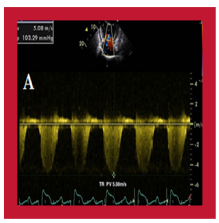
ISSN 2490-3329

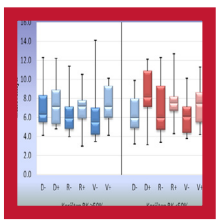
SCRIPTA MEDICA 48

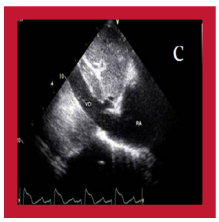
Journal of the Medical Society of the Republic of Srpska
Časopis Društva doktora medicine Republike Srpske

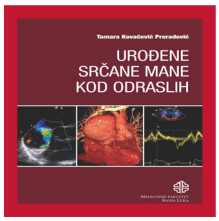
Vol. 48 • No 1 • April 2017.
Medical Society of the
Republic of Srpska

Godina: 48. • Broj 1 • April 2017.
Časopis Društva doktora medicine
Republike Srpske









www.scriptamedica.com

Scripta Medica (Banja Luka)

Journal of the Medical Society of the Republic of Srpska

Publishers: *Društvo doktora medicine RS*
Medicinski fakultet, Banja Luka
Printed by: *Grafix s.p., Banja Luka*

ISSN 2490-3329 (Print)
ISSN 2303-7954 (Online)

Tiraž: 1 000

All articles of the Scripta Medica journal are registered at the National and University Library of the Republic of Srpska, upon which analytic processing of the article was conducted in the international mutual catalogue Cobiss.

This issue of the SM is printed on acid-free paper

Content

ORIGINAL ARTICLE

doi: 10.18575/msrs.sm.e.17.01
UDC 616.727.2-009.7:616.155.2
COBISS.RS-ID 6395928

Platelet-Rich Plasma in the Treatment of "Frozen Shoulder"

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ABSTRACT

Introduction: This paper presents the results of treating the condition known as frozen shoulder with the platelet-rich plasma method (PRP). Frozen shoulder (periarthritis humeroscapularis, adhesive capsulitis) is the third most common condition (after back pain and knee arthrosis) occurring in middle-aged people (40 to 60 people).

Aim of the Study: To establish to which extent is platelet-rich plasma efficient in the treatment of painful shoulder syndrome.

Patients and Methods: In the period between January 2013 and December 2015, in the HI Hospital for Surgical and Internal Medicine "S.tetik", 54 female patients with clinical manifestations of a frozen shoulder were treated. The treatment consisted of three PRP administrations at 7-day intervals. The Quick Dash questionnaire was used for the results at the beginning of the treatment, as well as 30 days and 3 months after its completion. A checkup was conducted after a year.

Results: In total, we treated 54 female patients whose average age was 52 years (37 – 72). Pain in the left shoulder was experienced by 37 patients, while 17 of them experienced pain in the right shoulder. The Quick Dash score prior to the PRP administration was 42 (35-52), while after the PRP treatment the score was 18 (13-26) after 30 days and 13 (11-23) after 3 months.

Conclusion: By means of our protocol, implying the treatment of frozen shoulder with the platelet rich plasma method, it is possible to significantly reduce subjective difficulties of patients. Also, together with all other therapeutic procedures (analgesia, physical therapy, etc.), it may eventually lead to a complete recovery. Further work on the examination of pathophysiological effects of PRP and monitoring a large number of patients in multiple centers could result in scientifically proven standards for the application of PRP as a method of choice in the treatment of frozen shoulder.

Key words: Platelet-rich plasma, frozen shoulder, Quick Dash score

(Scr Med 2017:48:6-10)

Submitted: Decembar 1st, 2016
Accepted: Decembar 16th, 2016

Introduction

Frozen shoulder is a syndrome characterized by pain and limited active movements in the shoulder joint. The etiology of the condition itself has not been precisely defined yet. It is most common in the population aged between 40 and 65, occurring much more frequently in women (4:1).[1] In Japan, it is known as "50s shoulder". The term "frozen shoulder" was first described by Codman in 1934. He regarded this condition as a painful condition associated with shoulder stiffness and pain when sleeping on the affected shoulder. He also described the phenomenon of limitation of external rotation and elevation, which are standard diagnostic criteria still used to this day. Long before Codman, in 1845, Duplay named this condition periarthritis, while in 1872, Naviesar coined the term adhesive capsulitis. Pain in the shoulder joint is the third most common cause of disability, after lower-back pain and pain in the knee. The incidence of frozen shoulder in the total population is 2% and up to 11% in diabetics, occurring on both sides in 16% of patients.[1] The disease has been described as passing through three stages and each of these is specific and characteristic in terms of symptoms indicated by the patient. Stage 1 – painful phase – the patient describes an insidious onset of pain, with the pain usually occurring at night, without causal factors. The pain is not associated with activity, even though the slightest movement can increase it. As the disease progresses, patients feel pain even at rest. In this stage, which can last anywhere between 2 and 9 months, there is no loss of range of motion, while clinical presentation may be diagnostically unclear. Stage 2 – frozen phase – painful symptoms from the stage 1 may still be present, but they are most often improved. The loss of range of motion occurs as capsular pain (in all directions of movement). Daily activities may be rather affected. This stage of the disease may last between 2 and 9 months, and even up to 24 months. Stage 3 – thawing phase or regression phase – the pain begins to fade gradually and shoulder motion is slowly improved in the next 12 to 24 months.

The diagnosis is established based on a medical history and clinical examination, as well as standard X-ray imaging and possibly CT and MR. In the differential diagnosis, the diseases which should be taken into account are rupture and tendinitis of the two-headed muscle of the upper arm (m. biceps brachii), brachial plexus neuritis, cervical spine disorders (arthrosis, bulging or prolapsed cervical disc, torticollis), Parkinson's disease and primary and secondary tumors, Pancoast lung tumor in particular. The treatment involves rest, non-steroidal anti-inflammatory drugs, physical therapy, intra-articular administration of corticosteroids and arthroscopic procedures. In addition to the above mentioned treatment methods, a relatively new method used to treat bone and joint diseases is the platelet-rich plasma – PRP method. The first serious attempts at treatment by this method were described in the 70s of the last century and the first works dealing with this subject were published at the beginning of the 21st century, for soft tissue injuries and healing of skin defects, treatment of knee injuries after arthroscopic surgery (Sánchez et al. 2003), for muscle injuries (Sánchez et al. 2005), for tendon treatment (Sánchez et al. 2007), for knee arthrosis (Sánchez et al. 2008) and hip arthrosis (Sánchez et al. 2011).[3] PRP works through growth factors which play a key role in initiating and controlling the complex process of rehabilitation of injuries and diseases. This treatment method relies on an increased concentration of platelets which contain high concentrations of growth factors. Growth factors stimulate repair and healing of tendons, ligaments and cartilage, epithelialization and formation of new connective tissue. It is highly important that growth factors are derived from the patient's own blood, in order to avoid an autoimmune reaction of the organism during their administration.[4] Growth factors involved in PRP are: PDGF - *platelet-derived growth factor*, TGFB - *transforming growth factor beta*, FGF - *fibroblast growth factor*, ILGF 1 - *insulin-like growth factor 1*, ILGF 2 - *insulin-like growth factor 2*, VEFR - *vascular endothelial growth factor*, EGF - *epidermal growth factor, interleukin 8*, CGF - *Keratinocyte growth factor*, and CTGF - *connective tissue growth factor*. Worldwide, there are dozens of commercial systems for the preparation of plasma in which platelet count is 2 to 10 times higher compared with whole blood. Based on the results achieved in terms of platelet count, PRP is categorized.[5,6] Some of the systems used to categorize PRP are:

1. PAW (platelets, activators, white blood cells) provides an absolute white blood cell count and platelet count, as well as information about whether activators are used or not.

2. Mishra and Pavelko, together with colleagues from Stanford University in the United States of America, published the first-in-human study on the use of PRP in 2006 and they categorized PRP according to platelet count, white blood cell count, and presence or absence of PRP activators (Table 1).[7]

Table 1. Mishra et al. PRP classification

Type	Platelet concentration	White blood cell count	Activator
1	A) 5 or more times higher compared with whole blood B) up to 5 times higher compared with whole blood	increased	Without an activator
2	A) 5 or more times higher compared with whole blood B) up to 5 times higher compared with whole blood	increased	With an activator
3	A) 5 or more times higher compared with whole blood B) up to 5 times higher compared with whole blood	Minimal or without white blood cells	Without an activator
4	A) 5 or more times higher compared with whole blood B) up to 5 times higher compared with whole blood	Minimal or without white blood cells	With an activator

Aim of the Study

To establish to which extent is platelet-rich plasma efficient in the treatment of painful shoulder syndrome.

Patients and Methods

In the period between January 1st,2013 and December 31st, 2015 we treated 54 female patients with clinical manifestations of a frozen shoulder. All the patients experienced pain in one shoulder only. Pain lasted for three or more months and standard treatment procedures had been previously applied, including the use of analgesics, physical therapy and the administration of at least two doses of corticosteroids to the painful shoulder. The diagnosis was established on the basis of clinical presentation and X-ray imaging. Before the treatment was initiated, the patients filled in the Quick Dash score (The Disabilities of the Arm, Shoulder and Hand Score), which consisted of 11 questions about daily activities, providing 5 possible answers (no difficulties, slightly impaired function and pain, moderate pain and impaired functions, severe pain and all functions significantly impaired, and constant pain without any movement) (Table 2).[8]

Table 2. Quick DASH Score

Function	No difficulty	Mild	Moderate	Severe	Extreme
Opening a jar	1	2	3	4	5
Doing heavy household chores (washing walls and floors)	1	2	3	4	5
Carrying a shopping bag	1	2	3	4	5
Washing your back	1	2	3	4	5
Using a knife to cut food	1	2	3	4	5
Recreational activities or repeated use of the arm	1	2	3	4	5
Difficulties with prolonged sitting or moving, not using the arm, during the past week	1	2	3	4	5
Limitations in the work during the past week	1	2	3	4	5
Shoulder pain	1	2	3	4	5
Tingling, unpleasant feeling in the shoulder	1	2	3	4	5
Pain during sleep	1	2	3	4	5

The protocol of platelet-rich plasma administration was unique and the administration was carried out three times at 7-day intervals. Blood was prepared and PRP was administered in the morning. We collected 10 to 40 ml of blood from the patients; the blood was treated without an anticoagulant in a centrifuge, at a speed between 600 and 3,000 rpm, for 6 to 10 minutes. Upon the preparation of an area for administration using a preparation of iodine, the administration was done using a hypodermic needle to the subacromial bursa with aspiration and the application of PRP. Local anesthetics were not given. Following the administration, puncture site was covered with sterile gauze which was removed after one hour. The results were monitored with the *Quick Dash*, whose outcome was categorized as follows: no difficulty (up to 11 points), mild difficulty (12 to 22 points), moderate difficulty (23 to 33 points), severe difficulty (34 to 44 points), and extreme difficulty (45 to 55 points) (Table 3).

Table 3. Valorization of the Quick DASH score

	Quick Dash Score	Difficulty level	Function
1	11	No difficulty	Excellent
2	12-22	Mild difficulty	Good
3	23-33	Moderate difficulty	Satisfactory
4	34-44	Severe difficulty	Poor
5	45-55	Extreme difficulty	Unable to move

DASH:Disabilities of Arm, Shoulder and Hand

Results

In total, we treated 54 female patients whose average age was 52 years (37 – 72). Pain in the left shoulder was experienced by 37 patients (68%), while 17 of them (32%) experienced pain in the right shoulder. The mean amount of blood collected from the patients was 21.8 ml (10 – 40). The amount of produced PRP was 3.1 ml (1.7 to 4.9) using 10 ml of whole blood, with a mean value of platelets in whole blood of 198,000 ml (131 to 259), and of 708,000 platelets per ml (314 to 943) in the platelet-rich plasma. White blood cell count in whole blood amounted to 6.2 (3.1 to 8.7) on the average, while in the platelet-rich plasma it amounted to 11.3 (7.3 to 14.9). According to the Mishra et al. classification, our produced PRP belonged to the 1B group. The Quick Dash score prior to the PRP administration was 42 (35-52), while after the PRP treatment, the score was 18 (13-26) after 30 days and 13 (11-23) after 3 months. From a total of 54 female patients, after three months of the PRP treatment, 48 (89%) experienced no difficulties which would require further treatment, in 3 patients (5.5%), the PRP treatment was repeated after three months and their state was good afterwards, while 3 patients (5.5%) were treated by administering corticosteroids three months after PRP and upon physical treatment their state was orderly, without difficulties. A year after the PRP treatment, none of the 51 patients who were monitored experienced a recurrence of the disease.

Discussion

Treatment of a condition known as "frozen shoulder" represents a challenge faced by a team of orthopaedists and physiatrists. Taking into consideration that this condition affects working-age population, a successful recovery is even more important. Since standard methods applied by orthopaedists and physiatrists sometimes do not achieve satisfactory results, the platelet-rich plasma treatment appears to be a possible solution.[9,10] In a study by Aslani et al. entitled "Platelet-Rich Plasma for Frozen Shoulder: A Case Report", published in Arch Bone Jt Surg. in 2016, 70% improvement for function was reported after the treatment using PRP.[11] Our paper demonstrates that the treatment of "frozen shoulder" using the platelet-rich plasma method results in a subjective and objective improvement immediately after the drug administration, and particularly after three months. Obviously, this treatment method should be accompanied with all other standard treatment methods applied by orthopaedists and physiatrists.

Conclusion

In order to confirm the success of the treatment with this method, it is necessary to carry out extensive multicentre trials and to set standards in terms of the number of administrations, the frequency of administration, the number of platelets, with or without white blood cells, etc. Given the fact that this is a relatively painless method, with a small number of possible complications and good initial results, this method is becoming an indispensable part of the treatment for "frozen shoulder".

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Plazma obogaćena trombocitima u liječenju "zamrznutog ramena"

SAŽETAK

Uvod: U radu su prikazani rezultati liječenja oboljenja ramena poznatog kao zamrznuto rame metodom plazme obogaćene trombocitima(PRP)."Zamrznuto rame"(frozen shoulder, periarthritis humeroscapularis, adhezivni kapsulitis) je treće (nakon bolnih leđa i artroza koljena) najčešće oboljenje koje se javlja kod osoba srednje životne dobi (40-60 godina).

Cilj rada: Utvrditi koliki efekat liječenja bolnog sindroma ramena ima plazma obogaćena trombocitima.

Ispitanici i metode: U Bolnici "S.tetik" Banja luka, u periodu od januara 2013. do decembra 2015., liječili smo ukupno 54 osobe ženskog pola sa kliničkim manifestacijama "zamrznutog ramena". Tretman se sastojao od 3 aplikacije PRP u razmaku od po 7 dana. Za rezultate smo koristili Quick Dash upitnik na početku liječenja te 30 dana i 3 mjeseca po završenom liječenju. Kontrolni pregled smo radili nakon godinu dana.

Rezultati: Ukupno smo liječili 54 osobe ženskog pola, prosječne starosti 52 godine (37-72). Kod 37 pacijentkinja bolno je bilo lijevo rame, a kod 17 desno rame. Vrijednost Quick Dash scora prije započinjanja terapije PRP je bila 42 (35-52). 30 dana nakon primjene tretmana PRP, vrijednost je bila 20 (13-26), dok je nakon 3 mjeseca primjene tretmana vrijednost bila 18 (11 – 23).

Zaključak: Primjenom našeg protokola liječenja "zamrznutog ramena" metodom plazme obogaćene trombocitima, subjektivne tegobe oboljelih je moguće značajno smanjiti te uz primjenu svih ostalih terapijskih procedura (analgezija,fizikalna terapija...) dovesti do potpunog izlječenja. Daljim radom na ispitivanju patofiziološkog djelovanja PRP te praćenjem većeg broja oboljelih u više centara mogli bismo postići naučno dokazane standarde za primjenu PRP kao metode izbora u liječenju "zamrznutog ramena".

Ključne riječi: Plazma obogaćena trombocitima, zamrznuto rame, Quick Dash score

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.02
UDC 371.12.011.3:159.947.5.072-057.857
COBISS.RS-ID 6396184

The Relationship between Personality Traits and Job Satisfaction of Teachers

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ABSTRACT

Introduction: Persons that show discontentment with their work have a bigger chance to experience burnout syndrome, depression, anxiety and the lack of self-esteem. Paul Spector defined job satisfaction as „the thing that people feel about their job and different aspects of the same." There is much research in a domain of industrial-organizational psychology that has been questioning relationship between job satisfaction and specific personality traits. Most of those researches confirm the fact that some of personality traits are significant predictors in job satisfaction.

The Aim of the Study: To examine the level of job satisfaction and conduct research if there is a connection between certain aspects of it and personality traits among teachers in primary and secondary schools.

Patients and Methods: The research was designed as a cross-sectional study. The sample included 280 teachers. Personality traits have been tested by Zuckerman-Kuhlman Personality Questionnaire (ZKPQ-50-CC), short version for estimating five dimensions of personality. For evaluation of job satisfaction, Job Satisfaction Survey (JSS) questionnaire, which serves for evaluating nine aspects of job satisfaction, was used. The examination was done according to ethical principles. Statistic treatment included descriptive and correlation analysis.

Results: Results show the middle level of job satisfaction. They are not satisfied with their pay but they are pleasant with „Supervision", „Coworkers", „Nature of Work" and „Communication" while they are ambivalent on aspects of „Promotion", „Fringe Benefits", „Contingent Rewards" and „Operating Procedures". In this group of respondents, personality traits, Neuroticism and Sociability are rarely connected with job satisfaction. Neuroticism seems to be in negative and Sociability in positive correlation. Correlation among other personality traits with some aspects of job satisfaction is negligible.

Conclusion: Results of this research are showing that there is a poor connection between some personality traits and job satisfaction.

Keywords: Job satisfaction, personality traits, neurotic, sociability

(Scr Med 2017:48:11-16)

Submitted: January 18th, 2017
Accepted: February 4th, 2017

Introduction

Thanks to work, a man provides an economic security and his place and position in society by forming his personality and identity and developing skills and talents. A man finds its personality and meaning of life through work. First of all, a job considers performing job assignments in a specific work conditions that seem to be far from ideal; it also considers an interaction with coworkers and supervision, respecting the rules of organisation and seizing specific level of productivity.[1] A person capable of work spends more than half of his life, while he is awake, at the same. If a person spends time at work that doesn't fulfil its criterions, it will produce negative consequences not only for his job, but for its health condition and general satisfaction as well. People who spend their time working what they don't like have a bigger chances to experience a burning syndrom, depression, anxiety and lack of a self-esteem. Nowadays, there are two basic concepts related to job satisfaction. First approach is holistic, which means that it has got only one dimension. It implies person's attitude and feeling related to job. Second approach is multidimensional. According to this approach, job satisfaction appears to be a pleasure in certain aspects of job. This kind of approach is mostly accepted and was founded by Paul Spector. He defines job satisfaction as "something that person feels about job and different aspect of it."[2] There are lots of successful organizations worldwide considering employees as a primary developing resource. Motivation and staff satisfaction are becoming basics of modern organization. Understanding a question of motivation and job satisfaction is also essential for designing working environment, organizational culture and climate, awarding and progressing system and supervision style. There is a lot of research in the area of industrial and organizational psihology that questions a relation between job satisfaction and individual personality traits. Most of those research finding confirm that some of personality traits seem to be a significant predictors of job satisfaction.[3,4] Personality traits represent relatively permanent characteristics that determine a specific person to feel and act appropriate in similar situations. Nowadays, they are mostly defined according to five factorial model of personality.[5]

The Aim of the Study

The goal of this research is to examine a level of job satisfaction, and discover if there is a connection between specific aspects of job satisfaction and personality traits among teachers in primary and secondary schools.

Patients and Methods

This type of research, which was conducted with two reliable instruments, was designed as cross-sectional survey study in order to examine two key variables: job satisfaction and personality traits. The research included 280 teachers from primary and secondary schools in three towns in the Republic of Srpska. The research was conducted according to ethical principles, anonymously and voluntarily.

Personality traits were examined by Zuckerman-Kuhlman Personality Questionnaire (ZKPQ-50-CC), a questionnaire that included 50 items with a binary format of answers.[6] Questionnaire represented a short version of ZKPQ questionnaire and consisted of five ten-item scales intended for evaluation dimensions of alternative five factorial model of personality. Dimensions (characteristics) are next: Activity – Act (includes general activity, relates to general need for activity, but also to uneasiness in situation where possibility to fulfil that need doesn't exist. Second group of items related to tendency for heavy and challenging jobs); Aggression-Hostility – Agg-Host (relates to verbal aggression, rough and careless behavior, envyness, malice and impatience); Neuroticism-Anxiety – N-Anx (susceptibility to psychological exhaustion, inability to control urges, propensity to unrealistic ideas and stress dealing inability); Impulsive Sensation Seeking – Imp SS (includes two subscales: impulsivity and sensation seeking. The first one relates to tendencies of impulsive reacting and plan absence. The second one includes need for excitement, getting involved in unpredictable situations and need for constant changes and innovations); Sociability – Sy: extraversion-introversion (propensity to association and amusement relates to enjoying parties and contacts with lots of people, isolation tolerance relates to a tolerance level of social isolation whereas more sociable persons achieve lower scores).[6]

An instrument for the evaluation of job satisfaction, by which we evaluated nine aspects of job satisfaction was a scale called Job Satisfaction Survey (JSS).[7] There are nine aspects of job satisfaction being measured by this scale: Pay, Promotion, Supervision, Fringe Benefits, Contingent Rewards, Operating Procedures, Co-workers, Nature of Work and Communication. JSS consisted of 36 items (contentions) that was scored by Likert scale from 1 (I disagree) to 6 (I completely agree). Items proclaimed in negative context were scored in the opposite way. Each aspects was represented with four items. This was the way to calculate nine independent individual scores that were capable to move from 4-24 and the 10th score was an examinee's total on items. It represented total satisfaction with the results between 36 and 216. The score from 4-12 for individual aspect signifies dissatisfaction, >12-16 medium satisfaction – ambivalence and the score between >16 and 24 represents satisfaction. For a total

score: a score 36-108 represents dissatisfaction, >108-144 signifies medium level of satisfaction – ambivalence, while >144-216 satisfaction. Statistic treatment includes descriptive and correlational analysis.

Results

There were 180 (64,29%) examinees, that is, 80 teachers in primary and 100 (35,71%) professors in secondary schools. 89 (31,79%) males and 191 (68,21%) females were examined. An average age of examinees was M=39.01 (SD=4.81), an average years of service M=14.36 (SD=10.12), and an average expositional years of service M=11.84 (SD=10.12).

Table 1. Job Satisfaction Survey (JSS)

Job Satisfaction Questionnaire - subscale	Arithmetic Average (M)	Standard Deviation (SD)	Span	Cronbach Coeficient α
Pay	11.97	4.28	20	0.7019
Promotion	12.62	4.54	20	0.7641
Supervision	17.24	4.09	19	0.7844
Fringe Benefits	12.24	4.25	20	0.6975
Contingent Rewards	13.00	4.31	20	0.7316
Operating Procedures	13.52	3.29	17	0.4212
Coworkers	18.64	2.94	17	0.6096
Nature of Work	20.26	2.72	13	0.7334
Communication	17.26	3.26	17	0.5599
Total	**136.74**	**24.60**	**133**	**0.9217**

From Table 1. we can see results of applying the JSS which show a middle level of satisfaction or ambivalence in total score. Some of those results on a subscale show that this group of educators is dissatisfied with their pay, they are ambivalent to the aspect of "Promotion", "Fringe Benefits", "Contigent Rewards" and "Operating Procedures". Those results also show satisfaction in the aspects of "Supervision", "Coworkers", "Nature of Work" and "Communication". They are most satisfied with "Nature of Work". Internal consistency reliability coefficient α of total is 0.92. Reliability of subscales is between 0,42 and 0,78.

Table 2. Personality Questionnaire by Zuckerman – Kuhlman (ZKPQ-50-CC)

Personality Survey - subscale	Arithmetic Average (M)	Standard Deviation (SD)	Span	Cronbach Coeficient α
Activity (Act)	5.50	2.53	10	0.7407
Aggression-Hostility (Agg-Host)	2.55	1.79	9	0.4985
Impulsive Sensation Seeking (ImpSS)	3.37	2.13	10	10
Neuroticism-Anxiety (N-Anx)	2.30	2.09	9	0.7179
Sociability (extraversion and introversion) (Sy)	6.00	2.22	10	0.6452
Total	**19.72**	**5.19**	**33**	**0.6656**

In Table 2., we can see results of Zuckerman – Kuhlman (ZKPQ-50-CC) personality questionnaire: the highest score occurred in subscale Sociability (extraversion and introversion), then Activity, but the lowest result was subscale Neuroticism. Internal consistency reliabilities (coefficient alpha) of total was 0,66, of subscale was between 0,49 and 0,74.

Table 3. Correlation coefficient – Personality Questionnaire – Job Satisfaction Survey (JSS)

ZKPQ-50-CC-subscales	Act	Agg-Host	ImpSS	N-Anx	Sy
JSS			Corelation coefficient – r		
Pay	0.0640	-0.1803	-0.1600	-0.0990	0.1628
Promotion	0.0911	-0.1897	-0.1503	-0.1658	0.2317
Supervision	-0.0387	-0.1766	-0.1676	-0.1857	0.2724
Fringe benefits	0.0436	-0.1192	-0.0707	-0.1068	0.1709
Contigent Rewards	-0.0300	-0.1808	-0.1942	-0.2138	0.2269
Operating Procedures	0.0458	-0.1277	-0.1587	-0.2968	0.1616
Coworkers	-0.0387	-0.1897	-0.1868	-0.2117	0.2102
Nature of Work	0.1140	-0.0755	-0.1118	-0.2126	0.2145
Communication	0.0200	-0.1908	-0.2179	-0.2466	0.2588

Legend: JSS – Job Satisfaction Survey; ZKPQ-50-CC – Personality Questionnaire; Act – Activity; Agg-Host – Aggression-Hostility; ImpSS – Impulsive Sensation Seeking; N-Anx – Neuroticism-Anxiety; Sy – Sociability (extraversion and introversion)

In Table 3., we can see the correlation between some of the subscale personality and some of the aspects of job satisfaction. There was no significant correlation between Activity (Act) and aspects of job satisfaction, nor positive and negative. Negative correlation was present between Activity and aspects of "Supervision", "Contingent Rewards" and "Coworkers", which means if result is higher on dimension personalty Activity, satisfaction "Supervision", "Contingent Rewards" and "Coworkers" is lower.

When we talk about Agg-Host personality dimension with all aspects of job satisfaction, the correlation is in negative direction, but insignificantly, which means there was no connection. The correlation between ImpSS and all aspects of job satisfaction was negative, but also insignificantly.

When it comes to the N-Anx dimension of personality, there was a weak, but also insignificant correlation with all aspects of job satisfaction. There was also a weak negative correlation with aspects of "Operating Procedures" and "Communication".

When we talk about Sociability (Sy), there was a weak to insignificant positive correlation with all aspects of job satisfaction. There was also a weak correlation between two of those personality dimensions and job satisfaction aspects: "Communication" and "Supervision".

Discussion

Research results (JSS) have shown that there is a middle level of job satisfaction among examinees. The results on a specific subscale show that this group of educators is displeased with pay, middle pleased (ambivalent) according to aspects of "Promotion", "Fringe Benefits", "Contingent Rewards" and "Operating Procedures". They seem to be satisfied with aspects of "Supervision", "Coworkers", "Nature of Work" and "Communication". They are most satisfied with "Nature of Work".

