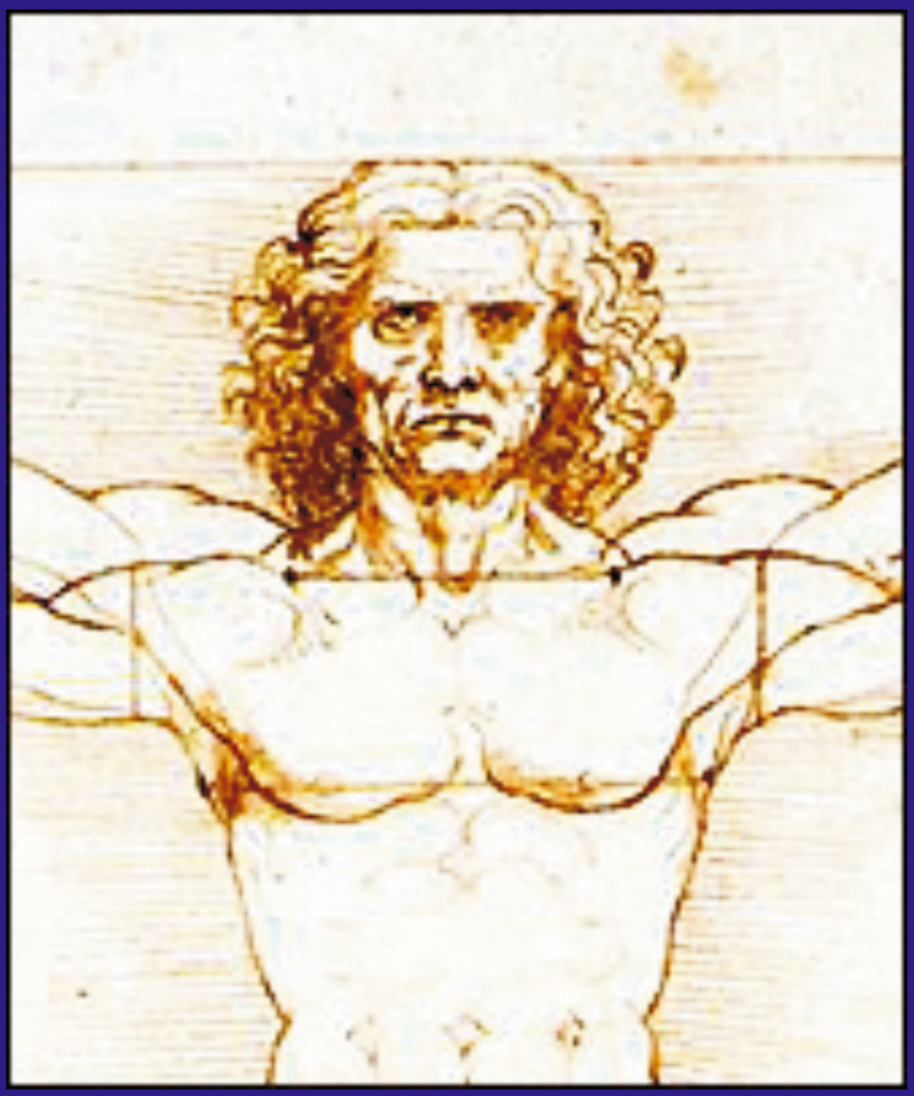
SANAMED

ISSN-1452-662X



Vol 13 (2) 2018.

MEDICINSKI ČASOPIS LEKARA

NOVI PAZAR

SANAMED

ISSN-1452-662X

Izdavač

Udruženje lekara Sanamed, Novi Pazar

ČASOPIS IZLAZI TRI PUTA GODIŠNJE

Adresa uredništva

„SANAMED“, Ul. Palih boraca 52, 36300 Novi Pazar, Srbija
email: sanamednp2006@gmail.com, www.sanamed.rs

Štampa

„ProGraphico“, Novi Pazar

Tiraž

500

Pretplata

Godišnja pretplata: 4500 din. za domaće ustanove; 1500 din. za pojedince; za inostranstvo 75 eura (u dinarskoj protivrednosti po kursu na dan uplate). Pretplatu vršiti na račun 205-185654-03, Komercijalna banka. Za sve dodatne informacije kontaktirati Uredništvo.

SANAMED

ISSN-1452-662X

Publisher

Association of medical doctors “Sanamed”, Novi Pazar

ISSUED THREE TIMES A YEAR

Editorial address

“SANAMED”, St. Palih boraca 52, 36300 Novi Pazar, Serbia
email: sanamednp@gmail.com, www.sanamed.rs

Print

“ProGraphico”, Novi Pazar

Circulation

500

Subscription

Annual subscriptions: 4500 RSD for domestic institutions and 1500 RSD for individuals. For readers abroad, annual subscription is 75 Euro (in Dinar equivalent at the exchange rate on the day of payment). For further instructions and informations, contact Editorial Board.

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• ORIGINALNI NAUČNI RAD

Čitaj da shvatiš

Piši da preneseš

Uradi da te pamte

* * *

Read to understand

Write to impart

Work to be remembered

Avdo Ćeranić

Phlebodia® 600mg

film tableta
(diosmin)

Olakšanje tokom celog dana

Venska insuficijencija

- Dugotrajno olakšanje[1]
  Dokazano dugotrajno delovanje
- Smanjenje bola za 55,6%[2]
  Značajno smanjenje bola prouzrokovanog venskom insuficijencijom nakon 4 nedelje terapije
- Poboljšanje simptoma kod 65% do 81% pacijenata[2]
  Poboljšanje ili nestanak svih simptoma nakon 4 nedelje terapije

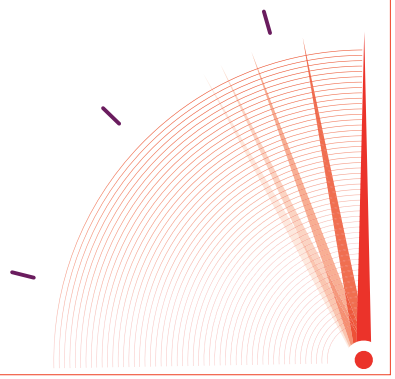
1 tableta dnevno,
ujutru
pre doručka[3]

Brzo olakšanje

Hemoroidalna kriza

- Ublažavanje bola tokom 3 dana[4]
  Statistički značajno smanjenje bola u poređenju sa placebom (p=0,042)
- Prestanak krvarenja za oko 3 dana[5]
- Zadovoljni pacijenti nakon 7 dana: 92%
  Zadovoljni do veoma zadovoljni pacijenti nakon 7 dana terapije

2 do 3 tablete
dnevno
uz obrok[3]



