan-Meier analysis, p=0.034)

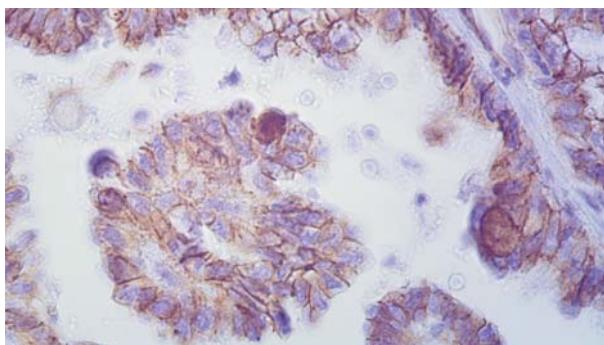


Fig. 2. Membrane and cytoplasmic expression of beta-catenin in a case of high-grade serous ovarian cancer (x400)

All stage III and IV cancers expressed a marked positivity for beta-catenin, independently of tumor grade. Indeed, 82% of the low-grade and 87% of the high-grade serous ovarian cancers manifested positive beta-catenin staining (Table 1).

During the follow-up period, 25 patients (62.5%) died as a result of causes associated with ovarian cancer. An increase of beta-catenin expression was significantly associated with poor overall survival (p=0.034) (Figure 1).

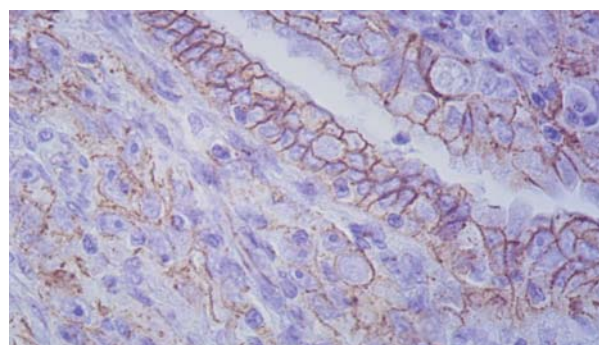


Fig. 3. Membrane expression of beta-catenin in a case of low-grade serous ovarian cancer (x400). A loss of expression is visible in a proportion of the cells

Discussion

Previous studies have associated beta-catenin with the oncogenic activity of human cancers. An increasing amount of data points towards the fact that beta-catenin is involved in the carcinogenesis, progression and metastatic spread of ovarian cancers [2, 7-10]. The role of the beta-catenin protein is different and depends on its location within the cell. E-cadherin plays an important role in the forming of cell junctions and is the key regulator of the differentiated phenotype of epithelial cells. E-cadherin forms complexes with other membrane proteins, including beta-catenin. The downregulation of membrane expression of beta-catenin is associated with poor histologic differentiation of cancer, increased risk of local invasion and metastatic spread of the tumor, which in turn is associated with poorer survival in such patients. The so-called Wnt pathway is another molecular mechanism that includes beta-catenin and is implicated in the process of tumor genesis. The activated beta-catenin within the Wnt pathway is accumulated in the cytoplasm and nucleus. In the current study, beta-catenin was primarily located on the surface membrane and cytoplasm of the ovarian cancer cells. We only found a small number of specimens with positive nucleus staining (2 out of the 40 serous ovarian cancers). We determined that

the loss of expression of beta-catenin on the cell membrane was often associated with a high tumor grade. We focused on the relation between beta-catenin presence in the cytoplasm and nucleus and the clinical and pathologic features of the cancer. Our data showed that the expression of beta catenin was positively associated with the advanced FIGO stage of disease, but was tumor-grade independent. Beta-catenin expression was also associated with significantly poorer prognosis. Lee *et al.* published that beta-catenin expression in the nucleus was associated with a high to moderate survival rate in patients with serous ovarian cancer [6]. Their data also illustrated that the beta-catenin distribution in the cell membrane, cytoplasm or nucleus was tumor-grade independent. Our data did not confirm that beta-catenin was exclusively present in the nucleus or the cell membrane of ovarian cancer cells.

In conclusion, increased expression of beta-catenin is detected more often in patients with advanced stage serous ovarian cancer. We did not find a significant association between beta-catenin expression and tumor grade. Positive beta-catenin staining points towards poorer survival rates. Therefore, beta-catenin could be used as a marker to determine the sub-group of serous ovarian cancer patients with increased risk of poor clinical outcomes.

Conflict of interest statement. None declared.

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Original article

PREVALENCE OF AUTOANTIBODIES AGAINST THE PLATELET GLYCOPROTEIN COMPLEXES GP IIb/IIIa, GP Ib/IX AND GP Ia/IIa IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

ПРЕВАЛЕНЦА НА АВТОАНТИТЕЛА СПЕЦИФИЧНИ ЗА ТРОМБОЦИТНИТЕ ГЛИКОПРОТЕИНСКИ КОМПЛЕКСИ GP IIb/IIIa, GP Ib/IX И GP Ia/IIa КАЈ БОЛНИ СО ХРОНИЧНА ЛИМФОЦИТНА ЛЕУКЕМИЈА

Marica Pavkovic, Lidija Cevreska, Aleksandar Stojanovic and Dimitar Efremov

University Clinic for Hematology, Medical Faculty, Skopje, Republic of Macedonia.

Abstract

Chronic lymphocytic leukemia (CLL) is a malignant disorder characterized by frequent occurrence of autoimmune hemolytic anemia (AИHA) and, less frequently, autoimmune thrombocytopenic purpura (ИТР). The pathogenic anti-platelet autoantibodies in patients with idiopathic (primary) autoimmune thrombocytopenia (ИТР) are usually directed against platelet glycoprotein complexes (GP). In this study we analyzed the presence of autoantibodies (auto-Abs) against the platelet GP complexes GP IIb/IIIa, GP Ib/IX and GP Ia/IIa in 55 consecutive CLL patients. All patients were simultaneously tested for the presence of anti-RBC auto-Abs. Antiplatelet auto-Abs against one or more GP complexes were detected in 25 patients (45%). Anti-RBC auto-Abs were detected in 18 patients (33%); 6 of them (11%) had evidence of AИHA at the time the sample was taken. The obtained data showed that platelet specific Abs developed frequently in patients with CLL, even more often than anti-RBC auto-Abs. The presence of platelet specific auto-Abs together with thrombocytopenia and normal Hb levels in 16% of the patients indicated that AИТР may be a more common cause of thrombocytopenia in CLL than previously anticipated.

Keywords: chronic lymphocytic leukemia, antiplatelet antibodies, GP complexes, autoimmune hemolytic anemia, autoimmune thrombocytopenia

Апстракт

Хроничната лимфоцитна леукемија (ХЛЛ) е малигно хематолошко заболување кај кое често се јавуваат автоимуни пореметувања, како што се: автоимуна хемолитичка анемија (АИХА) и автоимуна

тромбоцитопенија (АИТП). Патогените антиромбоцитни автоантитела, кај болните со идиопатска (примарна) автоимуна тромбоцитопенија, најчесто се специфични за тромбоцитните гликопротеински комплекси (ГК). Во оваа студија го анализиравме присуството на автоантитела, специфични за тромбоцитните ГК-GP IIb/IIIa, GP Ib/IX и GP Ia/IIa кај 55 пациенти со ХЛЛ. Кај сите пациенти истовремено го анализиравме и присуството на антиеритроцитни антитела. Антиромбоцитните антитела, специфични за еден или за повеќе гликопротеински комплекси (ГП) на површината на тромбоцитите, детектиравме кај 25 болни со ХЛЛ (45%). Антиеритроцитни антитела детектиравме кај 18 пациенти (33%), додека кај само шест од нив (11%) постоеја знаци за АИХА, во време на анализа, на присуството на антиеритроцитните антитела во крвта. Нашите податоци укажуваат дека антиромбоцитните автоантитела, почесто се јавуваат кај болните со ХЛЛ, во споредба со антиеритроцитните антитела. Присуството на антиромбоцитни антитела, придружено со тромбоцитопенија и нормално ниво на хемоглобин кај 16% од анализираниите болни, укажуваат на почеста појава на АИТП, од досега реферираниот во литература.

Клучни зборови: хронична лимфоцитна леукемија, антиромбоцитни антитела, гликопротеински комплекси, автоимуна хемолитичка анемија, автоимуна тромбоцитопенија

Introduction

Chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative disease, which is characterized by accumulation of morphologically small, mature CD5 positive B lymphocytes expressing low level of the surface immunoglobulin IgM or IgM/IgD antibodies. CLL is the most frequent type of adult leukemia in Europe; 90% of the patients are older than 50 years, and male patients are twice more often affected than

Correspondence to: Marica Pavkovic, University Clinic for Hematology, Medical Faculty, Skopje, Republic of Macedonia; E-mail: pavkovicm@yahoo.com

females. CLL is a malignant disorder characterized by the frequent occurrence of autoimmune disorders like hemolytic anemia (AIHA) and, less frequently, autoimmune thrombocytopenia (ITP).

The most common autoimmune disorder in patients with CLL is AIHA and it appears in 7-12% of CLL patients [1,2], but the incidence of red cell autoantibodies is even higher and in certain studies equaled 37% [1,2,3]. The appearance of autoimmune thrombocytopenia (ITP) is around 2%. However, ITP may appear much more frequently in patients with CLL [3,4], but it remains undetected due to lack of a sensitive and specific routine test for detection of anti-platelet antibodies.

The prevalence of anti-platelet antibodies in patients with CLL was analyzed in the study of De Rossi *et al.* [3] and they referred a very high prevalence of these antibodies (74%). They used a Dixon test, which detected the presence of platelet-associated antibodies. This test detects all antibodies that are present on the platelet surface, including non-pathogenic antibodies that are bound to the Fc receptor on platelets and do not cause their destruction. Therefore, this test has a very low specificity. Today it is known that pathogenic antiplatelet autoantibodies are most often specific for platelet glycoprotein complexes GPIIb/IIIa (fibrinogen receptor), GPIb/IX (receptor for von Willebrand factor) and GPIa/IIa (collagen receptor). Rarely autoantigens are other glycoprotein complexes on the platelet surface. These autoantibodies cause increased destruction and phagocytosis of opsonized platelets by the phagocytes in the RES system, especially in the spleen.

Nowadays, two methods are used for detection of autoantibodies specific to platelet glycoprotein complexes: MAIPA-monoclonal antibody immobilization of platelet antigens assay [5] and immunobead assay [6]. Both tests are used to detect free circulatory autoantibodies in the serum (indirect test) or to detect the antibody associated with the platelet complexes of the platelet membrane (direct test) in patients with idiopathic thrombocytopenia. The aim of our study was to investigate the prevalence of autoantibodies specific to GPIIb/IIIa, GPIb/IX and GP Ia/IIa in patients with CLL.

Materials and methods

The prevalence of anti-platelet autoantibodies specific to some of the three glycoprotein complexes (GPIIb/IIIa, GP Ib/IX, GPIa/IIa) was analyzed in 55 patients with chronic lymphocytic leukemia, diagnosed at the Clinic of Hematology, Medical Faculty in Skopje. The examined group consisted of 21(38%) women and 34(62%) men. The average age of the examined group was 63.6 ± 10.8 years. Patients were diagnosed on the basis of standard blood parameters at the Clinic of Hematology [7,8].

MAIPA-monoclonal antibody immobilization of platelet antigens assay is a method for detection of free

circulatory autoantibodies in the serum (indirect test) or for detection of antibody associated with platelet complexes of the platelet membrane (direct test) in patients with idiopathic thrombocytopenia. Complete kit (GTI-PAKAUTO) was used for detection of GP-specific anti-platelet autoantibodies based on the MAIPA assay. We used 10-40 ml of peripheral blood with anticoagulant-EDTA (depending on the number of platelets, complying with manufacturer's recommendations). Platelets were isolated by differential centrifugation (10 minutes at 800-1000 rpm). Then, the antiplatelet antibodies were eluted from the platelets with elution solution, and the eluate was frozen at -80°C or was used immediately.

Eluates were processed according to manufacturer's recommendations and incubated for 30-40 minutes in already coated wells with monoclonal antibodies specific for the three glycoprotein complexes (GPIIb/IIIa, GP Ib/IX, GPIa/IIa). After the incubation, they were eluted and PNPP p-nitrophenyl phosphate solution was added. The intensity of color (absorbance) was measured at 405 nm with standard ELISA reader.

Positive reactions (patients) were those in whom the coloration (OD-optical density value) was at least two times higher than the OD values of negative control (according to manufacturer's recommendation).

At the same time, all CLL patients were tested for the presence of anti-erythrocytes autoantibodies by using a direct antiglobulin test (DAT or Coombs test) and hemoglobin level and platelet count were measured.

Results

Our results from the direct MAIPA method showed that even 45% of patients with CLL (25/55) had antiplatelet autoantibodies specific to one or more of the glycoprotein complexes. In 9 patients (16%) we detected antibodies that had reacted with all three tested glycoprotein complexes (GPIIb/IIIa, GPIb/IX, GPIa/IIa); in other 9 patients (16%) we detected antibodies that had reacted with the two tested GP complexes, while 7 patients (12.6%) had antibodies specific to only one glycoprotein complex. At the same time, 33% of patients had a positive direct antiglobulin test, and 11% of these patients had parameters and signs for autoimmune hemolytic anemia.

In our study, we did not find a statistically significant difference in the number of platelets between MAIPA positive ($112 \pm 80 \times 10^6/\text{ml}$) and MAIPA negative patients with chronic lymphocytic leukemia ($137 \pm 90 \times 10^6/\text{ml}$), $p=0.62$. There was no statistically significant difference in the number of thrombocytopenic patients (platelet count less than $100 \times 10^6/\text{ml}$) between MAIPA positive (10/25) and MAIPA negative patients (15/30, $p=0.83$) (Table 1). However, in 10 of the 25 MAIPA positive patients thrombocytopenia with a normal hemoglobin level was isolated. This data indicates that the preva-

lence of autoimmune thrombocytopenia in our examined group equals 18% (10/55) (Table 2), which is much higher than reported in CLL patients (2-3%) and even higher than the prevalence of AIHA (11%).

Table 1. Number of platelets in MAIPA positive and negative patients with CLL

	Positive	Negative	
MAIPA	25 (45%)	30 (55%)	
Platelet count (n x 10 ⁶ /ml)	112 ± 80	137 ± 90	p=0.62
Thrombocytopenia	10/2	15/30	p=0.83

Table 2. Number of platelets and hemoglobin levels in MAIPA positive patients with CLL

MAIPA positive (n=25/55)	%
Plt < 100x10 ⁶ /mL	10/55 18%
Hb > 11g/L	
Plt < 100x10 ⁶ /mL	15/55 28%
Hb < 11g/L	

Discussion

Anemia and thrombocytopenia are common in CLL patients and they are negative prognostic factors (Rai *et al.*, Binet *et al.*) [7,8]. Anemia and/or thrombocytopenia may be caused by production defect (malignant infiltration of the bone marrow) or by the presence of autoimmune anti-platelet or anti-erythrocyte autoantibodies (causing the destruction of platelets and erythrocyte in the periphery), or both processes at the same time. Rarely the cause for anemia and/or thrombocytopenia is hypersplenism due to splenomegaly.

In addition, the mechanism and the cause of AIHA and the immune thrombocytopenia in CLL patients have been studied but are still not well known. One of the possible explanations for the common presence of anti-platelet and anti-erythrocytes antibodies in CLL patients is inhibition of some B or T lymphocytes clones that suppress autoreactive B cell clones [9,10]. The second possibility is that the malignant clone is producing auto-antibodies, after the process of isotypes switching and somatic hypermutation. The B-CLL cells are CD5+ B lymphocytes, and the CD5+ B lymphocyte often produce polyreactive, low affinity autoantibodies. There is a possibility for a malignant clone to go through the process of affinity maturation and after that to start to produce high-affinity mono-reactive autoantibodies [11,12]. The third possibility is that the malignant clone does not produce autoantibodies but it generates an autoimmune response by playing the role of an effective antigen presenting cell (APC) [11,13].

Our results regarding the MAIPA methods have shown high prevalence of antiplatelet autoantibodies (45%). At the same time, only 33% (18/55) of the CLL patients were positive for a direct antiglobulin test (DAT or Coombs test). The platelet count was not significantly different between MAIPA positive (112±80) and MAIPA negative patients (137±90), p=0.62. There was no statistically significant difference in the number of

We did not find any association between the positivity of the DAT test and the MAIPA test in our group of patients with CLL.

thrombocytopenic patients between MAIPA positive and MAIPA negative patients (p=0.83) (Table 1). These results have shown that very often antiplatelet autoantibodies are not pathogenic and do not cause thrombocytopenia [12]. It is interesting that 10/25 MAIPA positive patients had thrombocytopenia with a normal hemoglobin level, indicating that such isolated thrombocytopenia is most likely due to the presence of anti-platelet antibodies, rather than to the production defect. This data indicated that ITP was present in 18% of the CLL-tested patients in our study. This speaks of the much higher incidence of autoimmune thrombocytopenia than reported in the literature (2-3%) and it is higher than the incidence of AIHA (12%) in patients with chronic lymphocytic leukemia.

Conclusion

Our results indicated a high prevalence of anti-platelet antibodies (45%) in CLL patients and was higher than the prevalence of anti-erythrocyte antibodies (33%). In most cases, anti-platelet antibodies are not pathogenic, as they do not lead to a decrease in the platelet count. However, the presence of anti-platelet antibodies, accompanied by a reduced number of platelets and a normal hemoglobin level in 16% of patients with CLL, demonstrate that autoimmune thrombocytopenia is more common in CLL patients than it was assumed.

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Original article

MATERNAL OBESITY AS A PREDICTOR OF UNFAVORABLE PREGNANCY OUTCOME

ЗГОЛЕМЕНА ТЕЛЕСНА ТЕЖИНА КАЈ БРЕМЕНИ ЖЕНИ КАКО ПРЕДИКТОР ЗА НЕПОВОЛЕН ИСХОД НА БРЕМЕНОСТ

Ivo Kjaev¹, Dafina Kardjova¹, Ana Daneva¹, Rosa Spasova¹, Mile Tanturovski¹, and Silvana Jovanova²

¹University Clinic for Gynecology and Obstetrics, ²University Clinic for Cardiology, Medical Faculty, "Ss Cyril and Methodius" University, Skopje, Republic of Macedonia

Abstract

Introduction. Obesity is a growing concern worldwide. Maternal obesity has significant health implications, contributing to increased morbidity and mortality for both mother and baby. BMI is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults.

Aim. The aim of the study was to show a correlation between BMI, delivery mode, hypertension and prematurity.

Methods. The study was done at the University Clinic for Gynecology and Obstetrics. It was a case control observational prospective study, in which 63 pregnant women were evaluated. According to BMI pregnant women were divided in 3 groups: normal, overweight and obese. Women were recruited in the 28th gestational weeks and were followed until they delivered. Of interest were: hypertensive disorders in pregnancy, delivery mode, prematurity, Apgar score and newborns' weight.

Results. We found that increased BMI has strong association with hypertensive disorders in pregnancy, prematurity as well as with an increased caesarean section. We found that more than 76% of obese patients (BMI >30 kg/m²) were delivered with a cesarean section; median gestational age at delivery was 35.0 gestational weeks and hypertension in pregnancy was seen in 71% of these patients.

Conclusion. Maternal BMI shows strong associations with pregnancy complications and outcomes. Preventive strategies have to be introduced to reduce obesity and improve perinatal outcomes for both mother and baby.

Keywords: BMI, pregnancy, cesarean section, hypertension, prematurity

Апстракт

Вовед. Обезноста е феномен присутен насекаде низ

светот и е во постојан раст. Мајчината обезност има значителни здравствени импликации, придонесувајќи до зголемен морбидитет и морталитет за мајката и за детето. БМИ е едноставна вредност на тежина врз висина, која најчесто се користи да се класифицира зголемена тежина и обезност кај возрасни.