Results of correlational analysis show that there is a weak connection between Neuroticism-Anxiety (N-Anx) personality dimension with "Operating Procedures" and "Communication" aspects of job satisfaction. Connection is in a negative direction which means if the level of Neuroticism is higher, a job satisfaction in those aspects is weaker. Results also show a weak correlation between Sociability (Sy) dimension of job satisfaction and two aspects of job satisfaction: "Communication" and "Supervision". Direction of correlation is positive

which means if a scale of Sociability is higher, a higher level of job satisfaction would be achieved in the aspects of "Communication" and "Supervision". Basically, there is no correlation among others personality dimensions and aspects of job satisfaction or it is negligible. There is a negative correlation between personality dimensions Aggression-Hostility (Agg-Host), Impulsive Seeking Sensation (ImpSS), Neuroticism-Anxiety (N-Anx) and a positive direction between Sociability (extroversion and introversion) (Sy) and job satisfaction. When we talk about Activity (Act) dimension, there is a negative correlation according to three aspects of job satisfaction: "Supervision", "Contingent Rewards" and "Coworkers" and for the rest of six aspects the correlation is positive.

Therefore, Neuroticism and Sociability (extroversion and introversion) show a weak correlation with job satisfaction in this research, while other personality characteristics compared to "Big Five" do not show any connections with job satisfaction. Those results correspond with the results in the literature.[4,8-10] There is a possible explanation in the fact that persons with high score on a subscale of Neuroticism are disposed to experience negative emotions in all spheres of life, including a workplace. They get mad very often, get involved in disputes with others, they have violent reactions and probably have lots of conflicts on their workplace and this is the reason for developing job dissatisfaction. Contrary to the above mentioned, according to the results given in this research, emotionally stable persons, successful in the regulation of other people's emotions and their own, will develop higher job satisfaction. Emotional stability (low neuroticism) and extraversion are key aspects of "happy personality" so it could be expected that the factors which affect a person to feel happy will lead to job satisfaction.[11]

Conclusion

In this group of examinees, there is a weak connection between Neuroticism and Sociability personality traits with job satisfaction. Neuroticism is in negative, while Sociability is in a positive correlation. The rest of personality traits do not show important connection with job satisfaction.

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Povezanost osobina ličnosti i zadovoljstva poslom kod nastavnika

SAŽETAK

Uvod: Osobe koje nisu zadovoljne poslom imaju veću šansu da dožive sindrom sagorijevanja, depresiju, anksioznost i smanjeno samopoštovanje. Pol Spektor je zadovoljstvo poslom definisao kao ''ono što osoba osjeća prema svom poslu i različitim aspektima posla.'' Mnoga istraživanja u oblasti industrijsko-organizacijske psihologije ispituju odnos između zadovoljstva poslom i pojedinih osobina ličnosti. Većina istraživanja potvrđuju da su neke osobine ličnosti značajni prediktori zadovoljstva poslom.

Cilj istraživanja: Ispitati nivo zadovoljstva poslom, te istražiti da li postoji povezanost pojedinih aspekata zadovoljstva poslom sa osobinama ličnosti nastavnika u osnovnim i srednjim školama.

Ispitanici i metode: Istraživanje je dizajnirano kao studija presjeka. Uzorak je obuhvatio 280 nastavnika. Osobine ličnosti ispitivane su pomoću Zuckerman – Kuhlman Personality Questionnaire (ZKPQ-50-CC), skraćena verzija, za procjenu pet dimenzija ličnosti. Za evaluaciju zadovoljstva poslom korišćen je upitnik Skala Job Satisfaction Survey (JSS), kojom se procenjuje devet aspekata zadovoljstva poslom. Ispitivanje je obavljeno u skladu sa etičkim principima. Statistička obrada obuhvatila je deskriptivnu i korelacionu analizu.

Rezultati: Rezultati su pokazali da nastavnici u ukupnom skoru doživljavaju srednji (ambivalentan) nivo zadovoljstva poslom. Nezadovoljni su platom, zadovoljni su aspektima: „Nadređeni“, „Kolege“, „Obilježja posla“ i „Komunikacijske vještine“, a ambivalentni su u odnosu na aspekt „Napredovanje, „Pogodnosti“, „Nagrađivanje“ i „Uslovi rada“. Osobine ličnosti Neurotičnost i Socijabilnost su na nivou slabe povezanosti sa zadovoljstvom posla kod ove grupe ispitanika, Neurotičnost je u negativnoj korelaciji, a Socijabilnost u pozitivnoj. Korelacija ostalih osobina ličnosti sa pojedinim aspektima zadovoljstva poslom je zanemariva.

Zaključak: Rezultati ovog istraživanja pokazuju da postoji povezanost, istina slaba, između nekih osobina ličnosti i zadovoljstva poslom.

Ključne riječi: Zadovoljstvo poslom, osobine ličnosti , neurotičnost, socijabilnost.

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.03
UDC 616.34-007.43-089.5:616-007.43
COBISS.RS-ID 6396696

Complications During Surgical Treatment of Incarcerated Inguinal Hernia

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ABSTRACT

Introduction: An incarceration of inguinal hernia is a life-threatening condition and represents the most frequent complication, particularly in the elderly patients. It may compromise vascularisation of the contents of the hernia. A surgical treatment of the incarcerated inguinal hernia represents one of the most frequent surgical interventions in elderly patients and it grows proportionally with the age.

Aim of the Study: The aim of the study is to investigate some of the factors that may have an impact on the incarcerated inguinal hernias surgical treatment outcome in elderly patients.

Patients and Methods: The study included 149 patients classified in two groups: the study group (> 60 years of age), which included 96 patients, and the control group (≤ 60 years of age) , which included 53 patients, treated in the period from January 1st, 2012 to December 31st, 2016 at the Clinic of General and Abdominal Surgery UCC RS Banja Luka.

Results: Most of the patients had right inguinal hernia (51.16% in the study group, 60.37% in the control group). 82 patients (85.41%) of the study group suffered from some of the accompanying chronic diseases, opposite to 20 patients (37.73%) of the control group. Polypropylene mesh was implanted in 105 (70.47%) patients, while the tension technique was performed in 44 (29.53%) patients. The duration of incarceration longer than 24 h (p=0.015), previous abdominal surgery (p=0.001), the American Society of Anaesthesiologists physical status classification system (ASA classification) (p=0.033) and the presence of chronic diseases (p=0.01) appeared to be statistically significant risk factors for performing intestinal resection in the study group, while in the control group, they represented risk factors, but not at the level of statistical significance (p > 0.05), except for the duration of incarceration (p=0.007). A higher ASA stage (p=0.001), is the most important risk factor for lethal outcome in both groups of patients.

Conclusion: Incarcerated inguinal hernia is a very serious and demanding surgical problem, particularly in elderly patients. A higher ASA score and the presence of bowel resection are the most important risk factors related to very difficult complications.

Key words: Inguinal hernia, complications, risk factors, comorbidity

(Scr Med 2017:48:17-23)

Submitted: January 30th, 2017
Accepted: February 6th, 2017

Introduction

Due to abdominal wall weakness and conditions that increase intra-abdominal pressure, external hernia is more frequently seen in elderly patients.[1-4] The estimated incidence of the inguinal hernia in patients more than 60 years old is 13 per 1000.[5] Incarcerated external hernia repairs are one of the most common emergency procedures performed in elderly patients.[6] Males predominate among the patients up to 75 years of age, while females prevail in the older age.[7,8] The most recent data indicate that incarcerated inguinal hernia account for about 20% of all small bowel obstructions. Due to the fact that up to 30% of bowel incarcerations require intestinal resection, it is also associated with significant morbidity and mortality.[5] Up to 75 years of age, 10-15 % of men underwent surgical treatment of inguinal hernia.

Aim of the Study

The aim of the study is to examine some of the most important factors that may affect the outcome of incarcerated inguinal hernias surgical treatment in elderly patients.

Patients and Methods

SThe study included 149 patients that underwent surgery in the period from January 1st, 2012 to December 31st, 2016 at the at the Clinic of General and Abdominal Surgery UCC RS Banja Luka. The patients were divided into two groups: a study group (> 60 years of age) and a control group (≤ 60 years of age). During the research, the following parameters were followed: age, gender, type of incarceration (direct/indirect), the ratio of right to left incarcerated inguinal hernia, related chronic diseases, the duration of incarceration (0-24 h, > 24 h), The American Society of Anaesthesiologists (ASA) classification[6,16,20], type of surgical procedure (tension technique or tension-free technique).

In statistical analysis for comparing values of study and control group, parametrical tests (Student's t-test and ANOVA) were used. Analysis of variables not sorted by the type of normality was made by comparing the non-parametrical tests (Mann-Whitney, U-test, Spearmen Correlation, $\chi 2$ test, Fisher exact probability tests the null hypothesis). Analysis of the influence of some factors was made by inivariant logistic regression and the analysis of survival was made through Cox Regression models, where the invariant "Enter" method was used to determine hazard rate (HR). By means of this method (Enter), the level of risk factors was defined. The statistical analysis was implemented by software package SPSS (version 19) with a statistical probability of $p < 0.05$.

Results

The study included a total of 149 patients of whom 96 were in the study group and 53 in the control group. The patients in the study group (the average age of 68.23 ± 7.11 years), were significantly older than those in the control roup whose average age was 51.47 ± 13.87 years.

As expected, there was a statistically significant difference in the age of the study group and control group ($p = 0{,}008$). Out of 96 patients in the study group, 19 had direct and 75 indirect hernia. Out of 56 patients in the control group, 8 had direct and 45 indirect hernia. In both analyzed groups, no statistically significant differences was noticed in the frequency of occurence of the hernia ($\chi 2$: $p > 0{,}5$). The highest number of patients had a right inguinal hernia (84 or 56.38 % of the total number of patients, from which 52 or 61.9 % in the study and 32 or 38.1% in the control group). 82 patients in the study group (80.39 %) had some chronic diseases, which was significantly more than in the control group – 20 patients (37.73 %). (Table 1.)

Table 1. Characteristics of the Patients with Incarcerated Inuinal Hernia

Parameters	Study group n (%)	Control group n (%)	Total n (%)	p
Age (years), x ± SD	68.23 ± 7,11	51.47 ± 13.87		< 0.001
Men, n (%)	86 (96)	47 (53)	133 (100)	0.001
Women, n (%)	10 (96)	6 (53)	16 (100)	0.014
Direct hernia, n (%)	19 (70.37)	8 (29.63)	27 (100)	
Indirect hernia, n (%)	75 (62.5)	45 (37.5)	122 (100)	
Right hernia, n (%)	52 (61.9)	32 (38.1)	84 (100) (56.38)	
Left hernia, n (%)	30 (61.22)	19 (38.78)	49 (100) (32.89)	

Parameters	Study group n (%	Control group n (%)	Total n (%)	p
Billateral hernia, n (%)	14 (87.5)	2 (12.5)	16 (100) (10.74)	
Chronic diseases, n (%)	82 (80.39)		102 (100)	< 0.001

In most cases, the type of surgery in the case of incarcerated inguinal hernia was determined based on the individual assessment of surgeons. Out of 96 patients in the study group, 35 patients were subjected to tension surgical technique (without mesh – the intestinal resection was made), and of 53 patients in the control group, 9 were subjected to tension technique (without mesh – the intestinal resection was made). Synthetic material (polypropylene mesh) was embedded in 61 patients of the study group and 44 patients of the control group. It can be asserted that much higher percentage of the synthetic material was implemented in the control group in the comparison to the study group with the error level of $p < 0.001$ (Table 2.).

Table 2. Type of Surgical Treatment in the Patients of the study and control group

Type of surgical treaatment	Study group n (%)	Control group n (%)	Total n (%)	p
Tension technique	35 (36.45)	9 (16.98)	44 (29.53)	
Synthetic material	61 (63.55)	44 (83.02)	105 (70.47)	0.003
Total	96 (100)	53 (100)	149 (100)	

Table 3. Crude Odds Ratio (OR) of the Analyzed Risk Factors for Performing Intestinal Resection

Parameters	Study group OR (95% CI)	p	Control group OR (95% CI)	p
Type of incarceration				
Direct	1			
Indirect	6.923 (2.37 – 56.42)	0.079	30.859 (9.32 – 53.21)	0.852
Duration of incarceration				
0-24	1			
> 24	14.39 (2.56 – 96.38)	0.037	27.536 (14.82 – 43.59)	0.089
Previous abdominal surgery				
NO	1			
YES	3.341 (0.121 – 6.758)	0.005	2.319 (0.352 – 3.821)	0.796
ASA classification				
1-2	1			
3-4	8.764 (0.98 – 64.91)	0.041	25.389 (10.82 – 54.35)	0.93
Chronic diseases				
NO	1			
YES	3.112 (1.859 – 6.457)	0.007	7.428 (2.574 – 8.396)	0.387

CI – confidence interval

With the usage of inivariant binary logistic regression, in the study group, there were marked statistically significant risk factors for performing intestinal resection: incarceration over 24 h (OR = 14.39 95% CI = 2.56 – 96.38, p = 0.037), previous abdominal surgery (OR = 3.341 95% CI = 0.121 – 6.758, p = 0.005), ASA classification (OR = 8.764 95% CI = 0.98 – 64.91, p = 0.041) and the presence of chronic diseases (OR = 3.112 95% CI = 1.859 – 6.457, p = 0.007). Previously analysed factors in the control group represented the risk factors, but not at the level of statistical significance (p > 0.05) (Table 3.).

Table 4. shows the summary statistics of Cox regression model and log rank test of patients survival length. The patients' age in the study group did not represent a statistically significant risk factor for lethal outcome (p = 0,657). The length of survival in both groups did not seem to differ in age (p = 0,584). In the study group, gender did not represent a statistically significant risk factor for lethal outcome (p = 0,542). Also, there was no difference between the groups in terms of the length of survival by gender (p = 0,384). By increasing ASA stage for one, a chance for lethal outcome was increased 8,541 times at the level of significance (p = 0,016). The presence of intestinal resection was a statistically significant risk factor for lethal outcome, increasing the chance 7,358 times (p = 0,002). The duration of incarceration over 24h was a statistically significant risk factor, increasing the chance by 26 times (p = 0,04) and the patients with resection had significantly shorter survival time than the patients without resection (p = 0,043).

Tabela 4. **Cox regression model for survival analysis**

Factors	Hazard rate	95% CI	p	p (Log Rank)
Age (years)				
< 60	1			
> 60	4.935	0.928 – 23.459	0.657	0.584
Gender				
Male	1	0.693 – 3.514	0.542	0.384
Female	0.471			
*ASA				
Continuous	8.541	2.119– 45.874	0.016	0.001
Resections				
YES	1			
NO	7.358	3.628 – 19.987	0.002	0.001
Duration of incarceration				
< 24h	1			
> 24h	26.546	0.357 – 51.437	0.04	0.043

Discussion

Strangulation hernia is a condition in which the hernia cannot be returned to the abdomen. By putting emphasis on the increased risk of intestinal obstruction, strangulation incarceration is of a great importance.[8] Incarcerated external hernias are the second most important cause of intestinal obstruction.[9] In elderly people, about 40 % of inguinal hernias are surgically treated due to incarceration or intestinal occlusion. Although some earlier studies have presented data that only 5 % of all inguinal hernias require urgent surgical care[10], others have suggested that this percentage is slightly higher and amounts up to 13 %.[11] Since the anterior abdominal wall hernia incarceration, followed by incarceration of intestinal curves, is associated with high percentage of morbidity and mortality[10-12], urgent surgical intervention is necessary. There is a generally accepted view that hernia should be electively managed in order to avoid later complications.[13] However, many patients are undiagnosed, or consciously reject the proposed surgery, that resulting in occurrence of many emergency surgeries,

because of "neglected" cases of hernia. The incarceration percentage increases also by the waiting lists for elective surgery[1], as well as the fact that non-surgical medical staff did not provide enough information to the patients regarding the danger of incarceration. There were no significant differences in the occurrence frequency of inguinal hernia between the groups, as reported also by other published studies.[1,11] The published studies have shown that indirect herniasdominate over the direct ones in the proportion ranging from 7 : 3 : 10 : 1.[1] In our study, out of 96 patients in the study group, 19 patients had direct and 77 had indirect incarcerated inguinal hernia. Of 53 patients in the control group, 8 patients had direct and 45 patients indirect incarcerated inguinal hernia.

Our study has also shown that there was not more frequent occurrence of indirect hernia than direct incarcerated inguinal hernia concerning sex. Another important factor, contributing to the unwanted outcome in the patients with incarcerated inguinal hernia, is related to comorbidity of chronic diseases.[17,18] The chronic diseases are even more important factor when talking about mortality.[19]

Most of the patients in the study group had some chronic diseases, that is, 82 (85.41 %), which was statistically more significant that 20 (37.73 %) patients in the control group. Symptoms duration in the study group was accompanied by incarceration duration and lasted from one to three days. Duration increases with the age, which could be observed in other studies, too.[1] Late hospitalization is generally considered as an important factor for determining the level of intestinal resection and subsequent morbidity and mortality.[10,20-22] Incarceration and strangulation with or without intestinal obstruction are major complications.[23] Roughly speaking, about 15 % of all patients with incarcerated intestinal curve require resection due to intestinal necrosis caused by strangulation.[20,24] Manual reposition may be the method of choice without resection in incarcerated inguinal hernia, although there are no strict criteria to clearly differentiate strangulation, except for the obvious peritonitis.[24]

Higher number of patients studied in both groups who were without intestinal resection and had incarceration that lasted less than 24h (p = 0,002) is statistically significant.

Our study showed that, according to Cox's regression model and log-rank test on the patients with and without intestinal resection, the presence of intestinal resection was a statistically significant risk factor for lethal outcome, increasing the chance 7,4 times and the patients with resection had a significantly shorter survival time than those without resection. Open tension-free technique was the most common surgical technique type as in all previous studies,[25-27] and in both tested groups of our study.

This technique contributed to managing a total of 105 (70.47 %) patients. Considering a general attitude that synthetic material should not be implanted in patients younger than 30 years of age, because of the netting deformation during a young organism development, as well as because of the surgeons' fear to implant synthetic material in intestinal resection cases due to possible complications, we can argue with the level of error (p = 0,003), that much higher number of patients in the control group - 44 (83.02 %) had a built-in synthetic material, while the number of patients in the study group was 61 (63.55 %). In previous studies on patients with incarcerated inguinal hernias, it has been observed that a high ASA score is an independent predicting factor for small bowel gangrene.[28] Alvarez et al.[19] not only confirmed the higher rate of complications, but also showed a higher rate of mortality in patients with higher ASA grade. In our study, ASA grade was a risk factor for performing intestinal resection, but not at the level of statistical significance.

Conclusion

Our study showed that the incarcerated inguinal hernia is a very serious problem. If the inguinal hernia is not operated and managed at the right time, there is a risk that easy surgical problems may lead to various serious complications with a lethal outcome. The risk is higher in elderly patients because of the presence of associated chronic diseases. Statistically significant risk factors for performing intestinal resection in the study group patients were duration of incarceration longer than 24 h, previous abdominal surgery, higher ASA classification, whereas in the control group, the only risk factor was duration of incarceration more than 24h.

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Komplikacije hirurškog liječenja uklještenih preponskih kila

SAŽETAK

Uvod: Uklještenje je stanje kile opasno po život i predstavlja najčešću komplikaciju, posebno kod starijih osoba. Karakteriše je kompromitacija vaskularizacije sadržaja kilne kese. Hirurško liječenje uklještenih preponskih kila predstavlja jednu od najčešćih hirurških intervencija kod starijih osoba i procentualno raste sa godinama starosti.

Cilj rada: Cilj ove studije je ispitivanje faktora koji mogu uticati na ishod hirurškog liječenja uklještenih preponskih kila u odnosu na starosnu dob bolesnika.

Ispitanici i metode: Studija je obuhvatila 149 bolesnika, razvrstanih u 2 grupe: ispitivana (više od 60 godina), u kojoj je bilo 96 bolesnika i kontrolna grupa (manje od 60 godina), u kojoj je bilo 53 bolesnika, liječenih u periodu od 01.01.2012. do 31.12.2016. u Klinici za opštu i abdominalnu hirurgiju UKC RS Banja Luka.

Rezultati: Češće je bila zastupljena desna preponska kila (54,16% u ispitivanoj, 60,37% u kontrolnoj grupi). 82 bolesnika (85,41%) u ispitivanoj grupi su imala neko od pratećih hroničnih oboljenja, nasuprot 20 bolesnika (37,73%) u kontrolnoj grupi. Polipropilenska mrežica bila je ugrađena kod 105 (70,47%) bolesnika, a tenzionom tehnikom je zbrinuto 44 (29,53%) bolesnika. Dužina uklještenja preko 24h (p=0,015), prethodne abdominalne operacije (p=0,001), klasifikacioni sistem fizičkog stanja (ASA klasifikacija) (p=0,033) i prisustvo hroničnih oboljenja p=0,01) izdvojili su se kao statistički značajni faktori rizika od izvođenja resekcije crijeva u ispitivanoj grupi, dok su u kontrolnoj grupi predstavljali faktore rizika bez statističke značajnosti (p veće od 0.05), izuzev dužine uklještenja (p=0.007). Viši ASA stadijum (p=0.001) bio je najznačajniji faktor rizika od letalnog ishoda bolesnika u obe ispitivane grupe.

Conclusion: Uklještena preponska kila veoma je ozbiljan i zahtjevan hirurški problem, posebno kod starijih osoba. Viši ASA skor i resekcija crijeva predstavljali su najvažnije faktore rizika za teške komplikacije.

Ključne riječi: Preponske kile, komplikacije, faktori rizika, komorbiditet

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.04
UDC 664.641:633.12(497.6RS)
COBISS.RS-ID 6396952

Salt Content in White Bread in the Republic of Srpska

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ABSTRACT

Introduction: Numerous scientific studies have confirmed that the increased salt intake leads to increased blood pressure and increased risk of cardiovascular diseases. It has been found that the largest salt intake is achieved by eating cereals and their products, including different kinds of bread. The research of nutrition habits of the population of the Republic of Srpska has proven that white bread is most commonly consumed of all cereals.

Aim of the Study: The aim of this study is to determine the salt content in white bread and salt intake assessed by eating white bread, and to indicate the public health importance of regular control of salt in cereals and cereal products.

Patients and Methods: Determining the content of salt in white bread (n = 96) was conducted as cross-sectional study, using Mohr´s method and specific geographical distribution patterns. Descriptive statistic indicators were used in the survey (the number of samples, minimum and maximum values, standard deviation). T test and Sheffe's post-hoc test were used for testing the significance of differences in salt content in white bread.

Results: The results indicated that there was a statistically significant difference in the content of salt in white bread at the regional level as well as at the level of the region and the Republic of Srpska. The estimated intake of salt by eating white bread clearly pointed to the cardiovascular risk of the population of the Republic of Srpska and confirmed the importance of the public health necessity of the regulations that would ensure continuous monitoring of the salt content in white bread and other cereal products.

Conclusion: Salt intake assessed by eating white bread in population of the Republic of Srpska is bigger than the one in the population of the Republic of Serbia and Portugal. Estimated salt intake by eating white bread can be assessed as high and risky for cardiovascular health. It is necessary to continuously implement and educate the population in the area of proper nutrition.

Keywords: Salt, white bread, cardiovascular diseases

(Scr Med 2017:48:24-29)

Submitted: January 31st, 2017
Accepted: February 8th, 2017

Introduction

In his original environment, a man had insufficient nutritional intake of salt, and relied on the natural sodium content in food consisting of plants and animals.[1] The development of food industry and placing on the market large amounts of industrially produced food, has led to

an increased salt intake. After ingestion, salt dissociates in the digestive tract into sodium and chlorine ion. Absorption of sodium, ingested through food and water, takes place at the level of the small intestine and is about 98%, and similar amount of sodium is excreted from the body via kidneys.[1] Sodium helps maintain a membrane potential, in the absorption of nutrients from the small intestine, in the implementation of nerve impulses, in the maintenance of extracellular fluid volume and thus has a leading role in maintaining blood pressure.[2] The World Health Organization (WHO) recommends limiting salt intake to 5.0 g a day,[3] while the American Heart Association (American Heart Association, AHA) points out that the patients with hypertension need to reduce salt intake to 3.8 g a day.[4]

The main food source of sodium is salt, which is used in the production of food for human consumption. Sodium naturally found in food, participates in the total daily intake by 5 to 10%, the amount added during cooking or at the table by 10 to 15%, while sodium/salt from processed foods industry, accounts for about 75 to 80%.[5] The main sources of salt in the diet are bread and rolls, followed by meat and meat products, cheese, canned vegetables and dehydrated soups.[6,7]

Hypertension, cardiovascular (CVD) and cerebrovascular diseases are a global public health problem and the most frequent causes of death. In the Republic of Srpska, cardiovascular diseases in 2014 participated with 47% in causes of total mortality, while for the same year, the mortality due to neoplasms was 21.9%.[8] Although mass noncommunicable diseases (MND) affect mainly adult population, there is a great increase of the risk of their occurrence in children.[9] WHO points out that reducing salt intake to the recommended value of 5 g per day leads to the reduction of the risk of cardiovascular diseases by 17% and stroke by 23%.[5,10] Hypertension is the leading cause of MND. The prevalence of hypertension in many European countries is more than 40%, especially in those countries where the large population salt intake was found.[11-15] In the Republic of Srpska, salt used in diet is an important source of iodine.

Bread is the basic food from the grain group and the production of bread includes a specific type of flour, yeast, salt and water as basic raw materials.

Based on the analysed data in the Report on the results of studies of health of the Republic of Srpska population from 2010, the adult population of the Republic of Srpska uses white and semi-white bread in diet more often.[13]

Aim of the Study

Determining the salt content in white bread, determining the amount of salt according to Mohr´s method and evaluating salt intake by measuring white bread consumption of the adult population aged from 18 to 65. Raising awareness about the public health importance of regular controls of salt content in cereals and cereal products.

Patients and Methods

The survey was conducted as a cross-sectional study, in the period from January 20th 2016, to March 9th 2016. The survey included manufacturers of white bread on the territory of the Republic of Srpska that are subject to regular safety surveillance, in accordance to the Food Law of the Republic of Srpska (Food Law, Official Gazette of the Republic of Srpska No. 49/09). The study involved Public Health Institute Banja Luka and five Regional centers in Doboj, Zvornik, East Sarajevo, Trebinje and Foca.

The collection of samples was carried out in accordance with the sampling plan of the Department of Hygiene, the Institute for Public Health and the Section of Hygiene from the regional centre. The subject of sampling was white bread, made of "type 500" flour.

Testing of salt content was performed according to Mohr´s method and the principle of precipitation titration, in the Department of Sanitary Chemistry Institute for Public Health and in the laboratories for hygienic and microbiological analyses in regional centers.

After laboratory analyses, reports on the results of tests of salt content in white bread were delivered to the reception area of the Service or to the Department of Hygiene, where they were taken over after the research was completed.

Data processing and statistical analysis were conducted in the program SPSS 20. Statistical analysis determined the following data: number of samples (n), the minimum value (Min), the maximum value (Max) and average value (X), standard deviation (SD) and the coefficient of variation (CV). T-test was used for comparison of the groups. Scheffe´s post-hoc test was used to test the significance of multiple comparisons.

Results

The study included 88 subjects from the Republic of Srpska with 96 samples of white bread (90 unpackaged and 6 bulk packaged products). Interpretation of the results was conducted in accordance with the geographical

distribution of the samples from three regions. The first region of the distribution of the sample consisted of the cities of Banja Luka and Prijedor and municipalities of Gradiška, Kotor Varoš, Kostajnica, Mrkonjić Grad, Novi Grad, Prnjavor and Srbac, with the total participation of 50 samples in the research. The second region included the cities of Bijeljina, Doboj and Zvornik, municipalities of Bratunac, Brod, Derventa, Modrica, Ugljevik, Modriča, Teslić and Vlasenica, with the total participation of 33 samples in the research. The third region encompassed the cities of East Sarajevo and Trebinje, municipalities of Han Pijesak, Foca, Ljubinje, Nevesinje and Pale, with the total participation of 13 samples in the research. Results of salt content in white bread obtained by applying Mohr´s method are shown in Table 1.