Samo za stručnu javnost

1. IME LEKA Phlebodia®, 600mg, film tablet INN: diosmin **2. KVALITATIVNI I KVANTITATIVNI SASTAV** Jedna film tableta sadrži diosmin, čist, bezvodni 600 mg (izraženo na supstancu). Pomoćne supstance sa potvrđenim dejstvom: Ponceau 4R red (E124). Za listu svih pomoćnih supstanci videti odeljak 6.1. **3. FARMACEUTSKI OBLIK** Film tableta, ružičaste boje. **4. KLINIČKI PODACI 4.1. Terapijske indikacije** - Poboljšanje simptoma povezanih sa venolimfatičkom insuficijencijom: teške noge, bol, predekubitalna stanja. - Lečenje simptoma akutnih hemoroida. - Dodatna terapija funkcionalnih poremećaja pri kapilarnoj fragilnosti. **4.2. Doziranje i način primene** Oralna upotreba. - venska isuficijencija : 1 tableta dnevno, ujutru pre doručka - akutni hemoroidi: 2 do 3 tablete uz obrok. **4.3. Kontraindikacije** Ovaj lek se generalno ne preporučuje u periodu laktacije. (vidi odeljak 4.6.). Primena u periodu trudnoće i dojenja). Preosetljivost na diosmin ili neki drugi sastojak leka. **4.4. Posebna upozorenja i mere opreza pri upotrebi leka** Akutni hemoroidi: primena ovog proizvoda ne zamenjuje specifičnu terapiju ostalih analnih bolesti. Terapija treba da bude kratkotrajna.Ukoliko simptomi ne popuste brzo potrebno je sprovesti proktološki pregled i revidirati terapiju. Venska insuficijencija: Ovaj lek ima potpunu efikasnost uz odgovarajući higijenski režim: - treba izbegavati izlaganje suncu, toploti, produženo stajanje i višak kilograma; - duge šetnje, nošenje čarapa koje pospešuju vensku cirkulaciju. Proizvod sadrži propilenglikol koji može izazvati alkoholu slične simptome. Takodje, sadrži azo-agense za bojenje (Ponceau 4R (E 124)) koji može izazvati alergijske reakcije. Ovaj lek sadrži manje količine etanola. **4.6. Primena u periodu trudnoće i dojenja** **Trudnoća**: Eksperimentalne studije na životinjama nisu pokazale teratogeni efekat. Rizik na fetus ne može da se isključi zbog nedostatka klinički relevantnih informacija tako da se primena leka u periodu trudnoće ne preporučuje osim ako potencijalna korist od terapije nije jasno identifikovana. **Dojenje**: u odsustvu podataka koji se odnose na prelazak u majčino mleko, ne preporučuje se u periodu dojenja. **4.8. Neželjena dejstva** Mogući su povremeni gastrointestinalni poremećaji koji retko zahtevaju prekid terapije. U pojedinim slučajevima se mogu ispoljiti reakcije na koži (osip, urtikarija, pruritus). **5. FARMAKOLOŠKI PODACI 5.1. Farmakodinamski podaci Farmakoterapijska grupa:** Sredstva za stabilizaciju kapilara; bioflavonoidi **ATC kod:** C05CA03 Venotonik i vaskuloprotektiv koji izaziva vensku konstrikciju, povećavajući vaskularnu otpornost i smanjujući vaskularnu propustljivost **7. NOSILAC DOZVOLE** Predstavništvo Laboratoire Innotech International Beograd, Milentija Popovića 5 v, Beograd - Novi Beograd **8. BROJ PRVE DOZVOLE I OBNOVE DOZVOLE** Kutija sa 15 film tableta (jedan blister): 515-04-1584/03; 515-01-01172-13-001 Kutija sa 30 film tableta (dva blistera): 515-04-1584-1; 515-01-01173-13-001 **9. DATUM PRVE DOZVOLE I DATUM OBNOVE DOZVOLE** Kutija sa 15 film tableta (jedan blister): 29.07.2003.; 01.10.2013. Kutija sa 30 film tableta (dva blistera): 29.07.2003.;01.10.2013. **10. DATUM REVIZIJE TEKSTA** Mart, 2015. * Za dodatne informacije o leku Phlebodia 600 mg, molimo Vas pogledajte zadnji odobren Sažetak karakteristika leka. **Režim izdavanja leka** Lek se može izdavati samo uz lekarski recept.

1- R. Barbe Etude de la durée d'action du Diovenor avec contrôle par pléthysmographie. Tribune médicale, 1988, n°270, p49-50
2- Cazaubon M. *et al.* Etude comparative d'acceptabilité, d'efficacité et de tolérance de deux formes galéniques de diosmine 600 mg, dans le traitement des symptômes de la maladie veineuse chronique. Angiologie, 2011, vol.63,n°2
3- Sažetak karakteristika leka Phlebodia 600 mg, film tableta. Datum revizije teksta Mart, 2015.
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6- Debien P., Denis J. Traitement des signes fonctionnels de la crise hémorroïdaire aiguë : Essai multicentrique, randomisé, diosmine d'hémisynthèse *versus* association extrait de Ginkgo biloba-heptaminol troxérutine. Med Chir Dig 1996 ; 25 (5) : 259-64

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SANAMED
2018; 13(2): 117–121
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.219
UDK: 616.12-089
ID: 266985996
Original article

DOES BEATING HEART SURGERY TECHNIQUE REDUCE THE MORTALITY AND MORBIDITY AFTER REDO VALVE OPERATIONS?

Mungan Ufuk,[1] Demirdas Ertan,[2] Altinay Levent,[3] Cetin Erdem,[4] Atilgan Kivanc,[2] Katircioglu Fehmi,[5] Cicekcioglu Ferit[2]

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Primljen/Received 29. 03. 2018. god.

Prihvaćen/Accepted 21. 05. 2018. god.

Abstract: **Aim:** The aim of this study was to determine the effect of beating heart technique on mortality and morbidity after redo valve operations.

Material and Method: Fifty-two patients who had redo open-heart surgery between May 2005 and November 2006 in a Hospital included in this prospective study. All patients had a history of open-heart surgery with median sternotomy. Thirty-two patients who had redo open-heart surgery with beating heart technique were included in Group 1 and 20 patients who had redo open-heart surgery with conventional cardioplegic myocardial arrest technique were included in Group 2. Patients who had any cardiac surgery without median sternotomy were excl**uded. R**esults: Functional capacity according to New York Heart Association classification was significantly lower and number of patients with chronic obstructive lung disease was significantly higher in Group 1 ($p = 0.011$ and $p = 0.003$ respectively). There was no significant difference in other preoperative variables. Operation, cardiopulmonary bypass and aortic cross-clamping times were significantly higher in Group 2 ($p = 0.001$, $p = 0.003$, $p = 0.04$ respectively). Mechanical ventilation, inotropic agent support and hospitalization times were significantly higher in Group 2 ($p < 0.05$). Intensive care unit time was significantly longer in Group 1 ($p < 0.05$). Drainage volumes, blood product transfusion volumes, intra-aortic balloon pump support times were not significantly different between the groups.

Conclusion: Beating heart technique in redo heart valve operations has better outcomes than the conventional technique.

Key words: Redo operation; heart valve surgery; cardioplegia; beating heart technique.

INTRODUCTION

The success of the open-heart surgery is dependent on not only the technical abilities of the surgeon and the operation technique but also on the preservation of the myocardium. Development of cardiopulmonary bypass (CPB) and progression of elective cardiac arrest techniques provided time long enough to do an open-heart surgery safely and comfortably. The effect of hypothermia for myocardium preservation was firstly introduced by Bigelow and it was used solitarily or concomitantly with intermittent aortic cross-clamping (XCL) until 1970.

Diastolic arrest of the heart with potassium was first introduced by Melrose et al in 1955 (1). Improvements of cardioplegia solutions provided better myocardial preservation of the heart through the surgery.

The negative effects of CPB and myocardial ischemia time (XCL time) are two important predictors of morbidity and mortality after open-heart surgery. The most deteriorative effect of CPB on the heart is the reperfusion injury. Besides that, another major cause of myocardial dysfunction after open-heart surgery is myocardial oedema caused by diastolic arrest of the heart (2, 3).