Цел. Целта на оваа студија е да покаже постоење корелација меѓу БМИ, начинот на породување, хипертензија и прематуритет.

Методи. Студијата се изведува на Универзитетската клиника за гинекологија и акушерство во Скопје. Станува збор за опсервациона проспективна студија. Во студијата се вклучени 63 трудници. По влезот во студијата, трудниците се поделени во три групи, во зависност од БМИ: со нормална тежина, со зголемена телесна тежина и со обезитет. Трудниците се регрутирани во 28 гестациска недела и се следени до породување. Инклузиони критериуми се: хипертензија во бременост, начин на породување, прематуритет, Апгар вредност и тежина на бебето.

Резултати. Во нашата студија беше потврдена силна асоцијација на зголемениот БМИ со хипертензија во бременост, прематуритет, како и зголемен број царски резови. Во повеќе од 76% од обезните испитаници (БМИ>30 kg/m²), начинот на породување беше со царски рез, со среднагестациска старост на породување-35,0 гестациска недела, а хипертензија беше забележана во 71% од случаите. Нашите резултати се слични со други светски студии.

Заклучок. Мајчиниот БМИ покажува силна асоцијација со компликации во самата бременост, но и во исходот од бременоста. Идните истражувања треба да се насочуваат кон стратегии за превенција, со цел намалување на обезитетот и подобрување на перинаталниот исход кај мајката и бебето.

Клучни зборови: БМИ, бременост, царски рез, хипертензија, прематуритет

Introduction

Obesity is a growing concern worldwide. Obesity rates have doubled in the last 30 years [1]. What is even more alarming is that rates are also increasing among pregnant women [2]. The WHO defines obesity as a disease in which the excess of adipose tissue accumulates in such a degree that it endangers health. Maternal obesity has significant health implications, contributing to increased morbidity and mortality for both mother and baby. Obesity increases the risk of miscarriage, gestational diabetes, gestational hypertension, thromboembolism and preeclampsia [3]. The causes of obesity are many. The most common cause of obesity is the development of energetic imbalance; there is an increased intake of high-calorie food rich in fat and sugars combined with little or no physical activity. The extra energy in the body is stored as fat. Some evidence suggests that genetics is in part responsible for obesity [4]. Leptin is a hormone that is produced mostly by the adipose tissue. With a range of regulative mechanisms it leads to a lower storage and better usage of fat. Scientists now believe that obese people have leptin resistance because their levels of leptin are higher than the normal population [5]. An international symbol and measurement for obesity is the BMI (body mass index). BMI is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). BMI is a simple, cheap and safe method of estimating body mass and health [6]. The American Institute of Medicine defined normal body weight as BMI 18.5 -24.9 kg/m^2 , overweight 25-29.9 kg/m^2 and obese above 30 kg/m^2 [2-7].

Aim

The aim of the study was to show a correlation between BMI, delivery mode, hypertension and prematurity.

Materials and methods

The study was done at the University Clinic for Gynecology and Obstetrics. A total of 63 pregnant women were enrolled in the study, and they were recruited from the University Clinic for Gynecology and Obstetrics in Skopje. Before entry in the study each pregnant woman was given and signed an informed consent for participation in this study. This study has a seal of approval from the Ethics Committee of the Medical Faculty in Skopje. The study is a prospective case control study. Pregnant women were enrolled in this study between the 28th and 34th gestational weeks. Every woman was examined for basic demographic and clinical data and got an obstetric examination; the pregnancy was being monitored with Volvuson 730.

Furthermore, pregnant women were divided in three groups according to BMI:

- BMI (18.5-24.9 kg/m^2) normal
- BMI (25 – 29.9 kg/m^2) overweight
- BMI > 30 kg/m^2 obese

The pregnancy was followed up to delivery.

Obstetric characteristics of interest were: delivery mode, gestational week, hypertension disorders in pregnancy, Apgar score, birth weight, SGA, IUFD, perinatal mortality.

Exclusion criteria for enrolment in the study were: twin pregnancy, preexisting chronic condition, diabetes mellitus, chromosome or ultrasound suspected malformation.

Statistical analyses

Descriptive data are shown as means and standard deviations. Independent t-test with Bonferroni correction for continuous data and χ^2 tests for categorical data were used for comparison between groups (SPSS 8.0 Chicago, IL). A value of $P < 0.05$ was considered as significant.

Results

So far 63 pregnant women have been examined in the study. Basic demographic characteristics of pregnant

Table 1. Demographic characteristics of all pregnant women (n-63) at the time of inclusion in the study

	Pregnancy (n,%)
Age, y (mean \pm SD)	31.57 \pm 5.48
Gestational age of pregnancy, wk	32.3
weight, kg	74
height,cm	163
Body mass index, kg/m^2	
Normal BMI (n,%)	23.1
Increased BMI (n,%)	29.6
Body surface area, m ²	1.83
sysBP, mmHg	140
diasBP, mmHg	90
Heart rate, bpm	87
Gestational diabetes (n,%)	6
Hypertensive disorders	23
Hypertension in pregnancy (n,%)	16
Preeclampsia (n,%)	7
Proteinuria(n)	7

Table 2. Obstetrics characteristics and outcomes of all pregnant women (n-63) in the study

Index pregnancy	Pregnancy (n, %)
Primiparous (n,%)	25
Mode of delivery	
Section cesarean (n,%)	42
Spontaneous vaginal (n,%)	21
SGA/IUGR	10
IUFD	/
Perinatal mortality (n,%)	/
Gestational age at birth, wk	35
Birth weight, g	2690
Apgar score	7/8

women at the time of entry in the study are shown in Table 1. Mean age was $37 \pm \text{SD}$, mean BMI was $33.7 \text{ kg/m}^2 \pm \text{SD}$, mean gestational week was $32 \pm \text{SD}$. Basic data about obstetric characteristics and outcomes are shown in table 2.

Table 3 illustrates the correlation we made by Chi

Square and Student's t-test. We found significant difference in gestational age when women were delivered, as well as in values of both systolic and diastolic blood pressure. Furthermore, the test confirmed a strong correlation between hypertension in pregnancy and BMI.

Table 3. Differences in demographic characteristics among pregnant women with normal and increased body mass index

	normal BMI	Overweight	Obese	P value
Age, years (average \pm SD)	31.67 ± 4.23	30.43 ± 6.36	32.57 ± 4.96	$0.45; 0.51^\dagger$
Gest. age of pregnancy, wk (average \pm SD)	39 ± 1	36 ± 3	36 ± 2	$P < 0.001^\dagger; p < 0.001^\dagger$
weight, kg	63	74	92	
height, cm	165	168	167	
Body mass index, kgm^2	23,1	26,2	33,1	
Normal BMI (n,%)				
Increased BMI (n,%)				
Body surface area, m ²	1,7	1,86	2,07	
sysBP, mmHg (average \pm SD)	123 ± 21	145 ± 17	152 ± 14	$P < 0.001^\dagger; p < 0.001^\dagger$
diasBP, mmHg (average \pm SD)	86 ± 12	94 ± 10	99 ± 11	$P = 0.026^\dagger; p < 0.001$
Heart rate, bpm	85	88	90	
Gestational diabetes		1	5	$P = 0.076^*$
Hypertensive disorders (HTA/PE)	2	6	15	$P < 0.001^*$
Proteinuria(n)	2	2	3	$P = 0.837^*$

*Chi Square test; † Student's t-test

Table 4. Differences in obstetric characteristics among pregnant women with normal and increased body mass index

	normal BMI (n,%)	Overweight (n,%)	Obese (n,%)	p value
Primiparous (n,%)	12 (48%)	7 (28%)	6 (24%)	$p = 0.266^*$
Mode of delivery				$p < 0.001^*$
Section cesarean (n,%)	4 (17%)	7 (58%)	16 (76%)	
Spontaneous vaginal (n,%)	19 (83%)	12 (42%)	5 (24%)	
SGA/IUGR	1	2	7	$P = 0.024^*$
IUFD	/	/	/	
Perinatal mortality (n,%)	/	/	/	
Birth weight, g (average \pm SD)	2935 ± 580	2582 ± 649	2667 ± 646	$P = 0.07^\dagger; p = 0.15^\dagger$

*Chi Square test; † Student's t-test

In table 4, significant difference was found between delivery mode and BMI; increasing BMI increases the likelihood of cesarean section.

Additionally, we examined the hypertensive disorders in pregnancy (Table 5). Because of the small sample of patients we did not further divide them in either gestational hypertension or preeclampsia. Confirmation of hypertensive disorder was done (gestational hypertension) if in two measurements that were done 4 hours apart the blood pressure was $\geq 140/90$ in women

with no prior history of hypertension [8]. The diagnosis of preeclampsia was confirmed if increased blood pressure was measured twice $\geq 140/90$ and there was proteinuria of 0.3 mg/l or qualitative (+) present. Women with hypertensive disorders were further followed up at the University Clinic for Cardiology.

By using Chi Square test we found statistically significant hypertensive disorders in pregnancy related to increased BMI.

Table 5. Association of body mass index and hypertensive disorders in pregnancy

BMI	Normotensive pregnancy (n,%)	HTA/preeclampsia (n,%)	P value*
BMI ($18.5\text{-}24.9 \text{ kg/m}^2$)	21 (88.5%)	2 (10.5%)	$P < 0.001$
BMI ($24.9\text{-}29.9 \text{ kg/m}^2$)	13 (69%)	6 (31%)	$P < 0.001$
BMI ($>30 \text{ kg/m}^2$)	6 (24%)	15 (76%)	$P < 0.001$

*Chi Square test

Discussion

Obesity and increasing obesity in young women is a growing concern around the world, and Macedonia is no exception. Due to lifestyle habits obesity will be a problem of the coming generations. Economic, technologic, and lifestyle changes have created an abundance of cheap, high-calorie food coupled with decreased required physical activity. We are eating more and moving less.

Data from PRAMS has shown that the prevalence of prepregnancy obesity increased by 69% over a 10-year period, from 13% in 1993-1994 to 22% in 2002-2003. The normal values advocated by the Institute of Medicine are from 11.5-16 kg for normal weight, 7-11.5 kg for overweight and 5-9 kg for obese women [9]. We felt that this concern has to be put out towards our colleagues and the entire community. Pregnant women have to be advised and controlled about weight gains in pregnancy.

The results from our study have similar trends as other studies reported. The largest limitation of this study is the small sample size. Our results show the higher BMI level the higher likelihood of a cesarean section. We found that the rate of caesarean section among obese women was 76%; in overweight women it was 58% and in women with a normal BMI 17% ($p < 0.001$). These percentages are much higher than the overall percentage of caesarean section in our clinic which is around 35%. Similar studies have shown results like ours [10-13]. Our results are in agreement with those published in previous studies [14] showing a strong association between increasing BMI and gestational hypertension/preeclampsia ($p < 0.001$). The rate seen in our obese group of patients is extremely high 71%. The high number can be attributed to poor health care and poor prenatal control in our environment. These results coincide with the results of O'Brien TE *et al.* that showed that the risk of preeclampsia doubled with every 5 to 7 kg/m^2 increase. There is a linear relationship between increasing BMI and the risk of developing hypertensive disorders in pregnancy, induction of labor and cesarean section. Similar to the majority of studies, we found that overweight and obesity were responsible for the increased number of prematurity [15,16]. Namely, the median gestational week of delivery in overweight women in our study was 36.0 gestational weeks and in obese women group it was 35.1 gestational week ($p < 0.001$). The normal BMI group median delivery time was in the 39.1 gestational week. When the average weight of fetuses was examined in the three groups a small statistical significance was found, which is not the case in other studies [17]; namely, obesity is responsible for LGA babies. This is probably because of the small sample size of patients. It is worth mentioning that up to now gynecologists have failed to realize the complications of increased

BMI. This study found a statistical significance between obesity and SGA/IUGR fetuses ($p < 0.024$), but more patients are needed to establish a better correlation.

Most of the complications are curable with early recognition and control. Balancing the risks of fetal growth (in the large-for-gestational-age fetus and the small-for-gestational-age fetus), obstetric complications, and maternal weight retention is essential but will remain challenging until research provides evidence to further refine the recommendations for gestational weight gain, especially among women with high degrees of obesity. Thus, further studies are needed to find the optimal treatment for these women.

Conclusion

The nutritional status and weight gain of a mother during pregnancy are important healthcare indicators. Obesity and overweight before and in pregnancy have a negative influence on the health of the mother and the infant. Maternal BMI shows strong associations with pregnancy complications and outcomes. Future work should be concentrated in prevention strategies in order to reduce obesity and so improve perinatal outcomes for both mother and baby.

Conflict of interest statement. None declared.

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Original article

COMPARISON OF BENZODIAZEPINES AND OPIOIDS AS ORAL PREMEDICATION IN PEDIATRIC ANESTHESIA

КОМПАРАЦИЈА НА БЕНЗОДИЈАЗЕПИНИ И ОПОИДИ КАКО МЕДИКАМЕНТИ ЗА ОРАЛНА ПРЕМЕДИКАЦИЈА КАЈ ПЕДИЈАТРИСКИ ПАЦИЕНТИ

Ljupcho Donev, Mirjana Shosholcheva, Andrijan Kartalov, Albert Leshi, Marija Jovanovski-Srceva, Marija Toleska, Angela Trposka, Ana Stojkowska, Iskra Nedelkova and Filip Naumovski

University Clinic for Anesthesia, Reanimation and Intensive Care, Medical Faculty, "Ss Cyril and Methodius" University, Skopje, Republic of Macedonia

Abstract

Introduction. Premedication is widely used in pediatric anesthesia. Its objectives are to produce sedation, anxiolysis, to reduce emotional trauma and to facilitate induction of anesthesia. An ideal premedication drug should give consistent, predictable results, good patient acceptance and be free of side-effects. Different drugs can be used for premedication with different extent of side effects. The aim of this study was to determine whether premedication with opioids or with benzodiazepines is more acceptable for the patients, produces better sedation, anxiolysis, better compliance with induction of anesthesia and better emergence score postoperatively.

Methods. The study included 60 patients, aged 2-5 years, with body weight 10-20 kg and ASA (American Society of Anesthesiologists physical status) I/II, undergoing elective open surgical procedure for one-sided inguinal hernia. Preoperatively patients were randomly allocated to receive one of two medications for premedication. Group A (n=30) were patients who received 2.5 ml (oral solution of fentanyl) OF (15-20 µg/kg). Group B (n=30) were patients who received midazolam syrup (0.5 mg/kg). We assessed the score for acceptability of the premedication, the sedation and anxiety score. After arrival in the OR the child's compliance with induction of anesthesia was analyzed. Postoperatively in PACU (Post-Anesthesia Care Unit) 'emergence score' and the need for additional analgesia were analyzed in each child.

Results. OF was more effective in producing sedation and anxiolysis; it was more acceptable for patients and gave better compliance with induction of anesthesia compared to midazolam. Statistically significant difference was noticed in the emergence scores between the two groups. Patients who received fentanyl as a premedication required a reduced amount of opiates in the

postoperative period.

Conclusion. Oral fentanyl and midazolam used in their adequate doses 10-15 µg/kg and 0.5 mg/kg are safe and effective medications for premedication. The premedication with fentanyl reduces the need for opiates in the postoperative period.

Keywords: premedication, fentanyl, midazolam

Апстракт

Вовед. Премедикацијата е широко користена во педијатриската анестезија, со цел да обезбеди седација, анксиолиза, намалување на емоционалната траума и да овозможи мирен вовед во анестезијата. Идеалниот лек за премедикација треба да има постојани, предвидливи резултати, да биде добро прифатен од пациентот и да нема несакани ефекти. Различни лекови може да се користат како премедикација, со различен опсег на несакани ефекти. Целта на оваа студија е да се утврди дали премедикацијата со опиоиди или премедикацијата со бензодиазепини е поприфатлива за пациентите, дали обезбедува подобра седација, подобра анксиолиза, подобра кооперабилност на воведот во анестезија и подобар постоперативен резултат.

Методи. Во студијата беа вклучени 60 пациенти, на возраст од две до пет години, со телесна тежина од 10 до 20 килограми, ASA класификација 1 и 2, планирани за елективна отворена операција на еднострана ингвинална хернија. Предоперативно, пациентите, по случаен избор беа поделени во две групи, од по 30 пациенти: **Група А** - на кои им беше дадена премедикација 2,5 ml орален раствор - нафентанил (10-15 µg/kg) и **Група Б** - на кои им беше дадена премедикација - сируп мидазолам 0,5 mg/kg. Беше одреден резултатот за прифатливост на премедикацијата, резултат за седација и за анксиозност. По пристигнување во операционата сала беше анализирана кооперабилноста на пациентите за време на воведот во анестезија. По завршување на опера-

цијата, во PACU (Post anesthesia care unit) за секое дете беа анализирани резултатите за итност (emergence score) и потребите за дополнителна аналгезија.

Резултати. Оралниот фентанил беше подобро прифатен од пациентите, поефикасен, во однос на постигнатиот степен на седација и анксиолиза, и даде подобра кооперабилност на пациентите во текот на воведот во анестезија, во споредба со мидазолам. Сигнификантни статистички разлики имаше во резултатот за итност (emergence score) меѓу двете групи. Кај пациентите, кои беа премедицирани со фентанил, постоперативно беше забележана намалена побарувачка на опијати.

Заклучок. Оралниот фентанилот и мидазолам, употребени во соодветните дози, 10-15 μ g/kg и 0,5 mg/kg се безбедни и ефикасни средства за премедикација. Премедикацијата со фентанил ја намалува побарувачката на опијати во постоперативниот период.

Клучни зборови: премедикација, фентанил, мидазолам

Introduction

Premedication is widely used in pediatric anesthesia. Its objectives are to produce sedation, anxiolysis, to reduce emotional trauma and to facilitate induction of anesthesia [1]. Preoperative anxiety is correlated with various negative outcomes such as higher postoperative analgesic requirements, longer postanesthesia care unit and hospital stay, and delayed negative psychological effects. Additionally extreme anxiety and stress before surgery result in negative postoperative sequelae such as emergence delirium, maladaptive behavior (postoperative general anxiety, night time crying, enuresis, separation anxiety, decreased eating improvement, withdrawal, apathy) and increased postoperative pain [2,3,4]. Furthermore, preoperative anxiety activates the human stress response, leading to increased serum cortisol, epinephrine, and natural killer cell activity. This hormonal changes together with immunological and metabolic changes constitute the global surgical stress response which is considered a homeostatic mechanism for adapting to the perioperative injury [5,6]. Therefore, one of the major objectives of preanesthetic medication is to decrease the stress response with preservation of hemodynamic parameters [6].

The child's age, body weight, drug history, allergic status and underlying medical or surgical conditions are factors to be taken into consideration prior to administration of premedication. In most cases, medications administered without a needle are more pleasant to the children [4]. Literature reviews have published results that majority of children are premedicated via the oral route (80%), followed by the

intranasal route (8%), the IM route (6%), and the rectal route (3%) [6].

The most common question that clinicians and researchers argue about is "which premedication drug is more "ideal" for oral premedication in children?" This answer of the question remains controversial because of the different pharmacokinetic and pharmacodynamic characteristics those different drugs have as well as the individual response of the children to these drugs. The most commonly used sedative premedications in the preoperative holding area are midazolam (85%), followed by ketamine (4%), transmucosal fentanyl (3%), and meperidine (2%).