Table 1. Results of the Salt Content of White Bread Obtained by The Mohr´S Method (g of Salt in 100g of a Product)

Observation unit	Number (n)	Min	Max	X	SD
Region 1	50	1.18	2.12	1.51	0.227
Region 2	33	1.10	2.40	1.77	0.310
Region 3	13	1.25	2.06	1.50	0.223
Republic of Srpska	96	1.10	2.40	1.60	0.286

Legend: Min-minimal value, Max-maximal value, X-average value, SD-standard deviation

A The analysis of the salt content in white bread was carried out on 96 samples. Based on these results, it was found that the content of salt in white bread ranged from 1.10 g to 2.40 g (X = 1.60, SD = 0.286) for the Republic of Srpska. The minimum and maximum values of the salt content in white bread were recorded in Region 2. Given that for each of the regions, values of salt content in white bread were separately grouped, it was found that they were slightly higher in the bread from the Region 2 (X = 1.77, SD = 0.310) compared to the bread from the Region 1 (X = 1.51, SD = 0.226) and bread from the Region 3 (X = 1.50, SD = 0.223). Upon testing the statistical significance of these differences, it was found that the obtained value of F = 11.680 df = 2 exceeded the threshold for significance at the 0.01 level, and the conclusion was that at least one of three possible differences was statistically significant.

Scheffe's post-hoc test showed that the analysis of the obtained salt content in white bread from the Region 2 was statistically significantly different from the values obtained in Region 1 ($p = 0.000$, $p < 0.01$) and Region 3 ($p = 0.006$, $p < 0.01$).

Upon the analysis of statistical significance and the differences in the average salt content in bread for the Republic of Srpska and some regions, the existence of statistical significance of differences in salt content for Region 1 and Region 2 was found (Table 2 - 4).

The average salt content in white bread on the territory of the Republic of Srpska was higher (X = 1.60, SD = 0.286) than the salt content in white bread from Region 1 (X = 1.51, SD = 0.227). Upon statistical significance testing, it was observed that these differences were statistically significant ($t = -2.780$, $p = 0.008$, $p < 0.01$, $df = 49$); Table 2.

Table 2. Results of T-test for Region 1

	The Republic of Srpska	Region 1
X	1.60	1.51
SD	0.286	0.227
Min	1.10	1.18
Max	2.40	2.12
N	96	50
t test	t= -2.780, p=0.008, df=49	

Contrary to Region 1, the values of salt content in white bread determined from Region 2 were significantly higher (X = 1.77, SD = 0.310), compared to the average value determined for the content of salt in white bread for the Republic of Srpska (X = 1.60, SD = 0.286). After testing the statistical significance of the differences, it was observed that these differences were statistically significant ($t = 3.270$, $p = 0.003$, $p < 0.01$, $df = 32$); Table 3.

Table 3. Results of T-test for Region 2

	The Republic of Srpska	Region 2
X	1.60	1.77
SD	0.286	0.310
Min	1.10	1.10
Max	2.40	2.40
N	96	33
t test	t= 3.270, p=0.003, df=32	

Although the average value of the salt content in white bread, on the territory of the Republic of Srpska (X = 1.60, SD = 0.286) was slightly higher compared to the established value of the salt content in white bread from Region 3 (X = 1.50, SD = 0.223), it was observed that the difference was not statistically significant (t = -1.616, p = 0.132, p> 0.05, df = 12); Table 4.

Table 4. Results of t-test for Region 3

	The Republic of Srpska	Region 3
X	1.60	1.50
SD	0.286	0.223
Min	1.10	1.25
Max	2.40	2.06
N	96	13
t test	t= -1.616, p=0.132, df=12	

Based on the national survey in the Republic of Srpska, it was determined that, of all cereals,the adult population (≥18 years old to 65 years old) most commonly consumed white bread (89.5%), and they ate 4,8 slices of bread daily. The quantity of white bread eaten by an adult resident of the Republic of Srpska amounted to 2.30 g, and respectively for such a resident of Region 1, this amount was 2.16 g, for Region 2 it was 2.54 g and for Region 3 it was 2.16 g. The standard ration for a slice of bread was used, and according to the recommendations of the US Department of agriculture (United States Department of agriculture, USDA Food Guide) in 2010 it amounted to 30 g.

The average salt intake by white bread consumption was compared to the recommendations for salt intake given by world authorities. In relation to the recommendations of the WHO, the average intake of salt by white bread consumption in this study was 46.0% (at the regional level it ranged from 43.2 to 50.8%). Using AHA recommendations, it was found that the average salt intake in the study was 60.5% (at the regional level it ranged from 56.8 to 66.8%) of the recommended values.

Discussion

In the Republic of Srpska there is no legal act which regulates the amount of salt in bread and bakery products, hence it is rather left to the will of manufacturers.

According to this research, the residents of the Republic of Srpska, when consuming 100 g of white bread, averagely intake 1,60 g of salt (SD = 0,286) and 0.48 g of salt when consuming standard serving of white bread measuring 30 g. Looking at the results of the research by region, it can be noticed that the residents of Region 2 take the highest intake of salt by consuming white bread (X = 1.77, SD = 0.312). By analyzing the statistical significance of differences of salt content in white bread from the regions and in the total sample for the Republic of Srpska, using t-test, a statistically significant difference in the comparison of data for the Republic of Srpska and Region 1 (p = 0.008, p <0.01) (Table 2) and Region 2, (p = 0.003, p <0.01) (Table 3), was found, while the difference determined for Region 3 was not statistically significant (p = 0.132, p> 0.05) (Table 4).

If we compare this data to the data of the researchers who have used the same method of analysis in the Republic of Serbia, it can be concluded that the salt content identified in this study was higher by 0.39 g. However, it should be noted that in this study, white bread was not separated as a separate product group. The sampling lasted 8 years. Also, the analysis method was changed in the course of research in the potentiometric titration. By comparing the results of both methods, the researchers found that the difference in terms of results was negligible, and both methods could be considered precise.[14]

If we compare this data to the data of the researchers from Portugal, who determined sodium by flame photometry on 408 samples of white bread in 2006, it can be seen that the average value is 534 mg per 100 g of product with a range of concentrations from 344 to 718 mg. During the conversion of the quantity of sodium into the salt, the formula Na (g) x 2.5 = NaCl (g) (0.534 x 2.5 = 1.33 g) was used, and it was concluded that the average value of the salt in the white bread from Portugal researchers was lower for 0.27 g than our results. However, it should be noted that their method of analysis differs from the method of this research.[15]

Through epidemiological data on the intake of bread, it was found that, only by eating bread, the population of the Republic of Srpska intakes nearly half of daily intake of salt recommended by WHO[3], and three-fifths of the value compared to the recommendations given by AHA.[4] It should be noted that this was only white bread consumption and the intake of salt or sodium from all other sources was not considered. Since epidemiological studies have shown that every tenth inhabitant adds salt in food at the table and almost every second inhabitant twice a week eats dry meat products that contain two sources of sodium (from salt and preservatives sodium benzoate), it can be said that the population of the Republic of Srpska is at risk of developing CVD.

Even though the natural sodium content in food is

sufficient to maintain physiological needs of organism,[1,2] research on a population salt taking[5,10] confirm that the intake is far above the recommended daily values.[3,6]

Numerous studies have shown that high salt intake has a role in the development of high blood pressure[3,11] and thus less salt intake leads to the reduction of blood pressure.[16] The conclusion is that a balanced diet with the special emphasis on the amount of salt, and physical activity are key to reducing morbidity from hypertension.[12]

Key activity for the reduction of salt intake proposed by the WHO is the development of legislation that would allow the placing on the market of food with lower salt content.[3,5] Knowing that white bread is the food that the population of the Republic of Srpska daily consume,[13] reducing salt intake in this type of cereal products would result in total reduction of salt intake on a population level[5,11] and, consequently, would decrease morbidity from hypertension,[16] cardiovascular and cerebrovascular deseases.[8] For these reasons, the amount of salt not only in white bread, but also in other food products is public health problem that is preventable. The health system of the Republic of Srpska is burdened by treatment of MND, which is preventable, and therefore the reduction of population salt intake has a great significance in terms of cardiovascular health.

Reducing salt intake to the recommended value suggested by SZO[3] or AHA,[4] leads to reduced amount of iodine inserted into the body (100-150 μg of iodine in the event of WHO recommendations and 76-114 μg iodine in the case of recommendations AHA). Research in 2010 showed that residents of the Republic of Srpska eat fish rich in iodine only once a week or more rarely.[13]

In order to avoid the return of endemic goiter, it is necessary, along with education of the population on the amount of salt that is recommended by world authorities, to simultaneously educate people about the selection of food rich in iodine.

A balanced diet together with physical activity is the life style in which the risk of these factors is reduced to a minimum.

Conclusion

Values of salt content in white bread were in the range of 1.10 g to 2.40 g in 100 g of the product, and the highest value was obtained in Region 2 (1.77 g). Statistically significant differences in the content of salt in white bread between the Regions and the Region and the Republic of Srpska were found.

Residents of the Republic of Srpska consume 0.39 g per 100 g salt in white bread more than the population of the Republic of Serbia, and 0.27 g per 100 g salt more than the population of Portugal. On the basis of epidemiological data on the intake of white bread, salt intake estimated through this type of cereal in this study ranged up to three-quarters of the recommended daily salt intake proposed by world authorities. Estimated intake of salt by consuming white bread can be marked as high risk factor for the cardiovascular health problems of the residents of the Republic of Srpska aged from 18 to 65. The results clearly indicate that, in order to prevent the return of endemic goiter to our area, it is necessary to continuously educate the population regarding proper nutrition, which includes the reduction of salt intake, and the increase of the intake of food that is rich in iodine.

Reducing the amount of salt in bread and educating people about proper nutrition with an emphasis on reducing salt intake could lead to reduction in salt intake in population, which would be reflected in the reduction of the prevalence of hypertension and cardiovascular and cerebrovascular diseases.

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Sadržaj soli u bijelom hljebu u Republici Srpskoj

SADRŽAJ

Uvod: Brojne naučne studije su potvrdile da povećan unos soli hranom dovodi do povećanja krvnog pritiska i rizika od kardiovaskularnih bolesti. Utvrđeno je da se najveći unos soli postiže konzumiranjem žitarica i njihovih proizvoda, uključujući različite vrste hljeba. Istraživanjem životnih navika u ishrani stanovništva Republike Srpske dokazano je da se od namirnica iz grupe žitarica najčešće konzumira bijeli hljeb.

Cilj rada: Ciljevi rada su utvrditi sadržaj soli u bijelom hljebu, procijeniti unos soli putem bijelog hljeba i ukazati na javnozdravstveni značaj redovne kontrole soli u namirnicama iz grupe žitarica i proizvoda od žitarica.

Ispitanici i metode: Utvrđivanje sadržaja soli u bijelom hljebu (n=96) sprovedeno je kao studija presjeka, metodom po Moru (Mohr) i posebnom geografskom distribucijom uzoraka. Korišteni su pokazatelji deskriptivne statistike u istraživanju (broj uzoraka, minimalne i maksimalne vrijednosti, standardna devijacija). Za testiranje značajnosti razlike sadržaja soli u bijelom hljebu korišten je t test i Sheffeov post hoc test.

Rezultati: Rezultati ukazuju da postoji statistički značajna razlika u sadržaju soli u bijelom hljebu na nivou Regije, kao i na nivou između Regija u Republici Srpskoj. Procijenjen unos soli putem bijelog hljeba nedvosmisleno ukazuje na rizik u pogledu kardiovaskularnog zdravlja stanovnika Republike Srpske, te potvrđuje javnozdravstveni značaj potrebe za regulativom koja će obezbjediti kontinuirani monitoring nad sadržajem soli u bijelom hljebu i ostalim proizvodima od žitarica.

Zaključak: Stanovnici Republike Srpske unose više soli putem bijelog hljeba od stanovnika Srbije i Portugalije. Procijenjeni unos soli putem bijelog hljeba može se ocijeniti kao visok i rizičan po kardiovaskularno zdravlje. Neophodno je kontunirano provoditi i edukaciju stanovništva u oblasti pravilne ishrane.

Ključne reči: So, bijeli hljeb, kardiovaskularne bolesti

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.05
UDC 611.83.087:612.822
COBISS.RS-ID 6397208

Changes of Neurons and Blood Vessels of Human Substantia Nigra in Aging- Morphometric Study

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ABSTRACT

Introduction: With aging, populations of dopaminergic neurons in the central nervous system show prominent pathological changes compared to other brain regions. Previous studies on substantia nigra were performed in cases of Parkinson's disease and in old age.

Aim of the Study: Since Parkinson's disease is a disorder associated with age, it is important to examine how the relationship between neurons and blood vessels is associated with normal aging.

Patients and Methods: Ten brainstems were sliced into three strata. Each stratum was sliced in semiserial sections and stained by Mallory method. Studied phases were neurons and blood vessels of substantia nigra. The analysis was conducted by camera "Leica EC3" under the 40x magnification of light microscope „Leica" DM 1000, using ImageJ software (version 1.42 e). Determined morphometric parameters of neurons and blood vessels were: volume and surface density, and absolute numbers per visual field. Statistical analysis was performed using SPSS software, version 16.0, using Student's t-test and Pearson correlation coefficient.

Results: Volume and surface density, and total number of neurons per visual field of substantia nigra significantly decreased with age, while the volume and surface density and absolute number of blood vessels per visual field significantly increased ($p < 0.05$).

Conclusion: Decrease in size and number of neurons occurs with aging, which is compensated by the increase of vascular network. This affects the supply of nutrients from the blood to neurons, as well as the availability of blood cells or toxic substances, but also the susceptibility to neuronal diseases.

Key words: Aging; substantia nigra; humans.

(Scr Med 2017:48:30-38)

Submitted: January 31st, 2017
Accepted: February 9th, 2017

Introduction

With aging, populations of dopaminergic neurons in the central nervous system show more prominent pathological changes compared to other regions of the brain. This is especially pronounced with the pars compacta of the substantia nigra. Buchman and associates have showed that one-third of elderly persons without clinically diagnosed Parkinson's disease (PD) have a moderate to significant loss of neurons in this part of the substantia nigra (SN).[1] Between the older and younger groups of patients, Rudow and associates found a difference in the number of neurons of 28.3%, or a loss of 4.35% for a period of 10 years.[2] This loss is lower compared to a loss of 48% till 60 years of age, found by McGeer and associates and 35% loss till 90 years, as reported by Mann and associates.[3,4]

In a stereological study of SN pars compacta, Ma and co-workers found a significant age-dependent reduction in the total number of pigmented neurons.[5] The study of Vaillancourt and associates provided the first in vivo evidence that the microstructural integrity of the dorsal part of SN depended on age, while this was not true for ventral SN.[6] In their study, Fearnley and Lees took a step further and found that the ventral and lateral part of the SN pars compacta during the aging process was relatively spared (a loss of 2.1% at ten years) compared to the dorsal part (6.9% per decade).[7] The authors concluded that pigmented nigral cells in the ventral part of the SN pars compacta were most affected by PD, while aging affected pigmented cells in the dorsal SN.

In terms of arteries, SN is supplied (going rostrally) by anteromedial and anterolateral perforant and penetrating branches of superior cerebellar artery, posterior cerebral artery, collicular artery, posterior medial choroid artery, posterior communicant artery, and anterior choroidal artery.[8-11] Indeed, anteromedial arteries are typical perforating, interpeduncular (thalamoperforating) arteries,[11] and only their lateral arteries supply the most medial part of SN. Anterolateral arteries, also known as peduncular arteries, supply the largest part of SN.[11] Perfusion branches of posterior cerebral arteries also supply SN.

Vascular lesions of SN are extremely rare because of a large number of neurons, but also because of the large number of arteries that supply SN from various sources. Barcia and colleagues used the stereological methods and noticed an increase in the number of neurons with positive expression of vascular endothelial growth factor (VEGF), and an increase in the number of blood vessels and their volume in the SN pars compacta in monkeys.[12] These changes do not only occur by the increase of blood vessels, but by neuromicroangiogenesis, and they can affect the supply of nutrients to neurons from the blood, as well as on the availability of blood cells or toxic substances and the susceptibility of neurons to PD.

Neovascularization occurs in the brain after various insults cause the neuron loss.[13] Neuropathological analysis showed that PD patients had an increased number of nuclei of endothelial cells,[14] which could be related to the increased number and density of blood vessels or changes in the thickness of the wall of blood vessels. Morphometric studies on animal tissues, in which the PD was induced, showed an increase in the area occupied by blood vessels by 25% in SN pars compacta.[15,16] Increased vascularization in Parkinsonism seems to be induced by loss of dopaminergic cells. Angiogenesis in the affected parenchyma could also be related to the high demands for metabolites by surviving neurons.

Aim of the Study

Previous morphometric studies of SN were performed in cases of PD and in old age. Since this disease is related to age, it is important to examine how the relationship between neurons and blood vessels is associated with normal aging.

Patients and Methods

The research was done with the permission of the Ethics Committee of the University Clinical Center of the Republic of Srpska, on 10 brains of adults without diagnosed neurological diseases. Using an ordinary autopsy technique, brains were extracted from the cranial cavity, and then immersed in a 10% formalin solution for fixation. In order to reach SN samples, brainstems were separated from forebrain, by cutting the brain mass at the level of the posterior edge of mammillary bodies and from the cerebellum, by cutting cerebellar pedicles. After fixation, brainstems were cut in 3 mm thick strata in the transverse plane (stratified sampling), going caudally from the level of: middle of the superior mesencephalic colliculus (5 samples) and the caudal border of the superior mesencephalic colliculus (5 samples). Obtained strata were used to make semiserials sections (5, 10, 15, 20), 4 μm thick, which were stained by the Mallory method. Control of proper verification of blood vessels was carried out by the immunohistochemical method of antigen factor VIII.

In objects with a complex structure, at the beginning of morphometric analysis, the place and significance of their individual components should be determined, as well as their mutual relations. This will create a hierarchical model of the object.[17] For this research, we built the hierarchical model of SN. Reference space in all cases was

SN. Studied phases were nerve cells and blood vessels of SN. Images of objects of the research were taken with the camera "Leica EC3" in RGB format, 24-bit resolution of 2048 x 1536 pixels, under a 40x magnification of light microscope „Leica" DM 1000. For the analysis, resulting images were processed in the Adobe Photoshop 7.0 using "Auto Color" image adjustments and "unsharp mask" filter. For morphometric analysis, the program for the analysis and processing of digital images ImageJ (version 1.42 e) was used. In sample selection procedure, we picked up every second field, and the sample sizes, i.e. the required number of measurements for each variable and for each group was determined according to the formula:[18]

$$n = (200/ y \times s / x)^2$$

n– number of visual fields that should be analyzed, x – mean of the orientation sample, s– standard deviation of the orientation sample, y– allowed mean tolerance. Calculated number n represents the number of tests fields which should be morphometrically analyzed with a 95 % confidence interval.

Prior to analysis, the spatial calibration was done, using images of object micrometer taken at the same magnification as samples in this research. On a 24-bit image of object micrometer for a given magnification (400x), we measured the distance between the two notches on the object micrometer (10 μm), using the "straight line" selection. Option "set scale" in the software menu was used to convert the values from pixels to microns. By selecting the option "global", obtained calibration was applied to all images analyzed in one occasion.

After calibration, the parameters of the test system A100 were determined. Based on those parameters and software option"grid" we formed the grid of test system A100. Basic parameters of this system were: the total number of test points 100, the length of a line of test system was 0.020386 mm, and the surface of the test area was 0.04156 mm².

After grid superimposing, such image was analyzed with the cell counting tool ("cell counter"). For the analysis of the blood vessels SN we used the following morphometric variables: the volume density, surface density and an absolute number of blood vessels per field. On the same samples, the volume density, surface density and an absolute number of nerve cells per field were determined. For these tests, we used the conventional morphometric procedures.[19-21]

For calculation of volume density (Vv), the following formula was used: Vv = Pf / Pt (mm0), where Pf was the number of points of the test system falling on the studied phase, and Pt – total number of points within the test system A100.[18]

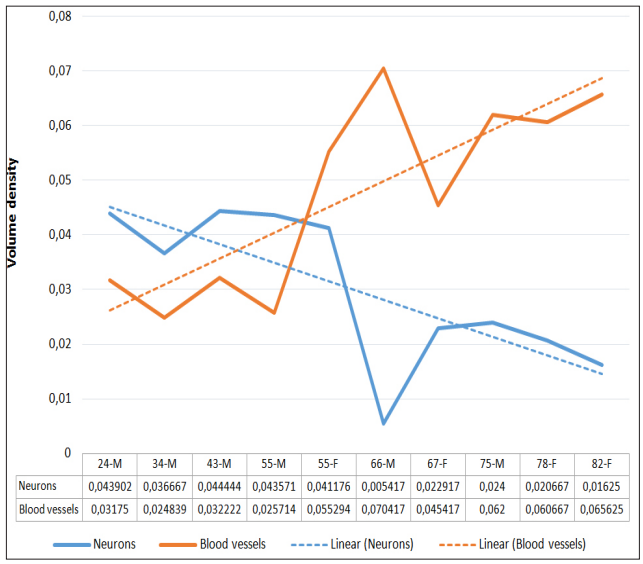
Surface density (Sv) was determined by the formula: Sv = 2×If / Lt (mm^{-1}), where If stood for the number of intersections of test system lines with studies phase, and Lt represented the total length of test lines.[17]

Statistical analysis of the results was performed using SPSS software, version 16.0, using Student's t-test and Pearson's correlation coefficient. Statistical significance was tested for the level of statistical significance of 5%.

Results

Morphometric measurements were carried out on 10 human brains, aged from 24 to 82 years. Volume and surface density and an absolute number per visual field of neurons and blood vessels of adult SN were determined in samples without diagnosed neurological diseases. Figure 1 shows values of volume density, Vv (mm0) of SN neurons and blood vessels in relation to age.

Figure 1. Age-related changes of volume density, Vv (mm^0) of neurons and blood vessels of the substantia nigra.



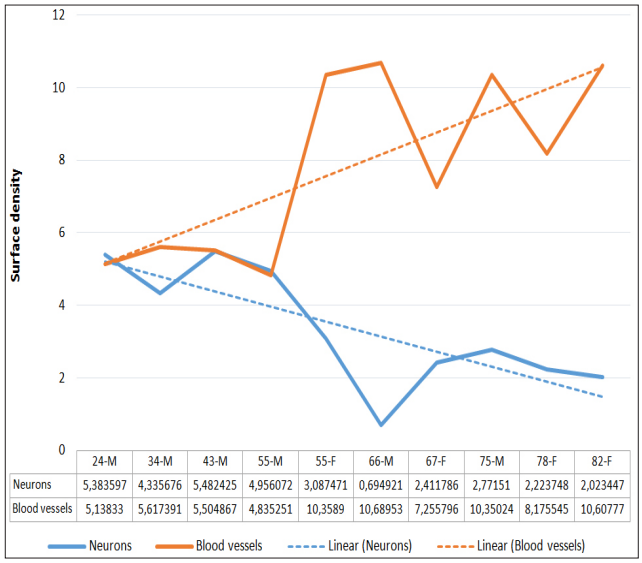
Pu Solid lines represent individual values, while dashed lines represent trendline of values of volume densities. Legend: M- male, F- female (afore values represent age)

By observing the linear trend of growth, it is obvious that the volume density of SN neurons decreased while SN blood vessels volume density increased with aging, as shown by Pearson's correlation coefficient of r = -0.82713. Using the t-test comparisons for different variances, we

obtained a statistical significance of p = 0.024.

Figure 2. shows the change of values of the surface density, Sv (mm^{-1}) of the blood vessels and neurons of SN according to age.

Figure 2. Age-related changes of surface density, Sv (mm^{-1}) of neurons and blood vessels of the substantia nigra.



	24-M	34-M	43-M	55-M	55-F	66-M	67-F	75-M	78-F	82-F
Neurons	5,383597	4,335676	5,482425	4,956072	3,087471	0,694921	2,411786	2,77151	2,223748	2,023447
Blood vessels	5,13833	5,617391	5,504867	4,835251	10,3589	10,68953	7,255796	10,35024	8,175545	10,60777

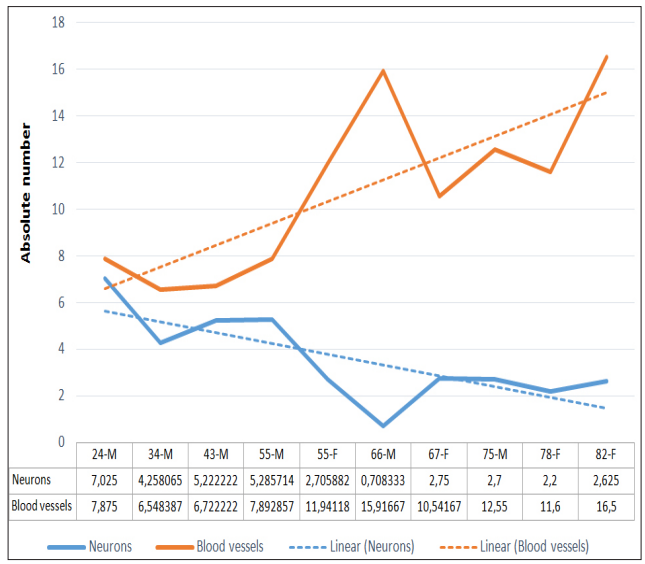
Solid lines represent individual values, while dashed lines represent trendline of values of surface densities. Legend: same as Figure 1.

By observing the linear trend of growth, it is obvious that the surface density of SN neurons decreased, while the surface density of SN blood vessels increased with aging, as shown by Pearson's correlation coefficient of r = -0.8468. Using the t-test comparisons for different variances, we obtained a significant statistical significance of p = 0.00022.

Figure 3.shows changes in the absolute value of the number of blood vessels and neurons of SN with aging.

Linear growth trend showed that the absolute number of SN neurons decreased, while SN blood vessels increased with aging, as shown by Pearson's correlation coefficient of r = -0.8025. Using the t-test comparisons for different variances, we obtained a significant statistical significance of p = 0.000056.

Figure 3. Age-related changes of absolute number, N of neurons and blood vessels per visual field of the substantia nigra.



	24-M	34-M	43-M	55-M	55-F	66-M	67-F	75-M	78-F	82-F
Neurons	7,025	4,258065	5,222222	5,285714	2,705882	0,708333	2,75	2,7	2,2	2,625
Blood vessels	7,875	6,548387	6,722222	7,892857	11,94118	15,91667	10,54167	12,55	11,6	16,5

Solid lines represent individual values, while dashed lines represent trendline of values of an absolute number of neurons and blood vessels. Legend: same as Figure 1.

Discussion

Detailed knowledge of SN microanatomy is necessary to explain the changes that occur during aging and in pathological conditions.[22-26] In the available literature, only a small number of data could be found, which were obtained by morphometric studies of neurons during the aging process, while the changes that occur in blood vessels are rarely documented. Available data are mainly related to the dimensions of the investigated nuclei and cells that are building them,[27] while changes in quantitative indicators with aging cannot be found. This paper presents data derived from the morphometric study of SN neurons and blood vessels of people who are classified into groups by age of both sexes without the diagnosis of a neurological disease. By processing the obtained results, we observed and described quantitative changes that occur in the neurons and blood vessels with the tissue aging.

The specificity of the SN is that this structure shows more pathologic changes with aging in comparison to other regions of the brain. The study on more than 750 older individuals without a defined PD revealed that in nearly one- third of the samples there was a serious loss of neurons in the SN, and 10% showed pathological Lewy bodies.1 It has been estimated that the loss of neurons in the SN per decade was 4.7 %,7 while older stereological techniques showed loss of up to 9.8%.[28]

Rudow and colleagues examined the relationship between the loss of neurons in the SN in normal aging and in PD.[2] They measured the total number and volume of the body of neuromelanin- containing pigmented neurons in the SN. The research was performed on younger control subjects (n = 7, average age: 19.9 years), middle- aged (n = 9, mean age: 50.1 years) and elderly control subjects from the Baltimore Longitudinal Study of Aging (n = 7, the average age: 87.6), as well as in patients with PD (n = 8, mean age: 74.8). On randomly, systematically selected paraffin sections stained by the Nissl method,they used an optical fractionator for estimation of the total number of neurons in SN on one side. Using nucleator, they measured the volume of these neurons. In younger and older control subjects, they also assessed the total number and volume of tyrosine hydroxylase-positive (TH +) nigral neurons. They observed a significant loss of pigmented (-28.3%, p< 0.01) and TH + (-36.2%, p< 0.001) neurons in older control subjects compared to the younger. The analysis of the distribution by size of pigmented and TH + neurons showed a significant degree of hypertrophy in elderly control subjects compared to the younger (p< 0.01). In contrast, a substantial atrophy of pigmented neurons in relation to all control groups was noticed in PD (p< 0.01). These data indicate that hypertrophy of neurons represents a compensatory mechanism within individual neurons in the SN, which allows normal motor function despite the loss of neurons during normal aging. It is assumed that in PD, this compensatory mechanism is out of order or it is in the shadow of pathological events caused by this disease, which leads to the appearance of the characteristic motoric disturbances.