The incidence of redo open-heart surgeries increases nowadays. The beating heart operation technique could be an alternative for conventional surgery technique in thishigh-risk patient population to reduce the mortality and morbidity rates. The aim of this prospective study was to determine the effect of beating heart technique on mortality and morbidity after redo valve operations.

MATERIAL AND METHODS

Fifty-two patients who had redo open-heart surgery between May 2005 and November 2006 in Türkiye Yüksek İhtisas Hospital were enrolled in this prospective study. All patients had a history of open-heart surgery with median sternotomy. Thirty-two patients who had redo open-heart surgery with beating heart technique were included in Group 1 and 20 patients who had redo open-heart surgery with conventional cardioplegic myocardial arrest technique were included in Group 2. Informed consent was taken from all of the patients and research approval was acquired from the local ethical committee.

Patients who had any cardiac surgery without median sternotomy were excluded.

Statistical analysis

All data were recorded as continuous and categorical variables. The SPSS 11. 05 (Statistical Package for the Social Sciences SPSS Inc., Chicago, IL) for Windows programme was used for statistical analysis of the data. Student t-test was used to calculate the statistical significance of the variables such as age, ejection fraction, aortic cross-clamping time, postoperative drainage volume and chi-square test was used to evaluate the data of the variables gender, functional capacity, preoperative patient condition, cardiac rhythm, morbidity and mortality.P value lower than 0.05 was accepted as statistically significant.

Operative technique

All operations were done under general anaesthesia. In Group 1, median sternotomy was done in all of the patients. CPB was initiated after systemic heparinization with proper dose heparin administration, standard aortic and unicaval cannulation. In five patients with ascending aortic aneurysm, arterial cannula was introduced through right femoral artery. A retrograde perfusion cannula was introduced through coronary sinus to perfuse the heart. The pressure on this retrograde cannula was monitored and it was used to supply oxygenated blood to the heart through a line from the CPB machine. Jostra HL 20 heart-lung machine was used for extracorporeal circulation and Dideco D 708 Simplex III oxygenator and systems was used in all of the cases. All operations were done with non-pulsatile flow. Patient body temperature was monitored with rectal heat probe and the body temperature of the patient maintained between 35–37 °C. Aortic, right pulmonary vein and apical venting was achieved. In 15 patients, aortic cross-clamp was applied and retrograde coronary sinus perfusion was initiated. The flow pressure on the retrograde coronary sinus perfusion line was maintained between 60–90 mmHg and the flow rate was maintained between 300–500 ml/min. In 17 patients, aortic cross-clamp was not applied and antegrade blood supply was achieved by holding the patient in Trendelenburg's position so retrograde perfusion was not utilized in these patients. Intraoperative electrocardiography (ECG) monitoring was done to follow the ischemia of the myocardium. Arterial pressure, blood oxygen saturation, central venous pressure and urine output was also monitored. While perfusing the heart through retrograde cannula, partial oxygen pressure (pO2) was measured in blood samples taken from the aortic vent line and coronary sinus perfusion line in every 20 minutes. Presence of anaerobic metabolism was checked by measuring pH and lactate levels in blood samples taken from the aortic vent line in every 5 minutes. Transcranial Doppler ultrasonography (DUSG) imaging and electroencephalographic monitoring was done intraoperatively to follow any air embolism of intracranial vessels. Preoperative transoesophageal echocardiography (TEE) imaging was done in all of the cases to evaluate the heart valve functionality and check the residual air bubbles in heart chambers. In postoperative period, transthoracic echocardiography (TTE) imaging was done to evaluate the myocardial functions and heart valve functions in all of the cases.

In Group 2, median sternotomy was done in all of the patients. After standard aortic unicaval cannulation, retrograde coronary sinus cannulation and aortic vent implantation, CPB was initiated. In two patients, arterial cannula was introduced through right femoral artery. Cardiac arrest was achieved with antegrade and retrograde infusion of cold crystalloid and blood cardioplegia solutions following the aortic XCL. Patient body temperature was monitored with rectal heat probe and it was maintained between 28–32 °C. Warm blood cardioplegia solution was administered before de-clamping the aorta. Myocardial and valve function evaluation was done with TTE imaging in all of the patients in this group.

RESULTS

Preoperative data is presented in Table 1. Functional capacity (New York Heart Association (NYHA) Functional Classification) was significantly lower and number of patients with chronic obstructive lung disease (COLD) was significantly higher in Group 1 when compared with Group 2 ($p = 0.011$ and $p = 0.003$ respectively). There was no significant difference in other preoperative variables. The initial operations that the patients had and redo operation types are presented

Table 1. *Preoperative data*

	Group I (n = 32)	Group II (n = 20)	P
Age (Mean ± sd)	50 ± 15	47 ± 9	> 0.05
Male	16	12	> 0.05
BMI (Kg/m^2) (Mean ± sd)	25 ± 5	27 ± 4	> 0.05
BSA (m^2) (Mean ± sd)	1,7 ± 0,1	1,8 ± 0,1	> 0.05
LVEF (%) (Mean ± sd)	53 ± 10	52 ± 7	> 0.05
SPAP (mmHg) (Mean ± sd)	57 ± 20	51 ± 15	> 0.05
NYHA Class ll/lll/IV	9/14/9	13/6/1	**0.011**
Diabetes mellitus(n)	6	3	> 0.05
Hypertension(n)	5	3	> 0.05
COLD(n)	8	1	**0.003**
CVI(n)	2	1	> 0.05
Active Endocarditis(n)	7	4	> 0.05

COLD: Chronic obstructive lung disease, BMI: Body mass index,BSA: Body surface area, SPAB:Systolic pulmonary arterial pressure, NYHA: New York Heart Association Classification, SVI: Cerebrovascular incident, LVEF: Left ventricle ejection fraction, sd: Standard deviation

Table 2. *Distribution of the initial operations of the patients undergoing redo operations*

Initial operations	Group I (n = 32)	Group II (n = 20)
MVR(n)	11	6
AVR(n)	3	4
AVR + MVR(n)	4	–
CABG + MVR(n)	1	–
CABG + AVR(n)	1	–
Open mitral valvulotomy(n)	8	6
Atrial septal defect(n)	1	1
Ventricular septal defect(n)	1	–
CABG(n)	2	3

MVR: Mitral valve replacement, CABG: Coronary artery bypass graft, AVR: Aortic valve replacement

in Table 2 and 3. Operative data of the groups are shown in Table 4. Operation, CPB and aortic XCL times were significantly higher in Group 2 when compared with Group 1 (p = 0.001, p = 0.003, p = 0.04 respectively). Postoperative data of the patients is presented in Table 5. Mechanical ventilation, inotropic agent support and hospitalization times were significantly higher in Group 2 when compared with Group 1 (p < 0.05). Intensive care unit time was significantly longer in Group 1 when compared to Group 2 (p < 0.05) Drainage volumes, blood product transfusion volumes, intraaortic balloon pump (IABP) support times were not significantly different between the groups.