Novel data and some inspirational studies show results that fentanyl given in a form of lozenge, syrup or tablet has a potential to abolish oral midazolam as a drug of choice for premedication in children. Although these studies are limited in aspects of material and method they might open new door in the premedication aspects in children [7-14]. The aim of this study was to determine whether premedication with opioids (fentanyl) or with benzodiazepines (midazolam) is more acceptable for patients, produces better sedation, anxiolysis, better compliance with induction of anesthesia, better emergence score postoperatively and less analgesia.

Materials and methods

The study included 60 patients, aged 2-5 years, with body weight 10-20 kg, ASA (American Society of Anesthesiologists physical status) I/II, undergoing elective open surgical procedure for one-sided inguinal hernia. Pre-operatively patients were randomly allocated to receive one of two medications for premedication. Group A (n=30) comprised patients who received 2.5 ml oral fentanyl solution (15-20 μ g/kg), and group B (n=30) patients who received midazolam syrup (0.5 mg/kg). (Table 2).

Children were given instructions for the premedication and the score for acceptability of premedication was monitored (Table 1). Heart rate and saturation (by pulse oximetry) and respiratory rate were measured and recorded every 5 minutes once premedicated. After 30 min an assessment of sedation and anxiolysis was made using a scoring system (Table 4 and 5)). After arrival in the OR the child's compliance with induction of anesthesia was assessed (Table 6).

After entering the operating room the children were put on continuous monitoring of heart rate, ECG, arterial blood pressure, oxygen saturation and capnography. The standardized anesthesia started with fentanyl at a dose of 2 μ g/kg and propofol at a dose of 2-3 mg/kg. To facilitate intubation 0.6-1 mg/kg rocuronium bromide was used. After intubation, children were ventilated in controlled manner with oxygen and air (50%; 50%). The parameters of tidal volume and frequency were

determined according to the age with a target of end-tidal CO₂ of 35-40 mmHg. In all children anesthesia was maintained with a continuous propofol infusion of 125-300 µg/kg/min.

All children received antiemetic ondansetron dose of 0.1 mg/kg. Fifteen minutes before the anticipated end of surgery, 15 mg/kg paracetamol was given to all children. At the termination of surgery neuromuscular blockade was reversed with neostigmine of 50 µg/kg and atropine 20 µg/kg and the trachea was extubated at

resumption of spontaneous ventilation and return of reflexes.

Immediately after surgery the children were monitored in PACU (Post-Anesthesia Care Unit) and each child was awarded an overall 'emergence score" (Table 7). Postoperative analgesic requirements were assessed during the two hours in PACU and the next four hours in the hospital ward. Morphine of 0.5-1 mg/kg was administered as an analgesic drug of choice.

Table 1. Scoring systems for acceptability of premedication, anxiety, sedation, compliance with anesthetic induction and emergence in recovery

Score	Acceptability of premedication
1	Accepts – with enthusiasm
2	Accepts
3	Accepts – but needs encouragement/persuasion
4	Accepts – but then spits out or rejects
5	Refuses
Score	Anxiety
1	None
2	Little-slight expression of fear/apprehension, easily reassured
3	Moderate – clearly fearful, cries but becomes quiet with reassurance
4	Excessive – crying, uncooperative, combative
Score	Sedation
1	Asleep – not easily arousable
2	Asleep – slowly responds to verbal commands or gentle stimulation
3	Drowsy – readily responds to verbal commands
4	Awake – calm and quiet
5	Awake – alert/active
Score	Compliance with anesthetic induction
1	Excellent – fully cooperative
2	Good – mostly cooperative, responds to reassurance
3	Fair – moderate fear/crying, not quiet with reassurance
4	Poor – stormy, crying, need for restraint
Score	Emergence
1	Excellent – quiet
2	Good – occasional crying
3	Fair – crying but able to be quieted
4	Poor – thrashing, unable to be quieted

The data was analyzed using the Statistical Package for Social Sciences (SPSS), version 17.0. Structure of numerical series was analyzed using measures of central tendency (average) and measures of dispersion (standard deviation). Testing of significance of differences between scores in groups A and B was done with the non-parametric Mann-Whitney U Test. A *p*-value less than 0.05 was considered statistically significant.

Results

Demographic and clinical data in both groups of patients were homogenous.

Average age in both groups was 3 years (min=2; max=5), while on average children weighted 15.8 kilos in group A and 14.5 kilos in group B. More children in group A were male -17 (56.7%) while more patients in group B were female-16 (53.3%).

Table 2. Demographic and clinical characteristics

Variables	Group A (n=30)	Group B (n=30)
Age (years) (mean)	3.10±1.06	3.15±0.89
Body weight (kg) (mean)	15.8±2.68	14.5±2.59
Ratio M/F (n)	17/13	14/16

Majority of the children in both groups were classified as ASA I (Table 2).

The acceptability scores were significantly better in oral fentanyl group (Mann-Whitney U test: $Z = -3.311$ $p = 0.0009$). Twenty-seven (90%) children accepted fentanyl without any need for persuasion, and 18 (60%) the syrup. One child who refused the oral fentanyl had been persuaded to take the syrup. Five children refused the syrup but all of them accepted the oral fentanyl (three enthusiastically, two with persuasion).

Table 3. Acceptability scores awarded for the syrup and the oral fentanyl given to each child

Score	(Oral Fentanyl) (n=30)	(Midazolam Syrup) (n=30)
1	22 (73.3%)	8 (26.7%)
2	5 (16.7%)	11(36.6%)
3	2 (6.7%)	6 (20.0%)
4	1(3.3%)	3 (10.0%)
5	0 (0%)	2 (6.7%)

Thirty minutes after the active premedication was given to the children, a better sedation score was noted in the children who received fentanyl. In group A, 17 (56.7%) children were asleep, slowly responding to verbal commands or gentle stimulation and in group B only 12 (40%) children.

Table 4. Sedation scores recorded 30 min after taking an active pre-med

Score	Group A (n=30)	Group B (n=30)
1	0 (0%)	0 (0%)
2	1 (3.3%)	1(3.3%)
3	7 (23.3%)	10 (33.3%)
4	17(56.7%)	12 (40.0%)
5	5 (16.7%)	7 (23.4%)

Thirty minutes after the active premedication was given to the children, a better anxiolysis, reduced incidence of crying and fear was noted in the children who received fentanyl compared to the children who received midazolam.

Table 5. Anxiety scores recorded 30 min after taking an active pre-med

Score	Group A (n=30)	Group A (n=30)
1	18 (60.0%)	16 (53.4)
2	10 (33.3%)	11(36.6%)
3	2 (6.7%)	3 (10.0%)
4	0 (0%)	0 (0%)

During the induction to anesthesia, 25(83.4%) children from group A and 20(66.7%) children from group B showed an excellent cooperation, which indicates the premedication with fentanyl affected the improvement of cooperation of the children.

Table 6. Compliance score with anesthetic induction

Score	Group A (n=30)	Group B (n=30)
1	25 (83.4%)	20 (66.7%)
2	4 (13.3%)	6 (20.0%)
3	1(3.3%)	4 (13.3%)
4	0 (0%)	0 (0%)

The children in group A had significantly better emergence scores than those in the midazolam group (Mann-Whitney U test: $Z = -2.398$ $p = 0.0164$).

Table 7. Emergence scores recorded after surgery

Score	Group A (n=30)	Group B (n=30)
1	20 (66.7%)	10 (33.3%)
2	3 (10.0%)	6 (20.0%)
3	5 (16.7%)	10 (33.3%)
4	2 (6.7%)	4 (13.3%)

In the children who received fentanyl as a premedication, postoperatively the time interval of pain appearance was prolonged, and these children required less analgesics (opiates).

Table 8. Postoperative analgesic requirements of opiates within the first 2 hours in PACU (Post-Anesthesia Care Unit) and within the next 4 hours in hospital ward

Opiates requirement	Group A (n=30)	Group B (n=30)
In PACU	0 (0%)	2 (6.7%)
On ward – within first 2 hours	3 (10.0%)	4(13.3%)
On ward – within next 2 hours	4 (13.3%)	5 (16.6%)
Total	7(23.3%)	11(36.6%)

Discussion

This study shows that opioids, oral fentanyl (OF), are more effective medicaments for sedation, anxiolysis and cooperation for anesthetic induction compared with benzodiazepines (midazolam). Furthermore, opioids have certain advantages over benzodiazepines in that they are more appealing to children, have better emergence characteristics and most important they have advantages over postoperative analgesic requirements in comparison to children who required analgesics and prolonged time interval of pain appearance.

The optimal premedication dose for oral fentanyl is 10-20 $\mu\text{g}/\text{kg}$, although some authors recommend doses of 10-15 $\mu\text{g}/\text{kg}$ [12-15]. Based on clinical experience we felt that a dose of 10-15 $\mu\text{g}/\text{kg}$ oral fentanyl was appropriate for an undergoing elective open surgical procedure for one-sided inguinal hernia. We chose to compare OF with one of the most commonly used sedative premedication in the preoperative holding area, midazolam as a syrup at a dose of 0.5 mg/kg [3]. The ideal pediatric premedication should be acceptable or preferably appealing to children. It should have a predictable onset and a reliable anxiolytic effect. It should be safe and have no, or minimal, upsetting side-effects, and cause no postoperative sequels [15] As expected, the acceptability scores were significantly better in the OF group. Twenty-seven children (90%) accepted the OF without any need for persuasion, and 18 children (60%) accepted the syrup. One children who refused the OF had been persuaded to take the syrup. Five children refused the syrup, but all of them accepted the OF (three enthusiastically, two with persuasion). Thirty minutes after the active premedication was given to the children, a better sedation score, a better anxiolysis, reduced incidence of crying and fear was

noted in the children who received fentanyl. In group A 17 children (56%) were asleep, slowly responding to verbal commands or gentle stimulation and in group B only 12 children (40%). Previous studies have shown different outcomes in sedation scores: Schechter (5) and Freisen [7] found OF not to be sedative, whereas several other studies [12,14,15] have found OF to be sedative.

During the induction to anesthesia, 25 children (83%) from group A and 20 children (66%) from group B showed an excellent cooperation, which indicates the premedication with fentanyl affects the improvement of cooperation of the children.

OF is safe and effective for use in relieving the pain of pediatric procedures, but frequent vomiting may restrict its clinical usefulness. Significant differences in pain ratings between the OF and placebo groups were noted on the pain scores [15]. In our study the children in the OF group had significantly better emergence scores than those in the midazolam group. In group A, where children received fentanyl as a premedication, post-operatively the time interval of pain appearance was prolonged, and these children required fewer analgesics (opiates). Seven children (23%), who received fentanyl as a premedication required analgesics: 0 in PACU (Post-Anesthesia Care Unit), 3 within the first two hours in the hospital ward and 4 the next two hours on ward.

Conclusion

Oral fentanyl and midazolam used in their adequate doses, 10-15 µg/kg and 0.5 mg/kg respectively, are safe and effective medications for premedication. The opioids used as a premedication in pediatric anesthesia produce better sedation and anxiolysis than benzodiazepines. The premedication with fentanyl reduces the requirement of opiates in the postoperative period.

Conflict of interest statement. None declared.

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Original article

MAINTENANCE OF THERAPEUTIC EFFECT OF ORAL ANTICOAGULANTS (ACENOCOUMAROL) IN LONG-TERM TREATMENT OF PATIENTS WITH PULMONARY EMBOLISM - REALITY OR VISION

ОДРЖУВАЊЕ НА ТЕРАПИСКИ ЕФЕКТ НА ОРАЛНИ АНТИКОАГУЛАНСИ (АЦЕНОЦОУМАРОЛ) ПРИ ДОЛГОТРАЕН ТРЕТМАН НА БОЛНИ СО ПУЛМОНАЛНА ЕМБОЛИЈА- РЕАЛНОСТ ИЛИ ВИЗИЈА

Marija Zdraveska, Deska Dimitrievska, Dejan Todevski and Elena Janeva

PHO University Clinic of Pulmology and Allergy Skopje, Macedonia

Abstract

Introduction. Pulmonary embolism (PE) is a potentially lethal condition associated with acute morbidity and propensity to recurrent episodes. Tailoring of the long-term treatment is essential. This retrospective study comprised 105 patients treated at the University Clinic of Pulmology and Allergy in Skopje for acute PE. Demographic data, risk factors, INR values during follow-up, number of attempts and time to achieving therapeutic INR were registered.

Results. In only 34.6% of the overall time of follow-up INR was in the optimal range of 2-3. Two or more than 2 attempts were made in most of the patients, leading to prolongation of hospitalization, and the average time to optimal INR was 1.012 months.

Conclusion. Although still treatment of choice, it is usually difficult to obtain therapeutic range of INR with acenocoumarol, which by itself represents a problem in maintaining a low risk of recurrent thrombotic incidences. New, more efficient and safer modes of treatment, which offer stable therapeutic effect with better patient compliance, are necessary.

Keywords: pulmonary embolism, treatment, acenocoumarol

Апстракт

Вовед. Пулмоналната емболија (ПЕ) е потенцијално летална состојба, асоцирана со висок морбидитет и морталитет и повторувачки тромботични епизоди. Од клучно значење е правилно моделирање на терапијата на одржување кај овие болни. Прикажуваме ретроспективна студија на 105 болни со ПЕ, третирани на Клиниката за пулмологија и алергологија, Скопје. Следени се демографските пода-

тоци, факторите на ризик, вредностите на ИНР, во тек на целокупниот третман, бројот на обиди, како и потребното време до постигнување оптимални вредности на ИНР.

Резултати. Само во 34.6%, ИНР бил во оптималните рамки 2-3. Бројот на обиди до постигнување тераписки ИНР беше 2 и повеќе, кај најголем број од болните; во просек 1.012 месеци беа потребни за постигнување оптимален ИНР.

Заклучок. При терапија со аценокумарол е често тешко да се постигне тераписки ИНР, поради што е зголемен ризикот за рекурентни тромботични епизоди. Потребни се нови, поефикасни и побезбедни модалитети на третман, кои би понудиле поголема тераписка стабилност, и подобра соработка на болните.

Клучни зборови: пулмонална емболија, третман, аценокумарол

Introduction

Pulmonary embolism (PE) and deep venous thrombosis (DVT) as the two features of venous thromboembolism (VTE) are common causes of morbidity and mortality. Often presenting with occult clinical signs and symptoms, they have traditionally been a major diagnostic and therapeutic problem of health care providers in various clinical settings. The incidence of PE is approximately 60 to 70 per 100,000, and its management remains a problem in spite of numerous guidelines published [1]. For the purpose of alleviating the decision-making process, the treatment of VTE and PE is usually presented as treatment of the acute phase, encompassing the first 5-10 days of diagnosis, followed by "long-term", or maintenance treatment in the period of 3, 6 or more months after the acute incident [2]. Estimation of the optimal duration of the long-term treatment and proper choice of medication represents a balance between risk and benefit (estimation of risk of bleeding versus risk of recurrent VTE) and must

Correspondence to: Marija Zdraveska, PHO University Clinic of Pulmology and Allergy. „Bul Mother Theresa” 17, 1000 Skopje, R. Macedonia; Phone: 071 40 07 65; E-mail: mzdraveska@gmail.com

be individualized for each patient and clinical setting. Exactly this period of long-term treatment still contains paucities and is consequently a matter of ongoing debate, even in the contemporary guidelines [3].

One of the major problems of the long-term treatment of PE is the possibility to tailor the adequate dosage of the administered drug in order to keep the international normalized ratio (INR) values in therapeutic range and to optimize the prevention of recurrent VTE.

Aim of the study

The primary goal of this retrospective study was to evaluate the effect of the long-term treatment with oral anticoagulants (Vitamin K antagonists, especially acenocoumarol-AC) in patients with pulmonary embolism, as well as to estimate the possibility of long-term maintenance of the therapeutic effect of the drug, measured by the INR, throughout the whole duration of treatment. As a secondary outcome we present the risk for recurrent deep venous thrombosis (DVT) and pulmonary embolic (PE) incidents in the evaluated patients, as well as the incidence of complications associated with the long-term treatment in our patients.

Material and methods

The study analyzed the data obtained from 105 patients, who were treated at the PHI University Clinic of Pulmology and Allergy during the past 5 years with diagnosis of acute pulmonary embolism.

Only patients with objectively confirmed diagnosis by ventilation/perfusion scan or CT angiography were included in the study. All patients received acute treatment for venous thromboembolism (VTE) with either low molecular weight (LMW) or unfractionated heparin, according to the current guidelines and the local protocol of the University Clinic of Pulmology and Allergy, followed by a long-term treatment with acenocoumarol. Long-term treatment was continued with LMWH when Vitamin K antagonists were contraindicated, or with rivaroxaban in several cases, by the preference of the patient. General baseline data were registered consisting of demographic data, current smoking status, presence of concomitant diseases, duration of hospitalization, presence of risk factors for VTE, as well as parameters concerning the treatment with acenocoumarol, such as duration of treatment, repeated measurements of INR, the time to first established therapeutic range of INR (2-3), number of trials (venepunctures to estimate INR) before establishing INR of at least 2. Patients were followed throughout their long-term treatment, registering the periodic values of INR, taken according to the indication and preference of the transfusiologist in charge, at the regional Center for Transfusiology, closest to each individual patient. Data were collected by phone or during regular visits

at the Clinic. The presence of thrombophilia, incidence of recurrent VTE episodes and sequelae were also registered for each patient.

Basic statistical analysis was done with Microsoft Excel, measuring demographic statistics, frequency and average calculations.

Results

A total of 176 patients were evaluated or hospitalized at the PHI University Clinic of Pulmology and Allergy, during a 5-year period, under clinical suspicion of pulmonary embolism. PE was confirmed by objective methods of visualization in 105 patients and they represent the pool of patients who were retrospectively evaluated. Eighty-five of these patients were followed for a period of at least 3 months, up to 54 months, and 20 were lost during the follow-up period and were analyzed only for the acute treatment during their hospitalization.

Demographic characteristics: Of the total number of evaluated patients, 54(51.43%) were male and 51 (48.57%) were female. The average age of the patients was 56.07 (range from 20 to 87). The number of current smokers was 41(39.04%), and 64(60.95%) never smoked, or had stopped smoking for at least 1 year previous to enrolment in the study.

Concerning the presence of co-morbidities, 45(42.85%) had no known co-morbid situations at the moment of diagnosis. Thirty (28.57%) patients suffered from hypertension, 1(0.95%) from chronic obstructive pulmonary disease (COPD), 3(2.86%) had diabetes, 2(1.90%) had previous cerebrovascular insult, 4(3.80%) had depression, 6(5.71%) were miscellaneous and 14(13.33%) had combined more than one underlying diseases.

Eighty-three (79.84%) of the patients had pulmonary embolism alone, and 22 (20.95%) had concomitant deep venous thrombosis of the lower limbs. Thrombophilia was diagnosed in 4(3.8%) out of 105 patients. For 83(79.05%) patients the PE incident was their first episode, 20(19.05%) of them had previously experienced a DVT/PE episode within 12 months from the actual episode, and for two (1.90%) patients this was their third episode of VTE.

In 44(43.810%) of the evaluated patients no evident risk associated with the acute thrombotic incident was found. Yet, 13(12.38%) had active cancer at the time of diagnosis, 18(17.14%) underwent recent surgery, 3 (2.89%) had trauma, 8(7.62%) had prolonged immobilization due to traveling or neuro-psychiatric disorders and 17(16.19%) had history of DVT.

The hospital stay of patients ranged from 1 to 25 days, with an average of 12.47 days. The length of treatment with acenocoumarol ranged from one day to 54 months (Figure 1), depending on the condition, estimated risk of thrombotic recurrence versus risk of bleeding and compliance of the patient. The average duration of treatment was 6.87 months. Three of the patients were

treated with LMWH because of active cancer, 5 patients chose to continue their treatment with rivaroxaban, 6 patients died and 9 were lost during the follow-up period,

therefore their data were excluded from further evaluation of maintenance of the therapeutic effect of the treatment. A total of 82 patients were subject to further analysis.