There is no consensus on a change in the volume of neurons in the SN during normal aging. Nonstereological studies provided different results. Although Ma and colleagues have noted the existence of finer pigmented neurons in the SN in elderly subjects,[28,29] Cabello and colleagues, using the rotator method, have found that there is an increased volume of these neurons.[30] Measures, made with nucleator,2 the different kind of stereological test compared to the one used by Cabello and colleagues, agree with the estimates in the aforementioned study.[30] Our observations also showed an increased volume of neurons in the SN in older subjects and that, in addition to the fact that comparison of mean values of the volume of neurons in younger compared to older control subjects did not reach the level of statistical relevance, the analysis of frequency of certain volumes of neurons showed a significant difference between the younger and older subjects. Also it is important that the examination of the histogram of frequencies of distribution showed that an increase in the average volume of SN in elderly subjects was not the result of a selective loss of small size neurons, but a real hypertrophy of pigmented nerve cells bodies. Morphological study of TH + neurons in the human SN has not been able to establish the correlation between cellular body size and age.[31] Although in stereological studies of SN the volume of TH + neurons in normal aging has not been studied, there is evidence that pigmented and TH + neurons behave similarly.[32] Accordingly, the hypertrophy of TH + neurons in normal aging would be expected. This is exactly what the studies have found - TH + neurons with normal aging become larger, a change similar to the one observed in pigmented neurons. The mechanism behind this hypertrophy of neurons is not directly examined in this study, but in addition, some theoretical possibilities could be considered. Progressive accumulation of neuromelanin and other pigments may be the reason for neuronal enlargement. However, this does not explain why the hypertrophy of neurons is present in elderly subjects, but there was no difference when the younger group and middle-aged subjects were compared. Another possibility is that the enlargement of neurons is a result of damage.[33] The third option, which seems most likely, is that since with aging there is a loss of neurons in the SN, the remaining neurons take over and re-innervate deafferented target zones, especially in the striatum. This hypothesis of the compensation, proposed by Cabello and co-workers,[30] is supported by the observation that the total volume (multiplication of volume and the number of neurons) of pigmented neurons in the SN is constant for all age groups. A similar mechanism is also mentioned when it comes to the hypertrophy of cortical nerve cells in asymptomatic dementia. Hypertrophy associated with aging was observed for the pigmented neurons in the locus coeruleus in humans, in a study conducted by Iwanaga and associates[34], attributing the spread of cytoplasm of neuronal bodies to the embrace of the synaptic terminals.

Vaillancourt and colleagues examined the effects of aging on the ventral and dorsal part of SN by diffuse tensor imaging (DTI).[35] Measurements collected by DTI images of 16 young adults (19- 27 years) and 15 older adults (55-71 years) have shown that in the dorsal SN fractional anisotropy is decreased, and radial diffusivity increased with age. In the ventral SN and the red nucleus measurements, using DTI did not show differences depending on age. DTI represents a non-invasive technique that accurately reflects the established pattern of cell loss caused by the aging of the dorsal and ventral part of the SN, which indicates the great potential in the use of DTI to describe degeneration of nigrostriatal pathway in healthy and diseased persons. Studies showed that the loss of neurons with age occurred in the dorsal, while in extrapyramidal disorders, the loss is in the ventral part of the SN.

Some studies have measured the loss of neurons with

aging in other regions of the brain that have not shown a similar degree of neuronal loss as in SN. It turned out that the number of neurons remains relatively stable over the life in the hippocampus, the putamen, the medial mammillary body and Meynert's nucleus, while neocortical neurons have a loss of 10% through the lifetime. But in other dopaminergic populations involved in the ventral tegmental area and retrorubral area loss is up to 50%. These studies show that the dopaminergic neuron populations are increasingly vulnerable to age-dependent loss compared to other brain regions.[36]

It is known that age induces changes in angiogenesis in the brain and other tissues, and that the vascular endothelial growth factor (VEGF) is a powerful regulator of angiogenesis and is thought to be involved in age-related changes of angiogenesis.[37] It is noted that physical exercise improves the effect of age-induced angiogenesis in many tissues.[37,38] Also, studies have shown that VEGF is also a neuroprotective molecule for dopaminergic neurons.[39]

In a study on experimental mice, divided into three age groups and two subgroups,[40] one group was assigned to physical activity on the treadmill and the other one was a control group. The results showed that age was likely related to chronic ischemia and therefore induced the reduction in the density of blood vessels and VEGF levels in the SN, which may have increased the vulnerability of dopaminergic neurons to additional injury.

Uchida and colleagues examined how transient focal brain ischemia could lead to neuronal damage in remote areas, including the thalamus and SN on the affected side, as well as in the ischemic core.[41] These researchers studied the long-term changes in rats SN that occurred between the first and twentieth week on the same side affected by 90-minute attack of transient focal brain ischemia, through immune marking using TH, protein NeuN, Iba-1, glial fibrillary acidic protein (GFAP) and brain-derived neurotrophic factor (BDNF). The results showed that transient focal cerebral ischemia in rats could cause serious and lasting damage to neurons in the striatum of the affected hemisphere. Also, the results obtained on the basis of TH immunolabeling and NeuN showed that atrophy of SN hemisphere affected by transient focal ischemia of the brain was not static, but had a progressive character. In addition, the double-immunohistochemical labeling indicated that the NFMP, released by GFKP-positive astrocytes, could play a key role in the preservation of dopaminergic neurons in the SN during the chronic phase on the same hemisphere affected by transient focal ischemia of the brain, although the surface of SN on that side was progressively reduced after ischemia. Thus, this study provided additional information on the pathogenesis of neuronal damage after transient focal cerebral ischemia.

Do the changes in vascularization have positive or negative effects during aging, or is it a combination of both? Manipulation by vascularization on experimental models can help finding the answer to this question and possibly identify new targets for treating diseases of SN. The formation of blood vessels may, in fact, come through a variety of processes and inflammation stimulates angiogenesis in various diseases. Numerous mechanisms can be the basis of such kind of neovascularization. There are several types of angiogenic factors which are released from the cells, which stimulate neovascularization. One of these mechanisms might be associated with the loss of neurons in the SN pars compacta since in parkinsonism, the existence of cytokines that promote an inflammatory process and the infiltrating cells of blood origin has been described.[12]

It was observed that with age- dependent decline of degrees of nigral vascularization and nigral VEGF, both degrees have increased after the implementation of locomotor exercises.[40] One study showed a beneficial effect that exercise had on neuroprotection in cerebral ischemic damage,[38] while other study suggested that exercises of the locomotor system induced an increase in VEGF expression, probably as a compensatory mechanism leading to increased capillary surface in response to the increased demand for oxygen and energy.[40]

Our previous studies of neurons in the magnocellular part of the red nucleus have shown the reduction of the quantitative parameters with age, but the decrease did not reach statistical significance.[42] We mention this because of the proximity of red nucleus and SN at the same midbrain level, and there is a joint functional activity in the extrapyramidal system. This is confirmed by Lambert and associates, who showed the reduction in the number of neurons, gliosis and increased levels of iron with aging in SN and red nucleus.[43] The increase in iron in vivo results in an increase neuromelanin, the substance that is then released by dying neurons and causes new neuronal damage.

One of the limitations observed in our study is that we do not have information whether the analyzed human tissue belonged to individuals whose lifestyle in the older age included intense physical activity and exercise, which could be accounted for increased angiogenesis in the elderly. This fact opens the door for further research on whether the increased activity of the musculoskeletal system in old age significantly affects the maintenance activity of the nervous system.

Conclusion

With aging, there is a statistically significant increase in the values of morphometric parameters of blood vessels and reduction of values of morphometric parameters of SN neurons. Taking into account that we have studied healthy individuals at different ages, this relationship explains that, with aging, there is a decline of neurons which is compensated by the increase of vascular network of SN.

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Promjene neurona i krvnih sudova substantiae nigrae kod čovjeka tokom starenja - morfometrijsko istraživanje

SAŽETAK

Uvod: Populacije dopaminergičkih neurona u centralnom nervnom sistemu sa starenjem pokazuju izraženije patološke promjene u poređenju sa drugim dijelovima mozga. Dosadašnja ispitivanja substantiae nigrae su rađena kod Parkinsonove bolesti i u starosti.

Cilj rada: S obzirom na to da je Parkinsonova bolest poremećaj povezan sa starosnom dobi, važno je ispitati na koji način je odnos neurona i krvnih sudova povezan sa normalnim starenjem.

Ispitanici i metode: Deset moždanih stabala je rezano u tri stratuma, od kojih su pravljeni semiserijski rezovi bojeni Mallory metodom. Proučavane faze su neuroni i krvni sudovi substantiae nigrae. Analiza je rađena kamerom "Leica EC3", pri povećanju objektiva 40x svjetlosnog mikroskopa "Leica" DM 1000, korišćenjem programa ImageJ (verzija 1.42 e). Određivani morfometrijski parametri neurona i krvnih sudova su bili: volumenska i površinska gustina, i apsolutni broj po vidnom polju. Statistička analiza je urađena pomoću softvera SPSS, verzija 16.0, upotrebom Studentovog t-testa i Pearson-ovog koeficijenta korelacije.

Rezultati: V Volumenska i površinska gustina neurona, te apsolutni broj neurona po vidnom polju substantiae nigrae su se statistički značajno smanjivali sa godinama života, dok su se volumenska i površinska gustina krvnih sudova i apsolutni broj krvnih sudova po vidnom polju statistički značajno povećavali ($p < 0,05$).

Zaključak: Starenjem dolazi do smanjenja dimenzija i broja neurona koji kompenzuje porast vaskularnog korita jedra i utiču na snabdijevanje neurona nutrijentima iz krvi, kao i na dostupnost krvnih ćelija ili toksičnih supstanci, ali i na podložnost neurona bolesti.

Ključne riječi: Starenje, substantia nigra, čovjek

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.06
UDC 612.822:611.83.087
COBISS.RS-ID 6397720

Age- Related Changes of Quantitative Parameters of Neurons in Extraocular Motor Nuclei

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ABSTRACT

Introduction: Extraocular motor nuclei are located in the midbrain (principal oculomotor and trochlear nucleus) and in the pons (abducens nucleus). With aging, there are significant changes in eyeball mobility.

Aim of the Study: The aim was to determine whether the quantitative parameters of neurons (volume and surface density, and the absolute number per mm^2 of the surface) in these nuclei significantly change with aging.

Patients and Methods: The study was done on 30 adult brainstems, both male and female, without diagnosed neurological disturbances. Three-millimeter-thick strata were taken in transversal plane and cut in 0.3 micrometer semi-serial sections stained with Mallory method. The images of studied nuclei were taken by „Leica" DM 1000 microscope and „Leica" EC3 digital camera under 400x magnification and analyzed by ImageJ software with A100 grid. The statistical analysis was performed by Statistical Package for the Social Sciences software using Pearson's correlation coefficient with 5% level of significance.

Results: The volume density of neurons had highly statistically significantly increased with age in principal oculomotor nucleus ($r = 0.571$, $p = 0.001$) and trochlear nucleus ($r = 0.581$, $p = 0.001$), while abducens nucleus showed no change in neuron volume. Changes of values of surface density and absolute number of neurons per mm^2 with age did not reach statistical significance.

Conclusion: Volume of neurons of extraocular motor nuclei located in the midbrain increase with age, while their surfaces and absolute number do not change significantly. These changes are not observed in the nucleus located in the pons.

Key words: Aging; oculomotor nuclear complex; trochlear nerve/anatomy and histology; abducens nucleus.

(Scr Med 2017:48:39-44)

Submitted: February 1st, 2017
Accepted: February 15th, 2017

Introduction

The first morphological signs of aging in the brain are found in the white mass at an early age (20- 40 years) and later (40-50 years) in the gray matter. As a result of these changes, the sensory-motor and cognitive skills will weaken.[1] The age-related changes in brain volume, weight

and the number of nerve cells vary in different parts of the brain. Macrostructural cerebral alterations progress with age without visible pathological changes,[2] while the microstructural changes, such as iron accumulation and loss of neurons, occur much earlier.

Extraocular motor nuclei (principal oculomotor, trochlear and abducens nucleus) belong to the group of general somatic efferent nuclei and contain motoneurons and internuclear neurons, which are a part of the circuit which coordinates eye movements.[3,4] Research of Clark and Isenberg has shown that with aging, between 30 and 90 years of age, there is the largest decline in the eyeball elevation, to an intermediate degree adduction and abduction, and the version least affected by age is depression.[5] A possible reason for these changes is the reduction in the number and size of myelinated axons, which leads to paralysis or neuropathy. Morphological study of Moriyama and associates showed that with age came a significant reduction in the total number of myelinated axons in the oculomotor nerve, while this change was not observed in the abducens nerve.[6] Stereological study of Sharma and associates showed a slight reduction in the total number of myelinated fibers and the surface of the axon with aging, whereby there was a significant increase in the thickness of the myelin sheath.[7] Reductions of the total number of myelinated fibers and surface of axons were not observed in the trochlear and abducens nerve with age, but even in their case there was an increase in the thickness of the myelin sheath.[8] In the available literature, we did not find any data on quantitative changes in extraocular motor nuclei with aging.

Aim of the Study

The aim was to determine whether quantitative parameters of neurons in extraocular motor nuclei (volume and surface density, and an absolute number per mm^2 of the surface) change significantly with aging.

Patients and Methods

The research was done with the permission of the Ethics Committee of the University Clinical Center of the Republic of Srpska, on samples of 30 adult brains, both sexes (11 female and 19 male), aged 21 to 83 years (average age 57.07 years) who died without diagnosed neurological diseases. Using conventional autopsy technique brains were extracted from the cranial cavity, and then immersed in a 10% formalin solution for fixation. After the fixation, brainstems were separated from forebrain, by cutting brain masses at the level of the posterior edge of mammillary bodies and from the cerebellum, by cutting cerebellar pedicles. After fixation, brainstems were cut in 3 mm thick strata in the transverse plane (stratified sampling), going caudally from the level of:

1. the middle of the superior mesencephalic colliculus (principal oculomotor nucleus),
2. caudal border of the superior mesencephalic colliculus (trochlear nucleus), and
3. caudal border of the facial colliculus (abducens nucleus).[9]

Obtained strata were used to make semi-serial sections (for principal oculomotor and abducens nuclei: 5,10,...,100; and for trochlear nucleus: 5,10,...,120), 0.3 μm thick, which were stained by the Mallory method. Referent space of the research in all cases was extraocular motor nuclei, and the study phase was nerve cells.

Images of objects of the research were taken by the camera "Leica EC3" (Leica Microsystems CMS GmbH, Wetzlar, Germany) in JPEG format, 2048 x 1536 pixels resolution, under a 400x magnification of light microscope „Leica" DM 1000 (Leica Microsystems CMS GmbH, Wetzlar, Germany) and 0.7 magnification of the camera's c-mount. In the sample selection procedure, we have picked up every second field, and the sample sizes, i.e. the required number of measurements for each variable and for each group was determined according to the De Hoff's formula: $n = (200 / y \bullet s / x)^2$

n– number of visual fields that should be analyzed, x – mean of the orientation sample, s– standard deviation of the orientation sample, y– allowed mean tolerance.

Quantitative analyses were done using ImageJ software, version 1.49 j (National Institutes of Health, Bethesda, USA). Prior to analysis, the spatial calibration with objective micrometer was done, and the parameters of the test system A100 were determined. Based on these parameters and software option "grid" we have formed the grid of test system A100 (table 1)..

Table 1. Basic parameters of the test system

A-100	Objective 40x
Pt	100
d	0.020386 mm
Lt	4.0772 mm
At	0.04156 mm^2

Pt - total number of points of the test system; d - length on one line of the test system; Lt - length of all test lines; At - area of the test system; Lt=Pt•d•2; At=Pt•d²

After grid superimposing, images were analyzed with the cell counter tool. For the analysis of neurons, the following variables were determined: volume density, surface density and an absolute number of nerve cells per mm² of the surface were determined. A total number of analyzed test fields was: for principal oculomotor nucleus 2060, for trochlear nucleus 2354 and for abducens nucleus 2080.

For calculation of volume density (Vv), which represents the amount of space occupied by analyzed phase, the following formula was used: Vv (mm⁰)= Pf / Pt (Pf is the number of points of the test system falling on the studied phase; Pt – total number of points within the test system A100).[10]

The second analyzed parameter, surface density (Sv), indicated the size of a certain inner or outer surface in a volume unit. It was determined by the formula: Sv (mm⁻¹)= 2•If/Lt (If- number of intersections of test system lines with studies phase; Lt- total length of test lines).[10]

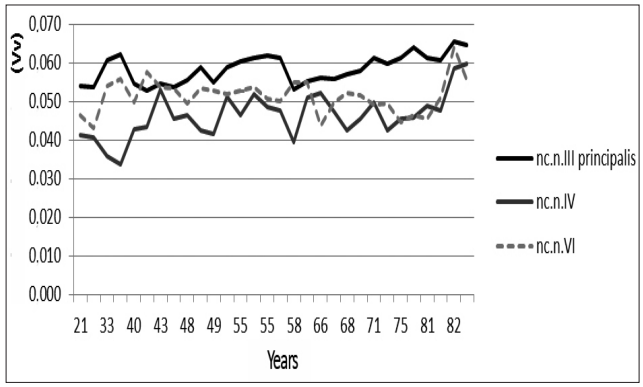
The absolute number of neurons per mm² of the surface was calculated by the formula: Nf= N/At (N- the number of neurons within test system; At- area of the test system).

Statistical analysis of the results was performed using SPSS software (SPSS Inc, Chicago, USA), version 16.0, using methods of descriptive statistics and Pearson's correlation coefficient. Statistical significance was tested for the level of statistical significance of 5%.

Results

The impact of aging on the volume density of neurons has differed in the investigated nuclei. There was a strong, positive correlation between the volume density of neurons and age in two extraocular motor nuclei- principal oculomotor nucleus (r = 0.571, p = 0.001) and trochlear nucleus (r = 0.581, p = 0.001). For the abducens nucleus, the correlation was weak and positive (r = 0.032, p = 0.865). Changes in values of volume density with aging are shown in Figure 1.

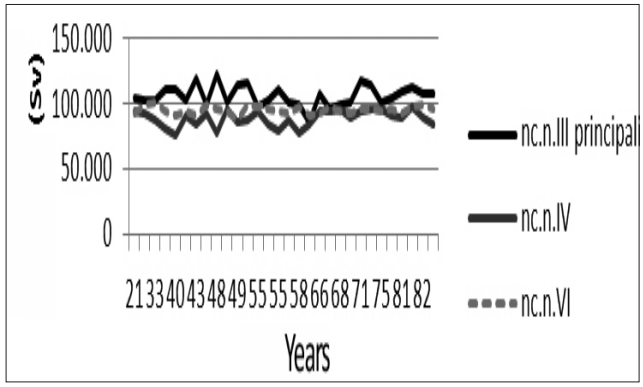
Figure 1. **Age-related changes in volume density (Vv) of extraocular motor nuclei**



Nc.n.III principalis- principal oculomotor nucleus; nc.n.IV- trochlear nucleus; nc.n.VI- abducens nucleus

The second tested parameter, surface density, did not significantly change with age, and correlations had a low intensity and were positive- for the principal oculomotor nucleus r = 0.086, p = 0.650, trochlear nucleus r = 0.081, p = 0.671, and for abducens nucleus r = 0.005, p = 0.979. Changes in values of the surface density with aging are shown in Figure 2.

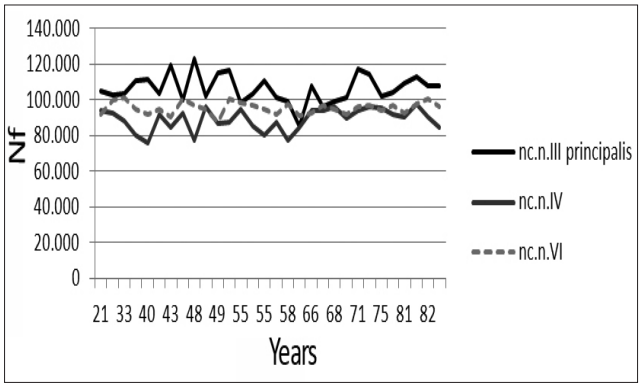
Figure 2. **Age-related changes in surface density (Sv) of extraocular motor nuclei**



Nc.n.III principalis- principal oculomotor nucleus; nc.n.IV- trochlear nucleus; nc.n.VI- abducens nucleus

Similar to the surface density, the absolute number of neurons per mm2 of surface with aging in all three extraocular motor nuclei did not significantly change, and correlations had a weak intensity- for principal oculomotor nucleus r = 0.010, p = 0.956, for trochlear nucleus r = 0.275, p = 0.142, and for abducens nucleus r = 0.043, p = 0.820. Changes in values of the absolute number of neurons of the extraocular motor nuclei per mm2 of surface with aging are shown in Figure 3.

Figure 3. Age-related changes in absolute number of neurons per mm2 of surface (Nf) of extraocular motor nuclei



Nc.n.III principalis- principal oculomotor nucleus;
nc.n.IV- trochlear nucleus;
nc.n.VI- abducens nucleus

Discussion

In the available literature, there is a little amount of quantitative data on changes in neurons of extraocular motor nuclei with aging. Available data are mainly related to the dimensions of the investigated nuclei and cells that are building them,[11-15] while changes of quantitative indicators with aging were not found. On the other hand, there are a number of diseases caused by changes in the cells of the extraocular motor nuclei, leading to certain clinical symptoms.[16-18]

In the present study, we have analyzed the changes of quantitative parameters of nerve cells of extraocular motor nuclei with aging. It was observed that neurons in the principal oculomotor nucleus and trochlear nucleus increase in volume with age, while this change was not observed in abducens nucleus. The mechanism related to this neuron hypertrophy has not been directly investigated in the present study, but observed increase in volume can be explained by the accumulation of neuropigments with aging, which occupy an increasing cell surface, which is particularly pronounced in large neurons and in brain regions involved with motor function.[19]

The values of Pearson's correlation coefficient showed that there was no statistically significant change in the number of neurons in extraocular motor nuclei. These results correspond to the results of Vijayashankar and Brody, who studied changes in the number of trochlear nucleus neurons and noted that from newborns to 87 years of age, there is no statistically significant change in the number of neurons in this nucleus. The only difference observed by these authors was that the neurons are more densely arranged in a newborn and that the adult nuclei were slightly longer (0.2 to 1.3 mm).[20] The same authors have studied the changes in abducens nucleus with aging.[21] In addition to individual variations, no significant reduction in the number of nerve cells in this nucleus was observed as well, but the length of adult nuclei was almost doubled. In studies on age changes in the number of neurons of all three extraocular motor nuclei in mice, Sturock also noted that there was no statistically significant difference in the number of neurons. However, the author states that in the neurons of these nuclei there is no significant loss of Nissl's substance with aging and that there is a very little accumulation of lipofuscin, and no variation in the diameter of the nucleus of neurons with aging.[22,23] Given the fact that extraocular muscles are very active throughout life, the nuclei of the nerves which innervate these muscles also have huge activity. It is assumed that muscle activity prevents loss of neurons in these nuclei, since activity can delay the occurrence of deterioration in certain nerve cells groups.[24]

Although principal oculomotor nucleus is not functionally directly related to the red nucleus and substantia nigra, because of the close topographic position in the same transverse plane, the research results were compared with the values of quantitative parameters of age- changes of neurons in aforementioned structures. Analyzed quantitative parameters of neurons in the magnocellular part of the red nucleus decrease with age, but the decrease does not reach statistical significance.[25] Quantitative measurements of substantia nigra show decrease in the value of quantitative parameters of neurons with age, with the exception that the analysis of histogram of the frequency distribution has shown that the increase of the average volume of substantia nigra neurons in older subjects is not the result of a selective loss of small size neurons, but a real hypertrophy of bodies of pigmented nerve cells.[26]

Conclusion

With aging, there is an increase of the volume of cells in principal oculomotor and trochlear nucleus, while this enlargement is not seen in abducens nucleus cells. Surface density and an absolute number of neurons do not change significantly with aging.

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Promjene kvantitativnih parametara neurona ekstraokularnih motornih jedara sa starenjem

SAŽETAK

Uvod: Ekstraokularna motorna jedra se nalaze u srednjem mozgu (nucleus nervi oculomotorii principalis, nucleus nervi trochlearis) i u moždanom mostu (nucleus nervi abducentis). Starenjem dolazi do značajnih promjena u pokretljivosti očne jabučice.

Cilj rada: Cilj rada je da se odredi da li se kvantitativni parametri (volumenska i površinska gustina, i apsolutni broj po mm^2 površine) neurona ovih jedara značajnije mijenjaju sa starenjem.

Ispitanici i metode: Istraživanje je obavljeno na 30 preparata moždanih stabala odraslih lica, oba pola, bez dijagnostikovanih neuroloških oboljenja. Stratume debljine 3 milimetra smo uzimali u transferzalnoj ravni i rezali u semiserijske rezove debljine 0,3 mikrometra koji su bojeni Mallory metodom. Fotografije istraživanih jedara su slikane pomoću mikroskopa Leica DM1000 i digitalne kamere Leica EC3 pod uvećanjem 400x, i analizirane softverom ImageJ uz korišćenje mrežice A 100. Statistička analiza je obavljena programom SPSS korišćenjem Pearson-ovog koeficijenta korelacije uz nivo značajnosti razlike od 5%.

Rezultati: Volumenska gustina neurona se visoko statistički značajno povećavala sa godinama života kod nucleus nervi oculomotorii principalis (r=0,571, p=0,001) i nucleus nervi trochlearis (r= 0,581, p=0.001), dok kod nucleus nervi abducentis nije bilo promjene u volumenu neurona. Promjene vrijednosti površinske gustine i apsolutnog broja neurona po mm^2 sa starenjem nisu dostigle statističku značajnost.

Zaključak: Volu Neuroni ekstraokularnih motornih jedara locirani u srednjem mozgu se volumenski povećavaju sa starenjem, dok im se površina i apsolutni broj značajnije ne mijenjaju. Ova promjena se ne uočava u jedru lociranom u moždanom mostu.

Ključne riječi: Starenje; okulomotorni jedarni kompleks; trohlearni nerv/anatomija i histologija; abducensno jedro.

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.07
UDC 616-002-074:612.018
COBISS.RS-ID 6398232

The Importance of the Bolus Calculator Use for Improving Glycemic Control in Patients on the Insulin Pump Therapy

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ABSTRACT

Introduction: Bolus calculator is an advanced function of insulin pump (IP). The use of bolus calculator increases the accuracy of calculation of the proper meal or corrective dose of insulin in patients with type 1 diabetes (T1D).

Aim of the Study: Compare the difference in the parameters of glycemic control (HbA1c, postprandial increase of blood glucose and number of hypoglycemic episodes per week) between the group of patients who use bolus calculator for <50% of the total daily boluses, and the group of patients who use bolus calculator for ≥50% of total daily boluses.

Patients and Methods: This study included 36 patients aged over 18 years with T1D on IP therapy in the Republika of Srpska. All patients used IP for at least one year prior to participation in the study. Before the IP therapy was initiated, all the patients were trained for carbohydrate counting in course of flexible insulin therapy training (FIT). Professional software, CareLink Pro® Software (Medtronic Inc., Northridge, CA, USA) was used to download data from insulin pumps to a personal computer. The default frequency of bolus calculator use was ≥50% of total daily boluses.