Table 3. *Distribution of the redo operations*

Reoperation	Group I (n = 32)	Group II (n = 20)
MVR(n)	22	10
AVR(n)	4	4
AVR & MVR	2	–
Thrombectomy of Mechanical Heart Valve(n)	2	–
AVR + AorticSupracoronary Graft Implantation	2	3
Benthall procedure	–	2
Paravalvular leak repair	–	1

MVR: Mitral valve replacement; AVR: Aortic valve replacement

Table 4. *Comparison of the operative data*

	Group I (n = 32)	Group II (n = 20)	P value
Operation time	146 ± 52	346 ± 87	0.001
CPB time	83 ± 34	123 ± 56	0.003
XCL time	54 ± 39	84 ± 41	0.04

CPB: Cardiopulmonary bypass; XCL: Aortic cross-klemp

Table 5. *Comparison of the postoperative data*

	Group I	Group II	P
Mechanical ventilation (hr) (Mean ± sd)	12 ± 9	33 ± 11	< 0.05
Drainage volume (ml) (Mean ± sd)	824 ± 478	725 ± 437	> 0.05
Blood product transfusion volume (ml) (Mean ± sd)	1680 ± 870	1600 ± 820	> 0.05
Inotropic agent administration time (hr) (Mean ± sd)	22 ± 18	47 ± 32	< 0.05
IABP support(n)	1	1	> 0.05
ICU time (days) (Mean ± sd)	3.5 ± 2.9	2.7 ± 2.2	< 0.05
In-hospital mortality (n)	2	1	> 0.05
Hospitalization time (days)	7.7 ± 5.6	13 ± 12	< 0.05

IABP: Intra-aortic balloon pump; ICU: Intensive care unit; sd: Standard deviation

DISCUSSION

The incidence of redo operations in heart valve diseases was increased in the last decades. As it was presented in the literature, the need for redo operations after heart valve reconstructional interventions –especi-

ally after mitral and tricuspid valve surgeries- increased in different rates. This increase might be caused by the increased mean age of general population, prosthetic heart valve disadvantages as well as the heart valve repair techniques or surgeon related factors (4).

The mortality and morbidity rates are higher in redo open-heart surgeries and they are technically more challenging according to primary operations. Multiple factors such as myocardial injuries due to adhesion of scar tissue on the heart during exploration, pulmonary hypertension accompanying heart valve dysfunction, excessive bleeding and blood product transfusions, inappropriate myocardial protection increase the morbidity and mortality rates after redo open heart surgeries (5). Due to improvements in myocardial preservation techniques, operative mortality risk and postoperative morbidity rates were decreased (6). Retrograde perfusion of the heart through coronary sinus cannula and achievement of myocardial preservation was already presented in the literature (7, 8, 9).

The number of valve operations done with beating heart technique increased in the last few years. The blood itself is the most effective myocardial protective agent despite the improved myocardial preservation techniques. Cardioplegia techniques inevitably cause myocardial reperfusion injury. Besides that, myocardial dysfunction after open-heart surgery is a result of myocardial oedema accumulated in the heart during diastolic arrest period. Thus, keeping the heart beating during the surgery will lessen the myocardial oedema and improve the postoperative cardiac functions (10).

Due to recent improvements in beating heart coronary bypass and heart valve surgeries, we decided to utilize these techniques and use them in our operations with some improvements. Continuous blood perfusion throughout the surgery reduces the uncontrolled myocardial perfusion risk. In recent studies, the beating heart valve surgery was proved a practicable and logical technique with reasonably positive results (11).

In this study we found that, the morbidity and mortality rates were lower in the patient group undergoing redo heart valve surgery operated with beating heart on-pump technique when compared with the patient group who were operated with conventional technique in which the heart was arrested with cardioplegic solutions. In addition, we found that our technique provided better myocardial preservation and resulted with better postoperative outcomes especially in the patients who had impaired myocardial functions.

In conclusion, lower mortality and morbidity rates, practicability, lower hospitalisation times, lower costs and improved quality of life after the operations are the positive results of beating heart valve surgery technique. Although these encouraging outcomes, we think that more studies should be conducted including larger patient series and more centres.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

DA LI OPERACIJE NA KUCAJUĆEM SRCU REDUKUJU MORTALITET I MORBIDITET NAKON OPERACIJE ZAMENE SRČANIH ZALISTAKA

Mungan Ufuk,[1] Demirdas Ertan,[2] Altinay Levent,[3] Cetin Erdem,[4]
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Cilj: Cilj ove studije bio je da se utvrde efekti operativnih tehnika na kucajućem srcu na mortalitet i morbiditet nakon operacije zamene valvula.

Materijal i metode: U ovoj prospektivnoj studiji učestvovalo je 52 pacijenta koji su bili podvrgnuti operacijama na otvorenom srcu između maja 2005. i novembra 2006. godine u Türkiye Yüksek İhtisas bolnici. Svi pacijenti su imali operaciju na otvorenom srcu sa medijalnom sternotomijom. 32 pacijenta, koja su imala operaciju tehnikom na kucajucem srcu svrstani su u grupu 1, dok je 20 pacijenata bilo podvrgnuto operaciji sa konvencionalnom kardioplegijskom metodom zaustavljanja rada srca i svrstani su u grupu 2. Pacijenti koji su imali bilo koju kardiohirursku operaciju bez medijalne sternotomije su bili isključeni iz studije.

Rezultati: Kod pacijenata u grupi 1, funkcionalni kapacitet srca prema NYHA klasifikaciji je bio značajno niži, a komplikacije hronične opstruktivne bolesti pluća su bile značajno više u (p = 0,011 i p = 0,003 respektivno). Nije bilo statistički značajne razlike između drugih preoperativnih varijabli. Operacija, kardiopulmonalni bypass kao i vreme klemovanja aorte su bili statistički značajno viši u grupi 2 (p = 0,001, p = 0,003, p = 0,04 respektivno). Mehanička ventilacija, inotropni agensi kao i vreme hospitalizacije je bilo značajno više u grupi 2. (p < 0,05). Vreme provedeno u jedinici intenzivne nege je bilo statistički značajno duže u grupi 1. Kada je u pitanju zapremina drenaže, zapremine elemenata za transfuziju krvi, vreme provedeno u korišćenju intra-aortne balon pumpe nije bilo statistički značajne razlike među grupama.

Zaključak: Operativna tehnika srčanih zalistaka kod kucajućeg srca, ima bolji ishod u poređenju sa konvencionalnim tehikama.

Ključne reči: operacije, hirurgija srcanih zalistaka, kardioplegija, tehnika kucajućeg srca.

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SANAMED
2018; 13(2): 123–129
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.224
UDK: 616.24-008.4-053.2
ID: 266979596
Original article

OXYGEN SATURATION INDEX FOR ASSESSMENT OF RESPIRATORY FAILURE IN NEONATES

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Primljen/Received 14. 04. 2018. god. Prihvaćen/Accepted 21. 05. 2018. god.

Abstract: **Introduction:** Acute respiratory failure (ARF) is the most common problem seen in the preterm and term infants admitted to neonatal intensive care units. Etiology is not uniform, and mostly depend on gestational age. For adequate treatment is certainly important to recognize and treat underlying disease, but at the same time, we have to supply adequate respiratory support, tissue perfusion and oxygen deliveries. For a good outcome we need reliable estimation method for functional state of respiratory system, as well as monitoring the effects of treatment. Current assessment ARF is with blood gas, chest X-ray and Oxygenation index (OI). OI is quite aggressive assessment method for neonates, because it involves arterial blood sampling. Promoted in recent studies, Oxygen saturation index (OSI) measured by pulse oximetry, attempts to objectively score respiratory disease with parameters available non-invasively. **The aim** of our research is to evaluate correlation between OSI and OI in neonates with ARF requiring mechanical ventilation. **Material and methods:** In a retrospective cohort study 101 neonates were selected, treated at the Department of intensive therapy and care, Pediatric clinic of Tuzla, due to ARF requiring mechanical ventilation.