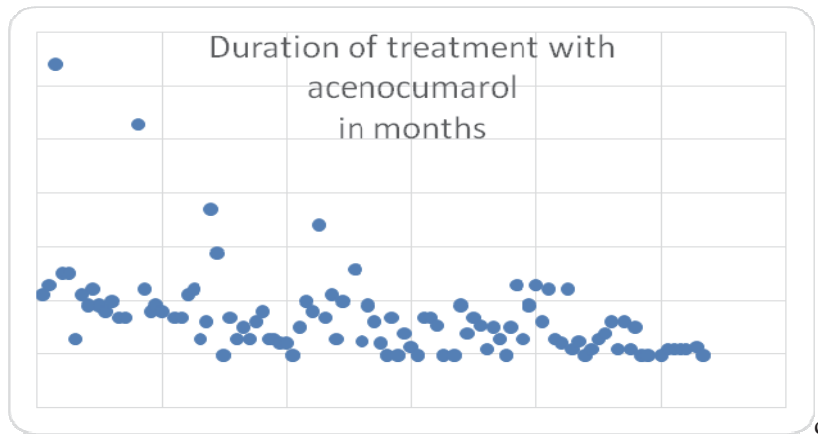


Fig. 1. Duration of treatment with AC in months

Table 1. Attempts required to achieve INR 2-3

Number of patients	Number of attempts				
	1	2	3	4	5
	18(25.7%)	30(42.8%)	14(20.0%)	6(8.6%)	2(2.9%)

The evaluation of the time to first achieved therapeutic INR showed a range of values from 5 days to 6 months; average time to therapeutic INR was 1.012 months. In 12 patients, INR between 2 and 3 was not registered in none of the control visits. In Table 1, we

are presenting data from the number of attempts to achieve therapeutic INR. A total of 356 values were measured during the follow-up period of the 82 evaluated patients, depending on the duration of treatment and their com-

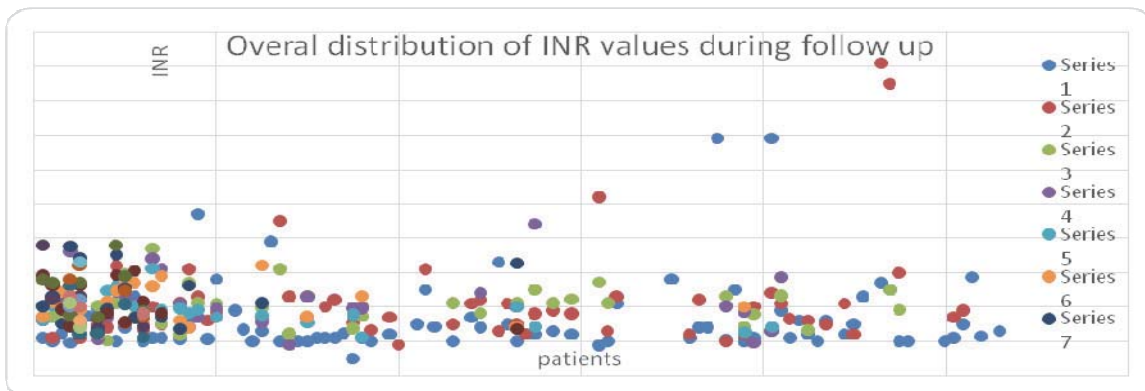


Fig. 2. Overall distribution of INR values during follow-up

Table 2. Distribution of INR values (No of patients)

Attempt number	1	2	3	4	5	6	7	TOTAL
INR<2	66(81.5%)	35(58.3%)	18 (46.2%)	14 (38.3%)	16 (69.5%)	7 (38.9%)	7(43.8%)	201 (56.5%)
2-3	10(12.3%)	18(30.0%)	19(48.7%)	12(40.0%)	6(26.1%)	10(55.5%)	5(31.2%)	123 (34.6%)
>3	5(6.1)	7(11.7%)	2(5.1%)	4(13.3%)	1(4.3%)	1(5/5%)	4(25.0%)	32 (9.9%)
Subtotal	81	60	39	30	23	18	16	356

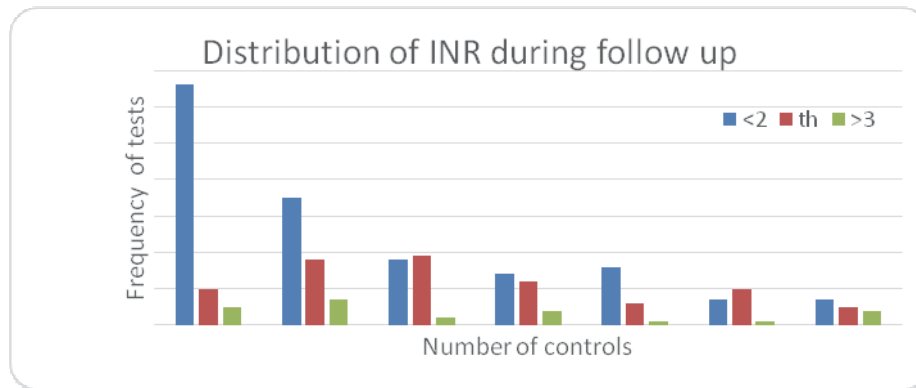


Fig. 3. Distribution of INR values by number of attempt

Table 3. Distribution complications (No of patients)

Complication	None	Bleeding	Bone fracture	Acute renal failure	Death	VTE recurrence
No of subjects	92(87.6%)	4(3.8%)	2(1.9%)	1(0.9%)	6(5.7%)	8(7.6%)

pliance. The distribution of the INR values is presented in Figure 2, showing that in a significant portion of situations ($56.5+9.9=66.4\%$ of the measurements), the values of INR were not in the therapeutic range (Table 2). Figure 3 shows the frequencies of the INR values, by each attempt (most of the INR values obtained were clustered in the first 7 measurement moments, and in only 12 patients in more than 7 measurement points (from 7 up to 18 points were registered); the total of these 89 measurements are not presented in Table 2.

As a secondary outcome, incidence of complications of treatment and VTE recurrence were registered in all 105 subjects (Table 3).

The analysis of the data of the 8 patients with VTE recurrence showed that all of the INR values taken during their follow-up (a total of 32 measurement points) were below the value of 2, and except for one patient who had recurrent DVT with INR of 3.9 at the moment of occurrence.

Discussion

Management of long-term treatment of PE and VTE contains many unresolved issues, even in the era of extended research and recently published guidelines [2-4]. Duration of treatment and choice of optimal treatment is still a matter of debate.

The incidence and prevalence of PE is still very high worldwide. While no exact epidemiological data are available, the incidence of PE is estimated to be approximately 60 to 70 per 100,000. However, the actual figures are likely to be substantially higher because silent PE can develop in up to 40% to 50% of patients with deep vein thrombosis (DVT) [5,6], and is generally sub-diagnosed in a high percentage of patients. Exact data about the incidence and prevalence of PE in Macedonia are not available, but in the period of conducting this study, from a total of 9223 hospitalized patients, 176 were treated as PE, 105 of which were confirmed

with imaging methods. Smoking is considered a risk factor for PE, associated with an absolute risk increase of 24.3 (95% CI 15.4-26.7) cases per 100,000 person-years in some studies [7]. On the other hand, direct association of smoking to thrombin generation has not been confirmed [8]. In our material, 39.04% of the patients with acute PE were smokers, which confirm the association of PE and smoking. Although male gender is an independent risk factor, the almost equal distribution by gender in our material did not confirm any significant differences (51.42% male versus 48.57% female).

Determining the presence of individual risk factors for PE/VTE (permanent or transitional) at the time of diagnosis is crucial for determination of the type and duration of long-term treatment, longer therapy needed (6-12 months) for cases with unidentified reason for VTE [3]. Forty of our patients (43.81%) had no evident risk associated with the acute thrombotic incident. In the other 60% of the patients evident risk factors were as follows: 13(12.38%) had active cancer at the time of diagnosis, 18(17.14%) underwent recent surgery, 3 (2.89%) had post-traumatic PE, 8(7.62%) had prolonged immobilization due to traveling of neuro-psychiatric disorders and 17(16.19%) had history of DVT. Thrombophilia was detected during follow-up in 4 patients. Duration treatment is directly associated to permanent factors of risk [9-11]. The duration of long-term treatment in our study was up to 54 months, with an average of 6.87 months. Concerning the presence of comorbidities, 45(42.85%) patients had no known comorbid situations at the moment of diagnosis; 30 (28.57%) patients suffered from hypertension, 1(0.95%) from chronic obstructive pulmonary disease (COPD), 3(2.86%) had diabetes, 2(1.90%) had previous cerebrovascular insult, 4(3.80%) had depression, 6 (5.71%) were miscellaneous and 14(13.33%) had combined more than one underlying disease. Hypertension, congestive heart failure, COPD and obesity are recognized as independent risk factors for PE [12] and psychiatric and

neurologic disorders are associated to a 3-fold increased risk, which is in accordance to the results of our study. Once risk stratification and acute treatment is determined, it is necessary to determine the duration and type of long-term treatment for each individual patient. The primary goal of the long-term treatment is maintaining the INR in therapeutic range from 2 to 3. The possibility to achieve this goal has been shown to be much more difficult than it seems. Multiple factors influence the effect of treatment including compliance of the subject, hepatic and renal function, presence of Vitamin K in diet, frequency of sampling and personal experience and judgment of the prescribing physician. Visser describes over-coagulation (INR up to 6) associated with the use of antibiotics [13]. In our study we had registered two patients with INR 6.9, and two with 8.5 and 9.1 respectively, treated with beta-lactam antibiotics at the time of sampling. Still, most of the data in the literature report sub-dosing of patients on long-term oral anticoagulation treatment. Stephan *et al.* compared the INR values in patients treated with acenocoumarol and phenprocoumon, stating that INR was in therapeutic range in 42% versus 50% of the time for the tested drugs, respectively [14]. van Schie *et al.* reported achieving therapeutic INR in 72% of the treatment period, using pharmacogenetic data to tailor the doses, still in almost 30% of the treatment time, patients were not adequately anticoagulated [15]. Our results showed that in 66.4% of the time the patients were out of therapeutic range (56.6 with INR < 2 and 9.9% INR > 3). Even more, the time to achieving the first therapeutic INR was relatively long (average of 1.012 months), this taking more than 2 attempts in most of the patients. Prandoni *et al.* suggest that despite adequate treatment, up to one quarter of patients with symptomatic deep vein thrombosis (DVT) and/or pulmonary embolism (PE) will experience recurrent venous thromboembolism (VTE) within the subsequent 5 years [16,17], and the odds are higher in non-optimal treatment. According to this, the number of detected recurrent VTE in our study was 8 out of 105 patients, and in 7 of them INR was < 2 at the time of recurrence. The current tendencies of the treatment of PE are to provide possibilities for home treatment or shortening of the duration of hospitalization for the acute episode of PE, thus reducing the costs of treatment and risk of intrahospital complications. Dentali *et al.* reported to have treated 53% of the DVT and 17% of the PE in their study entirely at home, and the hospitalization for PE was ≤ 5 days [18]. An additional shortening of the duration of hospitalization was referred with introduction of direct oral anticoagulants for acute phase treatment [19]. In contrast to these findings, the results in our study showed that our patients were hospitalized for an average of 12.47 days (1-25 days), mostly due to the need of several attempts to achieve therapeutic values of INR, as described previously. These facts signifi-

cantly increase the cost of treatment of patients, and call for a need of revising the current local protocols for management of PE, as for frequency of sampling for INR during hospitalization or revision of the type of treatment [20].

Conclusion

Our study shows that during treatment with Vitamin K antagonists for PE, it is usually difficult to obtain therapeutic range of INR, which by itself represents a problem in maintaining a low risk for recurrent thrombotic incidences. This justifies the quest for new, more efficient and safer modes of treatment, which offer stable therapeutic effect with better patient compliance.

Conflict of interest statement. None declared.

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Original article

COMPARISON OF TISSUE OXYGEN SATURATION (SpO₂) OF FLAPS AND SKIN GRAFTS

КОМПАРАЦИЈА НА САТУРАЦИЈАТА СО КИСЛОРОД НА ТКИВАТА (SpO₂) КАЈ РЕЗАНКИТЕ И ТРАНСПЛАНТАТИТЕ

Boro Dzonov¹, Lazo Noveski¹, Suzana Nikolovska², Elizabeta Mircevska-Zhogovska¹, Vladimir Ginoski¹ and Darko Daskalov¹

¹University Clinic for Plastic and Reconstructive Surgery, ²University Clinic for Dermatology, Skopje, Republic of Macedonia

Abstract

Infrared spectroscopy is a non-invasive technique that enables monitoring of tissue oxygenation and perfusion. This method is safe and reliable in examining the success in flaps, especially in examining the microvascular flap thrombosis, which compromise the success of the flaps, and thus, it is an alarm for a prompt response by the surgeon. For example, in a venous occlusion, larger quantities of deoxygenated blood and a greater blood volume are detected. It can also detect changes in tissue oxygenation in deeper layers. Oximetry is one of the better modes of monitoring flaps and skin grafts in the preoperative, intraoperative and postoperative period. Thrombosis, being the main cause for flap failure, is usually monitored clinically, and at the onset of the first symptoms, actions are taken to eliminate them, whether conservative or surgical. With the aid of an oximeter, it is possible to detect the decrease in tissue oxygenation even in the first phase. Determination of tissue oxygenation and hemoglobin concentration are regulated by the difference in intensity between the emitted and absorbed light in a specific wavelength established according to Beer-Lambert law postulates. The study was performed at the University Clinic for Plastic and Reconstructive Surgery in Skopje, Republic of Macedonia. It was a randomized prospective study in which two groups of subjects, with 30 patients each, were examined. Each patient was required to sign a permission for reconstructive surgery and an informed consent for participation in the study. A specially designed questionnaire (non-standardized) was filled in for all patients.

1. First group (I) of patients treated with flaps.

In this group a type of reconstructive technique with skin flaps or complex flaps was applied.

2. Second group (II) of patients treated with skin grafts (split-thickness skin grafts).

In this group the applied reconstruction involved appli-

cation of skin grafts with partial thickness. There was a significant difference between the saturation level in the arm flap on zero day, day 7 and day 30, and the multiple comparison test showed a significant difference between zero day and day 7. A significant difference was registered between the saturation level in split-thickness skin graft (STSG) to the leg, on zero day, day 7 and day 30, and the multiple comparison test showed a significant difference between zero day and day 7. There was a significant difference between the saturation level in split-thickness skin graft (STSG) to the arm on zero day, day 7 and day 30, and the multiple comparison test showed a statistically significant difference between zero day and day 7, zero day and day 30, and day 7 and day 30. A significant difference was registered between the saturation level in the leg flap on zero day, day 7 and day 30, and the multiple comparison test showed a statistically significant difference between zero day and day 7, zero day and day 30, and day 7 and day 30. The difference in the average oxygen saturation between patients with arm flap and in split-thickness skin graft (STSG) to the arm on zero day was insignificant. The difference in the average oxygen saturation between patients with arm flap and in split-thickness skin graft (STSG) to the arm after day 7 was significant. The difference in the average oxygen saturation between patients with arm flap and in split-thickness skin graft (STSG) to the arm after day 30 was significant. The difference in the average oxygen saturation between patients with leg flap and in split-thickness skin graft (STSG) to the leg on zero day was insignificant. The difference in the average oxygen saturation between patients with leg flap and in split-thickness skin graft (STSG) to the leg after day 7 was significant. The difference in the average oxygen saturation between patients with leg flap and in split-thickness skin graft (STSG) to the leg after day 30 was significant. Assessment of tissue oxygenation with infrared spectroscopy showed better results in the lower limbs with application of flaps compared to application of a skin transplant.

Correspondence to: Boro Dzonov, University Clinic for Plastic and Reconstructive Surgery, "Mother Theresa" 1000 Skopje, R. Macedonia; E-mail: bdzonov@yahoo.com

Keywords: infrared spectroscopy, flap, grafts

Апстракт

Инфрацрвена спектроскопија е неинвазивна техника што овозможува мониторинг на оксигенацијата на ткивата и перфузијата. Оваа метода е сигурна за испитување на успешноста кај резанките, посебно при испитување микроваскуларни тромбози во самите резанки, кои ја загрозуваат успешноста на резанката, а со тоа и аларм за навремено реагирање на хирургот. При венска оклузија, на пример, се детектираат поголеми количества деоксигенирана крв, а поголем волумен на крв. Позитивна страна е што може да ги детектира промените на оксигенацијата на ткивата и во длабина. Оксиметријата спаѓа во еден од поидеалните начини на мониторинг на резанките и трансплантатите пред, за време и во постоперативниот период. Тромбозата, како главен фактор за неуспех на резанките, најчесто е клинички мониторирана и при појава на првите симптоми се преземаат мерки за нејзино отстранување, дали конзервативно или хируршки. Со помош на оксиметарот е возможно да се детектира намалување на оксигенацијата на ткивото уште во првиот стадиум. Мерењето на оксигенацијата на ткивата и содржината на хемоглобинот е детерминирано со разликата на интензитетот меѓу емитираната и апсорбирана светлина на специфична бранова должина, одредена според Beer-Lambert законските постулати. Студијата е работена на Клиниката за пластична и реконструктивна хирургија. Изработена е рандомизирана проспективна студија. Во текот на студијата се формирани две групи испитаници од по 30 пациенти. Од секој пациент е барана дозвола за оперативен реконструктивен зафат и информативна согласност за учество во студијата. За сите пациенти е пополнуван специјално конструиран прашалник (нестандардизиран).

1. Прва (I) група пациенти третирани со резанки.

Во оваа група е применет тип на реконструктивна техника со кожна или со сложена резанка.

2. Втора (II) група пациенти третирани со трансплантати (split thickness grafts).

Во оваа група, применетата реконструкција се состои од апликација на кожни трансплантати со делумна дебелина на кожа. Разликата, која се регистрира меѓу вредностите на сатурација на резанка на рака, кај вредностите од - нултиот, 7. и 30. ден е сигнификантна, со тест на повеќекратна споредба, разликата е сигнификантна меѓу нулта денот и седмиот ден. Разликата, која се регистрира меѓу вредностите на сатурација на тирш на нога, кај вредностите нултиот, 7. и 30. ден е сигнификантна, со тест на повеќекратна споредба, разликата е сигнификантна меѓу нултиот и седмиот ден.

Разликата, која се регистрира меѓу вредностите на сатурација на тирш на рака, нултиот, 7 и 30 ден е сиг-

нификантна, со тест на повеќекратна споредба, разликата е статистички сигнификантна меѓу нултиот и седмиот ден, нултиот и 30. ден, седмиот и триесеттиот ден.

Разликата, која се регистрира меѓу вредностите на сатурација на резанка на нога, кај вредностите нултиот, 7 и 30 ден е сигнификантна, со тест на повеќекратна споредба, разликата е сигнификантна меѓу нултиот ден и седмиот ден, нултиот и 30. ден, седмиот и триесеттиот ден.

Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на рака и тирш на рака нултиот ден е несигнификантна.

Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на рака и тирш на рака по 7 ден е сигнификантна. Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на рака и тирш на рака по 30 ден е сигнификантна. Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на нога и тирш на нога-нултиот ден е несигнификантна. Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на нога и тирш на нога по 7 ден е сигнификантна. Разликата на просечните вредности на сатурација со кислород меѓу пациентите со резанка на нога и тирш на нога по 30 ден е сигнификантна. Испитувањето на оксигенацијата на ткивата со инфрацрвена спектроскопија покажа значајност кај долните екстремитети при примена на резанки, споредено со примена на кожен трансплантат.