Results: No statistically significant difference was found in HbA1c (6.61 ± 1.10 vs. 0.84 ± 6:56, p = 0.896) or the number of hypoglycemic episodes (2.00 (1.00, 4.00) (1.0 - 6.0) vs 3.00 (2.00, 4:00) (1.0 - 5.0), p = 0.298) between the group of patients who used bolus calculator for <50% of the total daily boluses, and the group of patients who used bolus calculator for ≥50% of total daily boluses. Patients who used bolus calculator had significantly lower postprandial increase in blood glucose after breakfast.

Conclusion: In order to maximize all the advantages of IP therapy, a regular re-education of both patients and diabetologists about advanced IP functions is needed for improving the glycoregulation in T1DM.

Keywords: Insulin pump, bolus calculator, T1D, glycoregulation

(Scr Med 2017:48:45-52)

Submitted: February 8th, 2017
Accepted: February 22th, 2017

Introduction

Insulin pump therapy (IP) represents one of two ways of applying intensive insulin therapy in type 1 diabetes (T1D), allowing precise dosing of basal and bolus insulin doses.[1]

Technological improvement of IP and use of rapid acting insulin analogues with faster onset of action has provided the development of a system that integrates IP and the system for continuous glucose monitoring (CGM), so-called sensor-augmented IP. This system has proved superiority in lowering HbA1c in comparation with conventional intensive insulin therapy.[2,3] By creating a control algorithm that automatically adjusts insulin delivery according to the measured blood glucose or assumed glucose values (i.e. insulin and glucagon in dual-hormone model of artificial pancreas), an artificial pancreas function is set.[4,5] In the last decade, the effectiveness of different models of artificial pancreas was demonstrated in order to achieve optimal glycemic control, with a lower incidence of hypoglycemia.[6-8] However, more studies with a larger number of participants will be needed to confirm the safety home use of artificial pancreas in unexpected situations.[9]

Automatic bolus calculators are integrated into commercial insulin pumps, and based on preprogrammed settings of specific algorithms, they suggest bolus dose, and thereby increase the accuracy of calculations in relation to mental calculation. These settings should be individually adjusted for each person, and should refer to the same parameters required for calculating bolus doses manually: insulin/carbohydrate ratio, corrective factor, active insulin time, target values of blood glucose and actual value of glycemia.[10,11]

"Smart" IPs have integrated bolus calculators which in the process of calculating the bolus dose also calculate the active insulin of the preceding dose. In that way, the bolus calculator use can accurately determine the bolus dose or the dose of insulin needed to correct high blood glucose. Furthermore, most commercial pumps with integrated bolus options provide three different types of bolus: 1) normal bolus- the pump delivers the entire bolus at once; 2) square bolus- the pump delivers equally required dosage of insulin during a period of time, and 3) combined bolus – the pump has options of two above mentioned pumps.[12] The previous studies who studied the impact of advanced IP functions on glycoregulation, have showed that the use of bolus calculator had no impact on the value of glycosylated hemoglobin HbA1c, but it could contribute to the glycemic excursion and postprandial glycemic decrease, and thus contribute to the improvement of glycemic control.[13]

Aim of the Study

To compare the parameters of glycemic control between the group of patients who use bolus calculator for <50% of the total daily boluses and the group of patients who use bolus calculator for ≥50% of total daily boluses based on the difference between the HbA1c, postprandial increase of glucose and the number of hypoglycemic episodes per week.

To compare the difference in the use of bolus option between the group of patients who use bolus calculator for <50% of the total daily boluses and the group of patients who use bolus calculator for ≥50% of total daily boluses.

Patients and Methods

This study included 36 patients aged over 18 years with T1D on IP therapy in the Republic of Srpska. The models used for the purpose of this study were IP MiniMed® Paradigm 722 (Medtronic Inc., Northridge, CA, USA) and MiniMed® Paradigm 754 (Medtronic Inc., Northridge, CA, USA) which had an integrated bolus calculator (Bolus Wizard) as an advanced insulin pump option. Most patients used MiniMed® Paradigm 754 (n = 20), while other patients preferred MiniMed® Paradigm 722 (n = 16). All patients used insulin pump for at least one year prior to participation in the study along with the therapy with short-acting insulin analogues. Implantation of insulin pumps was performed during the period from 2008 to the 2012 at the Department of Endocrinology, Diabetes and Metabolic Diseases at Clinical Center of Banja Luka. Before initiation of IP therapy the patients were trained for carbohydrate counting in course of flexible insulin therapy training (FIT).

Medtronic "CareLink® Pro" (Medtronic Inc., Northridge, CA, USA) is a software for professional management and monitoring of diabetes treatment for use on a personal computer. This software has been approved by the FDA for the market use in September 2010. In our study, the data from the insulin pumps were downloaded on a personal computer by Medtronic "CareLink® Pro 4.0c" (Medtronic Inc., Northridge, CA, USA) software. The amount of data within each pump varied depending on the degree of use of all insulin pump's technical possibilities and ranged from 63-266 days. For the purpose of our study, a period of nine weeks (63 days) was analyzed for each patient. The data were downloaded with a USB CareLink® (Medtronic Inc., Northridge, CA, USA) upon arrival of patients for a regular check-up, at the Department of Endocrinology of the Clinic for internal diseases of UCC Republika Srpska in Banja Luka. Body weight, demographic data and variables related to diabetes (the data of chronically complications

presence, the data on the duration of diabetes, duration of pump therapy, the average number of hypoglycemia per week, the value of HbA1c and glucose profiles were gathered from patients. During the study, none of the patients used the CGM.

The default frequency of bolus calculator use was ≥50% of the total daily boluses.[15,16] Obtained value of HbA1c referred to the period of the observed 9 weeks within which the profile of preprandial and postprandial glycaemia was made. All patients on insulin pump therapy measure HbA1c at the Institute of Laboratory Diagnostic at the University Clinical Centre of Republic of Srpska using the Cobas c 501, Roche Diagnostics (Basel, Switzerland) apparatus, which is certified as having a documented trace ability to the Diabetes Control and Complications Trial reference method by the National Glycohemoglobin Standardization Program (NGSP). Glycemic profiles were measured using the Accu-Chek ® Performa glucometers, Roche Diagnostics (Basel, Switzerland), which has the possibility of wireless transmission of stored values to a computer via Accu-Chek Smart Pix devices (Basel, Switzerland). Informed written consent was obtained from all participants before enrollment in the study, providing patients' personal data protection in the case of the publication of results.

For statistical analysis, IBM SPSS Statistics 21.0 software was used. In order to compare the differences in the frequency of observed characteristics between the groups of respondents, the Pearson's $\chi 2$ contingency test was used. Distribution normalcy of the observed characteristics was tested with Kolmogorov-Smirnov normalcy test. In order to compare the average values of characteristics between the groups of respondents, the Student t test for independent samples was used (observed characteristics that have a normal distribution) and non-parametric Mann-Whitney U test for independent samples (observed characteristics that do not have a normal distribution). When using Student's t test for independent samples, F test was used in order to grasp the significance of differences in the variances of observed characteristics. As statistically significant, all the values in which $p < 0.05$ were taken/used.

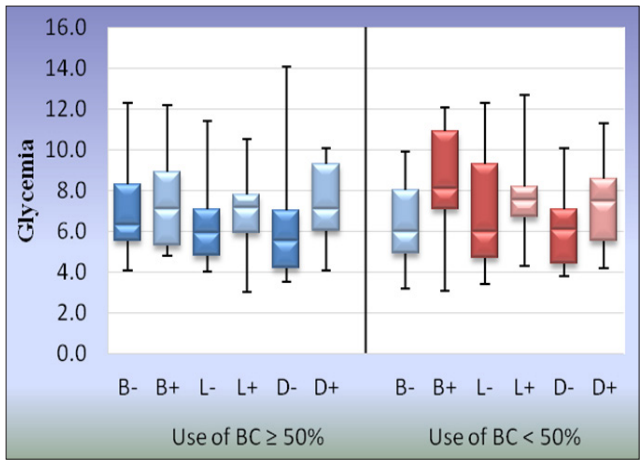
Results

A total of 36 adult patients with T1D on insulin pump therapy were included in the study and divided into two groups by bolus calculator use. The first group consisted of 17 patients (47.22%) used bolus calculator <50% of all daily boluses , and the other group consisted of 19 patients (52.78%) in which ≥50% of all given daily bolus were given by bolus calculator. Patients older than 30 years were majority in both groups (76.47% in the first group and 52.63% in the second group). The observed difference between the groups was not statistically significant. There was no statistically significant difference in duration of insulin pump therapy between the groups (4.35 vs. 3.74 ± 1:46 ± 2.13, $p = 0.300$).

Mean HbA1c was not significantly different between the two groups, although bolus calculator users had slightly lower HbA1c. (Table 1).

Patients who used bolus calculator for ≥50% of all given daily bolus, had a lower postprandial increase in blood glucose. Bolus calculator users had a significant lower postprandial increase after breakfast ($p < 0.034$). The difference between postprandial increase for lunch and dinner was not statistically significant. (Chart 1).

Figure 1. **Box-plot diagram for preprandial and postprandial for two groups of patients**



B- preprandial breakfast glycemia
B+ postprandial breakfast glycemia
L- preprandial lunch glycemia
L+ postprandial lunch glycemia
D- preprandial dinner glycemia
D+ postprandial dinner glycemia

The patients on IP therapy had an average of three hypoglycemic episodes per week. There was no statistically significant difference between the two groups, although bolus calculator users had slightly more frequent hypoglycemic episodes than bolus calculator non-users (Table 1).

Bolus calculator users had a lower average number of total boluses during the day and statistically significantly higher average number of boluses given with food as compared to bolus calculator non-users. A higher number of corrective boluses was observed in the group of bolus calculator users but with no statistically significant

Table 1. The parameters of glycemic control and "bolus option" parameters for bolus calculator users (BC+) and bolus calculator non-users (BC-)

	BC-	BC+	p
HbA1c (%)	6.61 ± 1.10	6.56 ± 0.84	p=0.896
The average number of hypoglycemic episodes/week	2.00 (1.00, 4.00) (1.0 - 6.0)	3.00 (2.00, 4.00) (1.0 - 5.0)	p=0.298
The average number of total boluses/day	7.35 (4.90, 7.79) (2.5 - 16.0)	5.30 (3.92, 6.59) (2.0 - 9.4)	p=0.136
The average number of manual boluses/day	5.46 (3.70, 6.63) (2.5 - 14.3)	0.11 (0.00, 1.00) (0.0 - 3.8)	**p<0.001**
The average number of boluses with food/day	0.11 (0.00, 1.67) (0.0 - 3.2)	3.03 (2.25, 4.13) (0.1 - 8.2)	**p<0.001**
The average number of corrective boluses/day	1.07 ± 1.06	1.90 ± 1.39	p=0.055
The average number of boluses given by BC	1.14 ± 1.12	4.63 ± 1.69	**p<0.001**
"Normal" boluses (%)	100.00 (97.60, 100.00) (64.8 - 100.0)	100.00 (93.12, 100.00) (55.6 - 100.0)	p=0.791
"Dual Wave" boluses (%)	0.00 (0.00, 1.08) (0.0 - 35.2)	0.00 (0.00, 6.88) (0.0 - 44.4)	p=0.873
"Square Wave boluses (%)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	p=0.530

differences between the groups. There was a statistically significant difference between the average number of bolus given by bolus calculator between two groups. The patients who used bolus calculator for <50% of total boluses were using bolus calculator to some extent.

According to the use of different types of bolus that bolus option offers, both groups of patients most commonly used "normal" boluses, but bolus calculator users had a slightly higher percentage of using "dual" and "square" boluses than bolus calculator non-users. This difference was not statistically significant (Table 1).

Discussion

Bolus calculator is used in 86% among all patients with T1D on IP therapy in Republic of Srpska. This result is significantly higher compared to results in other studies in which the use of bolus calculator varied from 16% to 58%.[16-18] The reason for high percentage of patients who use the bolus calculator in the Republic of Srpska could be the result of five days FIT education program, which is obligatory for all patients before IP therapy is initiated. In course of FIT program, all the patients were trained for carbohydrate. Observation of a large number of patients who used bolus calculator and who were at FIT program, was also available.[19]

The results from this study regarding the use of bolus calculator correspond to the results from other studies. Klup and colleagues have showed the effect of the use of bolus calculator on postprandial blood glucose level but not on HbA1c level.[20] In 12-months-long controlled randomized study, the use of bolus calculator did not show HbA1C decreasing but there was an effect on postprandial blood glucose level decreasing.[21] In contrast to the above mentioned, there are studies which confirm the effect of the use of bolus calculator on HbA1C decreasing.[22]

The effects of the use of bolus calculator on postprandial glucose decreasing level have been confirmed in numerous studies.[21,23,24] In our study, significant postprandial glucose decreasing level after breakfast was observed, which is a very important result since the highest glucose levels are usually after breakfast. One possibility for improving metabolic control among patients with DT1 could be prevention of postprandial glucose peaks after breakfast.[25]

When it comes to the total number of given bolus, patients who use bolus calculator give more bolus during meal, which should provide more food consumption. However, the patients who used bolus calculator used corrective boluses more frequently. This data is inconsistent with the statement about lesser postprandial increases with bolus calculator use.[15,24]

Walsh and colleagues have emphasized that imprecise insulin/carbohydrate ratio, corrective factor and active insulin time could diminish success of IP therapy, and also the use of "magical numbers" for preprogrammed settings by general practitioners. Due to the above mentioned patients who follow instructions of bolus

calculator have to give more corrective bolus.[10] This point of view can explain a number of corrective bolus among bolus calculator users in our study.

Furthermore, a lack of education of patients who are not educated enough to change upgraded options of bolus calculator as well as the lack of interests and knowledge of professional team to constantly adjust upgraded settings of an insulin pump[26] may explain a low use of different bolus types in our study. In comparison to the normal boluses, the use of combined and square boluses showed a greater effect on the reduction of postprandial excursions for meals composed of fats and those that were composed of slowly absorbed ingredients.[27-30] Well educated patients used a combined bolus more often, and with its usage, the value of HbA1c may be decreased.[27] The results of our study, with patients who mostly used normal type of bolus and who did not use combined or squared boluses, do not confirm good education.

One of the reasons for not using the bolus calculator is avoiding self-control of blood glucose due to the lack of test stripes which patients treated with insulin pump receive from the Health Insurance Fund of the Republic of Srpska. The number of test stripes (100 units/month) is insufficient for the required number of glucose measurements for bolus calculator use. Furthermore, there is no bolus calculator on the market that account the impact of proteins and fats to glycoregulation[30, 31] which could be the point for improved effectiveness of bolus calculator and for the increase of number of patients who have enough confidence in the usage of bolus calculator.

The use of CareLink® Pro (Medtronic, Inc., Northridge, CA, USA) could practically facilitate therapeutic decision for diabetologists. This software can precisely determine how to use an insulin pump and to improve the compliance of patients. It is possible to define the main points in self-control and therapy and to modify certain parameters (basal rates, insulin/carbohydrate ratio, and corrective factor). Furthermore, it could provide precise instructions for nutrition adjustment and physical activity. Using professional software, less time would be spend on data interpretation and more time on conversation with patients about the everyday IP treatment challenges, which could contribute to improving glycemic control.

Conclusion

In order to maximize all the advantages of IP therapy, a regular re-education of both patients and diabetologists about advanced IP functions is needed for improving the glycoregulation in T1DM.

The professional team for IP management should be formed in the Endocrinology Department. The use of CareLink® Pro (Medtronic, Inc., Northridge, CA, USA) could practically facilitate therapeutic decision for diabetologists by giving precise instructions for insulin adjustment, nutrition and physical activity in order to improve the glycemic control.

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Značaj upotrebe bolus kalkulatora za poboljšanje glikoregulacije kod pacijenata na terapiji inzulinskom pumpom

SAŽETAK

Uvod: Bolus kalkulator predstavlja naprednu funkciju inzulinskih pumpi (IP) čijom se upotrebom povećava preciznost izračuna odgovarajuće doze inzulina za obrok, odnosno korektivne doze inzulina, kod pacijenata sa tipom 1 dijabetesa (T1D).

Cilj rada: Uporediti razliku u parametrima glikoregulacije (HbA1c, postprandijalnog porasta glikemije, broja hipoglikemijskih epizoda u nedelji dana) između grupe pacijenata koji bolus kalkulator koriste za <50% ukupno datih dnevnih bolusa i grupe pacijenata koji bolus kalkulator koriste za ≥50% ukupno datih dnevnih bolusa.

Ispitanici i metode: U studiji je učestvovalo 36 pacijenata starijih od 18 godina koji su liječeni IP najmanje godinu dana prije početka istraživanja. Prije inicijacije terapije IP, obavljena je strukturisana edukacija po principima fleksibilne inzulinske terapije u okviru koje su pacijenti obučeni za korištenje metode »brojanja ugljenih hidrata«. Profesionalni softver, CareLink Pro® Software (Medtronic Inc., Northridge, CA, USA) za praćenje liječenja T1D je korišten za preuzimanje podataka sa IP na personalni računar. Podrazumijevana frekvenca upotrebe bolus kalkulatora iznosila je ≥50% svih datih bolusa tokom dana.

Rezultati: Nije uočena statistički značajna razlika ni u HbA1c (6.61 ± 1.10 vs. 6.56 ± 0.84, p = 0.896) niti u broju hipoglikemijskih epizoda (2.00 (1.00, 4.00) (1.0 - 6.0) vs 3.00 (2.00, 4.00) (1.0 - 5.0), p = 0.298) između grupe pacijenata koji su koristili bolus kalkulator za <50% ukupno datih bolusa i grupe pacijenata koji bolus kalkulator koristili za ≥50% ukupno datih bolusa. Pacijenti koji su koristili bolus kalkulator imali su signifikantno manji postprandijalni porast glikemije nakon doručka.

Zaključak: Da bi se maksimalno iskoristile sve prednosti IP, potrebne su redovne reedukacije i pacijenata i dijabetologa o naprednim funkcijama IP, što bi doprinijelo njihovoj redovnoj upotrebi i poboljšanju glikoregulaije u T1D.

Ključne riječi: Inzulinska pumpa, bolus kalkulator, T1D, glikoregulacija

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.08
UDC 618.33-07(497.6RS)
COBISS.RS-ID 6398488

Analysis of the Results of Amniocentesis Performed at the University Clinical Centre of the Republic of Srpska

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ABSTRACT

Introduction: Due to its reliability and relatively low risk, amniocentesis is the most widely used method of prenatal diagnostics, primarily for diagnostics of chromosomal aberrations. Each country has its own specificity in the indications for amniocentesis, and therefore different results.

Aim of the Study: The aim of the study was to analyze the results of amniocentesis performed at the University Clinical Centre of the Republic of Srpska by investigating the distributions of indications for amniocentesis, cytogenetic findings and abnormality rate according to indications.

Patients and Methods: The study retrospectively and prospectively analyzed 3994 cases of amniocentesis performed at the University Clinical Centre of the Republic of Srpska between 2009 and 2014. Cytogenetic findings were grouped according to referral indication. The positive predictive value was calculated for each indication.

Results: The most common indications for amniocentesis were advanced maternal age and abnormal screening markers in maternal serum. Overall abnormality rate was 2.35%. The most frequently found chromosomal aberrations were trisomy 21 and balanced reciprocal translocation. The highest positive predictive value had indications parent carrier of chromosomal aberrations and abnormal ultrasound findings. Far from the expected positive predictive value had indications abnormal screening markers in maternal serum and a family history of chromosomal aberrations or congenital anomalies.

Conclusion: Amniocentesis is a feasible tool for detecting fetal chromosomal aberrations and is mostly performed because of advanced maternal age. Analyzing the results of amniocentesis could help us to improve prenatal detection rate of chromosomal aberrations and give us useful database for proper genetic counseling of pregnant women.

Key words: Amniocentesis, indication, chromosomal aberration, positive predictive value.

(Scr Med 2017:48:53-60)

Submitted: January 18th, 2017
Accepted: February 24th, 2017

Introduction

Chromosomal aberrations are changes in the number or structure of chromosomes. They play an important role in human morbidity and mortality.[1,2] They occur in 4% of all clinically recognized pregnancies, in half of miscarriages, in 5% of stillbirths and in 0.5% - 1% of live births.[3] Chromosomal aberrations cause 20%-30% of all infant deaths. The most common prenatally detected chromosomal aberrations are: trisomy 21 (syndrome Down), trisomy 18, trisomy 13, monosomy X and 47,XXY. Other numerical and various structural chromosomal aberrations, balanced and unbalanced, are rare.

The definite diagnosis of chromosomal aberration in the prenatal period is only possible by performing invasive procedures. Because of its reliability and relatively low risk, the most commonly used procedure is amniocentesis, in which a sample of the amniotic fluid is collected using a needle guided by an ultrasound. The sample is then used for cytogenetic analysis, i.e. analysis of fetal chromosomes. Since there is a possibility of fetal loss or other complications associated with amniocentesis (0,3% - 1,0%), it should be performed only in pregnant woman when the risk of a fetal chromosomal aberration is higher than the risk of the procedure.[4,5]

In order to determine this „high risk" group of pregnant women, some screening approaches have been developed during the years. Advanced maternal age was the most common indication for amniocentesis in the past. Penrose has proved the connection between a maternal age and the prevalence of the Down syndrome in 1933.[6] However, if maternal age over 35 is used as the only indication for amniocentesis, the incidence of Down syndrome will be reduced for less than 20%. Therefore, during the past forty years, many studies have aimed at developing other methods of screening, using maternal serum markers and ultrasound, as well as increased awareness of risk based on an adverse family and obstetric histories.

Each country has its own specificity in the indications for amniocentesis, and therefore different results. University Clinical Center of the Republic of Srpska is the only institution in our country that performs genetic counseling and prenatal cytogenetic diagnostics. Analyzing the results of amniocentesis could help us to improve prenatal detection rate of chromosomal aberrations by upgrading the screening methods. Also, identification of major epidemiological characteristics of prenatally detected chromosomal aberrations may provide us with valuable information for genetic counseling followed the pregnant woman's decision in electing appropriate testing options.

Aim of the Study

The aim of the study was to analyze the results of amniocentesis performed at the University Clinical Centre of the Republic of Srpska in the first five years, from January 2009 to January 2014, by investigating the distributions of indications, cytogenetic findings and abnormality rate according to indications.

Patients and Methods

This study retrospectively and prospectively analyzed 4036 cases of amniocentesis performed at the University Clinical Centre of the Republic of Srpska from January 2009 to January 2014. Clinical data were obtained from records of the genetic counseling. Every woman referred for amniocentesis was initially counseled with a clinical geneticist and signed an informed consent. Amniocentesis was done by trained gynecologist, between 16 and 18 weeks of pregnancy. A sample of the amniotic fluid is collected using a needle guided by an ultrasound. The amniotic fluid cells were in-situ cultured on, at least, two coverslips, harvested by conventional G-banding technique, and then analyzed. In some cases, analysis was completed by the C-banding technique. Some parents were required to analyze lymphocyte karyotype to help judging the origin of abnormal fetal karyotype. Subsequently, 42 specimens were further excluded from this study because of unsuccessful cultivation or contamination with mother cells.

A total of 3994 cytogenetic findings were grouped according to referral indications: advanced maternal age (age 35 years and older), abnormal screening markers in maternal serum, abnormal ultrasound findings, family history of chromosomal aberrations or congenital anomalies, previous fetus/child with chromosomal aberration, parent carrier of chromosomal aberration, history of recurrent abortions or unexplained death in utero and other indications (advanced paternal age, parental diseases, personal reasons, etc.). In cases with multiple indications, the leading one has been taken into account.

All detected chromosomal aberrations, according to ISCN 2009,7 including mosaic cases, have been classified into the following categories: numerical autosomal chromosomal aberrations, numerical sex chromosomal aberrations, marker chromosomes, polyploidies, structural balanced and structural unbalanced chromosomal aberrations. Variant chromosomes were considered to be normal.

Positive predictive value (PPV), which represents overall frequency of chromosomal aberrations among groups, was calculated for each indication. Also, frequencies and

proportions of different categories of aberrations were calculated by referral indications.

Data used for analysis contained no identifiable personal information in order to protect individuals' privacy. Statistical analyses were performed using the statistical package SPSS. The obtained data were expressed as counts and percentages. Differences among groups were evaluated by using chi-square test. A two-tailed $p \leq 0.05$ was considered to indicate statistical significance.

Results

The most frequent referral indication for amniocentesis (Table 1) in our study was advanced maternal age (60.7%), followed by an abnormal screening markers in maternal serum (24.01%), family history of chromosomal aberrations or congenital anomalies (4.51%) and abnormal ultrasound findings (4.38%).

Table 1. Distribution of indications for amniocentesis and their PPV in detection of chromosomal aberrations

Referral indication	Number of amniotic fluid specimens		Chromosomal aberrations	
	N	%	N	% (PPV)
Advanced maternal age	2435	60.97	57	2.34
Abnormal screening markers in maternal serum	959	24.01	13	1.36
Family history of chromosomal aberration or congenital anomalies	180	4.51	1	0.55
Abnormal ultrasound findings	175	4.38	12	6.86
History of recurrent abortions or unexplained death in utero	108	2.70	5	4.63
Previous fetus/child with chromosomal aberration	51	1.28	0	0
Parent carrier of chromosomal aberration	17	0.43	6	35.29
Other	69	1.73	0	0
Total	3994	100	94	2.35

A total of 94 chromosomal aberrations were found among 3994 amniotic fluid specimens, so overall abnormality rate was 2.35%.

Parent carrier of chromosomal aberration showed the highest PPV in prenatal detection of chromosomal aberrations among indications (35.29%) (Table 1). Abnormal ultrasound findings showed the second highest PPV (6.86%). There was only one case of chromosomal aberration among those pregnant women who underwent amniocentesis because of family history of chromosomal aberrations or congenital anomalies, so PPV of this indication was 0.55%.

Numerical aberrations were seen in 57 (59.57%) and structural in 38 cases (40.43%). The majority of chromosomal abnormalities were numerical autosomal aberrations (39.36%). Trisomy 21 was the most common abnormality (32.98%). The second most frequent findings were structural balanced aberrations (24.47%), with balanced reciprocal translocation accounting for over half of this group (12.77%) (Table 2).

The most of chromosomal aberrations were found among advanced maternal age group (57/94; 60.4%), followed by groups with abnormal screening markers in maternal serum (13/94; 13.83%) and abnormal ultrasound findings (12/94; 12.77%) (Table 1).

The advanced maternal age accounted for over half of the referral indications (60.97%). There was no significant difference in overall frequency of chromosomal aberrations between this group and the group of younger women who done amniocentesis because of other reasons. Only three categories of chromosomal aberrations had significantly different proportions between these groups: balanced and unbalanced reciprocal translocations had significantly lower proportion ($p=0.011$ and $p=0.030$) and inversions had significantly higher proportion ($p=0.049$) in the advanced maternal age group (Table 3).