We reviewed data such as gestational age, birth weight, gender, Apgar scores, values of Score for Neonatal Acute Physiology-Perinatal Extension, all the parameters from the arterial blood gas analysis, pulse oximetry values, Oxygenation Index and Oxygenation Saturation Index, that were calculated by the formulas. OSI and OI were calculated and correlated. Mean values of OSI and OI correlated with *Pearson's* coefficient of 0.76; $p < 0.0001$ (95% CI = 0.66-0.83). OSI correlated with SNAP-PE with *Pearson's* coefficient of 0.52; $p < 0.0001$ (95% CI = 0.36-0.65). Comparing the values of OSI between patients who died and those who survived, we found that OSI correlated with the outcome with Spearman's coefficient of -0.47; $p < 0.0001$ (95% CI = -0.16 - -0.31). Bland-Altman plot confirmed correlation between OSI and OI in mean values, identifying discrepancy between two indices for extreme values. **In conclusion**, OSI correlates significantly with OI in infants with respiratory failure. This noninvasive method of oxygenation assessment, utilizing pulse oximetry, can be used to assess the severity of ARF and mortality risk in neonates.

Key words: neonates; respiratory failure; Oxygenation Index; Oxygenation Saturation Index.

INTRODUCTION

Due to the high predisposition to problems with breathing, acute respiratory failure (ARF) is the most common reason for admission to Neonatal intensive care units (NICU). The causes of neonatal respiratory failure mostly depend on gestational age. In premature neonates, the most common cause is respiratory distress syndrome due to surfactant deficiency. Acute respiratory failure in term and near term infants is usually a result of aspiration syndromes, infections and congenital anomalies, including primary pulmonary hypertension. The pathophysiological sequence is represented as combination of primary surfactant deficiency and surfactant inactivation process with various factors, such as pneumonia, sepsis and asphyxia (1).

In the treatment of respiratory insufficiency it is very important to recognize and treat the underlying disease. But before that and at the same time, we have to realize, as soon as possible, adequate respiratory support and adequate tissue perfusion and oxygen deliveries. Mechanical ventilation in children is still a more complex area, whose safe and successful application requires good knowledge of pulmonary physiology and pathophysiology that is changing with age, and

ventilation should be designed according to the age, lung disease characteristics and pathophysiological process causing respiratory insufficiency. Currently, there are many options, strategies and modalities, suggesting that we have not created the ideal mode yet, that would maximally respond to the needs of the child, expedite recovery with minimal complications (1).

The requirement for a good outcome is a reliable estimation method for functional state of respiratory system, as well as monitoring the effects of treatment. Neonates with respiratory insufficiency need continuous expansion pressure to menage appropriate functional residual volume and capacity. Deterioration and improvement in the severity of disease and pulmonary functional status is reflected as a change in need for pressure / volume and oxygen expressed through the distending pressure or fraction of inspired oxygen (FiO2) or both. Estimation method that incorporates these parameters would potentially help in objective assessment of the severity of the pulmonary disease. Current assessment of pulmonary disease is with blood gas, chest X-ray and Oxygenation index (OI). OI has its limitations and resource implications (2). A noninvasive alternative assessment tool would allow clinicians to use it more frequently. Promoted in recent studies, Oxygen saturation index (OSI) attempts to objectively score respiratory disease with parameters available with less invasive procedures (3).

Optimizing oxygenation is crucial for neonates on the intensive treatment. Transcutaneous pulse oximetry is usually used to assess oxygenation, whereas the Oxygenation Index, one of the respiratory indices, is used to categorize the severity of oxygenation failure and pulmonary status of neonates requiring mechanical ventilation (2, 3). Respiratory indices are preferred over pulse oximetry in categorizing the severity of illness and have been used in many neonatal trials (4, 5, 6, 7). Traditional respiratory indices, including OI, require indwelling arterial lines, which in turn is associated with multiple complications (4). In order to avoid complications related to invasively obtained respiratory indices, some of noninvasively obtained indices of oxygenation failure and pulmonary status have been proposed in recent studies (3-7), such as the Oxygenation Saturation Index that has been used as a substitute to OI (3, 4). In our study we sought to determine the relation between OSI and OI in intubated, critically ill neontes. **Our primary objective** was to evaluate if the Oxygen saturation index correlates with Oxygenation index in neonates with respiratory failure requiring mechanical ventilation support.

PATIENTS AND METHODS

Our study was retrospective and was conducted at the Department of intensive therapy and care, Pediatric clinic of University Clinical Center of Tuzla and included patients treated during the one-year period (from January 2017 through December 2017). Our including criteria were: neonatal age, involve term and preterm neonates, admited to the NICU, because of respiratory failure requiring mechanical ventilation and had an ABG available for review. We analyzed medical records of all consecutive neonates who were admitted to the NICU during the one-year period, and from total of 332 patients we selected those who were appropriate for the study, and thus extracted the final sample out of 101 neonates. We reviewed data such as gestational age (GA), birth weight (BW), gender, Apgar scores at the first and fifth minutes. We also reviewed values of Score for Neonatal Acute Physiology-Perinatal Extension (SNAP-PE), as already been measured for each individual neonate, received in the NICU, next all the parameters from the arterial blood gas (ABG) analysis, next Oxygenation Index (OI) calculated by the formula, and next Oxygenation Saturation Index (OSI), also calculated according to its own formula. Characteristics of the patients are shown in Table 1. For each selected participant only one blood gas was evaluated. Oxygen saturation (SaO_2) was noted on the ABG analysis and by transcutaneous pulse oximetry recorded in the List of respiratory parameters,which is an integral part of medical records for each patient treated in our Department, and also includes recorded data about the set respiratory parameters continuously for a period of mechanical ventilation (Flow, Fraction of inspired oxygen (FiO_2), Peak inspiratory pressure (PIP), Positive end-expiratory pressure (PEEP), Mean Airway Pressure (MAP), inspiratory time (IE), expiratory time (ET), respiratory rate (spontaneous and given), pulse rate, pulse oximetry, ABG parameter). In our institution for ABG monitoring for neonates who require respiratory support, we attempt to get an ABG on admission and blood test sample in these cases is taken from umbilical arterial catheter or a peripheral arterial line or by arterial puncture. The OI was calculated according to the following formula: value of Fraction of inspired oxygen multiplied by value of Mean airway pressure divided by value of arterial partial pressure of oxygen ($FiO_2 \times MAP / PaO_2$ (3). OSI were calculated according to formula: value of Fraction of inspired oxygen multiplied by value of Mean airway pressure divided by value of peripheral capillary oxygen saturation ($FiO_2 \times MAP/SpO_2$ (3). OSI and OI, according to the above formulas, were calculated, and subsequently, were correlated mutually. We did not stratify the patients according to gestational age. The study was approved by the institutional review board (Ethics Committee of the Institution). Statistical analysis used by the standard methods of descriptive statistics. The significance of differences between samples was tested using parametric

and nonparametric tests of significance and methods of linear correlation, using a statistical program Arcus QuicStat and Systat software.

RESULTS

During 2017, 462 children were treated at the Intensive Care Unit, including 332 neonates, 150 term and 182 preterm infants. 148 children were treated with mechanical ventilation, including 101 neonates, who are involved in the study.

A total of 101 patients were analyzed, 51 (50.5%) male and 50 (49.5%) female, 25 term and 76 preterm. Most infants, 64 (63.3%), were admitted to the NICU in the first day of life. The average age of the patients at the time of admission was 1 day,with the interquartile range of 1-3 days, and with a minimum of 1 hour and a maximum of 14 days. Gestational age ranged around the average of 36 weeks, with the interquartile range 32-36 weeks, and with a minimum of 26 and maximum of 41 weeks. Descriptive characteristics of the patients are shown in Table 1.