Клучни зборови: оксиметрија, резанка, трансплантат

Introduction

Infrared spectroscopy is a non-invasive technique that enables monitoring of tissue oxygenation and perfusion [1]. This method is safe and reliable in examining the success in flaps, especially in examining the microvascular flap thrombosis, which compromise the success of the flaps [2,3], and thus, it is an alarm for a prompt response by the surgeon (Figure 1). For example, in a venous occlusion, larger quantities of deoxygenated blood [4], and a greater volume of blood are detected. It can detect changes in tissues oxygenation in deeper layers [5]. Oximetry is one of the better modes of monitoring flaps and skin grafts in the preoperative, intraoperative and postoperative period. Thrombosis, being the main cause for flap failure, is usually monitored clinically, and at the onset of the first symptoms, actions are taken to eliminate them, whether conservative or surgical. With the aid of an oximeter, it is possible to detect the decrease in tissue oxygenation even in the first phase. Determination of tissue oxygenation and hemoglobin concentration are regulated by the difference in inten-

sity between the emitted and absorbed light in a specific wavelength established according to Beer-Lambert law postulates. The magnitude is determined by the emitted light intensity equivalent to the quantity of the substance and the amount of the absorbed light from one unit of the same substance. This is defined as the coefficient of absorption, and this factor varies depending on the wavelength of light and on the structure of the substance. The deepness of light penetration across the substance is proportional to the mean distance of photons penetration through the tissue. Transmission of light through the tissue is determined by the reflection, fragmentation i.e. dispersion and the absorption effects of the tissue. Reflection is a function between the angle at which the light falls and the regularity of the surface of the tissues on which the light falls. Reflection is decreased by increasing the wavelength, which goes in favor of infrared light instead of to visible light. Dispersion depends on the composition of the tissue, while absorption depends on the composition of the tissue molecules that will come across in the route of light. Beyond 1300 nm, water (H₂O) absorbs all photons in the length of several millimeters, with an intermediate high value between 950 and 1050 nm increasing light dispersion, and more intense absorption portions of hemoglobin prevent further effective transmission of light. In the range of 700-1300 nm, infrared light penetrates the biological tissue several centimeters. Within the scope of infrared light, the light-absorbing molecules in the tissue are metallic complexes: hemoglobin, bilirubin, and cytochrome. The absorption spectrum of deoxyhemoglobin (Hb) ranges between 650-1000 nm, oxyhemoglobin (HbO₂) values range between 700-1150 nm, and cytochrome oxidase aa₃ (Caa₃) has a wide peak in 820-840 nm. The wavelengths of infrared light used in commercial devices are chosen to be sensitive to these biologically important chromophores; they generally use wavelengths between 700 and 850 nm, where the absorption spectra of Hb and HbO₂ are hugely divided and have minimal overlaps with H₂O. As noted earlier, the absorption of infrared light in the tissue is determined by the Beer-Lambert law that connects the pathway of infrared light to the concentration and absorption spectrum of the tissue and is conventionally written as:

$$\Delta A = L \times \mu$$

Where **A** is the amount of light attenuation, **L** is the differential path of the photons through the tissue, and **μ** is the absorption coefficient of the chromophore X.

In general, the amount of light received by the detector shows the amount of oxygen bound to hemoglobin in the blood. Oxyhemoglobin absorbs more infrared light than red light, while deoxygenated hemoglobin absorbs more red light than infrared light. By comparing the

quantities of red and infrared light received on the detector, the instrument can calculate SpO₂ (Figure 2).

Taking into consideration that the application of these measurements of oxygen saturation in our study was to indicate the oxygenation of the flaps, and respectively skin transplants, due to the limitations of this measuring instrument to determine the deep perfusion of the tissues (Figure 3), a color Doppler (i.e. Doppler ultrasound) can furthermore be used to determine the circulation in the major blood vessels of the limbs.

Material and methods

The study was performed at the University Clinic for Plastic and Reconstructive Surgery in Skopje, Republic of Macedonia. It was a randomized prospective study that included two groups of subjects, with 30 patients each. Each patient was required to sign a permission for reconstructive surgery and an informed consent for participation in the study. A specially designed questionnaire (non-standardized) was filled in for all patients.

1. First group (I) of patients treated with flaps.

In this group a type of reconstructive technique with skin flaps or complex flaps was applied.

2. Second group (II) of patients treated with skin grafts (split-thickness skin grafts).

In this group the applied reconstruction involved application of skin grafts with partial thickness.

The study included patients with defects of the skin and soft tissues, who had an indication for reconstructive surgical procedure.

These patients were excluded from the study: children under 14 years of age, adults over 75 years old, patients with systemic diseases that could have impact on the results of reconstructive interventions and patients with bone-like surface defect without periosteum as contraindication for skin grafting.

The results of the reconstructive procedures according to the objectives set were clinically analyzed into three periods: preoperative, postoperative day 7 and postoperative day 30. The following examinations were carried out: determination of oxygen saturation of the blood and tissues in the flaps, pre- and postoperatively (day 7 and day 30), as well as in the skin grafts pre- and postoperatively (day 7 and day 30), using infrared spectroscopy, i.e. determining oxyhemoglobin concentration.

Results

The average preoperative saturation level in the arm flap was 95.3±2.3, after 7 days it decreased to 89.2±1.3, and on day 30 it increased to 95.5±2.0 (Table 1).

The average preoperative saturation level in the leg flap was 96.7±1.4, after 7 days it declined to 89.7±1.1, and on day 30 it increased to 93.2 ± 2.0 (Table 1).

The average preoperative saturation level in the split-thickness skin graft (STSG) to the arm was 96.9±1.1,

after 7 days it dropped to 82.4 ± 1.9 , and on the 30th day it rose to 88.9 ± 1.1 (Table 1).

The average preoperative saturation level in the split-thickness skin graft (STSG) to the leg was 96.1 ± 1.4 , after 7 days it dropped to 83.3 ± 0.9 , and on the 30th day it rose to 88.9 ± 1.0 (Table 1).

The registered difference between the saturation level

in the arm flap on zero day, day 7 and day 30 according to the ANOVA test was statistically significant for $p < 0.05$ ($p = 0.000000$). The Tukey HSD test, a multiple comparison test, that aims to detect which difference (among multiple) is "credited" for the overall score, showed statistically significant difference between the zero day and the seventh day for $p < 0.05$ ($p = 0.000119$).

Table 1. Average tissue oxygen saturation (SpO₂) in arm and leg flaps, and split-thickness skin graft (STSG) to the arm and leg preoperatively, and day 7 and day 30 postoperatively

Localization/tissue saturation - SpO ₂	average	number	Stand.dev.	minimum	maximum
Preoperatively					
Arm flap	95.33333	15	2.257263	92.0	99.0
STSG to the leg	96.66667	15	1.447494	93.0	98.0
STSG to the arm	96.875	16	1.087811	95.0	98.0
Leg flap	96.14286	14	1.406422	94.0	99.0
Postoperatively - day 7					
Arm flap	89.20	15	1.264911	87.0	91.0
STSG to the leg	89.73333	15	1.099784	88.0	92.0
STSG to the arm	82.43750	16	1.860779	80.0	88.0
Leg flap	83.28571	14	0.913874	82.0	85.0
Postoperatively - day 30					
Arm flap	95.53333	15	2.03072	89.0	98.0
STSG to the leg	93.20	15	11.98332	50.0	98.0
STSG to the arm	88.86667	15	1.12546	87.0	90.0
Leg flap	88.92857	14	0.99725	87.0	90.0

The registered difference between the saturation level in the split-thickness skin graft (STSG) to the leg on zero day, day 7 and on day 30 according to the ANOVA test was statistically significant for $p < 0.05$ ($p = 0.033642$). The Tukey HSD test, a multiple comparison test, that aims to detect which difference (among multiple) is "credited" for the overall score, showed statistically significant difference between the zero day and the seventh day for $p < 0.05$ ($p = 0.025639$).

The registered difference between the saturation level in the split-thickness skin graft (STSG) to the arm on zero day, day 7 and day 30 according to the ANOVA test was statistically significant for $p < 0.05$ ($p = 0.000000$). The Tukey HSD test, a multiple comparison test, that aims to detect which difference (among multiple) is "credited" for the overall score, showed statistically significant difference between the zero day and the seventh day, between the zero and day 30, and between the seventh and thirtieth day for $p < 0.05$ ($p = 0.000130$).

The registered difference between the saturation level in the leg flap on zero day, day 7 and day 30 according to the ANOVA test was statistically significant for $p < 0.05$ ($p = 0.000000$). The Tukey HSD test, that aims to detect which difference (among multiple) is "credited" for the overall score, showed statistically significant difference between the zero day and the seventh

day, between the zero and day 30, and between the seventh and thirtieth day for $p < 0.05$ ($p = 0.000123$).

There was a statistically insignificant difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm on zero day ($p > 0.05$).

There was a statistically significant difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm after day 7 ($p < 0.05$) ($p = 0.000004$).

There was a statistically significant difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm after day 30 ($p < 0.05$) ($p = 0.000015$).

There was a statistically insignificant difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg on zero day ($p > 0.05$).

There was a statistically significant difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg after day 7 ($p < 0.05$) ($p = 0.000005$).

There was a statistically significant difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg after day 30 ($p < 0.05$) ($p = 0.000071$).



Fig. 1. (a, b, c) Demonstration of pulse-oximeter measurements intraoperatively

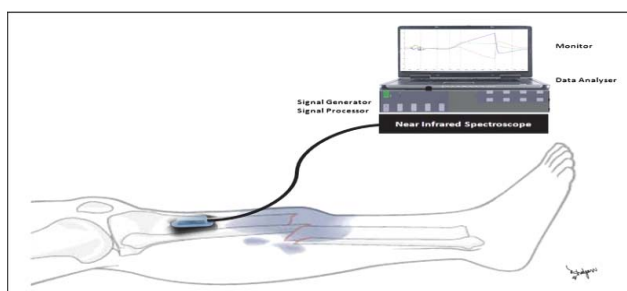


Fig. 2. Schematic representation of the measurement



Fig. 3. Probe used for pulse oximeter

The average preoperative saturation level in the arm flap was 95.3 ± 2.3 , after 7 days it decreased to 89.2 ± 1.3 , and on the 30th day it rose to 95.5 ± 2.0 .

The average preoperative saturation level in the leg flap was 96.7 ± 1.4 , after 7 days it declined to 89.7 ± 1.1 , and on the 30th day it rose to 93.2 ± 2.0 .

The average preoperative saturation level of split-thickness skin graft (STSG) to the arm was 96.9 ± 1.1 , after 7 days it dropped to 82.4 ± 1.9 , and on the 30th day it rose to 88.9 ± 1.1 .

The average preoperative saturation level of split-thickness skin graft (STSG) to the leg was 96.1 ± 1.4 , after 7 days it dropped to 83.3 ± 0.9 , and on the 30th day it rose

to 88.9 ± 1.0 .

A significant difference was registered between saturation levels in the arm flap on zero day, day 7 and day 30, and the multiple comparison test showed a significant difference between the zero day and the seventh day.

A significant difference was registered between the saturation levels of split-thickness skin graft (STSG) to the leg, on zero day, day 7 and day 30, and the multiple comparison test showed a significant difference between the zero day and the seventh day.

A significant difference was registered between the saturation level of split-thickness skin graft (STSG) to the arm, on zero day, day 7 and day 30, and the multiple comparison test showed a statistically significant difference between the zero day and the seventh day, the zero day and the 30th day, and the seventh and the thirtieth day.

A significant difference was registered between the saturation levels in the leg flap, on zero day, day 7 and day 30, and the multiple comparison test showed a statistically significant difference between the zero day and the seventh day, the zero day and the 30th day, and the seventh and the thirtieth day.

The difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm on the zero day was insignificant.

The difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm after day 7 was significant.

The difference in the average oxygen saturation between patients with arm flap and split-thickness skin graft (STSG) to the arm after day 30 was significant.

The difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg on the zero day was insignificant.

The difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg after day 7 was significant.

The difference in the average oxygen saturation between patients with leg flap and split-thickness skin graft (STSG) to the leg after day 30 was significant.

Discussion

Oximetry for experimental objectives is utilized primarily for postoperative monitoring of flaps. In this study, a modification was made with a comparative analysis between flaps and skin grafts. The results we obtained in 95% correspond to similar studies related to flap monitoring. Scheufler *et al.* demonstrate monitoring of pedicle TRAM flaps in breast reconstruction, where the values of tissue oxygenation in the first seven days dramatically fall, but normalize completely by day 30 [6]. Holzle *et al.* illustrate measurements of oximetry, which show that 15% of the flaps have an early detection of thrombosis within the blood vessels in the flap, before the onset of clinical symptoms [7]. Kamolz *et al.* verify that reducing the oxygenation in the tissue of the flap is followed by an occlusion of the arterial vessel in the pedicle of the flap, while the reduced oxygenation in the skin grafts in the later postoperative period is due to the formation of an abundant connective tissue, which is less oxygenated [8]. Edwards *et al.* apply oximetry in delayed pedicle flaps, for their early separation and thus shortening the surgical procedure, as well as reducing the complications related to the flap survival [9]. Lin *et al.* with the application of oximetry in the monitoring of the flaps have increased by about 20% the survival rate of the flaps [10].

Conclusion

Assessment of tissue oxygenation with infrared spectroscopy showed better results in the lower limbs with application of flaps compared to application of a skin transplant.

Conflict of interest statement. None declared.

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Case report

CONGENITAL LEFT VENTRICULAR APICAL ANEURYSM IN 20-YEAR-OLD MALE: CASE REPORT

КОНГЕНИТАЛНА АНЕВРИЗМА НА ЛЕВАТА КОМОРА: ПРИКАЗ НА СЛУЧАЈ

Sasko Jovev¹, Vangjel Zdraveski¹, Kujtim Qelili¹, Andreja Arsovski⁴, Suzana Dameska¹, Lidija Popovska³ and Omer Dzemali²

¹PHI University Clinic of State Cardiac Surgery - Clinical Center "Mother Teresa" Skopje, Republic of Macedonia, ²Clinical Hospital - "Triemli" - Cardiac Surgery Department - Birmensdorfesstrasse 497 - Zurich - Switzerland, ³PHI University Clinic of Cardiology - Clinical Center "Mother Teresa" - Skopje - Republic of Macedonia, General Hospital „Re-Medika” – Skopje, Republic of Macedonia

Abstract

Introduction. Congenital cardiac ventricular aneurysm is a rare cardiac malformation, defined as a protrusion from the left ventricular cavity with a wide connection and without contractility, which may be presented with anakinetic or dyskinetic movement during systole.

According to a recent review congenital left ventricular aneurysms are asymptomatic in 41.8% of patients.

Case report. In this case report we present a 20-year-old male with a rare apical aneurysm on the left ventricle. This patient was treated by a multidisciplinary team at the UC of State Cardiac Surgery in Skopje. At PCI there was no significant stenosis in coronary arteries. MR revealed a giant left ventricular apical aneurysm. The surgical method that we performed was aneurysmectomy by Dorr technique. After 7 days the patient was in a good condition and was dismissed from the hospital.

Conclusion. This is the first patient with congenital left ventricular aneurysm treated surgically in a public health institution in the Republic of Macedonia.

Keywords: congenital LV aneurysm, ventricular tachycardia, heart failure, arrhythmia, aneurysmectomy

Апстракт

Вовед. Кардијална конгенитална вентрикуларна аневризма е ретка малформација, дефинирана како испакнување (протрузија) на ѕидот на левата комора, кој е без контрактилност и/или може да се презентира со акинетично и/или дискинетично движење на срцето за време на систола.

Конгениталните левовентрикуларни аневризми се асимптоматски кај 41,8 проценти од пациентите.

Приказ на случај. Во овој приказ на случај е опи-

шана ретка апикална аневризма на лев вентрикул кај 20 годишно момче, кое преку мултидисциплинарен пристап беше хируршки третиран на УК за државна кардиохирургија-Скопје.

На коронарографскиот наод не биле евидентирани стенози на коронарните крвни садови.

Кај пациентот беше направена и магнетна резонанца на срце, на која се евидентира левовентрикуларна гигантска апикална аневризма. Оперативната техника со која се реши случајот е аневризмектомија по Дор.

По седумдневна хоспитализација, пациентот во добра здравствена состојба ја напушти Клиниката за државна кардиохирургија во Скопје.

Заклучок. Значењето на овој приказ на случај е во тоа што ова е прв пациент со конгенитална аневризма на лев вентрикул, кардиохируршки третиран во јавното здравство во Република Македонија.

Клучни зборови: Конгенитална левовентрикуларна аневризма, вентрикуларна тахикардија, срцева слабост, аритмии, аневризмектомија

Introduction

Congenital left ventricular aneurysm was described for the first time in 1816.

It is a rare cardiac malformation, defined as a protrusion from the ventricular cavity with a wide connection (ratio of the connection to the body of the anomaly >1) and without contractility, which may be presented with akinetic or dyskinetic movement during systole.

Left ventricular aneurysms (LVA) can be associated with heart failure, arrhythmias, systemic embolization, coronary artery stenosis and sudden death due to ventricular rupture.

According to a recent review congenital left ventricular aneurysms are asymptomatic in 41.8% of patients [1-3].

Ventricular aneurysms are among many complications that may occur after a heart attack.

Correspondence to: Sasko Jovev, PHI University Clinic of State Cardiac Surgery-Clinical Center "Mother Teresa" Skopje, R. Macedonia; E-mail: jovev99@yahoo.com

Ventricular tachycardia may be present in 18.4% of patients with LVA.

Treatment of ventricular tachycardia (VT) in patients with congenital aneurysms is usually complex-multidisciplinary and involves electrophysiological monitoring or cardiac surgical intervention [4].

In this case report we describe a rare apical congenital aneurysm in a young, 20-year-old male patient, who was successfully surgically treated with a multidisciplinary approach at UC of State Cardiac Surgery in Skopje.

The three main indications for surgery of left ventricular aneurysm are angina (40%), cardiac failure (35%), and arrhythmias (10%) [5,6].

Case report

A 20-year-old male, with anamnesis for chest pain, dyspnea, palpitations and dizziness, was admitted at UC of State Cardiac Surgery in Skopje for cardiac surgical intervention - aneurysmectomy.

He had no family history of sudden death, ventricular arrhythmia or cardiomyopathy. The patient had no additional diseases and no allergic reactions to food and drugs.

The first symptoms started in November 2015, when the patient presented with chest pain and was admitted to the Clinical Hospital in Tetovo. Coronarography was then performed, and it showed no significant stenosis of coronary arteries.

Since symptoms persisted for 3 months the patient was admitted to the University Clinic of Cardiology, Clinical Center "Mother Teresa" in Skopje.

On admission the patient had irregular heart rhythm with monomorphic ventricular tachycardia VT and a ventricular rate of 220 bp/min.

The patient was admitted to ICU where transthoracic echocardiogram was performed showing EF- 53% (Simpsons), LVEDd- 55 mm, LVEDs- 40 mm, RV-26 mm, LA-32 mm, Ao-31 mm, and hypokinesia of the middle and distal part of IVS and apical dyskinetic part of apex of left ventricle was also described. With this TTEcho Takotsubo cardiomyopathy-was excluded.

In order to exclude structural cardiac abnormalities, cardiac magnetic resonance imaging was performed that showed a left ventricular giant apical aneurysm.

After establishing the diagnosis, electrophysiological examination was performed in order to determine the origin of the VT and catheter ablation was done. It was also decided to implant a subcutaneous implantable cardioverter - defibrillator (ICD).

After the patient was stabilized and having in mind the cardiologist's recommendations, he was prepared for cardiac surgery- aneurysmectomy.