Table 2. Frequency of chromosome aberrations

CHROMOSOMAL ABERRATIONS	N	%
NUMERICAL	56	59.57
Numerical autosomal chromosomal aberrations	37	39.36
Trisomy 21	31	32.98
Trisomy 18	4	4.26
Trisomy 13	2	2.13
Numerical sex chromosomal aberrations	13	13.83
45,X	3	3.19
45,X/46,XX	3	3.19
45,X/46,XY	1	1.06
chi 46,XX/46,XY	1	1.06
47,XXX	2	2.13
47,XXY	3	3.19
Marker chromosomes	5	5.32
In all cels	2	2.13
Mosaic	3	3.19
Poliploidy	1	1.06
STRUCTURAL	38	40.43
Structural balanced chromosomal aberrations	23	24.47
Robertsonian translocations	5	5.32
rob(DqDq)	2	2.13
rob(DqGq)	3	3.19
Reciprocal translocations	12	12.77
Inversions	6	6.38
Structural unbalanced chromosomal aberrations	15	15.96
Robertsonian translocations	1	1.06
Reciprocal translocations	3	3.19
Deletions	2	2.13
Duplications	8	8.51
Isochromosomes	1	1.06
Total	**94**	**100**

Table 3. Comparison of proportion of chromosomal aberrations between the group with indication of advanced maternal age and the group with other indications

CHROMOSOMAL ABERRATIONS	Referral indications:				p
	Advanced maternal age		Other		
	N	% of CA	N	% of CA	
NUMERICAL	35	61.40	21	56.76	0.813
Numerical autosomal CA	22	38.60	15	40.54	0.850
Trisomy 21	21	36.84	10	27.03	0.437
Trisomy 18	1	1.75	3	8.11	0.140
Trisomy 13	0	0	2	5.41	0.077
Numerical sex CA	9	15.79	4	10.81	0.541
45,X	2	3.51	1	2.70	0.839
45,X/46,XX	1	1.75	2	5.41	0.326
45,X/46,XY	1	1.75	0	0	0.424
chi 46,XX/46,XY	0	0	1	2.70	0.211
47,XXX	2	3.51	0	0	0.258
47,XXY	3	5.26	0	0	0.166
Marker chromosomes	3	5.26	2	5.41	0.965
In all cells	2	3.51	0	0	0.258
Mosaic	1	1.75	2	5.41	0.326
Poliploidy (69,XXX)	1	1.75	0	0	0.424
STRUCTURAL	22	38.60	16	43.24	0.697
Structural balanced CA	13	22.81	10	27.03	0.661
Robertsonian transl.	4	7.01	1	2.70	0.383
Reciprocal transl.	3	5.26	9	24.32	0.011
Inversions	6	10.53	0	0	0.049
Structural unbalanced CA	9	15.79	6	16.22	0.939
Robertsonian transl.	0	0	1	2.70	0.211
Reciprocal transl.	0	0	3	8.11	0.030
Deletions	2	3.51	0	0	0.258
Duplications	7	12.28	1	2.70	0.124
Isochromosomes	0	0	1	2.70	0.211
Total	57	100	37	100	0.949

CA = chromosomal aberration(s)

Numerical autosomal aberrations and sex chromosomal aberrations were the most frequently identified when the indication was abnormal ultrasound findings, followed by advanced maternal age and abnormal screening markers in maternal serum (Table 4).

Table 4. The frequency and the proportion of chromosomal aberrations by referral indications

Indication	Numerical autosomal CA	Numerical sex CA	Marker chromoso-mes	Poliploidy	Structural balanced CA	Structural unbalanced CA
Advanced maternal age	0.90%	0.37%	0.12%	0.04%	0.53%	0.37%
	22/2435	9/2435	3/2435	1/2435	13/2435	9/2435
Abnormal screening markers in maternal serum	0.73%	0.20%	0%	0%	0.21%	0.21%
	7/959	2/959	0/959	0/959	2/959	2/959
Abnormal ultrasound findings	4.57%	1.14%	0.57%	0%	0%	0.57%
	8/175	2/175	1/175	0/175	0/175	1/175
Family history of CA or congenital anomalies	0%	0%	0%	0%	0%	0.56%
	0/180	0/180	0/180	0/180	0/180	1/180
Previous fetus/child with CA	0%	0%	0%	0%	0%	0%
	0/51	0/51	0/51	0/51	0/51	0/51
Parent carrier of CA	0%	0%	0%	0%	29.41%	5.88%
	0/17	0/17	0/17	0/17	5/17	1/17
History of recurrent abortions or unexplained death in utero	0%	0%	0.93%	0%	2.78%	0.93%
	0/108	0/108	1/108	0/108	3/108	1/108
Other	0%	0%	0%	0%	0%	0%
	0/69	0/69	0/69	0/69	0/69	0/69

CA = chromosomal aberration(s)

Therefore, these indications had the highest PPV in detection numerical chromosomal aberrations. On the other hand, structural, both balanced and unbalanced, chromosomal aberrations were the most frequently identified when the parent was carrier of chromosomal aberration, followed by history of recurrent abortions or unexplained death in utero (Table 4).

Discussion

The most frequent referral indication for amniocentesis performed in University Clinical Center of the Republic of Srpska between January 2009 and January 2014 was advanced maternal age. This indication had 60.97% of pregnant women. This result is similar to result in the report of Milošević Đerić et al. (63%) and smaller than results of the other studies from region (78% - 80%).[8-10] In contrast, in countries in which prenatal screening policy is adopted, the proportion of this indication was even smaller (18% and 45%).[9,10] This is due to the fact that most developed countries have national guidelines that recommend fetal aneuploidy screening prior to invasive testing. In the Republic of Srpska, however, fetal aneuploidy screening using a screening ultrasound and markers in maternal serum hadn't been routinely offered to pregnant women before 2012.

A total of 94 chromosomal aberrations were found among 3994 amniotic fluid specimens, so overall abnormality rate was 2.35% which was similar to that reported in previous studies in other countries (1.67% - 3%).[7-10,12-17] However, it varied by indication. The highest PPV in detection of chromosomal aberrations had indication parent carrier of chromosomal aberration (35.29%). Women with this indication have the highest risk of getting a child with unbalanced karyotype. Abnormal

ultrasound findings showed the second highest PPV (6.86%) which was similar to that reported in other studies (5.9% and 11.8%).[10,12] This indication had the highest PPV in detection numerical autosomal (4,57%) and sex chromosomal aberrations (1.14%). PPV of indication advanced maternal age was 2.34% which was similar to that reported in other studies (2.3% - 4.5%).[9,10,12,18] This indication had the highest PPV in detection numerical autosomal aberrations (0.90%).

PPV of indication abnormal screening markers in maternal serum was 1.36%, which was similar to that in studies in region (1.40% and 1.60%),[8,9] but was significantly smaller to that reported in countries with long history of prenatal screening (2.60% and 3.50%).[12,13]

Indication family history of chromosomal aberrations or congenital anomalies had very small PPV in prenatal detection of chromosomal aberrations in our study (0.55%). Other studies have reported significantly higher PPV of this indication (1. 0% and 3.70%).[9,10] In order to do less amniocentesis among this group of women, we should better investigate family history and recommend fetal aneuploidy screening prior to invasive testing, especially in those women who have a family member with free trisomy 21. In the group with other indications (advanced paternal age, parental diseases, personal reasons, etc.), there were no detected chromosomal aberrations. This result was similar to that reported in other numerous studies.[18-20]

The most frequent abnormal finding was trisomy 21 (32.98%), followed by balanced reciprocal translocation (12.77%). There was no significant difference in overall frequency of chromosomal aberrations between the group with indication advanced maternal age and the group with the other indications.

Conclusion

Amniocentesis is a feasible tool for detecting fetal chromosomal aberrations. It is mostly done because of advanced maternal age and it should be offered to pregnant women over 35. Amniocentesis is particularly useful when a parent is a carrier of chromosomal aberration or when fetal ultrasound is abnormal, because these cases belong to the group with the highest risk.

Analyzing the results of amniocentesis could help us to improve prenatal detection rate of chromosomal aberrations and give us useful database for proper genetic counseling of pregnant women. Our results suggest that we should improve screening method that uses markers in maternal serum and better inform pregnant women who come for other reasons, especially for family history.

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Analiza rezultata amniocenteza urađenih u Univerzitetskom kliničkom centru Republike Srpske

SAŽETAK

Uvod: Zbog svoje pouzdanosti i relativno malog rizika, amniocenteza je najrasprostranjenija metoda prenatalne dijagnostike, prvenstveno dijagnostike hromozomskih aberacija. Svaka zemlja ima svoje specifičnosti po pitanju indikacija za amniocentezu, a samim tim i različite rezultate.

Cilj rada: Cilj rada je bio analizirati rezultate amniocenteza urađenih na Univerzitetskom kliničkom centru Republike Srpske, kroz prikaz distribucije indikacija za amniocentezu, rezultata citogenetskih analiza i stope detekcije hromozomskih aberacija prema indikacijama.

Ispitanici i metode: Retrospektivno-prospektivnom studijom smo analizirali rezultate 3994 amniocenteze urađene na Univerzitetskom kliničkom centru Republike Srpske između 2009. i 2014. godine. Citogenetski nalazi su podijeljeni u grupe zavisno od indikacije za amniocentezu. Za svaku indikaciju smo izračunali pozitivnu prediktivnu vrijednost.

Rezultati: Najčešće indikacije za amniocentezu su bile starija dob majke i rezultat skrininga na osnovu markera u krvi majke. Patoloških nalaza je bilo 2,35%. Najčešće hromozomske aberacije su bile trizomija 21 i balansirana recipročna translokacija. Hromozomska aberacija kod roditelja i odstupanje u ultrazvučnom nalazu su bile indikacije sa najvećom pozitivnom prediktivnom vrijednosti. Daleko manju pozitivnu prediktivnu vrijednost od očekivane su imale indikacije: rezultat skrininga na osnovu markera u krvi majke i porodična anamneza o hromozomskim aberacijama ili kongenitalnim anomalijama.

Zaključak: Amniocenteza omogućava uspješnu prenatalnu detekciju hromozomskih aberacija i najčešće se izvodi zbog starije dobi trudnice. Analiza rezultata amniocenteze može nam pomoći da povećamo prenatalnu stopu detekcije hromozomskih aberacija i daje nam korisnu bazu podataka za genetičko savjetovanje trudnica.

Ključne riječi: Amniocenteza, indikacije, hromozomske aberacije, pozitivna prediktivna vrijednost.

ORIGINAL ARTICLE

doi:10.18575/msrs.sm.e.17.09
UDC 615.381:616.9-036.22
COBISS.RS-ID 6399512

First Results in Genotyping for Blood Donors of the Republic of Srpska with Serological Weak D Antigen

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ABSTRACT

Introduction: The Rh system is very complex, polymorphous and the most significant for clinical practice, along with the ABO blood group system. The D antigen is the most important antigen in the Rh system and the most immunogenic one, following the ABO antigens. The D antigen, which consists of a mosaic of epitopes, is determined in all the blood donors and patients. Most people are either RhD positive or RhD negative, but there is a certain number of people who have a variation of the D antigen, which are called weak D, partial D and DEL phenotypes.

Aim of the Study: The objective is to use molecular methods to determine whether blood donors in the Republic of Srpska (with whom a serological weak D antigen has been detected) really have the weak D antigen, partial D, a combination of these two variants or if their D antigen is normally present, but the used anti-D serum tests did not have the avidity needed to prove the presence of this antigen in blood donors.

Patients and Methods: Blood samples were used from regular blood donors, who had been determined as persons with a weaker D antigen (based on the agglutination strength) using serological techniques, the test tube method, the microplate method and the gel method. To determine the blood groups and red blood cell/erythrocyte antigen typing, the following methods were applied: a) test tube method or agglutination in an aqueous environment, b) gel method, c) microplate method and d) molecular determination of blood groups.

Results: Blood group samples were collected from April 2016 to February 2017 in the Institute for Transfusion Medicine of Republika Srpska. During this period, blood was collected from 8153 voluntary donors. It was serologically proved that 40 donors (0.49%) had the weak D antigen. All results where the weak D antigen was determined serologically were confirmed by molecular testing. 23 respondents were proved to have weak D type 3 (0.28%), while 17 had weak D type 1 (0.20%).

Conclusion: The results from the first molecular testing of our population is in accordance with the results of frequency of weak D antigen in the populations of other European countries, though it did show a small advantage of weak D type 3 over weak D type 1.

Keywords: The Rh system, antigen D, weak D, partial D, molecular method

(Scr Med 2017:48:61-67)

Submitted: February 18th, 2017
Accepted: March 2nd, 2017

Introduction

The Rh system is very complex, polymorphous and the most significant for clinical practice, along with the ABO blood group system. The D antigen is the most important antigen in the Rh system and the most immunogenic one, following the ABO antigens. The terms "Rh positive" and "Rh negative" refer to the presence or absence of the D antigen. In the mid-1940s, four anti-liquid antigens were identified: C and c, E and e. They were named by Fisher, who continued the nomenclature established by Landsteiner with the ABO system. Even though 50 antigens of this system have been discovered to this date, a set of five basic antigens, which are also called the Rh phenotypes, are responsible for the formation of the most clinically significant antibodies with Rh specificities.[1,2]

The Rh system consists of 50 antigens, ranging from Rh 1 to Rh 57. There are several theories regarding the genetic inheritance of the system Rh antigens. At present, it is clear that there are two gene structures, RHD and RHCE and that the RHD gene encodes the formation of the D antigen, while the RHCE gene is responsible for the formation of the CE antigen in various combinations (ce, cE, Ce or CE). Molecular testing has confirmed the accuracy of this theory. These two genes have opposite orientations on the chromosome, but are identical to a large extent (approximately 97%), and each consists of ten exons. As opposed to most blood groups systems whose antigens are coded by one gene, the Rh system antigens are coded by two genes. The fact that these genes are located on the same chromosome very close to one another refers to the possibility of exchange of material between them and explains the complexity of the Rh system. The exchange between the genes RHD and RHCE leads to the formation of new polymorphic proteins which are responsible for the emergence of a multitude of antigens belonging to the Rh system.[1,2,3-6] Genes which control the Rh system antigens are located on the chromosome 1p36.13-p34.3.[1,6]

Approximately 85% of Caucasians are RhD positive, as well as approximately 95% of the population in Africa south of Sahara and approx. 99,5% of people in the eastern Asia. It has been determined that between 1 and 2% persons of European descent has one of the D antigen variants, while their frequency is higher with the African population.[7,8]

The D antigen is presented as a mosaic of epitopes, and those individuals whose erythrocytes do not have some of those parts (following the exposure to the complete D antigen) form anti-D antibody against the missing epitope. In contact with a normal D antigen, this antibody behaves as any other anti-D antibody. Most people are either RhD positive or RhD negative, but there is a certain number of people who have a variation of the D antigen, which are called weak D, partial D and DEL phenotypes.

The term D weak has traditionally meant the complete D antigen, which has all the epitopes, but weakly expressed. For a number of years, it was considered that separation of the phenotype D weak from partial D had significance for clinical practice, because individuals with the phenotype D weak will not form anti-D antibody after receiving D-positive blood, given that their D antigen is normally built, even though being weaker.

As opposed to them, it was considered that individuals with partial D can form anti-D antibody (after receiving D-positive blood) against the part of the D antigen which they are lacking, and should therefore only receive D-negative blood.[9-11]

However, this assumption was diminished after it was noted that polypeptide D with individuals with the phenotype weak D is not normally built and that people with this phenotype can form anti-D antibody. Many examples of D weak and partial D have been examined on the molecular level.[12-17]

This is how it was concluded that it is very important to separate weak D from partial D antigen in the clinical practice, because patients with the D weak phenotype will not form anti-D antibody after the application of D-positive blood (given that their antigen is weakened, but normal). As opposed to them, patients with partial D antigen can be immunized on the part of the D antigen they themselves are lacking and should therefore receive transfusion with D-negative blood.

Detection of anti-D antibodies with individuals with weak D type 15 has shown that this division is not sufficiently clear or precise, if the definition that only individuals with partial D form anti-D antibody is correct. Essentially, the phenotype D weak type 4.2 is functionally identical to the partial antigen DAR, even though it has been described that erythrocytes of these two phenotypes lead to formation of anti-D antibodies in recipients.[10]

Erythrocytes of the phenotype weak D type 2 have the lowest density of all the most common types of the weak D antigen. Therefore, Flegel and associates have recommended that erythrocytes of the phenotype weak D type 2 are a threshold for the detection of anti-D test reagents and should be used as part of quality control in routine immunohematological work.[18] This is why the validity of the assumption that individuals with a weak D phenotype cannot form anti-D antibody and that they cannot cause an immune response with D-negative recipients has been questioned.[10-11]

With his research, Flegel has showed that there are different nucleotide substitutions in the RHD gene which lead to the exchange of amino acids in the erythrocytes samples with the weak D phenotype, which indicate their abnormal structure and individuals with this phenotype can form anti-D antibody.[12-17]

The so-called partial RhD phenotypes were marked as DIIIa, DVI, DBT and DFR. They can mutually be separated by serological and molecular methods, and at least one individual with any of these phenotypes has formed anti-D antibody. Forms of weaker D phenotype are marked with numbers from weak D type 1 to weak D type 135, and there are also a number of intermediate forms (for instance, weak D.-4.2), where the number continues to rise.

These weaker forms of D antigen are connected to a smaller expression of the D antigen on the erythrocyte membrane, but they can be mutually differentiated exclusively by the application of molecular test methods. More to this, some forms, especially the phenotypes of the weak D.-4.2, -15 and -21 have been determined in patients who have formed anti-D antibody.[19-20] The most common of the weakly expressed D antigen is type 1, which is determined by the mutation 809T>G in exon 6.

Single nucleotide polymorphism, SNP, in the gene is expressed as the mutation V270G in the RhD protein and together with types 2 and 3, it represents approximately 90% of the weak D phenotypes found in individuals of European descent.[21-22] The weakly expressed D antigen type 3 is determined by the mutation 8C>G in exon 1. The SNP in the gene RHD is expressed as the mutation S3C in the RhD protein.

According to the published data, from the overall number of tested samples, the frequency of weak D type 1 is 70%, for weak D type 2 18%, while the frequency for weak D type 3 is 5%. Weak D type 1 and type 3 are typical for the haplotype DCe, weak D type 2 for the haplotype DcE.[19,20-23]

Aim of the Study

1. The objective is to use molecular methods to determine whether blood donors in the Republic of Srpska (with whom a serological weak D antigen has been detected) really have the weak D antigen, partial D, a combination of these two variants or if their D antigen is normally present, but the used anti-D tests serum did not have the avidity needed to prove the presence of this antigen in blood donors;

2. The objective is to determine the frequency of the proven weak and partial D antigens and compare it with the frequency of weak and partial D antigens in other Caucasians.

Patients and Methods

Blood samples were used from regular blood donors, who had been determined as persons with a weaker D antigen (based on the agglutination strength) using serological techniques, the test tube method, the microplate method and the gel method, according to the instruction of the manufacturer of the test serum.

Two blood samples were taken from each respondent, with EDTA in the amount of 5ml: one for the serological confirmation of the respondent's RhD status, and the other for molecular examinations.

To determine the blood groups and red blood cell/ erythrocyte antigen typing, the following immuno-serological methods were applied, according to the described standard operating procedures:

1. The test tube method or agglutination in an aqeous environment. The reaction takes place in the test tube following the mixing of erythrocyte suspension and test serum. Test serums of a known specific quality are used to determine the presence or absence of a certain antigen (serums of Biognost Zagreb, Novaclone Canada, Sanguin Holland, Immucor USA, Diagast France),whereas erythrocytes of an identified phenotype are used to detect antibodies.[24-26]

2. Gel method.This method is based on the use of microtube plates containing gel particles. Each gel card contains six microtubes for six equal or different tests. Gel particles are spherical and have the function of reaction media and filter in the test. They contain erythrocyte agglutinate, and they let free erythrocytes through to the bottom. In that way, the reading of the gel particles reaction result is easier and harmonized.[24-27]

3. Microplate method. This method is used to determine erythrocyte antigens or antibodies. Small amounts of erythrocytes and test serums are added to microplate tubes, which are then centrifuged. Erythrocytes are resuspended from the bottom of the recess by putting the plate on the mixer or by manually shaking the plate, whereafter the reaction is being read either manually or by an automatic reader. In the case of negative reaction, erythrocytes are fully resuspended in the serum, without any visible agglutinates. The advantage of this method as opposed to the conventional tube agglutination method is the lower

consumption of reagents.[24,25]

4. In this study, the process implies automatic examination of ABO and RhD antigen blood groups, as well as Rh phenotypes from the donor's samples on the device Techno (DiaMed),[28] by applying the microplate method, as well as the gel method, by DiaMed test serums. In this way we have electronic entry in the Lira reader and any error in copying is being avoided. Serological examination of D antigen variants with the commercial panel (BioRad, Diagast).

5. Molecular determination of blood groups, with special reference to the D antigen.

Molecular diagnostics in terms of proving the RHD gene will be done on all blood donors who are serologically confirmed to be RhD-negative, as is the case with individuals with Ccddee and ccddEe phenotypes, or those with serologically weak positive D antigen. FluoGene is a unique method for molecular examination of HLA, erythrocyte and thrombocyte genes, which combines all the SSP-PCR, i.e. polymerase chain reactions and speed the fluorescent detection has as the final point of examination. The analysis is based on the specifically modified system TaqMan® tests, fluorescence of which is detected in the Fluo Vistadevice of the manufacturer Inno-train Diagnostik, Germany. Advantages of this method are: a) PCR-SSP method without gel electrophoresis, by which the analysis is made in 90 minutes; b) there is no hybridization and washing processes; c) the overview of the gained results is fully objective, software-wise, immediately following the reading; d) there is no risk of post-PCR contamination; d) DNA consumption is low; e) the software is easy to use, it has a completely automatic reading of the results.[29]

Additional advantages of the Fluogene system include the testing possibility to get the results of low DNA concentration samples (c<10 ng / μL), as well as minimum risk of sample contamination, which remain sealed during the test.[29]

FluoGene tests: DNA is isolated by the isolation method on silicone colons that have the specific ability to connect and release the DNA in puffers of different pH (Ready DNA Isolation Spin Kit, Inno-train Diagnostik, Germany). Following the DNA isolation, the testing plate is prepared (tests: RBC-Fluogene Dweak/variant and RBC-Fluogene CDE,Inno-train Diagnostik, Germany) and it contains primers lyophilized in advance in the wells and TaqMan tests by pipetting the mixture FluoMixa (containing DNA, dNTP, Mg2+, Taq polymerase) and DNA according to the instructions of the manufacturer. The primers are dependent on the sequences of certain gene variants determined to, as a rule, have primers in each well for mutually different DNA segments. The prepared plate was put in the FluoVista apparatus to have the basic fluorescence intensity (pre-PCR) read. In accordance with the manufacturer's protocol, the polymerase chain reaction (PCR) was done in 40 cycles, in the apparatus Eppendorf – vapo.protect, mastercycler (Eppendorf, Germany). Following the PCR in the apparatus FluoVista, the fluorescence intensity was read after the PCR (post-PCR). For the positivity of the well, the difference between post-PCR and pre-PCR fluorescence intensity is essential. The wells where the difference in intensity is above the level defined by the manufacturer were marked as positive. The software automatically calculated the difference between the fluorescence intensity and determined the Dweak type, i.e. RHD and/or RHCE phenotype, which was displayed as the result for the envisaged phenotype on RHD and/or RHCE locus.

Results

Blood group samples were collected from April 2016 to February 2017 in the Institute for Transfusion Medicine of Republika Srpska. During this period, blood was collected from 8153 voluntary donors. It was serologically proved that 40 donors (0.49%) had the weak D antigen (Table 1).

Table 1. The number of tested RhD samples

RhD	n (%)
RhD +	6791 (83.29)
RhD -	1322 (16.22)
Rh D weak	40 (0.49)
TOTAL	8153 (100.0)

The respondents belonged to the category of regular blood donors, who had donated blood at least twice before this study and who were serologically proven to have weak D. Their results upon the testing of presence of the markers for transfusion transmissible diseases were negative.

In the sample, most of the blood donors were men (31 donor, 77.5%) while 9 donors were women (22.5%). This was expected given that men are more represented as blood donors in our population (approximately 82%) as opposed to women (approximately 18%).

The analysis of Rh phenotypes showed most of the respondents were found to have the Rh phenotype

CcDwee – 39 (97.5%) and only one had the phenotype CCDwee (2.5).

All results where the weak D antigen was determined serologically were confirmed by molecular testing. In 23 respondents, it was proved that they had weak D type 3 (0.28%), while 17 had weak D type 1 (0.20%) (Table 2).

Table 2. Molecular testing of the samples serologically proven as weak D

D weak	n (%)
D weak type 1	17 (0.20)
D weak type 3	23 (0.28)
TOTAL	8153 (100.0)

Discussion

The prevalence of the serologically proven weak D phenotype varies among races and nations.[30] These forms of the weak D antigen are most proven D variants in Europe and the USA. It is estimated that 0.2% to 1.0% Caucasians inherits the RHD gene, which encodes the formation of the serologically weak D phenotype.

Most often, these are weak D types 1, 2 and 3.[30-32] The results of the prevalence of the serologically weak D phenotype also vary depending on the applied test method (manual test tube method as opposed to automatic testing), the anti-D reagents used (polyspecific serum as opposed to monoclonal blend) and on the use of reaction enhancers (bromelin).[33] Most data on RHD alleles and risk of alloimmunization in serologically weak D phenotypes result from studies conducted in central Europe.[30,34-37] These studies indicate that blood recipients, who have weak D type 1, 2 or 3, in a homozygous or hemizygous form, do not have a risk of forming anti-D antibody after receiving RhD-positive erythrocytes with normally expressed D.[35,36]

Approximately 95% of Caucasians in central Europe who have been determined to have the weak D antigen have the weak D type 1, 2 or 3. They are treated as RhD-positive and can receive RhD-positive blood transfusions. The absence of anti-D antibodies in individuals of the mentioned phenotypes seems to be the consequence of the fact that different RHD alleles encode formation of all epitopes of the RhD antigen in these individuals, as opposed to individuals with normally expressed D antigen, even though the antigen density on the surface of the erythrocyte with weak D type 1, 2 or 3 is smaller than the ones with normal D.[30]

The alloimmunization to the D antigen and anti-D antibody was proven with some other types of weak D antigen, such as weak D type DAR,[30] type 11,[35,37] type 15,[35,37] type 21[38] and type 57.[39]

The results from the first molecular testing of our population is in accordance with the results of frequency of weak D antigen in the populations of other European countries,[30,34-37] although it did show a small advantage of weak D type 3 in relation to weak D type 1. This initial study has showed that the algorithm of serological testing of the D antigen in the Institute for Transfusion Medicine Republika Srpska correlates with the current immunohematological practice in the world.[40] Fluogene method is reliable, precise, fast and very suitable for molecular determination of RHD and RHCE phenotype. The results are available within 2 hours of the test start.[41]

Conclusion

The first molecular examinations on frequency of weak D antigens in the population of blood donors of the Republic of Srpska pointed to good results in the application of contemporary immunohematological recommendations in the testing of D antigens with blood donors in the Institute for Transfusion Medicine Banja Luka, but they also pointed to the necessity of applying the same testing mode in all centers in the Republic; it further pointed to the necessity of determining the frequencies of all RHD alleles in the Republic of Srpska, in order to improve the immunohematological testing of D antigens with blood donors and pregnant women by introducing molecular methods in the routine work; the first results showed that the donors who were molecularly confirmed to have the weak D antigen belonged to the categories that were treated as RhD-positive, and should they need blood they may also receive RhD-positive blood. This is how RhD-negative blood reserves are preserved. This examination should be expanded on all pregnant women with serologically weak D antigen, in order to determine those that have weak forms 1, 2 and 3 and do not have to receive RhD immunoprofylaxis, so that the reserves of this valuable immunoglobulin may be preserved.

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Prvi rezultati u genotipizaciji serološki slabih oblika D antigena kod davalaca krvi u Republici Srpskoj

SAŽETAK

Uvod: Sistem Rh je veoma kompleksan, polimorfan i, pored sistema ABO, najznačajniji za kliničku praksu. Antigen D je najvažniji antigen sistema Rh i najimunogeniji poslije antigena ABO. Svim davaocima krvi i pacijentima određuje se antigen D, koji je sačinjen od mozaika epitopa. Većina ljudi je ili RhD pozitivna ili RhD negativna, ali postoji i određen broj osoba sa varijantama antigena D, takozvanim slabim D (D weak), parcijalnim D i fenotipom DEL.

Cilj rada: Molekularnim metodama utvrditi da li davaoci krvi u populaciji Republike Srpske, kod kojih je serološki određen slab oblik antigena D, zaista imaju slabo izražen antigen D, parcijalni antigen D, kombinaciju ova dva tipa, ili je njihov antigen D normalno izražen, ali korišćeni anti-D test serum nije imao aviditet dovoljan za dokazivanje ovog antigena kod davalaca krvi;

Ispitanici i metode: Koristili smo uzorke krvi redovnih dobrovoljnih davalaca koji su, na osnovu jačine aglutinacije, serološkim tehnikama, metodom u epruveti, metodom u mikroploči i metodom u gelu određeni kao osobe sa slabije izraženim antigenom D. Za određivanje krvnih grupa i tipizaciju eritrocitnih antigena koristili su se sljedeće imunoserološke metode, prema opisanim standardnim operativnim procedurama: a) Metoda u epruveti ili aglutinacija u tečnoj sredini; b) Metoda u gelu; c) Metoda u mikropločama; d) Molekularno određivanje krvnih grupa.