Regarding the outcome, 87 neonates (86.1%) survived, and 14 neonates (13.8%) died, i.e. 11 premature and 3 term infants. The median for the full length of intensive treatment was 8 days, with interquartile range 5-13 days, and with a minimum of 2 and maximum of 50 days. There were no significant differences in the length of treatment between survivors and nonsurvivors (Mann-Whitney, Z = -1.74, p = 0.083).

In all of neonates with respiratory failure pulmonary gas exchange was variously impaired with estimation parameters as presented in Table 2.

Table 2. *Respiratory indices of analyzed neonates with respiratory failure*

Parameter	Meian	Minimum	Maximum
Oxygenation index (OI)	15.3	3.6	58.3
Mean alveolar pressure (MAP)	8.0	6.0	12.0
Fraction of inspired oxygen (FiO_2)	50.0	21.0	100.0
Arterial oxygen partial pressure (PaO_2)	48.6	22.3	58.4
Arterial oxygen saturation (SaO_2)	84.8	48.2	94.0
Pulse oxymetri saturation (SaO_2)	94.0	78.0	100.0
PaO_2/FiO_2	80.3	21.5	187.5
Oxygenation saturation index (OSI)	7.3	2.9	11.9

The aim of this study was to investigate if the Oxygen saturation index (OSI) correlates with Oxygenation index (OI) in neonates with respiratory failure requiring mechanical ventilation support. We compared these two indices and we found linear correlation between Mean values of OSI and OI in our sample with Pearson's correlation coefficient of 0.76; and $p < 0.0001$ (95%CI = 0.66-0.83) as shown in the Figure 1.

In order to evaluate OSI as assessment tools in neonates, we compared results of OSI with the values of SNAP-PE scores for each patient and we found that OSI correlated with SNAP-PE score with Pearson's correlation coefficient of 0.52; and $p < 0.0001$ (95%CI = 0.36-0.65).

We also compared the values of OSI between patients who died and those who survived and we found that OSI correlated with the outcome with Spearman's

Table 1. *Clinical characteristics of analyzed neonates and deliveries*

	X ± SD		Minimum	Maximum
Birth weight (grams)	2407.7 ± 923.9		590.0	4450.0
Birth length(centimeters)	47.3 ± 6.6		28.0	59.0
SNAP-PE score	34.8 ± 8.6		5	86
	Median	Interquartile range	Minimum	Maximum
Apgar score 1th minute	7	5-8	0	9
Apgar score 5th minute	8	7-9	1	9
Gestational age (weeks)	36	32-36	26	41
Postnatal age (days)	1	1-3	1	14
Gender (Number)	Male / female		51/50	
Delivery characteristics	n		%	
Caesarean section	42		41.6	
Multiple births	23		22.7	
Inborn	61		60.4	
Outborn	40		39.6	

X ± SD – Mean ± standard deviation

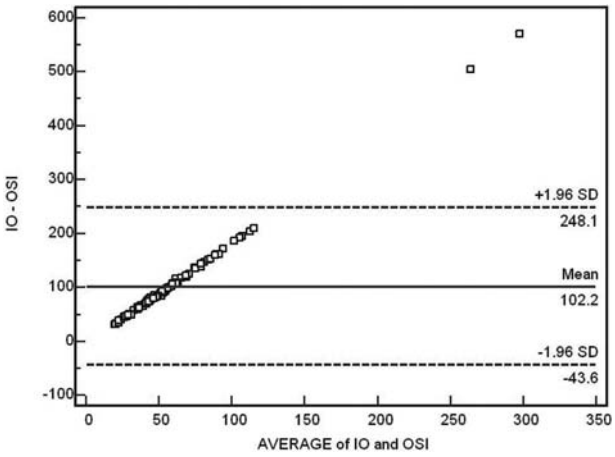

Figure 1. *Linear correlation between Oxygenation Saturation Index (OSI) and Oxygenation Index (OI)*

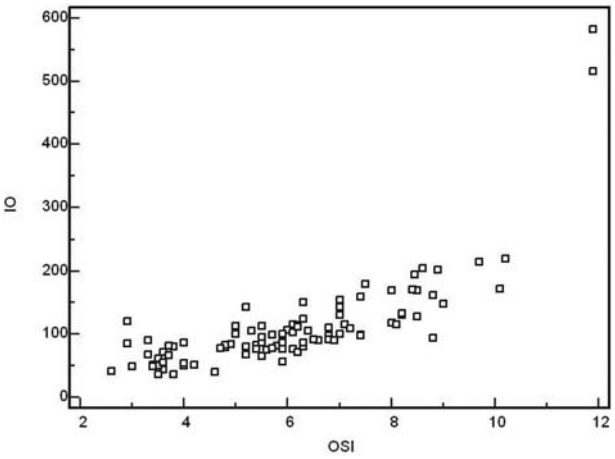

Figure 2. *Bland-Altman plot for Oxygenation Saturation Index (OSI) and Oxygenation Index (OI)*

correlation coefficient of -0.47; and $p < 0.0001$ (95%CI = -0.16- -0.31).

In addition we tested correlation between OSI and OI with the Bland–Altman plot as shown in the Figure 2. Bland-Altman plot shows the compatibility of two indices. According to the chart, the two indices are agreed in mean values, but there was disagreement in the extreme values and tendency to drop OSI in relation to IO at lower values, or to excess the value of OSI in relation to IO in the upper values. It means that OSI can be considered reliable in the average values for the parameters of assessment, but it is not highly reliable for limit values.

DISCUSSION

Hypoxic respiratory failure (HRF) can be defined as a relative deficiency of oxygen, often associated with insufficient ventilation. This deficiency can be reflected by progressive respiratory and metabolic acidosis and remains a persistent challenge in the management of some neonates. HRF accompany common respiratory diseases in neonates such as aspiration syndrome, pneumonia, respiratory distress syndrome (RDS), and congenital diaphragmatic hernia (2). Overall incidence of HRF in neonates, as measured by the overall rate of mechanical ventilation, is 18 per 1000 live births. This rate is 100-fold greater in very low birthweight infants and is also greater in males. Overall mortality rates ranged from approximately 10% to 15%. (8). In Bosnia and Herzegovina, HRF is a leading cause of neonatal mortality and is responsible for nearly 90 neonatal deaths each year, including preterm neonates (9).

HRF is a clinical problem that occurs in many different settings, including wide spectrum of diseases. Our sample of 101 neonates constitutes heterogeneous groups of gestational age, lung maturity, as well as of the underlying disease processes and postnatal interventions. The problem sometimes involves only one area of dysfunction, but most commonly several types of dysfunction are mixed in pathophysiology, including the effect of our treatment. Therefore, clinicians should continually reassess the underlying pathophysiology and adjust treatment accordingly (10).

In fact, the main task in HRF treatment is the best possible oxygenation for all tissues, in order to prevent and avoid hypoxemia. This is achieved through mechanical ventilation, often with the use of high concentrations of oxygen, which is almost inevitable. However, we must not cross the border when the damage outweighs the benefit in light of the fact that exposure to hyperoxia may result in opposite effect and cause complications. So, with equal care, we must avoid both hypoxia and hyperoxia in the treatment of critically ill neonates with respiratory insufficiency (2). Hypoxia causes pulmonary vasoconstriction, normoxia results in pulmonary vasodilation, but hyperoxia does not lead to additional vasodilation.