When the patient was admitted to the UC State Cardiac Surgery, he was clinically evaluated with new echocardiography where no intracavity thrombotic mass was

seen. He was again evaluated for the apex of the left ventricle where a giant aneurysm was located.

The ECHO showed that the left ventricle was remodeled with normal global systolic function, valves were morphologically normal, not stenotic and with normal flow. There was no pericardial effusion.

X-ray (AP/LL) was normal with no pleural effusion, myopathic heart and correct position of ICD. Dental check was normal, lung capacity was within normal range, and biochemistry findings were normal.

After a complete preoperative clinical evaluation the patient was operated in OETA, with central venous catheter, arterial line for monitoring the vital parameters. Transesophageal echosonography was used intraoperatively.

The surgical technique that we used was aneurysmectomy according to Dorr. Sternotomy, cannulation and aneurysm identification was first performed (Figure 1).

We continued with incision in the middle of the aneurysm (Figure 2). We continued with Dacron insertion (Figure 3) and double suture closure (Figure 4).

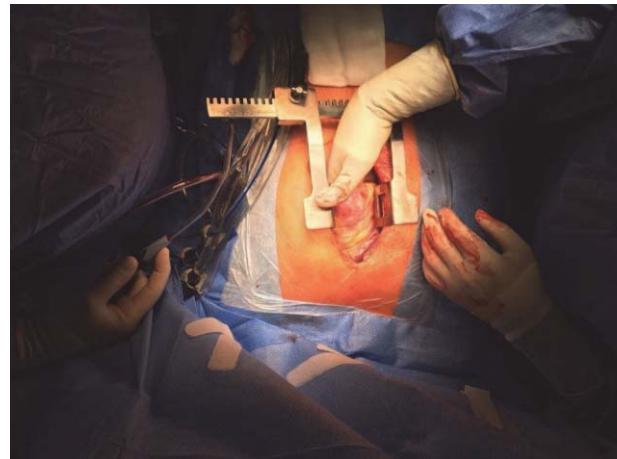


Fig. 1. Location of aneurysm at the apex of left ventricle

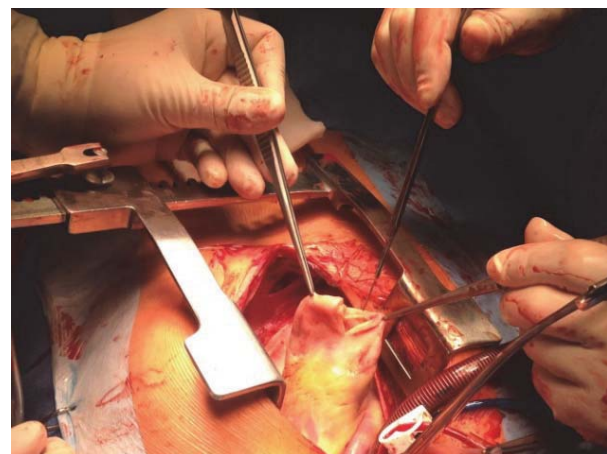


Fig. 2. Resection of dyskinetic and akinetic LV

During the surgery we performed TEE intraoperatively and there was no air in the heart cavity. There was a good contractility of the heart apex. After that we sto-

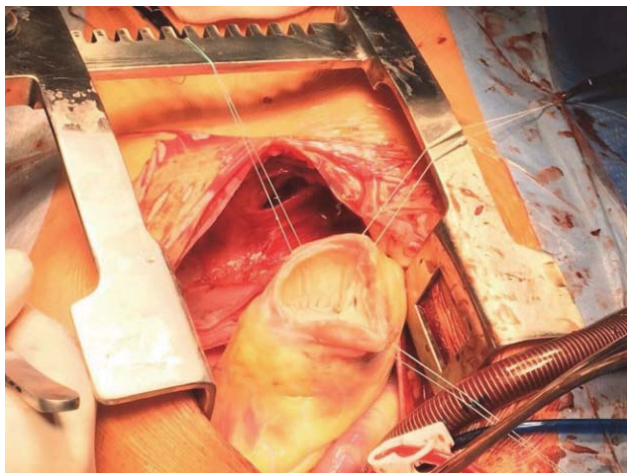


Fig. 3. Suture with Dacron patch lined in junction of the endocardial muscle and scarred tissue

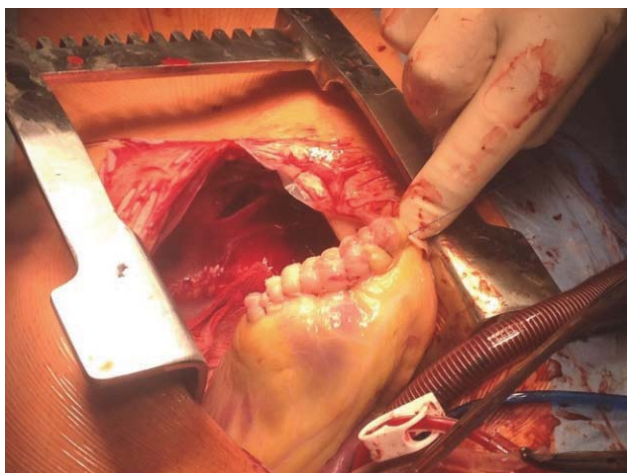


Fig. 4. Two lined sutures and closure of the heart and after going out from CPB.

pped CPB without inotropic support; we performed sternum closing and suturing. After the surgical intervention the patient was extubated in ICU only one hour after surgery. Drainage was 200 ml and we removed the tubes after 24 hours. On the third postoperative day we sent the patient to the Cardiac Surgical Department with TA -120/70 mmHg, with sinus rhythm, and saturation of 98% (spontaneous breath).

On the 7th postoperative day the patient was in a good physical condition, with stable sternum, without epicardial wires for pacemaker and with properly healed wounds. He was dismissed from the PHI University Clinic of State Cardiac Surgery-Clinical Center "Mother Teresa", Skopje, R. Macedonia.

Histopathological report included microscopic analysis showing hypertrophic fibrotic endocardium, under which the myocardium was changed with fibrotic tissue, and at some parts myocytes with hypertrophy were seen. This was in agreement with the changed myocardium by the aneurysm, probably from congenital origin.

Discussion

The prevalence of congenital LV aneurysm is less than 0.34% in adult population, although there is a lack of epidemiological data. The aneurysm is typical, located at the apex and is asymptomatic. It can be presented with serious complications such as supraventricular and ventricular arrhythmias, tamponade, cardiac rupture or sudden cardiac death. It has a large neck with a fibrous wall, and paradoxical motion. The LV diverticulum has predominantly a narrow neck and no paradoxical motion. It can also be detected in children, mostly as a part of Cantrell's pentalogy. These characteristics were present in our patient and they helped us to diagnose the aneurysm using echocardiography and MRI. In cases with an aneurysm, we can often see myocardial torsion and LV ejection fraction that is reduced.

Diagnosis and treatment of LVA are often a challenge [7]. In this case report we have described a patient with a left ventricular apical aneurysm without coronary disease and without acute coronary syndrome in the anamnesis. The coronary angiography was clear. These data were suggestive of an aneurysm. Surgical treatment was indicated in the already diagnosed patient who had chest pain and malignant ventricular arrhythmia [8,9]. The purpose of the surgery is to correct the size and geometry of the left ventricle, which can reduce wall tension pressure and improve the pump function, functional status and survival of the patients.

The congenital aneurysm has a wide neck with a fibrous wall and paradoxical movement, dyskinesia. On the other side, the diverticulum has a thin neck, muscular wall and synchronous contraction with the left ventricle. The congenital aneurysm is often isolated, and the diverticulum is a part of the so-called Cantrell's pentalogy. In our case there was no synchronous contraction with the left ventricle.

In general, the left ventricular diverticulum has a more benign prognosis.

The left ventricular congenital aneurysm is often associated with thromboembolic complications. Our patient had no coronary syndrome in the anamnesis and no changes at coronarography. There was a change in the left ventricular geometry, with small myocardial torsion and slight reduction in the ejection fraction, which increased after the surgery.

Cardiac surgery resection of the ventricular aneurysm was performed because of the persistence of clinical chest pain in spite of ICD implantation.

Dorr's surgery technique is a safe and effective method for akinetic and dyskinetic parts of the heart.

In a historic way of view with a small modification, the method includes placing of endovascular patch through ventriculotomy with cutting fibrotic part of akinetic/dyskinetic tissue. Thus, reduction and remodeling of

the size and geometry of the left ventricular is made, obtaining an elliptic shape of the ventricle.

It has to be emphasized that this patient is the first case with aneurysm of the left ventricle of the heart which was surgically treated in a public health institution in Macedonia.

In spite of the small number of patients with diagnosis of LVA, the importance of treating aneurysms of the left ventricle is huge because they are associated with heart failure, arrhythmias, systemic embolization, coronary artery stenosis and sudden death due to ventricular rupture. Punctual diagnosis and appropriate treatment usually increase the survival of the patient [10-12].

Conflict of interest statement. None declared.

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Case report

THE OCCURRENCE OF POSTOPERATIVE COMPLICATIONS IN PATIENTS UNDERGOING SURGERY DUE TO COMPLICATIONS FROM CROHN'S DISEASE: CASE REPORT

ПОЈАВА НА ПОСТОПЕРАТИВНИ КОМПЛИКАЦИИ КАЈ ХИРУРШКИ ТРЕТИРАНИ ПАЦИЕНТКИ ПОРАДИ КОМПЛИКАЦИИ ОД КРОНОВА БОЛЕСТ

Vladimir Joksimovic¹, Nikola Jankulovski¹, Svetozar Antovic¹, Ljubinka Manceva² and Marija Joksimovic³

¹University Clinic for Digestive surgery, Clinical Centre Mother Theresa, Skopje, ²General Hospital Strumica, Strumica, ³University Clinic for Gynaecology and Obstetrics, Clinical Centre Mother Theresa, Skopje, Republic of Macedonia

Abstract

Introduction. Various risk factors specific for patients with Crohn's disease (CD) can influence on the outcome of the surgical treatment, that is, the onset of complications in the postoperative period. Our hypothesis was that those risk factors associated with further postoperative complications can be elevated levels of preoperative laboratory inflammatory markers such as white blood cells-WBC and CRP values, anaemia, phlegmon of the anterior abdominal wall, preoperative interintestinal abscess and positive resection margins.

Case report. We are presenting a case of a patient who was surgically treated as emergent patient for Crohn's disease. He underwent through the standard preoperative clinical protocol, which includes complete preoperative biochemical blood and urine analysis with inflammatory parameters (white blood cell count-WBC and C reactive protein-CRP), total protein albumin and hemoglobin levels and ultrasound of abdomen. The results showed anemia, hypoproteinemia, and elevated CRP levels. Intraoperative findings showed presence of intraabdominal abscess and mild leukocytosis. After the primary surgery, on the 8th postoperative day under a suspicion of anastomosis dehiscence, with a clinical presentation of acute abdomen we set an indication for revision. The revision confirmed the assumed complication.

Conclusion. The determination of certain preoperative and intraoperative factors that may be associated with the onset of postoperative complications can help in their optimization in order to reduce postoperative complications in patients with Crohn's disease.

Keywords: Crohn's disease, risk factors, complications

Апстракт

Вовед. Кај пациентите со Кронова болест (КБ) постојат различни фактори на ризик, поврзани со самата болест, кои може да влијаат врз исходот од хируршкиот третман, односно врз настанување компликации во постоперативниот период. Нашата хипотеза беше дека токму тие ризик фактори, асоцирани со натамошните постоперативни компликации, би можеле да се покачени нивоа на предоперативните биохемиски инфламаторни маркери, како што се бројот на бели крвни клетки-WBC, и нивоата на Ц реактивниот протеин-ЦРП, анемијата, присуството на флегмона на предниот абдоминален ѕид, предоперативното постоење интеринтестинални апсцеси и позитивните ресекциски маргини.

Приказ на случај. Во овој труд, презентираме случај на пациент со КБ, примен како итен пациент и хируршки третиран. Кај пациентот беа направени стандардните предоперативни комплетни биохемиски анализи на крвта и урината, според клиничкиот протокол, кои вклучуваат инфламаторни параметри (број на бели крвни клетки-WBC и нивоа на Ц реактивен протеин ЦРП), протеинограм со вкупни протеини и албумини, хемоглобински концентрации и ултразвучен преглед на абдоменот. Резултатите покажаа анемија, хипопротеинемија, лесна леукоцитоза и покачени нивоа на ЦРП. Интраоперативниот наод покажа присуство на интраабдоминален апсцес. На осмиот постоперативен ден, се постави индикација за хируршка ревизија, поради сомневање за дехисценција на анастомозата од примарниот хируршки зафат, под клиничка слика на акутен абдомен. Ревизијата ја потврди претпоставената компликација.

Заклучок. Детерминирањето на одредени предоперативни, како и интраоперативни фактори, кои може да се асоцирани со настанувањето на постоперативните компликации, може да помогне во нивната навремена оптимизација, со цел редуцирање на пост-

Correspondence to: Vladimir Joksimovic, University Clinic for Digestive surgery, Clinical Centre Mother Theresa, 1000 Skopje, R. Macedonia; E-mail: vladimirjoksimovic@hotmail.com

оперативните компликации кај пациентите со Кронува болест.

Клучни зборови: Кронува болест, ризик фактори, компликации

Introduction

Crohn's disease (CD) is a chronic granulomatous inflammatory disease of the gastrointestinal (GI) tract with characteristic presentation of abdominal pain and diarrhea. Complications of the disease may include formation of intestinal fistulae or obstruction. This disease usually affects the small intestine, colon or anus. Rarely it can appear in other parts of the gastrointestinal tract -stomach, esophagus and mouth and may exist at several places simultaneously. Crohn's disease is an idiopathic disease and is believed to be a result of an imbalance between proinflammatory and anti-inflammatory mediators. Unpredictable flares and remissions characterize the long-term course [1-3]. Despite the significant progress in the medical treatment of Crohn's disease (CD), most patients require surgery. The combined approach in the treatment of this disease including drug treatment and timely surgical intervention, [4] is the optimal treatment of this disease, thereby improving the quality of patient's life and reducing the costs of treatment [5]. The aim of the surgical treatment is to achieve "long-lasting symptomatic relief".

Nearly 70-90% of Crohn's disease [CD] patients will undergo at least one operation during the course of CD [6,7]. Intestinal resection and surgery for perianal fistulas are the most common procedures. Indications for surgical intervention include obstruction, intra-abdominal or perianal abscess, enterocutaneous fistulas, and complex perianal disease. As medical therapies continue to improve, it is important to choose carefully surgical therapies [8]. The postoperative complication rate following intestinal resection for CD is higher than for other benign diseases, despite the fact that most patients with CD are young and without significant comorbidities. Postoperative septic complications, including anastomotic leakages, enterocutaneous fistulae, and intraabdominal abscesses, are especially troublesome, with incidence rates ranging between 5% and 20% [9-11] and are often the underlying causes of death following surgery [12]. However, the risk factors for intraabdominal septic complications [IASCs] which involve anastomotic leakage, intraabdominal abscess, or enterocutaneous fistula, still remain controversial. Some risk factors related to higher rates of IASCs include the following: preoperative steroids use [13,14], preoperative abscess [13,14], poor nutritional status [14,15], low albumin levels [16], advanced age [17,18], immune-modulating medications [19], the method of anastomosis [20], ope-

rating time [20,21], duration of symptoms leading to surgery [22,23], and a colo-colonic anastomosis.

According to Yung *et al.* [24], there are certain risk factors that influence on the postoperative outcome and on the occurrence of complications in Crohn's disease such as anemia and hypoproteinemia. According to Fasio *et al.* [25] positive resection margins on the postoperative histopathological analysis, affect the occurrence of postoperative complications and so do the duration of preoperative symptoms, poor nutritional status, etc. Also, according to Y. Pennington *et al.* [26] and Shental O. *et al.* [27], there are certain intraoperative risk factors that have an impact on the occurrence of postoperative complications as following: indication for surgery, intraoperative diagnosis, type of surgery, positive resection margins.

Aim

The aim of this paper was to present the case of a patient with CD, subject to surgical treatment and postoperative anastomosis related to intra-abdominal septic complication. We are going to discuss about certain perioperative conditions and parameters as possible risk factors for IASCs.

Case presentation

We report a case of a 19-year-old male patient who was presented as a medical emergency with severe pain in the stomach and stop of stool and winds for four days before admission to our hospital. From his medical history we found out that six months previously he was diagnosed with Crohn's disease following colonoscopy with ileoscopy and biopsy, after which CD was pathologically verified. Initial symptoms were pain in the right lower quadrant of the abdomen and diarrhea. The patient was treated with Salofalc (Mesalazine) 1000 mg per day, divided in two doses, for the past six months and had a considerable amelioration of the symptoms until this episode.

Table 1. Preoperative laboratory values

CRP	64.7 g/L
Le	11,3 x10 ⁹ /L
Er	2.95 x10 ¹² /L
Hct	0.261 L/L
TP	47 g/L
Albumin	20 g/L
Hgb	80 g/L

On the day of admission the patient was afebrile and looked severely ill. He underwent several diagnostic procedures: native radiography of abdomen, which revealed hydroaeric levels in formation; abdominal ultrasonography, which showed distended small bowel loops, without fluid in the abdomen, and finally compu-



Fig. 1. Intraoperative material



Fig. 2. Intraoperative material

terized tomography of the abdomen (CT) with intravenous contrast, which confirmed obstruction in the region of the terminal ileum. From the biochemical blood analyzes (Table 1) it is noteworthy mentioning mild leukocytosis ($11.3 \times 10^9/L$), significant anemia (Hgb 95 g/l, RBC 2.93×10^{12} , Hct 0.261 L/L), hypoalbuminemia (20 g/l) including low total protein level (47 g/l) and elevated marker of inflammation (CRP 64 mg/dl). After appropriate preoperative preparation and appropriate fluid and electrolyte substitution, the patient was sent to operating theatre. Intraoperative finding showed distended small bowel loops and a stenotic segment in the area of the terminal ileum, adhered to the mesocolon of colon ascendens. When we separated the mesocolon from the bowel loops we found a festering collection (Figure 1 and 2). It was followed by copious lavage of

the abdominal cavity and minimal bowel resection of the stenotic segment with creation of ileocolo latero-lateral manual anastomosis in two layers. On the 8th day postoperatively, intestinal contents appeared out of the abdominal drain, as well as fever and deterioration of laboratory parameters in terms of increased inflammation markers. We set an indication for revision. Intraoperative finding was a dehiscence of the anastomosis with development of diffuse peritonitis. We made a lavage and created unipolar ileostomy. Two weeks later the patient was sent home in good overall health condition and proper functioning of the ileostomy.

Discussion

There is vast scientific debate about preoperative risk factors which may affect postoperative IASC manifestation in Crohn's disease patients. In our case we encountered several of presumed risk factors-low total protein as well as albumin levels, anemia, elevated CRP levels and intraabdominal abscess.