Rezultati: Uzorci dobrovoljnih davalaca su prikupljeni u periodu od aprila 2016.god. do februara 2017.god. u Zavodu za transfuzijsku medicinu Republike Srpske. U tom periodu je prikupljena krv od 8153 davaoca. Kod 40 davalaca (0,49%) je serološki dokazan slab D antigen. Svi ispitanici kojima je serološki dokazan slab D antigen su potvrđeni i molekularnim testiranjem. Kod 23 ispitanika je dokazan D weak tip 3 (0,28%), a kod 17 ispitanika D weak tip 1(0,20%).

Zaključak: Prva molekularna ispitivanja naše populacije u skladu su sa rezultatima ispitivanja o učestalosti slabog antigena D u populacijama drugih naroda Evrope, mada malu prednost ima slabi D tip 3 u odnosu na slabi D tip 1.

Ključne riječi: Th Rh sistem, D antigen, slabi D (Dweak), parcijalni D, molekularna metoda

REVIEW ARTICLE

doi:10.18575/msrs.sm.e.17.10
UDC 616.12-008.331.1:616.24
COBISS.RS-ID 6399768

Diagnosis and Treatment of Pulmonary Hypertension

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ABSTRACT

Pulmonary hypertension (PH) is a hemodynamic and clinical state defined as an increase in mean pulmonary arterial pressure ≥25mmHg at rest. Five groups of patients have been defined: group 1 as pulmonary arterial hypertension (PAH), group 2 as PH due to left heart disease, group 3 as PH due to lung diseases, group 4 as chronic thromboembolic PH, and group 5 as PH of other causes. PAH is a rapidly progressive and fatal disease with an incidence of 3 cases per million whereas incidence of PH due to left ventricular dysfunction is as high as 60-70% of all cases. Pulmonary capillary wedge pressure, invasively measured at rest, has been used to distinguish between pre- (≤15mmHg) and post-capillary (>15mmHg) PH. The early clinical symptoms and signs are subtle and non-specific, such as exertional dyspnea, fatigue, pre-syncope and progressive limitation of exercise capacity so the vast majority of patients have an advanced disease with World Health Organization functional class of III or IV at first presentation. The diagnostic approach in PH has the goal to evaluate the two main anatomic components: pulmonary vasculature and right ventricle in order to establish the diagnosis and identify the group of PH. The therapy for PAH patients includes three main components: general measures and supportive therapy; initial therapy with calcium channel blockers in vasoreactive or specific drugs approved for PAH in non-vasoreactive patients either single or in combination, and lung transplantation. All patients with PAH should be referred to PH expert centers for comprehensive diagnostic and therapeutic assessment.

Keywords: Pulmonary hypertension,ventricle, pulmonary arterial pressure

(Scr Med 2017:48:68-76)

Submitted: February 10th, 2017
Accepted: February 28th, 2017

Definition

Pulmonary hypertension (PH) is an increase of the mean pressure in the pulmonary artery (mPAP) and its value ranges from 25mmHg upwards. Depending on the hemodynamics, it is crucial to differentiate between "pre-capillary" and "post-capillary" PH.

Pre-capillary PH implies mPAP ≥25mmHg and pulmonary capillary pressure (PCWP) ≤15mmHg, which is why this type of hypertension is referred to as pulmonary arterial hypertension (PAH). On the other hand, post-capillary PH is defined as an increase of mPAP ≥25mmHg, whereas PCWP >15mmHg and it is commonly the PH resulting from the left heart weakness.[1]

The increase of pulmonary vascular resistance (PVR) is the basis of vascular pathophysiology in PH and it results in the escalation of pressure in pulmonary artery, which further causes pressure-load in the right ventricle (RV). Compensatory mechanisms induce the right ventricular dilatation so the basic determinants of symptomatology and patient prognosis are actually the pulmonary arterial pressure values and RV function.

Classification of pulmonary hypertension

Over the last decade, there have been a few changes in PH classification, especially when it comes to specific groups of patients. The most recent classification was adopted at the 2013 World Symposium on Pulmonary Hypertension held in Nice, according to which PH is divided into five groups with clearly defined sub-categories (Table 1).[1]

Table 1. Clinical classification of pulmonary hypertension.

1. Pulmonary arterial hypertension
1.1 Idiopatic
1.2 Heritable
1.3 Drugs and toxins induced
1.4 Associated with:
1.4.1 Connective tissue disease
1.4.2 Human immunodeficiency (HIV) infection
1.4.3 Portal hypertension
1.4.4 Congenital heart disease
1.4.5 Schistosomiasis
1*. Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
1**. Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension due to left heart disease
2.1 Left ventricular systolic dysfunction
2.2 Left ventricular diastolic dysfunction
2.3 Valvular disease
2.4 Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
2.5 Congenital/acquired pulmonary veins stenosis
3. Pulmonary hypertension due to lung disease and/or hypoxia
3.1 Chronic obstructive pulmonary disease
3.2 Interstitial lung disease
3.3 Other pulmonary disease with mixed restrictive and obstructive pattern
3.4 Sleep-disordered breathing
3.5 Alveolar hypoventilation disorders
3.6 Chronic exposure to high altitude
3.7 Developmental lung diseases
4. Chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions
4.1 Chronic thromboembolic pulmonary hypertension
4.2 Other pulmonary artery obstructions
4.2.1 Angiosarcoma
4.2.2 Other intravascular tumors
4.2.3 Arteritis
4.2.4 Congenital pulmonary arteries stenoses
4.2.5 Parasites (hydatidosis)
5. Pulmonary hypertension with unclear and/or multifactorial mechanisms
5.1 Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis, neurofibromatosis
5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4 Others: pulmonary tumoral thrombothic microangiopathy, fibrosing mediastinitis, chronic renal failure (with/without dialysis), segmental pulmonary hypertension

Clinical features

Symptoms and signs of PH are usually nonspecific which frequently results in delayed diagnosis and adequate therapy application. A most common first symptom is the shortness of breath (dyspnea) during physical activity accompanied by general ailment and disability. Other symptoms which may appear later are as follows: vertigo, disturbance of consciousness, chest pain and pressure, leg swelling (a sign of right ventricular failure). Depending on to which extent the symptoms prevent patients from physical activities, we distinguish between four functional classes of patients (WHO FC) (Table 2). The functional class of patients suffering from PH is directly proportional to the survival rate so that patients from WHO FC IV have the rate of survival <40% on the three-year level.[2]

Table 2. World Health Organization (WHO) functional class of patients with PAH.

Functional Class	Symptoms
I	Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause dyspnoea or fatigue, chest pain, or near syncope.
II	Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
III	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
IV	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

Diagnosis

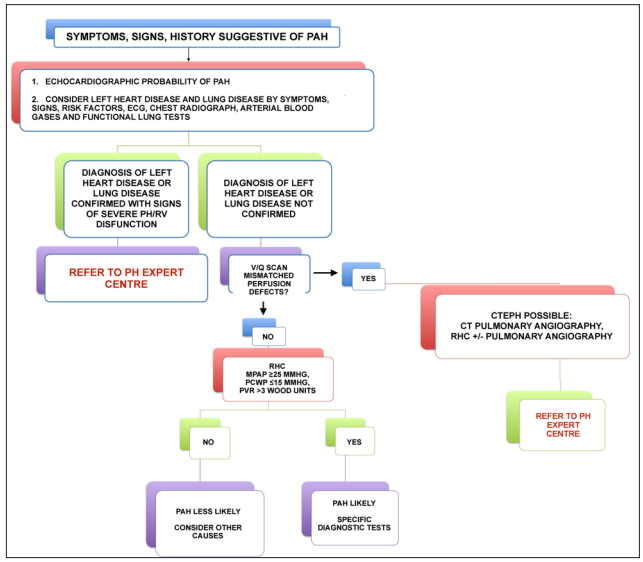
The PAH diagnosis begins with anamnesis, i.e. the estimation of symptoms which might indicate PH. Graph 1 displays an algorithm of procedures once we suspect of PAH. Electrocardiographic changes with patients suffering from PAH are nonspecific and they indicate the right ventricle ballast: right axis deviation, ST segment depression and/or negative T waves in right precordial leads (V1-V3), right bundle branch block. The patients showing signs of right ventricular failure suffer from the common malignant disorder of heart rhythm, which is why it is crucial to periodically perform the 24-hour ECG monitoring.

At the moment of PAH diagnosis, more than 90% patients have already experienced changes in heart and lung X-rays although these changes are of poor sensitivity.[3,4] Typical changes are as follows: "lifting" of heart top due to right ventricular hypertrophy, right ventricular enlargement, the expansion of the main branch of pulmonary artery and/or right interlobar artery, and significant reduction of peripheral pulmonary vascular network.

Pulmonary function tests and analysis of arterial blood gas may identify the presence of respiratory diseases and pulmonary parenchyma illness. Patients suffering from PAH usually have mild to moderate lung volume reduction depending on the disease severity. The carbon monoxide diffusing capacity (DLCO) less than 45% is a bad prognosis sign and its differential diagnosis with PAH patients may indicate pulmonary veno-occlusive disease, PAH associated with scleroderma and parenchymal pulmonary disease. The chronic obstructive pulmonary disease which causes hypoxic PH is diagnosed on the basis of the irreversible airflow obstruction accompanied by an increase of residual volume, DLCO decrease, partial oxygen pressure (PaO_2), and increase of partial pressure of carbon dioxide ($PaCO_2$).

The transthoracic echocardiogram (TTE) is a widespread non-invasive cardiovascular diagnostic procedure pertinent for PAH patients as it helps set diagnosis and monitor the patients. TTE enables us to acquire a whole range of information as follows: evaluation of systolic, mean and diastolic pulmonary artery pressures, analysis of morphology and function of right ventricle and estimation of echocardiographic predictors of clinical outcomes in PAH patients.

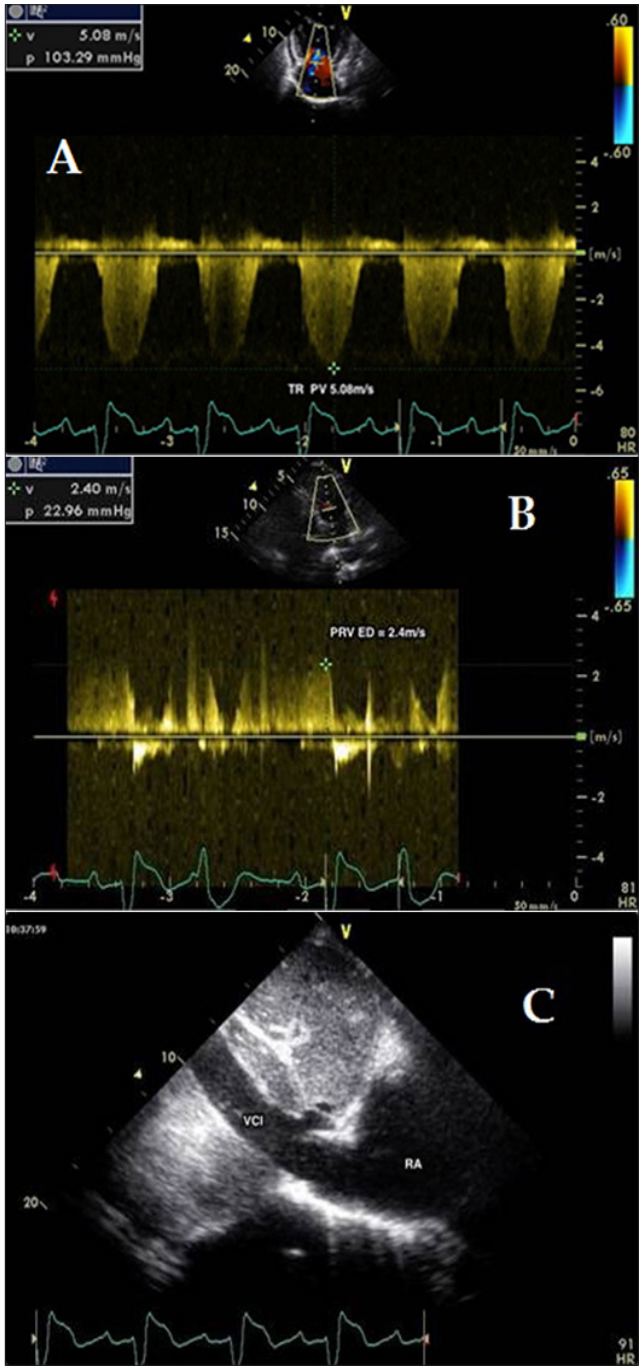
Graph 1. Algorithm for diagnosing pulmonary arterial hypertension (PAH).



**p *adapted from: Galie N et al. Eur Heart J. 2016;37(1):67-119.(4)*
PH, pulmonary hypertension; RV, right ventricle; V/Q scan, a lung ventilation-perfusion scan; RHC, right heart catheterization; mPAP, Mean Pulmonary Arterial Pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; CTEPH, Chronic thromboembolic pulmonary hypertension , CT, a

computed tomography

Picture 1. Echocardiographic evaluation of pulmonary artery pressure.



Picture 1 displays the usage of continuous-wave Doppler (CW) in order to show regurgitation of tricuspid (Picture 1, A) and pulmonary (Picture 1, B) valves in PAH patients. Systolic pressure in pulmonary artery (SPAP) equals the right ventricle systolic pressure (RVSP) and it is calculated on the basis of maximum speed of tricuspid regurgitation (TRPV) and estimated right ventricle pressure (RAP) in line with the following formula: RVSP = SPAP = 4xTRPV2 + RAP. The evaluation of the right atrial pressure (RAP) is performed on the basis of diameter of vena cava inferior (VCI) and respiratory variations of its diameter: with IVC >2.1 cm diameter and <50% diameter collapse with deep inspirium, the evaluated RAP is 15 mmHg (Picture 1, C). The diastolic pulmonary arterial pressure (DPAP) is calculated on the basis of the end-diastolic velocity of the pulmonary regurgitation (PRVED) in line with the following formula: DPAP = 4xPRVED2 + RAP (Picture 1, B). The mean pulmonary arterial pressure (MPAP) is calculated on the basis of systolic and diastolic pressures in line with the following formula: MPAP = 1/3 SPAP + 2/3 DPAP.[4-6]

High-Resolution Computed Tomography (CT) provides us with pertinent information on potential changes in pulmonary parenchyma and enables us to eliminate emphysema, bronchitis and interstitial lung disease diagnosis, infarction, and vascular and pericardial malformations. It may also be useful to diagnose pulmonary veno-occlusive disease with typical pulmonary abnormalities such as “milk glass image”, interstitial edema and bilateral interlobular septal thickening.[7]

Speaking of the acute pulmonary embolism diagnosis, the CT angiography has been a widely used method of choice and it has practically replaced the ventilation-perfusion lung scan. On the other hand, V/Q scan is a method of choice with diagnosing the chronic thromboembolic pulmonary hypertension (CTEPH).[8-10] The criterion for diagnosing CTEPH on a V/Q scan is at least one massive defect after a minimum three-month effective anticoagulation therapy. The V/Q scan is 90-100% sensitive and 94-100% specific for diagnosing CTEPH. Possible errors for diagnosing CTEPH are caused by minor perfusion-like defects or nonsegmental perfusion abnormalities typical of otherwise caused PAH and pulmonary veno-occlusive disease.[11] In addition, the classical segmental perfusion defects may disappear during the CTEPH terminal stage.[12] Perfusion scintigraphy appears nonsegmented in cases of large central thrombotic masses in Eisenmenger syndrome or thrombus within aneurysm of pulmonary trunk or pulmonary artery branches in idiopathic PAH.[13]

Laboratory tests of blood, and biochemical and immunological tests are an integral part of etiological treatment of patients suspected of PH and other organ failures. Other routine analyses are thyroid hormone values and transaminase values, particularly after introduction of endothelin receptor antagonist therapy (ERA). Serological tests are compulsory in order to

diagnose potential connective tissue disease (CTD), hepatitis and human immunodeficiency virus (HIV) hidden behind PH. Up to 40% of patients suffering from idiopathic PAH have high antinuclear antibodies but in low titers (1:80).[14]

In most cases, TTE is an initial diagnostic method when there is a suspicion of PH, whereas right heart catheterization (RHC) is a necessary invasive diagnostic procedure for the definite diagnosis and evaluation of pulmonary vascular reactivity.[4] The left heart catheterization is simultaneously performed with patients in risk of coronary heart disease or left heart insufficiency with preserved left ventricular systolic function (HFpEF). It is recommended to perform RHC at specialized clinics as the procedure itself is technically demanding and might cause serious complications. Basic parameters crucial for monitoring with RHC are as follows: mPAP (mean pulmonary arterial pressure), PCWP (pulmonary capillary wedge pressure) and PVR (pulmonary vascular resistance). If it is not possible to measure PCWP, we determine left ventricular end-diastolic pressure in left ventricle (LVEDP). Vasoreactivity tests are recommended in all patients with idiopathic PAH, hereditary PAH and PAH accompanied by usage of weight-loss medications in order to determine patients who might be introduced with calcium channel blockers (CCB). Positive vasoreactivity tests are defined as a decrease of mPAP ≥10mmHg and absolute values of mPAP ≤40mmHg with an increased or unaltered stroke heart volume. In order to perform a vasoreactivity test, it is advised to use nitrogen monoxide (NO) or intravenous epoprostenol, whereas we may use adenosine or inhaled iloprost as the alternative.[14-16] CCB therapy is absolutely contraindicated if the vasoreactivity test is negative.

Therapy

PAH therapy is a complex strategy which might be divided into three basic steps as follows: the initial approach and application of general measures, introduction of CCB therapy (only patients with positive vasoreactivity test) and/or specific PAH therapies, and finally, the third step which entails monitoring of the initial therapy response, introduction of combined PAH therapy, patient care during the terminal disease stage, and determining indications for lung transplantation.

General measures and supportive therapy. The recommendation is a regular physical activity which does not provoke symptoms and mandatory avoidance of severe physical exhaustion. It has been proven that the functional capacity and life quality of PAH patients who practise controlled physical activity are better that in those patients who are physically inactive.[15-17] PAH patients are advised to have influenza and pneumococcal pneumonia vaccines as these cause 7% of total deaths with this group of patients.[14,15]

PAH supporting therapy entails the oral anticoagulant therapy, oxygen therapy, right heart insufficiency therapy, and correction of anemia syndrome. The oral anticoagulant therapy is indicated only in patients with idiopathic and hereditary PAH and PAH due to weight-loss medication abuse.[16-18] It is well-known that PAH patients also suffer from coagulation disorder and physiological fibrinolysis so it is crucial to take into account risks of venous thromboembolism (heart weakness and immobilization) and hemorrhage before the introduction of oral anticoagulant therapy. Particular caution should be paid in patients suffering from Eisenmenger syndrome.[18-20] The oxygen therapy is indicated in all PAH patients with pO2 <60mmHg (8kPA) as hypoxia is one of major causes of vasoconstriction and this therapy decreases PVR. The usage of diuretics is indicated in all PAH patients with signs of right heart weakness or water retention. Digoxin improves stroke volume only in cases of acute aggravation in patients suffering from idiopathic PAH but its efficiency has not been proven for chronic usage.[21] Digoxin is administered to PAH patients with acute aggravation primarily in order to slow down ventricular response in cases of atrial cardiac rhythm disturbance. The usage of ACE inhibitors, sartans, beta blockers and ivabradine is not recommended except in cases when these are an irreplaceable comorbidity therapy (eg. arterial hypertension, coronary heart disease). Sideropenic anemia is registered with around 43% idiopathic PHA patients and 56% Eisenmenger syndrome patients, in which cases it also predicts mortality so it is crucial to monitor the iron values and substitute the treatment if necessary.[22-24] Eisenmenger patients are a particularly sensitive group because frequent and unfounded venipuncture cause these patients severe anemia syndrome, which further increases mortality rates.[25] Therefore, prior to venipuncture, these patients should be examined by cardiologists specialized in PAH treatments.[26]

Specific therapy. The specific PAH patient therapy covers the following classes of medications: CCB, endothelin receptor antagonists (ERA), phosphodiesterase type 5 inhibitors (PDE-5i) and guanylate cyclase stimulators (sGC), prostacyclin analogues and prostacyclin receptor antagonists. It is well-known that only few patients suffering from idiopathic PAH have positive vasoreactivity test, which is the only indication for CCB therapy. The CCBs used in PAH therapy are nifedipine, diltiazem and amlodipine. In addition, their regular dosage is 120-240mg for nifedipine, 240-720mg for diltiazem and up to 20mg for amlodipine, depending

on tolerance.4 Endothelin receptor antagonists (ERA) are widely used in PH therapy due to the fact that these patients also suffer from activation in endothelial cells in both plasma and pulmonary tissue although it is still not quite clear if the increase of plasma endothelin-1 level causes PH or results from it.[27,28] ERA medications used in current PH therapy for diagnostics and treatment of PAH are ambrisentan, bosentan and macitentan.[4]

The fact that pulmonary vascular network contains certain amounts of phosphodiesterase type 5 is the basis for the application of PDE-5i in PH therapy due to both consequential vasodilatation and antiproliferative effect. All three PDE-5 inhibitors approved for treatment of erectile dysfunction (sildenafil, tadalafil, vardenafil) cause massive vasodilatation of pulmonary vascular network.[29,30] Unlike PDE-5i, sGC (riociguat) increases cyclic guanosine monophosphate production (cGMP) and causes vasodilatation and antiproliferative effect.[31] The efficiency of riociguat application in PH therapy has been proven positive when combined with ERA or prostanoid therapy in 2.5mg three times per day dosage in sense that it improves functional capacities of patients as well as hemodynamic parameters.[32] Combination of riociguat and PDE-5i therapies is contraindicated due to strong hypotension.

The usage of prostacyclin analogues and prostacyclin receptor antagonists in PH therapy is based on the role and relevance of prostacyclin in PH pathogenesis. Prostacyclin, produced primarily by endothelial cells, is a potent vasodilator and endogenous inhibitor of platelet aggregation and it also has proven cytoprotective and antiproliferative properties. Clinical use of prostacyclin in PH therapy is enabled due to synthesis of stable prostacyclin analogues which have different pharmacokinetic properties but still similar pharmacodynamic effects. Medications from this class approved for PH treatment are beraprost, epoprostenol, iloprost and treprostinil and their most common indications are for treatment of WHO FC III and IV. Selexipag, a selective IP prostacyclin receptor agonist is available for oral usage as well as monotherapy or supplement to mono and dual therapy for PH patients in WHO FC II and III with ERA and/or PDE-5i in which cases it decreases morbidity and mortality per 40%.[33]

Combined therapy and transplantation. The combined PH therapy implies simultaneous usage of two or more PH medicaments from different classes in which process it is possible to initiate therapy with one medicament and then introduce medicaments from another class or initially start therapy with two medicaments from different classes. The initial combined therapy is justified by the fact that PH patients share high mortality rates with many other malignant diseases.[4]

Balloon atrial septostomy (BAS) is a palliative method of PH treatment which results in the interatrial right-left shunt targeting at the right heart decompression, improvement of left heart function, and enhancement of stroke volume. Some published studies demonstrate benefits of this treatment with patients in WHO FC IV who show signs of right heart weakness and who are refractory to optimal medication therapy and suffer from sever syncope.[34-36] In addition, the treatment is optional for patients awaiting lung transplantation who show no significant clinical improvement after the maximum combined medicament therapy.

Transplantation is a final therapy for PH patients who remain in WHO FC III or IV despite the maximum combined and supportive medicament therapy. Delayed transplantation combined with long waiting lists due to the pragmatic lack of organ donors increases mortality rates and causes clinical aggravation at the moment of transplantation. Recent data indicate additional 5-year life span with 52-75% of PH patients after transplantation and additional 10-year life span with around 45-66% of these patients.[37] PH patients are administered heart-lung transplantations as well as double-lung transplantations even though there is no information on the limits of irreversible stress of right ventricular systolic function and/or left ventricular diastolic function.[38] According to the International Registry for Heart and Lung Transplantation, most patients worldwide have double-lungs transplants.[39] Patients suffering from Eisenmenger syndrome and simple shunts have lung transplantations and surgical corrections of congenital heart defects or heart and lung transplantations.[40]

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Dijagnostika i terapija plućne hipertenzije

SAŽETAK

Plućna hipertenzija (PH) predstavlja hemodinamski poremećaj definisan kao porast srednjeg pritiska u plućnoj arteriji od 25mmHg ili više u miru. Razlikuje se pet grupa pacijenata: grupa 1- plućna arterijska hipertenzija (PAH), grupa 2- PH kao posljedica bolesti lijevog srca, grupa 3- PH kao posljedica bolesti pluća, grupa 4 - hronična tromboembolijska PH i grupa 5- PH drugih uzroka. PAH je brzo progresivna i fatalna bolest sa incidencom od oko 3 slučaja na milion stanovnika, dok je incidenca PH kao posljedice bolesti lijevog srca prisutna kod čak 60-70% ovih pacijenata. Plućni kapilarni pritisak, mjeren invazivno u miru, omogućava razlikovanje prekapilarne (≤15mmHg) i postkapilarne (>15mmHg) PH. Rani klinički simptomi i znaci su veoma diskretni i nespecifični u obliku dispneje pri naporu, slabosti, presinkope i progresivnog smanjenja tolerancije fizičkog napora te najveći broj pacijenata ima teži stadijum bolesti sa funkcionalnom klasom Svjetske zdravstvene organizacije III ili IV pri prvom pregledu. Dijagnostički proces PH ima za cilj evaluaciju dvije osnovne anatomske komponente: plućna vaskulatura i desna komora srca sa ciljem postavljanja dijagnoze i identifikovanja grupe PH. Terapija pacijenata sa PAH se sastoji iz tri osnovna koraka: opšte mjere i suportivna terapija; inicijalna terapija blokatorima kalcijumskih kanala kod vazoreaktivnih odnosno specifičnih lijekova za PAH kod ne-vazoreaktivnih pacijenata bilo pojedinačno ili u kombinacijama te transplantacija pluća. Sve pacijente sa PAH je potrebno uputiti u ekspertske centre za dijagnostiku i liječenje PH.

Ključne riječi: Plućna hipertenzija, desna komora, plućni kapilarni pritisak

SPECIAL ARTICLE

doi:10.18575/msrs.sm.e.17.11
UDC 612.018:543.3
COBISS.RS-ID 6401560

Chemism and the Role of Endocannabinoids in Physiological Processes

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ABSTRACT

Despite being known for 5000 years, after the records of imperial Chinese doctors, cannabinoids as a subject of scientific research experienced its rise after 1964, when delta nine tetrahydrocannabinol (Δ 9 THC) by Israeli scientists was identified. This was followed by the discovery of endogenous ligand / endocannabinoids, as well as receptors CB1 and CB2.
In a broader sense, endocannabinoids act as neuromodulators and immunomodulators. They are included in the various physiological processes such as: the occurrence of pain, cognition, memory formation and neuroplasticity, physical activity, respiratory processes, appetite regulation, control and heart rate, nausea and emesis, intraocular pressure, inflammatory and immune processes (antigen recognition).

Key Words: Endocannabinoids, anadamide, cannabinoid receptors.

(Scr Med 2017:48:77-81)

Submitted: October 22nd, 2016
Accepted: November 22nd, 2016

Introduction

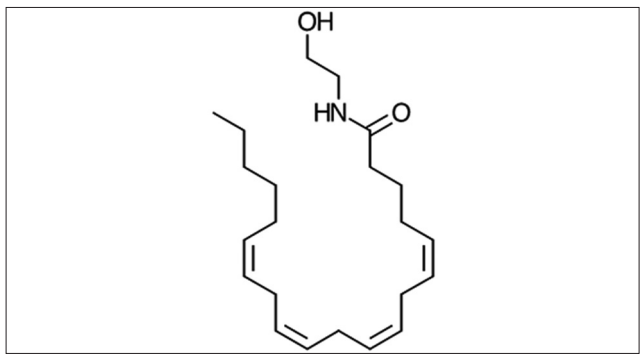
Endocannabinoids were named after a plant cannabis or fitocannabinoids. Studying fitocannabinoids has resulted in the development of the most important physiological endocannabinoid system (ECS) that is important for the establishment and maintaining homeostasis in humans.