The evaluation of the hypoxic infant is one of the most common problems for the pediatric clinician. The optimal PaO_2 in the management of HRF is not clear. Some recent findings suggest that gentle ventilation with avoidance of hyperoxia and hyperventilation results in good outcomes in neonates with respiratory failure (1).

Respiratory indices including oxygenation index (OI), mean airway pressure (MAP), FiO_2, PaO_2, arterial oxygen saturation (SaO_2) and PaO_2/FiO_2 values, have been used mostly in recent studies investigating neonates with HRF (2, 3, 4). These values were carried out using arterial blood gas values. For instance, Serdar et al (2) evaluate early outcomes of surfactant treatment in term neonates with HRF using exactly such indices.

The use of the PaO_2/FiO_2 ratio and OI to assess HRF is limited exclusively to the arterial sampling

ABG. In neonates, in whom we perform a maximum saving approach to diagnostic procedures, ABG and complementary noninvasive monitoring techniques, provide information essential for clinician assessment, therapeutic decisions, even predicting the outcomes (6). Rapid changes in physiology, difficult access to the sampling site, and small total blood volume, represent special challenges (7). With the advancements in pulse oximetry in recent years, this noninvasive measure of systemic oxygenation has become the fifth vital sign (7), and has likely led to the decrease in arterial blood gas measurements. The results of this study demonstrate that noninvasive methods of oxygenation assessment, utilizing pulse oximetry as a substitute for PaO_2, can be calculated and used as a substitute for assessment of respiratory failure in neonates.

Oxygen saturation as measured by pulse oximetry (SpO_2)/fraction of inspired oxygen (FiO_2) has demonstrated to be an adequate marker for lung disease severity in children under mechanical ventilation (3, 4). Lobete et al. (3) sought to validate the utility of SpO_2/FiO_2 ratio in a population of critically ill children under mechanical ventilation, noninvasive ventilation support, and breathing spontaneously. They conclude that SpO_2/FiO_2 ratio is an adequate noninvasive surrogate marker for PaO_2/FiO_2 ratio. They suggest SpO_2/FiO_2 ratio may be an ideal noninvasive marker for patients with acute hypoxemic respiratory failure. Khemani et al. (7) sought to validate the comparability of SpO_2/FiO_2 to PaO_2/FiO_2 and OSI to OI in children. They have found that noninvasive indices are a good substitute severity markers in children with respiratory failure specifically for SpO_2 between 80% and 97%.

The aim of our study was to investigate if OSI correlates with OI in neonates with respiratory failure requiring mechanical ventilation support.We compared these two indices and we found linear correlation between mean values of OSI and OI in our sample with Pearson's correlation coefficient of 0.76; and $p < 0.0001$ (95%CI = 0.66-0.83). Mean values of both indices in our study showed a significant correlation,which is consistent with reports of Khemani et al. (7) that also highlight conformity of two indices for SpO_2 between 80% and 97%.

We tested the correlation between OSI and OI with the Bland–Altman plot, showing the two indices were agreed in mean values, but there was disagreement in the extreme values and tendency to drop OSI in relation to OI at lower values, or to excess the value of OSI in relation to OI in the upper values. It means that OSI can be considered reliable in average values for the parameters of assessment, but it is not highly reliable for limit values. Other similar noninvasive indices, also show better compliance and strongest association with the OI for neonates ranking SpO_2 between 88% and 94% as Iyerand Mhanna report (5).

In order to evaluate OSI as assessment tools in neonates, we compared results of OSI with the values of SNAP-PE scores for each patient and we foundthat OSI correlated with SNAP-PE score with Pearson's correlation coefficient of 0.52; and $p < 0.0001$ (95%CI = 0.36-0.65). We also compared the values of OSI between patients who died and those who survived and we found that OSI correlated with the outcome with Spearman's correlation coefficient of -0.47; and $p < 0.0001$ (95%CI = -0.16- -0.31). Ghuman et al. (6) investigated the relationship between markers of oxygenation, PaO_2/FIO_2 ratio, SpO_2/FIO_2 ratio, OI, and OSI, and mortality in children with acute hypoxemic respiratory failure. They conclude that in pediatric acute hypoxemic respiratory failure, easily obtainable pulmonary specific markers for severityof disease (SpO_2/FIO_2 ratio and OSI) may be useful for the early identification of children at high risk of death.

There are some limitations to our study. Firstly, this was a retrospective study. A prospective study of data collected, with close attention to the variance of the pulse oximeter and the exact timing of the arterial blood gas measurement with the recording of the SpO_2, is required in future validation studies of these measures. Another limitation is that, again due to the use of SpO_2 data due to reliability report in the conduct of large-scale clinical trials. Thirdly, the oxygen-hemoglobin dissociation curve and thus the relationship between PaO_2 and SpO_2, are known to be affected by a variety of variables, including pH, temperature, $PaCO_2$, and concentration of 2,3 diphosphoglycerate (1). Compared to the other models, OSI would be the preferable method, as it has the advantage of including the Paw and also utilizing the noninvasive measure of oxygenation (SpO_2). Although these values require validation in a prospective trial, utilizing this new criterion clinically has the potential to allow more accurate diagnosis of respiratory failure in neonates.

CONCLUSION

OSI correlates significantly with OI in critically ill neonates with respiratory failure. This noninvasive method may be used to assess the severity of hypoxic respiratory failure and mortality risk in neonates without arterial access. These results additionally confirm that noninvasive oxygenation assessment method, utilizing pulse oximetry as a substitute for invasively obtained value of partial pressure of oxygen through the arterial puncture, can be calculated and used as a substitute for assessment of respiratory failure in neonates.

Abbreviations:

ABG — Arterial blood gas
ARF — Acute respiratory failure

BW — Birth weight
ET — Expiratory time
FiO_2 — Fraction of inspired oxygen
GA — Gestational age
HRF — Hypoxic respiratory failure
IT — Inspiratory time
MAP — Mean Airway Pressure
NICU — Neonatal intensice care unit
OI — Oxygenation index
OSI — Oxygenation saturation index
Paw — airway pressure
PaO_2 — arterial partial pressure of oxygen
PEEP — Positive end-expiratory pressure
PIP — Peak inspiratory pressure
RDS — Respiratory distress syndrome
SaO_2 — Oxygen saturation
SNAP-PE — Score for Neonatal Acute Physiology-Perinatal Extension
SpO_2 — peripheral capillary oxygen saturation