During an acute phase response, such as active Crohn's disease, albumin levels can fall [28], thus low albumin could be indicative of the disease state rather than nutritional status alone. There are studies that found albumin less than 3.0 mg/dL to be associated with an increased risk of IASC [29,30]. Other studies using the same cutoff value, albumin < 3.0 mg/dL, did not find a similar association [31-33]. These results are further complicated by a study including preoperative nutritional supplementation in patients with an albumin less than 3.0 mg/dL [31]. Moreover, albumin levels less than 3.5 mg/dL [34,35] and less than 4.0 mg/dL [36] were reported to have no association with IASC. A recent meta-analysis using many of these described studies found a correlation with low albumin and increased risk of IASC [37], but the definition of low albumin is quite inconsistent in these studies making even the pooled results difficult to determine. Yang *et al.* [24] suggested a careful consideration of appropriate interventions aimed at correction of preoperative anemia in most patients. Present guidelines recommend measurement of hematocrit concentration as close as possible to 28 days before the scheduled surgical procedure, and subsequent investigation and intervention in patients with anemia [38]. Yang *et al.* support these guidelines because anemia is the most significant preoperative risk factor for morbidity. At least in elective surgical cases, the treatment of preoperative anemia before surgical intervention should be strongly considered. Studies that investigated the presence of intraabdominal abscess at the time of surgery for Crohn's disease found these patients at an increased risk of IASC [29, 39-41]. In contrast, abscess was not associated with abdominal complications in other studies [35,36]. Some studies included abscesses that were drained preoperatively and found no association with abscess and post-

operative IASC [36,42]. A meta-analysis which included a large number of studies discussing intra-abdominal abscess, found an increased risk of IASC with intra-abdominal abscess. Thus, the risk of IASC is higher when an intraabdominal abscess is present, but the risk is likely ameliorated if the abscess is drained preoperatively.

Conclusion

The accent should be on the preoperative detection of the risk factors for IASC and preoperative preparation of the patients, particularly nutritional supplementation and abscess drainage. Furthermore, in patients with multiple risk factors that cannot be optimized preoperatively, a diverting stoma should be considered instead anastomosis as in the presented case.

Conflict of interest statement. None declared.

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Case report

HUMAN PAPILLOMAVIRUS INFECTION AND GENITAL COINFECTIONS IN PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AND CERVICAL CARCINOMA

ИНФЕКЦИИ СО ХУМАНПАПИЛОМА ВИРУС И ДРУГИ ГЕНИТАЛНИ КОИНФЕКЦИИ КАЈ ПАЦИЕНТИ СО ЦЕРВИКАЛНИ ИНТРАЕПИТЕЛНИ НЕОПЛАЗИИ И ЦЕРВИКАЛЕН КАРЦИНОМ

Irena Aleksioska Papestiev, Megi Micevska, Goran Dimitrov, Viktorija Jovanovska, Gligor Tofoski and Marija Joksimovik

University Clinic of Obstetrics and Gynecology, Skopje, Republic of Macedonia

Abstract

Aim. This study aimed to examine the association between human papillomavirus infection and genital co-infections with cervical intraepithelial neoplasia/ cervical carcinoma, and the correlation between human papillomavirus (HPV) infections and other genital co infections.

Methods. A total number of 9579 patients underwent liquid-based cervical cytology and histology verifications during the period 2013-2016. ASCUS, CIN I, CIN II CIN III, CIS or cervical carcinoma were considered abnormal results from cervical PAP smear. Histological verification was done only in the cases with cervical carcinoma or adenocarcinoma. There were 2309 positive patients, who were compared to 5308 patients in the control group with normal cytology on PAP smear. DNA was isolated in all samples. It was then analyzed with multiplex PCR, to confirm the presence of HPV DNA. We used reverse hybridization for HPV genotyping at HPV positive samples, 763 samples were analyzed by the method of Real time PCR for the presence of seven sexually transmitted (STD) pathogens.

Results. The overall rate of HPV infection was 27.6%. HPV infection was most common in patients with CIS, cervical carcinoma and adenocarcinoma (94.4 %, 98.0% and 100%, respectively). The most common genotype was HPV type, 16 in 30.1% of all patients, as self sufficient or in combination with other HPV type. The most common genital coinfections were *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU) and *Chlamidia trachomatis* (CT), with statistically significant association between HPV infections with UP, *Mycoplasma hominis* MH and CT infections.

Conclusion. There is strong correlation between HPV infection and cervical carcinomas. Associations between HPV infection with *Ureaplasma parvum*, *Mycoplasma hominis* and *Chlamydia trachomatis* can in part be explained as a result of the coinfection, taking into

consideration that they share the same route of transmission.

Infection with sexually transmitted pathogens is associated with separate cytological diagnoses ASCUS, CIN I, CIN II, but not with CIN III, CIS and invasive cervical cancer.

Keywords: HPV, coinfections, intraepithelial neoplasia, carcinoma

Апстракт

Цели. Да се испита поврзаноста меѓу инфекциите со хуман папилома вирус (ХПВ) и други генитални коинфекции, со цервикалните интраепителни неоплазии/цервикален карцином, како и корелацијата меѓу ХПВ инфекцијата со другите генитални коинфекции.

Методи. Студијата вклучи 9579 пациенти, кај кои беа земен цитолошки брисеви и хистолошки примероци од грлото на матката, во периодот од 2013 до 2016 година. Абнормални цитолошки наоди предмет на евалуација беа: ASCUS, CIN I, CIN II, CIN III CIS (цервикален карцином In situ), инвазивен цервикален карцином. Хистолошка верификација беше правена кај пациентите со цитолошки наод за инвазивен цервикален карцином/аденокарцином. Испитуваната група ја сочинуваа 2.309 пациенти со абнормални цитолошки или со хистолошки наоди, и тие беа компаративно анализирани со 5.308 пациенти со нормални наоди, кои ја сочинуваа контролната група. Од сите примероци беше изолирана ДНК. Потоа беше направена анализа со multiplex PCR за потврдување присуство на ДНК од ХПВ. Кај ХПВ позитивните примероци беше одреден типот на ХПВ со користење на методата на реверзна хибридизација. 763 примероци беа анализирани со методот на Real time PCR и за

Correspondence to: Irena Aleksioska Papestiev University Clinic of Obstetrics and Gynecology-Skopje, R. Macedonia; E-mail: irenaaleks10@gmail.com

присуство на некој од седум сексуално преносливи (STD) патогени.

Резултати. Вкупната стапка на ХПВ инфекција изнесуваше 27,6%. Истата беше најчеста кај пациентки со CIS, цервикален карцином или со аденкарцином (94,4%, 98,0% или 100% соодветно). Најчестиот изолиран ХПВ генотип, со стапка од 30,1% беше ХПВ тип 16. Најчестите генитални коинфекции, пак, беа *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU) и *Chlamydia trachomatis* (CT), при што се најде на статистички сигнификантна поврзаност меѓу ХПВ инфекцијата и инфекциите со UP, *Mycoplasma hominis* (MH) и CT.

Заклучок. Постои силна корелација меѓу ХПВ инфекциите со цервикалниот карцином. Поврзаноста, пак, меѓу ХПВ инфекциите, со инфекциите со *Ureaplasma parvum*, *Mycoplasma hominis* и *Chlamydia trachomatis*, може да е резултат на коинфекција, земајќи го во предвид заедничкиот пат на трансмисија. Инфекциите со сексуално преносливи патогени се асоцирани со следниве цитолошки дијагнози: ASCUS, CIN I, CIN II, но не и со CIN III, CIS и со инвазивен цервикален карцином.

Клучни зборови: ХПВ, коинфекции, интраепителни лезии, карцином

Introduction

Based on epidemiological and laboratory studies, the human papillomavirus (HPV) is considered a primary cause of uterine cervical cancers. The global prevalence of HPV in cervical cancers is over 99% which actually means that all cervical cancers have HPV infection [1]. Human papillomavirus is one of the most sexually transmitted infections. Most cases of genital HPV infections regress spontaneously, but some of them persist for a longer period and progress to cervical cancer, suggesting that other cofactors may contribute to cancer occurrence after HPV infection. Certain HPV types are considered as factors associated with the persistence of HPV infection and progression to cancer. There are several studies confirming the role of genital coinfections in the development of CIN and cervical cancer in HPV infected woman. It is the biological susceptibility for HPV infection and the immunological ability for resolving this infection that can be influenced and altered by the genital coinfections by a manner of breaking the inborn protective mechanisms against HPV infection. *Chlamydia trachomatis* is the most frequently investigated and has demonstrated association with HPV infection especially high risk HPV, CIN and invasive cervical carcinoma [2-6]. The role of *Chlamydia trachomatis* in cervical carcinogenesis might be restricted to squamous cell malignancy [2,7]. In addition, *Trichomonas vaginalis*, *Mycoplasma* and

Ureaplasma species have been associated with HPV infection and abnormal cervical cytology [8-11].

In this study tests for HPV DNA and for seven genital coinfections were performed in patients with abnormal cervical cytology in the obstetrics and gynecology outpatient clinic, and histological verifications were made in those with cervical squamous carcinoma/ adenocarcinoma. The same tests were performed in the control group. The aim of this paper was to investigate the presence of human papillomavirus and the distribution of HPV genotypes in patients with cervical intraepithelial neoplasia and cervical cancer, to investigate the correlation between HPV infection and cervical carcinoma as well as to examine the correlation between cervical HPV infections and infections with other sexually transmitted pathogens.

Materials

Cases

We used cervical liquid-based PAP smears/tissues taken from 7617 patients in the period 2013-2016. These samples were taken by the gynecologists in the University Clinic for Obstetrics and Gynecology in Skopje, and by the gynecologists in the primary care system in our country. There were 2309 liquid-based cervical smears with positive finding which included: ASCUS, CIN I, CIN II, CIN III, CIS, cervical carcinoma and adenocarcinoma. The cytological findings that showed cervical carcinoma or adenocarcinoma underwent histological verification. In the 2309 positive findings, we found the following distribution: ASCUS 483, CIN I 1193 CIN II 208, CIN III 299 CIS 51 cervical carcinoma 51 and adenocarcinoma 3.

Control group

This group included 5308 patients with normal cytology findings. Diagnoses included cervicitis, colpitis, condylomata accuminata and polypus cervicalis. Besides these two groups, we also performed analysis of patients with HPV cervicitis identified with cervical smear, 10 pregnant patients and 779 patients with conization of the uterine cervix.

Informed consent was obtained by all participants in the study.

Methods

DNA isolation

DNA was extracted from cervical cytology samples using an AccuPrep Genomic DNA Extraction kit (Bioneer) and the analysis was made according to the manufacturer's protocol. Cell pellet from 1.5 mL cervical cytology sample was washed in phosphate buffered saline (PBS) and reacted at 60°C for 20 minutes in 200 µL PBS buffer mixed with 20 µL proteinase K. 100 µL isopropanol was added and mixed well, followed

by transfer to a binding column for centrifugation, which was performed with two washes with a washing buffer. The binding column was transferred to a new tube, and elution buffer was added to elute the bound DNA, which was then centrifuged to finally obtain the DNA solution. DNA isolation from the tissue specimens was done with the commercial set QIA amp DNA mini Kit, according to the manufacturer's protocol. After mechanical homogenization, PBS and buffer and proteinase K were added to the tube, which was followed by an incubation at 56°C for 1-3 hours. The lysate was filtrated through silica gel which absorbs the DNA. At the end of the procedure the genomic and viral DNA was eluted with low ion strength solution. With each series of isolated samples, we made a blind probe containing only of PBS. Part of each sample was kept until the final results.

HPV screening

The HPV DNA test was performed using a Seeplex HPV4A ACE Screening kit (Seegen Inc., Seoul, Korea) following the manufacturer's protocol; 4 µL of 5× HPV4A ACE PM (primer mixture including primer pairs for HPV, a primer pair for internal control, and template of internal control), 3 µL of 8-methoxypsoralen solution, and 10 µL of 2× Multiplex Master Mix (containing DNA polymerase and buffer with deoxyribonucleotide-5'-triphosphate [dNTP] and dye) were mixed to form 17 µL of polymerase chain reaction (PCR) master mix, to which 3 µL of DNA solution extracted from the specimen was added to prepare a mixture with a total volume of 20 µL for the PCR reaction. Positive and negative controls were used from the kit. With this set we can simultaneously make screening for 16 high-risk HPV types (26,31,33,35,39,45,51,52,53,56,58,59,66,68,69,73 and 82) and 4 low-risk HPV types (6,11,42,43 and 44) and genotypization of HPV type 16 and 18, using the Dual Priming Oligonucleotide (DPO) for multiplex PCR amplification of the target DNA. We also performed positive control in order to determine the quality of the PCR reaction and a negative control to detect eventual contamination. The samples were analyzed with gel electrophoresis on a 2 % agarose gel with ethidium bromide. The visualization of amplicones was done by UV transluminator.

HPV genotypization

Positive samples were genotyped using a commercial set High+LowPapillomastrip (Operon), which is based on the principle of reverse hybridization. The assay was used for amplification and qualitative detection of regions E6-E7 of HPV DNA of 37 high and low risk HPV subtypes. To each PCR tube, a 38 µl of PCR premix, 5 µl of each primer, 2 µl of Taq enzyme and 5 µl of DNA were added and mixed. During PCR, the amplification of an internal control gene (β-globin or GADPH) also occurs, acting as an indicator of the

absence of PCR inhibitors in the amplified DNA samples. After amplification, PCR products were hybridized onto the High or Low Papillomastrip containing specific probes. The strip was interpreted with the help of the evaluation chart supplied with the kit

Genital coinfections testing

We performed testing for the presence of sexually transmitted (STD) pathogens: *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV) on 763 samples. They were analyzed by the multiplex Real time PCR, using Cyclic-CMTA (Cyclic-Catcher Melting Temperature Analysis) method, commercial set Anyplex TM II STI-7 Detection (Seegene).

Each PCR was performed in 5 µl of extracted DNA, 4x STI-7 TOM, and Anyplex PCR Mix in a 20-µl reaction. The thermal cycle conditions consisted of an initial incubation at 50°C for 4min to activate the UDG system and prevent contamination. The melting temperature was analyzed by increasing the reaction temperature from 55°C to 85°C (5 s/0.5°C). The whole process control was added to the samples immediately before the DNA extraction to confirm the DNA extraction and PCR inhibition

Statistical analysis

Statistical analysis was performed using SPSS- Statistical Package for Social Science, 18.0. Several statistical tests were used: Chi-square, logistic regression, Mc Nemar test and linear correlation. Values of p 0.05 and 0.01 were considered to be statistically significant. T-test and ANOVA variance analysis were used for results comparison.

Results

HPV distribution

Patients included in this study were 15 to 83 years old, majority in the age group 27 to 43. The highest rate of HPV positive patients was in the age group 24 to 48. The largest number of the patients had normal PAP smear (55.4%), which did not exclude existence of some benign conditions. The overall rate of HPV infection was 27.6%. HPV presence was most common in the group with cervical carcinoma, CIS, invasive cervical carcinoma and adenocarcinoma (94.4%, 98.0%, and 100%, respectively), and the smallest presence was in the cervicitis group (3.6%). We found only 25.8% HPV infection rate in PAP smear findings that suggested HPV infection (Table 1). There was a positive correlation ($p < 0.001$) between positive cytological findings and HPV infection. Patients younger than 25 years had mostly normal cytological finding, cervicitis, colpitis, Condylomata accuminata and HPV infection (PAP smear).

There were no CIS, invasive carcinoma or adenocarcinoma in the group under 20 years. Only 2 patients from this group were treated with conization. CIS, invasive cervical carcinoma and adenocarcinoma were most common in the age group 45 to 50 years. Our results showed a significant correlation ($p < 0.01$) between the patients' age and the grade of cervical dysplasia. HPV type 16 was most prevalent with 30.1% of all patients, as self sufficient or in combination with other type. Second in terms of representation was HPV type 31, and right after HPV type 52 with frequency of 7.6% and 7.5%, respectively. HPV type 18 was on the fifth place with 5.5% of all the participants, but it was most prevalent in the adenocarcinoma group-67% (Table 2). HPV positive samples were analyzed for coinfection with two or more HPV types. These coinfections did not show significant difference in the CIN I, CIN II, CIN III, CIS and patients with normal cytology findings. Coinfections that included high-risk type 16 were most prevalent in the ASCUS and invasive cervical cancer group-75% and 80% from the total number of coin-

fections in each group respectively, while coinfections with high-risk HPV type 18 were most prevalent in CIN II cytology smear finding -16.3% of the total number of coinfections in this group.

HPV and genital coinfection with sexually transmitted pathogens

763 samples were tested for sexually transmitted pathogens (*C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum* and *T. vaginalis*). We observed the highest prevalence of these genital coinfections in the ASCUS findings on PAP smear, which were not detected in patients with adenocarcinoma, condylomata acuminata and polypus cervicalis. The correlation between genital coinfections and cytology findings showed p value < 0.05 . There was also a statistically significant association between positive PAP smears and presence of one or more pathogens, compared to normal cytology findings.

Table 1. Distribution of patients according to cytological diagnosis and prevalence of HPV infection

Diagnosis	Number of patients	% of patients from the total number	HPV-positive patients	% of HPV positive patients per group
Negative PAP test	5308	55.4%	1203	23.7%
ASCUS	299	3.1%	89	29.9%
CIN 1	1193	12.5%	348	29.4%
CIN 2	483	5.0%	251	52.7%
CIN 3	208	2.2%	159	78.7%
CIS	72	0.8%	68	94.4%
Ca PVU Invasivum	51	0.5%	50	98.0%
Adenocarcinoma	3	0.03%	3	100.0%
Cervicitis	440	4.6%	93	3.6%
Colpitis	203	2.1%	32	16.8%
Condylomata acuminata	13	0.1%	9	75.0%
Polypus cervicalis	7	0.1%	2	33.3%
HPV from PAP test	510	5.3%	131	25.8%
Graviditas	10	0.1%	2	28.6%
St post conisatio	779	8.1%	124	16.0%
Total	9579	100.0%	2564	27.6%

Табела 2. Percentage of high-risk HPV types and low-risk HPV 6 and HPV 11 in various diagnosis

	6	11	16	18	31	33	35	39	45	51	52	53	58	66	68	82
Normal results	5.7%	0.6%	24.1%	4.4%	8.1%	3.3%	3.5%	4.4%	3.2%	6.7%	7.5%	9.5%	4.4%	5.4%	4.9%	1.4%
ASCUS	1.1%	0%	48.3%	10.1%	7.9%	2.2%	2.2%	1.1%	4.5%	5.6%	5.6B	1.1%	2.2%	3.4%	1.1%	4.5%
CIN 1	5.2%	0%	33.6%	4.9%	8.6%	3.2%	2%	3.4%	2.9%	4.3B	7.5%	6.6%	4.9%	5.7%	4.6%	3.2%
CIN 2	4%	1.6%	41.4%	7.2%	10%	4%	3.2%	2%	2%	7.2%	8%	3.6%	3.2%	1.6%	2.4%	0%
CIN 3	1.9%	0.6%	44.1%	5%	6.3%	5.7%	5%	5%	2.5%	3.1%	8.2%	1.9%	5.7%	1.3%	0.6%	0%
CIS	2.9%	0%	54.1%	10.3%	5.9%	8.5%	1.5%	1.5%	7.4%	0%	8.4%	4.9%	4.4%	0%	1.5%	2.9%
Ca invasivum	0%	0%	66%	10%	9%	5%	0%	2%	0%	0%	8%	2%	2%	2%	2%	2%
Adenocarcinoma	0%	0%	0%	66.7%	0%	0%	0%	0%	33.3%	0%	0%	0%	0%	0%	0%	0%

($p=0.0004$, OR (95%CI)=2.09 (1.39-3.15). The strongest association was observed in the ASCUS findings ($p=0.002$; OR (95%CI)=12.2 (1.54-97.11) followed by CIN II [$p<0.01$; OR (95%CI)=3.32 (1.5-7.34)] and CIN I [$p<$

0.05; OR (95%CI)=1.95 (1.02-3.77)]. Correlation between HPV infection and other genital coinfections was with $p<0.01$. Analyzing patients with HPV infection and those without HPV infection, regarding the presence of

Table 3. Results of STD-panel examinations in various diagnosis

Diagnosis	STD-panel total	STD-panel negative	STD-panel positive	% STD-panel positive
Negative PAP – test	369	214	155	42%
ASCUS	41	6	35	85%
CIN 1	79	34	45	57%
CIN 2	51	16	35	68%
CIN 3	59	26	33	56%
CIS	25	21	4	16%
Ca PVU Invasivum	34	24	10	29%
Adenocarcinoma	0			
Cervicitis	25	17	8	32%
Colpitis	25	13	12	48%
Condylomata acuminata	0			
Polypus cervicalis	0			
HPV ad PAP	39	14	25	40%
Graviditas	5	3	2	50%
St post conisatio	10	5	5	48%
Total	763	394	369	42%

one or two pathogens, these two infections showed statistically significant association [$p<0.0001$ OR (95% CI)= 4.34 (3.01 -6.26)] (Table 3).