In 1964, dr. Raphael Mechoulam, dr. Lumir Ondrej Hanus and their followers at the Hebrew University in Jerusalem identified Δ (delta) -9-tetrahydrocannabinol (THC) in cannabis. The following research led to the development of a receptor on which THC is connected as well as the development of endocannabinoid and endocannabioid system.[1]

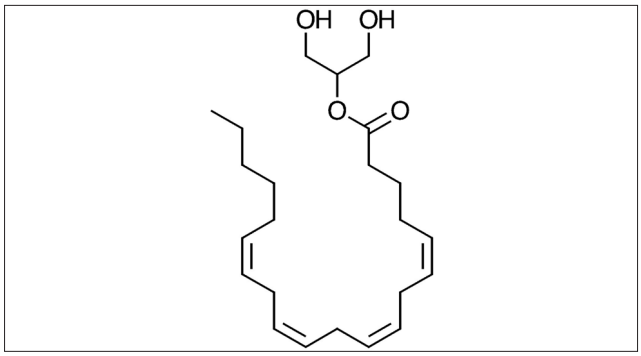
Endocannabinoids are endogen agonist/ligand of cannabinoid receptors CB_1 and CB_2 which are produced in mammalian tissues.

The first endogen cannabinoid anandamid (arahidoniletanolamid or AEA) was isolated and described by Czech chemist Ondrej Lumir Hanus and American molecular pharmacologist William Anthony Dekane in 1992.The name itself was given according to the Sanskrit word ananda which means blissfulness.[2] Shortly after the discovery of the first one, the other endogenous cannabinoid, 2-arahidonilglicerol (2-AG), was developedby Shimona Ben-Shabat, one of the first students of the above mentioned doctors.

Picture 1. Anandamide (AEA) (3).



Picture 2. 2-arachidonyl-glycerol(2-AG) (4).



Endocannabinoids are derived from arachidonic acid and they can be amids, ester or eter with long unsaturated fat acids. According to their own chemism, they are hydrophobic molecules and hence they do not migrate through the body so their engagement is local, surrounded by the space that is close to synthesis molecules endocannabinoids.[3-6]

The most examined endocannabinoids (Picture 1 and 2) are arachidonyletanolamid (anandamide, AEA) and 2- arachidonyl-glycerol (2-AG). The theory, accoridng to which their synthesis appears when neccessary, was refuted and it was proved that anandamide was and is contained in some cells. The other representatives of endocannabinoid are: 2-arachidonyl-glycerol (noladin), O- arachidonyletanolamin (virodhamine), N-arachidonyldopamine and others.

Synthesis of anandomide is carried out parallely in a few different ways upon which are contained different enzymes (phospholipase D, phospholipase C, α, β, hydrolase 4 and different phospholipases). Precursor for the synthesis of anandamide is a membrane phospholipid N- arachidonyle phosphatidyl- ethanolamine.[6]

Synthesis of anandomide in neuron is stimulated by a binding of a neurotransmitter that is released from presynaptic neuron in an adequate inotrope or metabotropic receptor on postsynaptic neuron.

This process causes increased cytosolic free calcium-ions concentration in postsynaptic neuron which represents a stimulant for synthesis and releasing endocannabinoids from their precursors in a membrane. The released endocannabinoids are bound to receptors CB1 on presynaptic membrane. After that, activated receptors inhibitate potassium canals that are dependent on power.

Due to the increased diffusion of potassium ions, depolarization of presynaptic membrane is decreased. The consequence is the inhibition of neurotransmitters release such as glutamate, dopamine and γ- aminobutyric acid (GABA).[7,8]

Termination of biological results of anandamide is carried out in two parts. In the first part, anandamide migrates into the cell, while in the second part, it is decomposed with the help of hydrolase amid fatty acid FAAH (Fatty Acide Amide-Hydrolase).[9] Bearing in mind that anandamide is a lipofiln molecule, it can migrate into the cell via passive diffusion in the direction of concentrated gradient. The transport anandamide in the cell is also enabled via selective transport molecule called AMT (anandamide membrane transporter), which is placed in the membrane plasma and acts reversible. There are two other known mechanisms by which it is possible to endorse anandamide in the cell, that is, with intracellular membrane vesiculum and with the help of endocytosis.[10-14]

Using transgenic animal models, without genes for receptors CB_1 and CB_2, other receptors on which endocannabinoid is carried out were gradually developed. Receptors $TRVP_1$ (transient receptor potential cation channel subfamily V member vaniloid receptor) participate in the regulation of body temperature and system of nocicepsys that medites signals to pain stimuli. Receptors GPR55 (protei-coupled receptor) has 13% of homology with receptors CB1 and 14 % of homology with receptor CB2. It is placed in the brain, liver, spleen, intestines, fetal tissues and placenta. It is a very serious candidate to be named receptor CB1.[15,16]

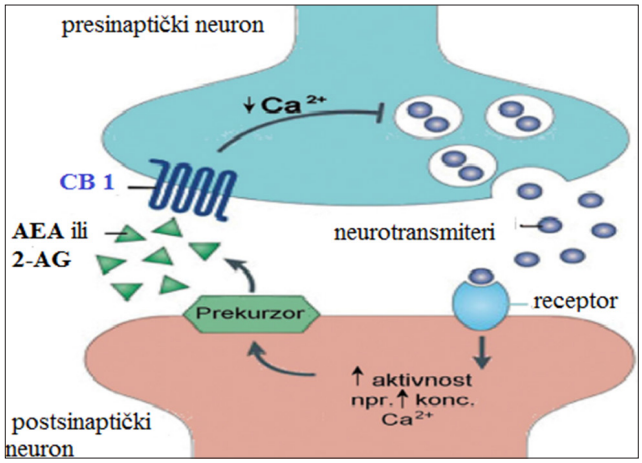
The role of endocannabinoid in physiological processes

In wide range, endocannabinoids work as a neuromodulator and immunomodulator. They are included in different physiological processes such as: pain, cognitive processes, memory formation and neuroplasticity, motoric activities, endocrine processes, regulation appetite, control and puls, nausea, intraocular pressure, inflammatory and immune processes (antigen).[17,18]

Endocannabinoids are intracellular transmitters (vectors) of signals close to the neurons in synapsis. Since they are lipophilic molecules, they are not contained in intracellular vasculum but after synthesis, they become a part of cellular membrane. It is characteristic for them to participate in retrograde signalling between neurons, which means that the signal, instead from presynaptic neuron, travels to postsynaptic neuron in another way (Picture 3). Endocannabinoids are released in the synaptic cut from the postsynaptic neuron and act on presynaptic nerve endings. Activation of cannabinoid receptors

on presynaptic neuron for the small amount of time inhibitates the release of the second neurotransmitter. The final result is dependent on the type of neuron which is inhibitated from cannabinoid such as, for example, inhibition of excitatory neurotransmitters such as glutamate leads to inhibition of an excilatory neuron, while inhibition of releasing inhibitive neurotransmitter GABA (gamma-aminobutyric acid) leads to the increasment of excitability.[19]

Picture 3. Schematic diagram of endocannabinoid activity in presinapse and retrogressive modulation of releasing neurotransmitters glutamate and GABA (gamma-amino fatty acid), AEA (arachidonyletanolamid), 2-AG (2-arachidonyl-glycerol) (20).



Endogenous cannabinoid receptors

The first proof of the presence of cannabinoid receptors was the evidence that THC inhibitates adenyl cyclase, after which studies that used radioligandi followed.[19,20] The first cannabinoid receptor CB1 was cloned in 1990 from the cell of a mouse brain, while the other cannabinoid receptor CB2 was cloned in 1993 from human cells of promyelocytic leukemia HL-60.[21,22]

Cannabinoid receptors are divided in two types - CB_1 and CB2. They are rated as high affinity 7-transmembrane receptors that are connected with G protein. Activation of receptors causes the induction of different intracellular processes: inhibition of adenyl cyclase and, as a consequence, concentration of CAMP decreases (adeonid cyclic 5- monophosphate), activation with mitogen- activated protein kinase ERK (extracellular signal-regulated kinase), amino terminal kinase, activation of phosphatidylinositol 3-kinase, degradation of sphingomyelin, as well as the ceramide occurence.[23-25]

Receptors CB_1 and CB_2 have just 44% conguence with the chain amino acids while in the domen of binding, they have more similarity in sequences of amino acids (68%), which is the reason why some ligands do not make difference in binding to CB_1 or CB_2 receptors. It is interesting that human receptors CB_1 have high similarity to other animal types (monkeys 100%, rats 97%, mice 96%).[26]

Receptors CB_1

Receptors CB_1 are present in the central nervous system as well as in the periphery tissues. In the brain, they are in the parts that control motor activities (basal ganglia and cerebellum), memory and cognitive functions (cortex and hypocampus), emotions (amigdala), sensory perceptions (thalamus), autonomy and endocrine functions (hypothalamus, pons and medul). Distribution of CB_1 receptors in the brain is in accordance with famous pharmacodinamic results regarding the effects of cannabinoid on memory, cognitive activities, pain and movement. Low concentration of receptors CB_1 is present in a brainstem, extended spinal cord and tilamus. The above mentioned explains why cannabinoids do not cause mortality due to dangerous acts on vital physiological functions in cases of ingestion of a very high dose of cannabinoid. According to the data published in American Scientist in December 2015, mortality assosiated with the use of cannabis in the USA was around 0, as well as in 2014 and, in contrast to alcohol, which caused 30700 deaths in 2015.[27] On the periphery, they are located in testicles, vascular endothelium, spleen and periphery nerves.[1,21,23,28]

Receptors CB_2

In comparison with CB_1 receptors, CB_2 receptors are primarily manifested in the cells of the immune system and they participate in modulation of functional ascpect of the immune system. The large number of receptors CB_2 is expressed in lymphocytes B and in natural cells killers (natural killers NK). Aditionallly, they are present in tonsils, spleen and in lymph nodes. Some cannabinoids act on validus receptors and T-type calcium channels. It is generally considered that psychoactive cannabinoid results are dispatched through CB_1 receptors, while immunomodular ones are dispatched through CB_2 receptors.[21,28-30]

Conclusion

The oldest records regarding the medical use of cannabis are known since the time of Chinese imperial herbalist Shen Nung 5000 years ago, who recommended cannabis for curing malaria, beriberi, constipation, rheumatological pains, shortage of concentration and woman problems. The famous doctor of that time in China, Hua Tuo, used the mixture of cannabis resin and

wine as analgesic in cases of surgery. Medical use of cannabis is also well known in all old civilizations such as India, Mesopotamia and Ancient Egypt.[30,31]

Although cannabinoids have been used for centuries, the range of studies that examines the consequences of cannabinoid has developed in the last 50 years, after the development of cannabinoid receptors and their endogen ligand. The research is not only conducted in the field of basic mechanism of cannabinoid activity but in the developing pharmacological and therapeutic results of cannabinoid. Until now, there are more than 150000 studies connected to the theme of endocannabinoid systems, endocannabinoid as well as pharmacological and therapeutic activity of phytocannabinoid. The fact that more and more countries all over the world legalize the use of medical cannabis speaks in favor of justification of clinical studies carried out with the usage of cannabinoid, as well as the validity of positive results that are obtained by these studies.

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Hemizam i uloga endokanabinoida u fiziološkim procesima

SAŽETAK

Iako poznati već 5000 godina po zapisima carskih kineskih ljekara, kanabinoidi kao predmet naučnih istraživanja doživljavaju svoj uspon nakon 1964. godine kada je od strane izraelskih naučnika identifikovan delta devet tetrahidrokanabinol (Δ 9 THC). Nakon toga uslijedilo je otkriće endogenih liganda kao i receptora CB_1 i CB_2 na koji se vežu endokanabinoidi.

Endokanabinoidi u širem smislu djeluju kao neuromodulatori i imunomodulatori. Uključeni su u različite fiziološke procese kao što su: pojava boli, kognitivni procesi, formiranje pamćenja i neuroplastičnost, motoričke aktivnosti, endokrini procesi, regulacija apetita, kontrola i broj otkucaja srca, mučnina i povraćanje, intraokularni pritisak, inflamatorni i imunološki procesi (prepoznavanje antigena).

Ključne riječi: Endokanabinoidi, anandamid, kanabinoidni receptori.

CASE REPORT

doi:10.18575/msrs.sm.e.17.12
UDC 616.717.2-001:616.833-009.11
COBISS.RS-ID 6401816

Medial Clavicular Epiphysiolysis with Anterior Dislocation

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ABSTRACT

Fractures of the medial portion of the clavicle occur infrequently in children and account for only about 5% of all pediatric clavicular fractures. In many males, a clavicular medial physis does not close completely until 24 to 26 years of age.1 In our case report, 17-year-old male was injured following a direct fall on shoulder. In the Emergency Department, we identified a medial clavicle physeal fracture with anterior dislocation after standard chest radiography and "serendipity" view. In total intravenous anesthesia, we reduced the fracture and immobilized with Desault cast for 21 days and figure- of- eight dressing 3 weeks more. We didn't notice any functional deficit nor cosmetic defect.

Keywords: Physeal clavicle fracture, Hobbs view, "serendipity" view.

(Scr Med 2017:48:82-84)

Submitted: February 20th, 2017
Accepted: March 1st, 2017

Introduction

Physes are a specific layer of tissue, unique to immature bone, providing longitudinal and diametrical bone growth. The growth of primary and secondary ossification center narrows the space of physis and eventually, it completely disappears and the growth stops.[1] Epiphysiolyses include all fractures that affect the coupling cartilage. In these cases, the fracture goes through physis and can affect metaphysis, epiphysis or both. The most widely used classification, however, is the Salter–Harris system. Physeal injuries have been reported to occur in approximately 30% of children's long bone fractures.[1]

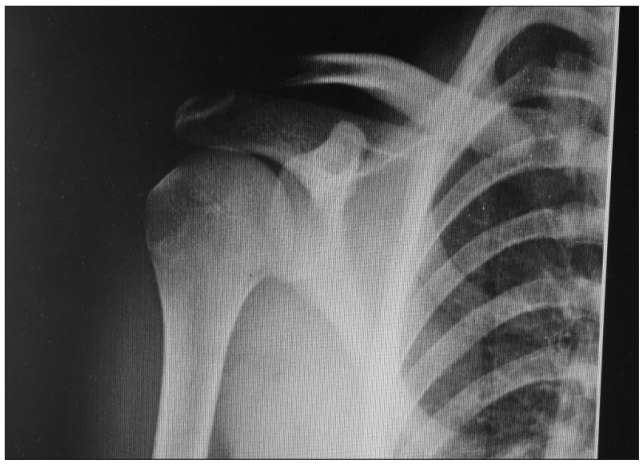
A fracture of the medial portion of the clavicle occurs infrequently in children and accounts for only about 5% of all pediatric clavicular fractures.[2] Medial physeal fractures are more common than medial shaft fractures, and the former can mimic sternoclavicular joint dislocations. Forces in children and adolescents usually produce a physeal injury rather than an actual dislocation of the sternoclavicular joint because the capsule of the sternoclavicular joint is more resistant to injury than the physis which does not close completely until 24 to 26 years of age in many males. The most common mechanism of injury is axial compression of the shoulder toward the midline.[1] Different imaging modalities are used such as plain radiography, specific radiographic views like Heinig, Hobbs and "serendipity" as well as CT and MRI.[2] Nondisplaced fractures of the medial portion of the clavicle can be managed symptomatically and have a good prognosis. In anteriorly displaced fractures, closed reduction can be attempted with longitudinal traction and direct pressure over the fracture. Usually, reduction is easily obtained but difficult to maintain, with frequent re-displacements.[1]

Case report

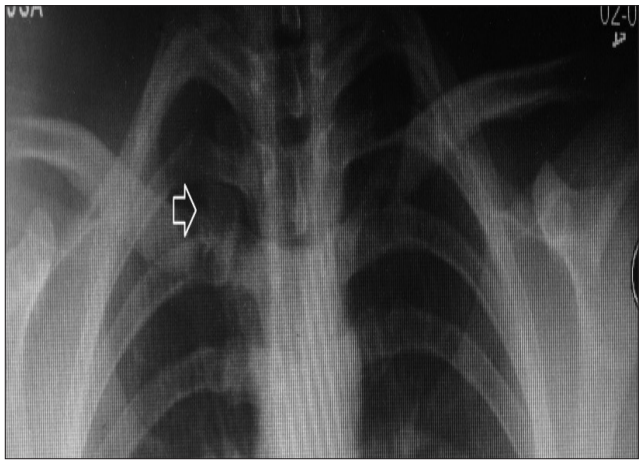
17-year-old male, a basketball player, presented at the Emergency Surgical Department of University Clinical Center Banja Luka with injury sustained in direct fall on right shoulder. An objective evaluation demonstrated

patients arm in fixed position along the body, flexed at the elbow, deformity and swelling in the projection of the medial clavicle and movements in the shoulder caused severe pain. Neurocirculatory status was intact. Standard chest radiography didn't reveal signs of fracture (Picture 1). Additional "serendipity" view identified a right medial physeal clavicle fracture (Picture 2).

Picture 1. Standard chest radiography

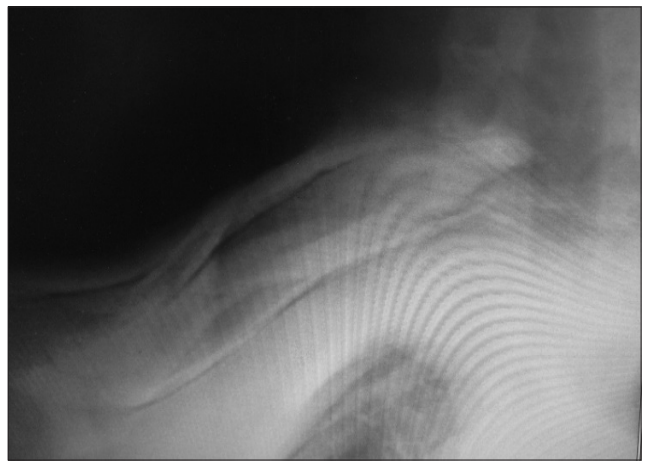


Picture 2. "Serendipity view"



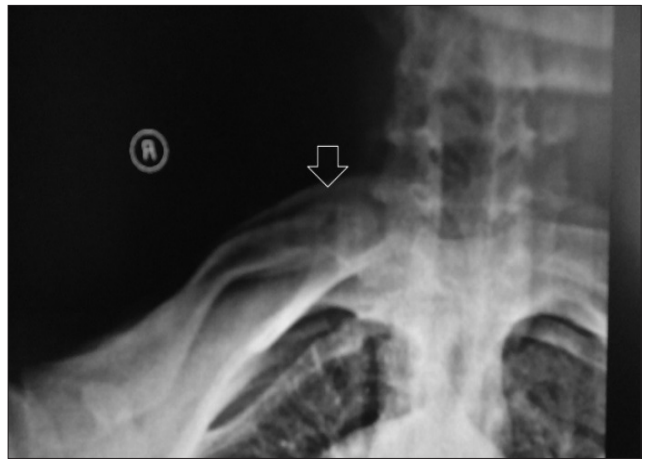
In total intravenous anesthesia, closed reduction was performed with longitudinal traction of arm and direct pressure over the fracture with utilization of a sandbag placed between the shoulders. After closed reduction, during total anesthesia, we noticed re-dislocation. We performed reduction again and set figure- of- eight dressing which didn't retain the achieved reduction. After the second reduction, we immobilized the shoulder with Desault cast and we set gauze as compression material on the site of the fracture due to the retention of fragments. Hobbs view showed good reduction (Picture 3).

Picture 3. Reduction of medial physeal clavicle fracture stabilized with Desault cast and gauze



We took immobilization off after 21 days. Clinical examination showed hematoma in resorption without swelling and no visible deformities. We set a figure- of – eight dressing for a period of 3 more weeks. The control examination, clinically and radiologically, confirmed a good position of fragments (Picture 4).

Picture 4. Radiography (Hobbs view) 6 weeks after reduction of medial clavicular epiphysiolysis



Instructions were given on the exercises at home. Eight weeks after the injury, the patient had no subjective complaint, with fully functional shoulder and without cosmetic defect. We recommended avoiding severe physical work another 6 weeks. An excellent clinical result was confirmed at the controls after 6 months, one and three years.

Discussion

Medial clavicle injuries are rare.[1-3] There are no specific

outcome scores that specifically evaluate sternoclavicular joint injuries. These fractures are uncommon, and as a result, there is little information available in the literature.[4] In a prospective study of 222 patients with radiologically proven fractures of clavicle, Nowak and al. showed that the risks for persistent symptoms following nonoperative treatment was far higher than expected.[5]

In our case report of medial physeal clavicle fracture with this method of treatment, excellent result was obtained.

There are no clear recommendations in the literature for this type of injuries. If reduction fails or re-displacement occurs, further intervention is rarely indicated.[1] Some authors believe that these injuries only cause a minor cosmetic defect.

Conclusion

If there are clinically insecure signs of a sternoclavicular joint injury, besides standard chest radiography, Hobbs and "serendipity" views need to be done. After the closed reduction, we recommend Desault cast with additional compression with gauze on the fracture site in the first 21 days, and another three weeks with figure- of- eight dressing. After three years follow-up, we got a result without neither functional nor cosmetic deficits.

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Luksacioni prednji epifiziolizarni prelom medijalnog dijela klavikule

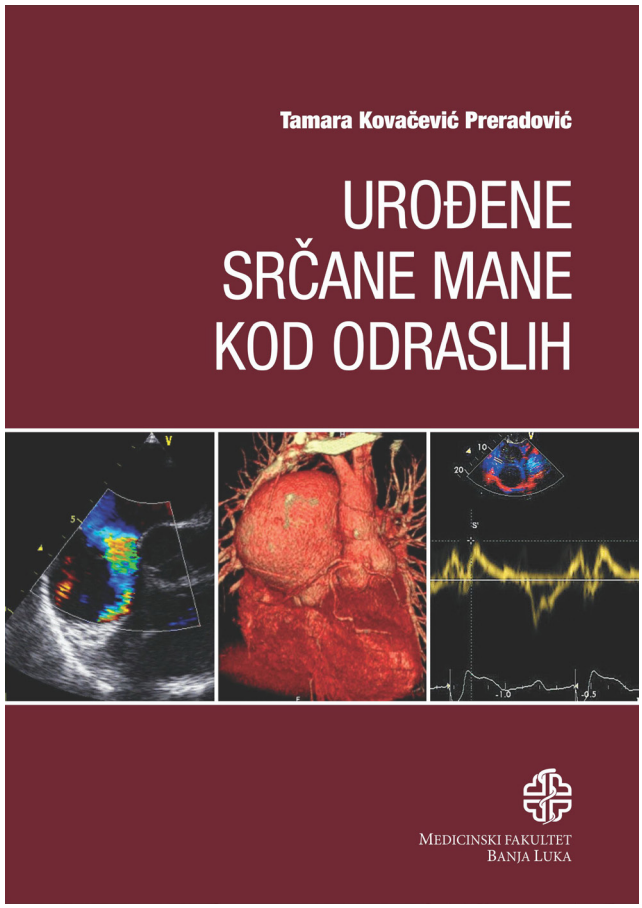
SAŽETAK

Povrede medijalnog okrajka klavikule su rijetke i učestvuju sa 5% svih povreda ove kosti kod djece. Ploča rasta se kod klavikule medijalno zatvara između 24-te i 26-te godine života. U prikazu ovog slučaja, povrijeđen je 17-godišnji muškarac prilikom direktnog pada na rame. Na prijemu su urađeni klinički pregled, RTG- standardni snimak ramena i „serendipity" snimak i dijagnostikovan fizarni prednji luksacioni prelom medijalnog okrajka desne klavikule. U kratkotrajnoj intravenskoj anesteziji uradili smo zatvorenu repoziciju i postavili imobilizaciju po Dezolu u trajanju od 21 dan, a potom „osmica" imobilizaciju u trajanju od 3 nedelje. U toku trogodišnjeg praćenja, nismo primjetili oštećenje funkcije niti estetske defekte.

Ključne riječi: Fizarni prelom klavikule,Hobsov snimak, „serendipity" snimak.

BOOK REVIEW

Congenital Heart Diseases in Adults



A monograph "Congenital Heart Diseases in Adults" by Tamara Kovačević – Preradović and coauthors Mirko Stantić, Peđa Kovačević and Bojan Stanetić sheds new light on this important health and social issue which has not been assessed in Serbian language in such a concise way. The authors from the University Clinical Center Banja Luka have explained pathology, pathophysiology, symptoms, signs, diagnostic tests, as well as treatment of such anomalies in a very concise and systematic way.

Congenital hears anomalies are usually the field of interest of pediatric cardiologists, if such anomalies are diagnosed at all during childhood, since very often, they can be diagnosed and become important health issue later in life. In that case, the care of such adult patients is the field of interest of adult cardiologists.

We are witnessing phenomenal technological and scientific advances in cardiology which have allowed such conditions to be diagnosed and adequately treated.

The book has a lot of illustrations collected during every day work at the Cardiology Clinic of the University Clinical Center, which is a handy indicator of the high scientific and medical standards of the Institution. Practically, all conditions which have been described in the book have appropriate ultrasound or radiological imaging, as well as other necessary ancillary test with excellent explanation of each illustration or diagram. Since the authors have used their own material, experience and knowledge from their patients, it is not surprising that the explanations for each anomaly have been done in such an easy-to-understand way. Therefore, for the keen reader, it is going to be much easier to get necessary knowledge from such a specific field.

Even superficial review of the monograph can show how concisely and systematically the topic is divided in chapters, from simple heart anomalies through complex ones, pulmonary hypertension, Eisenmenger syndrome, other pulmonary manifestations and heart rhythm disturbances in these patients.

It is especially important that the pregnancy and practicing sports in adults with congenital heart disease have been described in separate chapters.

Neatly sorted references at the end of each chapter are price worthy. The book has sufficiently comprehensive index which allows even easier usage and navigation when necessary information is needed.

The monograph "Congenital Heart Defects in Adults" is mostly intended for cardiologists and gynecologists, sport medicine specialists, pediatricians, internists and family physicians and can be of great help in understanding the complex issues of monitoring and treatment of these patients.

Prof. Dr Bosiljka Vujisić Tešić

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Scripta Medica (SM) is a peer-reviewed international journal published under the auspices of the Medical Society of the Republic of Srpska. The journal publishes original biomedical studies, including those addressing ethical and social issues. As a general medical journal, SM gives preference to clinically oriented studies over those on experimental animals. It publishes peer-reviewed original research papers, case reports, review articles, essays, special articles, clinical problem-solving, images in clinical medicine only in English. Book reviews and news are published only in Serbian. The full text of SM is available, free of charge, online at www.scriptamedica.com.

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De Lacey G, Record C, Wade J. How accurate are quotations and references in medical journals. BMJ 1985; 291:884-6.

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Croat Med J 2003; 44:770-83.

Huth EJ. How to write and publish papers in the medical sciences. Philadelphia: ISI Press, 1982.

Davidović L, Marković M, Čolić M, et al. Treatment of traumatic rupture of the thoracic aorta. Srp Arh Celok Lek 2008; 136: 498-504.

Curtis MJ, Shattock MJ. The role of the manuscript assessor. In: Hall GM, ed. How to write a paper. London: BMJ Publishing Group; 1994: 89-95.

Electronic publications:

International Society of Scientometrics and Informatics Web site. Available at: http://www.issi-society.info Accessed March 20, 2012.

Lock SP. Journalology: are the quotes needed? CBE Views. 1989:1257-9. Available at: http://garfi eld.libraryupenn. edu/ essays/v13p019y1990.pdf. Accessed April 25, 2012.

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