Further analysis of HPV positive with HPV negative group, and their correlation with each pathogen, showed a

statistically significant association only for UP and MH infections [$p<0.0001$; OR (95% CI)=2,98 (2,03-4,39)] and [$p<0.0001$ OR (95% CI)=4.48 (2.55-7.86)] respectively and in CT infections [$p=0.0001$ OR (95% CI)=4.2 (1.93-8.91)] (Table 4).

Table 4. Single pathogens out of total number of examined patients and in HPV positive patients

STD-pathogen	Number of patients with detected pathogen	%	Number of HPV-positive patients with detected pathogen
UU	88	25.4%	32
UP	183	52.9%	91
MG	10	2.9%	3
MH	86	24.9%	48
NG	6	1.7%	3
CT	48	13.9%	26
TV	12	3.5%	4

Analysis of the association between group with severe dysplasia (CIN III, CIS invasive carcinoma and adenocarcinoma) and some STD pathogen compared to the normal cytology findings showed no statistically significant association.

Discussion

There is ample scientific evidence on the strong correlation between HPV infections and cervical carcinoma incidence worldwide. HPV type 16, 18, 45, 33, 58, 31 and 52 are responsible for approximately 90% of the cervical carcinoma [12]. The most prevalent HPV type in our study was HPV type 16, followed by HPV type 31, 52, 53, 18 and 51. There was a similar type distribution among groups, although the smallest rate of HPV inci-

dence was found in CIN I group (24.1%), which gradually increased and reached the highest rate in the group with invasive cervical cancer. We observed a gradual growth of the percentage representation of the HPV types 33, 18 and 45 from the CIN I to the CIS group. This was due to the reduced rates of presence of low-risk HPV types that were absent in the cervical cancer group. Vidal *et al.* [13] conducted a study among women in Tanzania, where high risk HPV type presence was found in almost all invasive cervical carcinomas (prevalence of 89%), most commonly HPV type 16, followed by HPV type 35, 45, 18, 31, 52. On the other hand, HPV type 16 was seldom found in patients with CIN I, CIN II, CIN III findings. Ursu *et al.* [14] conducted a study among women in Romania and found that HPV type 16 was predominant in all the HPV po-

sitive groups, followed by HPV type 53, 51, 52, 18, 31. The presence of HPV infection in cervical carcinomas in the literature ranges from 90 to 100% of cases. This has also been confirmed by the World Health Organization according to which HPV infection increases with the severity of the lesion. HPV practically causes 100% of cases of cervical cancer, and the smaller prevalence of HPV in cervical cancers is most often due to limitations of methodologies. Our results are in accordance with the literature data, showing a clear correlation between infections with high-risk types of HPV and cervical carcinomas. In 94.4% of our patients with CIS and 98% of patients with invasive cervical cancer, there was a proven presence of HPV DNA and the few negative results are likely due to the insufficient sensitivity of the method. Analyzing the cytological and histopathological diagnoses and the prevalence of HPV infections in relation to the age of patients, it can be concluded that despite the high percentage of HPV positive patients up to 25 years, the percentage of patients with CIS and invasive cervical carcinoma was the lowest. The fact that in the youngest age group there were no patients with a diagnosis of CIS, invasive cervical cancer or adenocarcinoma, goes in favor that it takes several years for HPV infection to develop into severe dysplasia. HPV infections are associated with infections with other sexually transmitted pathogens [15,16]. Our results showed a significant association between HPV infections and infections with investigated genital coinfections ($p < 0.0001$). The association with individual cytological diagnoses is not very high, but is still significant with all diagnoses except for severe dysplasia and cancer (CIN III, CIS invasive cervical cancer). The data on which pathogen is most strongly associated with HPV infection or with some of the pathological cytological findings vary greatly, probably because of the differences in the representation of individual pathogens in different regions and countries; there was no sufficient number of infections with a particular pathogen to draw conclusions about their possible impact on HPV infection and cervical carcinogenesis. Thus, in most cases infections with *N.gonorrhoeae* are present in a very small number or are not present at all, and the situation is similar with *T. vaginalis* [16-18]. In an American study by Andre LP [19] *N. gonorrhoeae* and *T. vaginalis* were the pathogens most often correlated with HPV infection in patients with diagnoses > ASCUS. Our analysis showed no association between *N. gonorrhoea* and *T. vaginalis* infections with HPV infections or with positive cytological findings. A small number of competing infections with *N. gonorrhoea* and *T. vaginalis* could limit our ability to detect possible associations. In our patients, HPV infection was most frequently associated with infections with *U. parvum*, *M. hominis*, as well as with *C. trachomatis* infection. This association can be partly explained as a consequence of coinfection, given the fact that some of

the pathogens share a transmission group. Infection with one pathogen increases the risk of infection with others [20]. HPV type that showed a significant association with even two of the pathogenic microorganisms (*M. hominis*, *C. trachomatis*) was HPV type 39, found also in other studies, [21] although the most common and strongest association was between *C. trachomatis* and HPV type 33 [20]. In our study, the highest proportion of patients with *C. trachomatis* infection was observed in the 20-30 year group, while in patients over 45 years of age it was not detected at all. *C. trachomatis* infection may increase susceptibility to HPV infection at cellular level by providing an approach to the basal epithelium due to microabrasion or by changing the characteristics of the epithelial cells, increasing the viral load on the infection, and alleviating persistence. Alternatively, concomitant *C. trachomatis* infection could impair the clearance of HPV by induced changes in the immune response to HPV infections. Perhaps *C. trachomatis* infections require a younger age group that would have the potential to promote the persistence of HPV infection and progression to CIN I, CIN II, and to invasive carcinoma.

Our analyses showed no significant association between *M. genitalium* and *U. Urealyticum* with HPV infection and pathological cytological diagnoses, contrary to the results of Kim H C [16] and Dehon PM [22]. On the other hand, our analyses have shown a significant association between *U. parvum* and HPV infections. We found a significant association between *M. hominis* and HPV infections.

Conclusion

The most prevalent HPV types in our population were 16, 31, 52, 53, 18 and 51. HPV type 16 was found in as many as 66% of patients with invasive cervical cancer. The most prevalent sexually transmitted pathogens other than HPV in our population was *U. parvum*, followed by *U. urealyticum*, *M. hominis* and *C. trachomatis*, while *M. genitalium*, *T. vaginalis* and *N. gonorrhoeae* were less represented. HPV infections were associated with infections with other sexually transmitted pathogens examined in this study. Infection with sexually transmitted pathogens was associated with separate cytological diagnoses ASCUS, CIN I, CIN II, but not with CIN III, CIS, invasive cervical cancer. Hence, further research is needed aimed to further define groups with abnormal cytological findings and HPV status among our population, which will benefit most from the introduction of regular testing for other genital coinfections. This would improve treatment of low-grade dysplasia and abnormalities of indefinite meaning.

Conflict of interest statement. None declared.

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Case Report

CHRONIC LYMPHOCYTIC LEUKEMIA FOLLOWING SUCCESSFUL TREATMENT OF HODGKIN'S LYMPHOMA: REPORT OF TWO PATIENTS

ХРОНИЧНА ЛИМФОЦИТНА ЛЕУКЕМИЈА ПОСЛЕ УСПЕШЕН ТРЕТМАН НА ХОЧКИНОВ ЛИМФОМ: ПРИКАЗ НА ДВАЈЦА ПАЦИЕНТИ

Gazmend Amzai, Oliver Karanfilski, Tatjana Sotirova, Sonja Genadieva-Stavric, Arif Latifi and Aleksandar Stojanovic

University Clinic for Hematology, Medical Faculty, "Ss. Cyril and Methodius", - University, Skopje, Republic of Macedonia

Abstract

Over the past few decades, introducing effective therapy, improved staging procedures, and significant improvement of supportive measures, significantly improved the prospects for patients with Hodgkin's lymphoma, leading to a 75-90% cure rate.

Hodgkin's lymphoma survivors are at high risk of developing a large variety of second malignant neoplasms. It is crucial to maintain awareness regarding this issue. The subsequent development of Chronic Lymphocytic Leukemia (CLL) in patients after successful treatment of Hodgkin's lymphoma (HL) is an extremely rare possibility. The relationship between these two lymphoproliferative disorders is unclear.

Herein we describe two cases of CLL, developed in previously treated patients with HL, with characterization of two distinct lymphoproliferative diseases in these two patients. We also systematically reviewed the existing literature on this very rare occurrence of treatment-induced second hematological malignancies.

Keywords: Hodgkin's lymphoma, chronic lymphocytic leukemia, second malignancies

Апстракт

Воведувањето ефикасна терапија, подобрување на процедурите за дефинирање на распространетоста на заболувањето, односно „staging“ процедурите, како и значителното подобрување на супортивните мерки, во изминатите неколку децении во голема мера ги зголемуваат шансите за долготрајно преживување на пациентите со Хочкинов лимфом, достигнувајќи стапка на излекување од 75 до 90 проценти.

Кај преживеаните пациенти со Хочкинов лимфом постои висок ризик за развивање голем број дру-

ги секундарни малигни неоплазми. Одржувањето на свеста за ова прашање е од клучно значење.

Развојот на хронична лимфоцитна леукемија (ХЛЛ), после успешен третман на пациенти со Хочкинов лимфом (ХЛ) е исклучително ретка појава. Нејасна е поврзаноста меѓу овие две лимфопрлиферативни заболувања.

Во трудот претставуваме два случаи на ХЛЛ, развиени кај претходно третирани пациенти со ХЛ, со приказ на карактеристиките на двете различни лимфопрлиферативни заболувања кај овие двајца пациенти.

Освен тоа, приложуваме и систематски преглед на постојната медицинска литература за оваа многу ретка појава на тераписки предизвикани секундарни хематолошки малигноми.

Клучни зборови: Хочкинов лимфом, хронична лимфоцитна леукемија, секундарни малигноми

Introduction

Hodgkin's lymphoma (HL) is a clonal lymphoid malignant neoplasm. Patients with HL are treated with multi agent systemic chemotherapy, with or without radiation therapy. Generally, the most common chemotherapeutic regimen is a 4-drug combination called ABVD given every 28 days for 6-8 cycles, which consists of Doxorubicin, Bleomycin, Vinblastine and Dacarbazine [1]. Cure rates are high following treatment, but treatment-related toxicity is a major concern [2,3]. The occurrence of second malignancies after successful treatment of Hodgkin's lymphoma is now a well-recognized phenomenon [4,5]. Secondary neoplasms are defined as histologically distinct neoplasms developing at least 2 months after the completion of treatment for the primary malignancy [6]. Increased risk of hematological malignancies, especially chronic lymphocytic leukemia (CLL), is a very unusual finding in previously treated patients with HL. On the other hand, CLL transforming into Hodgkin's

Correspondence to: Gazmend Amzai, University Clinic for Hematology, "Mother Theresa" 17, 1000 Skopje, R. Macedonia; E-mail: dr.Gazmend_Amzai@hotmail.com

lymphoma is a recognized complication, defined as Richter's syndrome. CLL is considered to be a low-grade lymphoproliferative disorder. In 5-10% of patients the disease transforms into a more aggressive lymphoma, most common into diffuse large B-cell lymphoma and less frequently into HL [7,8,9].

We report two cases of exceedingly rare secondary CLL after HL, posing a major diagnostic challenge for the hematologist.

Case Presentation

Case 1

A 65-year old male presented with a mass on the right side of his neck (6 x 8 cm), night sweats, and weight loss. The patient underwent excisional biopsy in April, 2008. The H&E histology of the specimen as well as the immunohistochemical profile, confirmed the diagnosis of classical Hodgkin's lymphoma with a lymphocyte-depleted (LDHL) histopathological subtype. The clinical stage was assessed as IIB. Bone marrow biopsy was normal. The initial hemogram showed a hemoglobin level of 115 g/l, leukocyte count of $16.3 \times 10^9/l$ (42% of lymphocytes) and a platelet count of $155 \times 10^9/l$.

He received 6 cycles of initial treatment with ABVD in the Clinic Day Care Unit. The patient received his last cycle of chemotherapy in September, 2008.

Evolution of slowly progressive leukocytosis and lymphocytosis started 12 months after the completion of the treatment for HL. In March, 2011 white blood cell counts increased to $41,5 \times 10^9/l$, of which 81% were lymphocytes. After flow cytometric assessment, a CLL diagnosis was established. Clinical examination results were normal, with no noted disease-related symptoms or clinical manifestations. The disease was in the early stage (Rai 0, Binet A). We chose not to treat this patient at that moment, and the watch and wait strategy was implemented.

Follow-up of the patient continued, noting a significant disease progression during the next 2 years. In February of 2013, his leukocyte count increased to $135 \times 10^9/l$, consisting of 95% lymphocytes, platelets dropped to $70 \times 10^9/l$, and there was detectable splenomegaly, palpable more than 6 cm below the left costal margin (20 cm in diameter). He was treated with cyclophosphamide continuously (100 mg per day) for 6 months. The patient had an extremely satisfactory response to therapy.

Case 2

A 61-year old man was referred to our Clinic in August 2006 due to right cervical lymphadenopathy. Lymph node biopsy confirmed the diagnosis of Hodgkin's lymphoma-lymphocyte rich subtype. The patient was sta-

ged as early favorable stage I-A. The initial hematological parameters were as follows: hemoglobin 145 g/l, white blood cell count $11.1 \times 10^9/l$ (30% of lymphocytes), platelet count $256 \times 10^9/l$. No neoplastic infiltration was detected in the bone marrow biopsy specimen. The patient was started on combination chemotherapy with 3 cycles of ABVD, followed by local radiotherapy. The patient completed the treatment in November 2006.

Seven years later, in December 2013, cervical lymph node enlargement was detected again and a relapse was diagnosed. The patient was treated with the same regimen, with 6 cycles of ABVD chemotherapy and a second complete remission was achieved.

Thirty months after the second treatment, he was diagnosed with Rai II Binet B stage CLL. Leukocytosis was the main abnormality and a peripheral blood smear revealed typical CLL morphology (lymphocytes 62%). Flow cytometry analysis on peripheral blood specimens was performed, detecting a present circulating CLL clone, and a diagnosis of CLL was confirmed.

Histopathology of a lymph node biopsy from the right inguinal region corresponded to Hodgkin's lymphoma, confirming the coexistence of CLL and HL. The patient was treated with three additional cycles of ABVD chemotherapy, as for Hodgkin's lymphoma, rendering that it is a more aggressive and powerful option, and again achieved complete remission.

The patient is presently alive with no symptoms or disease manifestations.

Discussion

Hodgkin's lymphoma is highly sensitive to combination chemotherapy and/or radiotherapy. Patients with HL achieve long overall survival with standard frontline chemotherapy (ABVD). Most patients will survive the disease, but long-term complications of chemotherapy, which can occur years after treatment, including multi organ damage and secondary malignancies, have a negative impact on overall survival and quality of life of these patients. Chronic lymphocytic leukemia after Hodgkin's lymphoma is reported in a few studies and is extremely rare [10].

In our report we describe two patients with Hodgkin's lymphoma, with well-characterized clinicopathologic and immunophenotypic features and adequate Ann Arbor staging information. They were treated with ABVD chemotherapy. Following treatment, they both achieved complete remission. Late relapse was confirmed in the second case, and after six ABVD cycles a second complete response was obtained. After successful treatment, they were both subsequently diagnosed with CLL by morphology. In order to establish a definitive diagnosis, flow cytometry was performed. Patients revealed an immunophenotype typical of CLL.

The interval between the diagnosis of HL and the diagnosis of CLL was 12 and 30 months respectively. The outcome regarding CLL in our patients, arising as a second neoplasm after HL, is encouraging.

Although the link between CLL and transformation into HL is well established (Richter's transformation), the association between HL treatment and subsequent CLL is less elaborate. This is especially the case with the second patient, manifesting concomitant CLL and HL. A study by Weisenberg *et al.* reported 8 cases with the association of the two malignancies [11]. In another article, Kornberg *et al.* reported a similar case of CLL developing two years after inducing a remission in a patient with HL [12].

It is very difficult to understand the underlying pathogenesis of CLL arising after HD. Clinical significance of this association may imply disease specific risk factors, a pro-tumorigenic microenvironment in patients with HL. On the other hand, it could be related to chemotherapy induced factors, high risk carcinogenic agents, or it could also be rendered as purely coincidental.

As in other reports, and mostly due to a small number of patients, we were not able to identify a particular subgroup of patients at high risk for developing CLL, with regard to age, gender, type, or clinical stage of HL. There are some seemingly intriguing questions for which contemporary medical science is not able to offer a reasonable answer, primarily regarding the possible reasons or circumstances surrounding this peculiar phenomenon. Whether these disorders are coincidental findings, or are caused by one primary, or maybe common genetic alteration, which increases the genomic instability and susceptibility to further lymphoid clonal disorders, remain to be investigated.

Conflict of interest statement. None declared.

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УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед"(ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.

Списанието ги има следниве рубрики и категории на трудови:

- **Изворни трудови**
- **Соопштувања за клинички и лабораториски искуства**
- **Прикази на случаи**
- **Од практика за практика**
- **Едукативни статии**
- **Вариане** (писма од редакцијата, општествена хроника, прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката „Во сеќавање„ и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриците 2-5 имаат белези на стручни трудови.

Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.

Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.

Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

• **ТЕКСТ НА РАКОПИСОТ**

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Times New Roman големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.

Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспонденција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и соопштувањата го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со MeSH (Medical Subject Headings) листата на Index Medicus.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните СИ единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

• ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).

Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

• ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. [3-6]).

Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Index Medicus):

- **сѝаѝија во сѝисание** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор.*) Neglia JP Meadows AT, Robison LL *et al.* Second neoplasms after acute lymphoblastic leukemia in childhood. N Engl J Med 1991; 325:1330-6.

- **заеднички авѝор**

GIVIO (Interdisciplinary group for cancer care evaluation). Reducing diagnostic delay in breast cancer. Possible therapeutic implications. *Cancer* 1986; 58: 1756-61.

в) без авѝор - анонимно. Breast screening: new evidence. (*Editorial Lancet* 1984; i :1217-8).

г) ѝоѝлавје во книѝа или моноѝрафија

Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. Vo: Sodeman WA Jr, Sodeman WA, Ed. Pathogenic physiology: mechanisms of disease. Philadelphia; W B Saunders, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

**Уплата за испечатен труд во списанието ММП изнесува 3.000, 00 денари и се уплаќаат на жиро сметката на: Македонско лекарско друштво
300000000211884 – Комерцијална банка
со цел на дознака: уплата за стучен труд**

Адресата на Редакцијата

Даме Груев бр. 3
Градски сид блок II,
1000 Скопје,
Тел.: ++ 389 02 3162 577

Електронска адреса (Е-маил): mld@unet.com.mk

Известување за рецензентите за ММП

Во склад со правилникот на УКИМ рецензентите што навремено и одговорно ќе ја одработат рецензијата ќе добијат 0.4 бода кои се собираат за унапредување во академските звања. Бодовите можат да се добијат и ретроградно преку побарување во МЛД – 3162 557.