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

OKSIGENACIJSKI SATURACIJSKI INDEKS U PROCENI RESPIRATORNE INSUFICIJENCIJE NOVOROĐENČADI

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Uvod: Akutna respiratorna insuficijencija (ARI) je najčešći problem koji se sreće u neonatalnoj jedinici intenzivne nege. Etiologija nije uniformna i uglavnom zavisi od gestacijske starosti. Za adekvatan tretman je svakako važno prepoznati i lečiti osnovnu bolest, ali istovremeno moramo snabdeti odgovarajuću respiratornu podršku, perfuziju tkiva i isporuku kiseonika. Za dobar ishod potreban je pouzdan metod procene funkcionalnog stanja respiratornog sistema, kao i praćenje efekata tretmana. Prema važećim načelima težina ARI procenjuje se na osnovu nalaza gasnih analiza, radiografije pluća i Oksigenacijskog ndeksa (OI). OI je prilično agresivan metod procene novorođenčadi, jer uključuje uzimanje uzoraka arterijske krvi. Respiratorni indeks promovisan u najnovijim studijama pod nazivom Oksigenacijski saturacijski indeks (OSI) daje mogućnost objektivne procene respiratorne bolesti pomoću neinvazivne pulsne oksimetrije. **Cilj** našeg istraživanja bila je procena korelacije između OSI i OI kod novorođenčadi sa ARI na mehaničkoj ventilaciji. **Materijal i metode:** U retrospektivnoj kohortnoj studiji odabrano je 101 novorođenče, lečeno u Odseku za intenzivnu terapiju i negu Klinike za dečije bolesti u Tuzli koji su zbog ARI zahtevali mehaničku ventilaciju. Analizirani su klinički podaci kao što su: gestacijska dob, porođajna težina, Apgar skor, bodovna vrednost procene težine bolesti novorođenčadi (SNAP-PE), acidobazni status, Oksigenacijski indeks i Oksigenacijski saturacijski indeks, koji su izračunati i potom međusobno korelirani. Srednje vrednosti OSI i OI korelirale su sa Pearsonovim koeficijentom od 0,76; $p < 0,0001$ (95% CI = 0,66-0,83), a OSI je korelirao sa SNAP-PE sa Pearsonovim koeficijentom od 0,52; $p < 0,0001$ (95% CI = 0,36-0,65). Poredeći vrednosti OSI među pacijentima koji su umrli i koji su preživeli, utvrdili smo da je OSI korelirao sa ishodom sa Spearmanovim koeficijenta -0,47; $p < 0,0001$ (95% CI = -0,16 - -0,31). Bland-Altman metoda potvrdila je korelaciju između OSI i OI u srednjim vrednostima, identifikujući neusaglašenost između dva respiratorna indeksa za ekstremne vrednosti. **U zaključku**, OSI značajno korelira sa OI kod novorođenčadi sa respiratornom insuficijencijom. Ovaj neinvazivni metod procene oksigenacije, baziran na pulsnoj oksimetriji, može se koristiti za procenu težine ARI i rizika smrtnosti kod novorođenčadi.

Ključne reči: novorođenče; respiratorna insuficijencija; Oksigenacijski indeks, Oksigenacijski saturacijski indeks.

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SANAMED
2018; 13(2): 131–138
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.241
UDK: 615.357.035
ID: 267007244
Original article

A STUDY ON THE EFFECTIVENESS OF CLOMIPHENE CITRATE IN COMPARISON TO GnRH ANTAGONIST IN PREVENTING LH SURGE AMONG PATIENTS UNDERGOING OVULATION INDUCTION IN IVF-ICSI

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Primljen/Received 06. 07. 2018. god.

Prihvaćen/Accepted 01. 08. 2018. god.

Abstract: **Objective:** To determine the efficacy of clomiphene citrate (CC) in preventing luteinizing hormone (LH) surge without adding gonadotropin releasing hormone (GnRH) antagonist or GnRH agonist in stimulated first fresh intracytoplasmic sperm injection cycle by evaluating the outcome of oocytes and embryo quality. **Design:** Registry cohort study. **Settings:** Fertility Clinic Registry at Hospital University Kebangsaan Malaysia. **Patients:** A total of 235 fresh stimulated ICSI cycle for patients aged 18-40 years old using clomiphene citrate plus gonadotropin (n = 117) and GnRH antagonist plus gonadotropin (n = 118) were studied. **Intervention:** Comparing two different ovarian stimulation protocol. **Main outcome measure(s):** Social economical demographic, ovarian stimulation response and laboratory outcome. Fertilization rate as our primary outcome and our secondary outcome were oocyte retrieval rate, mature oocyte rate and top quality embryo rate. **Result(s):**There were no difference in the demographic and hormonal characteristic of the study groups. The primary outcome of fertilization rate has significant difference with p value of 0.003; 73.2% for CC group and 64.2% for GnRH antagonist group. The secondary outcome of OR rate (78.4% ± 17.6% VS 80.3% ± 13.4%, p = 0.368), mature oocyte rate (85.2% ± 19.0 VS 81.7% ± 16.7%, p = 0.130) and top quality embryo rate (79.4% ± 24.2% VS 74.9% ± 22.9%, p = 0.178) were comparable between both groups. There were significant difference between the endometrial thickness on the day of trigger and OHSS risk among both groups (8.5 mm ± 1.0 mm VS 9.4 mm ± 1.1 mm, p < 0.001 and 12.8% VS 44.1% respectively).

Discussion: Minimal stimulation protocol with CC and gonadotropin may be the answer to many infertile couples in need of IVF and yet having financial situation deterring them in attempting IVF treatment. GnRH antagonist could be safely replaced by CC by extending to 10 days as this protocol gives better primary outcome and comparable secondary outcomes with less OHSS. CC is recognized to induce thinning of endometrial lining and thus, may impair embryo implantation. However, with advancement of the vitrification system and higher success rate in frozen-thaw embryo transfer worldwide provides an excellent solution for this issue.

Key words: Clomiphene citrate, minimal stimulation, GnRH antagonist, premature LH surge, low cost IVF.

INTRODUCTION

Controlled ovarian stimulation (COS) is an important component in IVF in order to yield optimum number of oocytes with the aim of achieving the best pregnancy outcome (1). There are various protocol that were created to assist patient to conceive without compromising the outcome, such as cycle cancellation due to premature ovulation secondary to LH surge and unwanted complication such as ovarian hyperstimulation syndrome (OHSS). This maximum ovarian stimulation has been challenged by International Society for Mild Approaches in Assisted Reproduction (ISMAAR) in which minimal stimulation provides a simple, safe, cheap and less injec-

tion and stimulation days for patients undergoing ICSI (1, 2, 3). It has become a popular for patients with good prognosis, poor responders and women of advanced age as an alternative to conventional protocol (1, 4, 5).

Premature LH surge during ovarian stimulation has become the main concern for clinicians (6) as it would lead to poor quality of oocyte, poor fertilization and cycle cancellation. The incidence of LH surge was reported to be as high as 15-30% (6) leading to cycle cancellation which is unacceptable to the patients and clinicians. This lead to the role of adding GnRH antagonist or GnRH agonist protocol in IVF to prevent LH surge. Agonist protocol has become less popular as it required more injection and prolonged stimulation which was considered unfriendly to the patients. More than 60% of our ICSI patients undergoing ovarian stimulation using antagonist protocol and the rest were by natural or long agonist protocol for the past 10 years. This explained the reason of using GnRH antagonist protocol as a control in our study.

Clomiphene citrate (anti-estrogen) is used commonly in minimal stimulation protocol as an ovarian stimulation agent. It has a role acting as anti-estrogen blocking the negative feedback to hypothalamus pituitary axis in returns stimulating FSH to produce more follicle (7). Co-administration of CC and gonadotrophin is to reduce the dose requirement of gonadotrophin particularly for patient that prefer less injection. Many authors suggested the need of adding GnRH antagonist in preventing LH surge if CC was used in ovulation stimulation protocol (8) as it has showed to have higher cancellation rate due to LH surge. Extended use of CC till the day of trigger has been advocated recently as CC has its anti-estrogenic effect to block spontaneous LH surge replacing GnRH antagonist or GnRH agonist agents. Al-Inany (9) has proved the efficacy of CC from their research published in 2010 but in IUI patients. It was then further studied by Bhandari S (7) on IVF patients that the fertilization rate using this protocol was as high as 76.1% and it was a viable option without additional use of antagonists. But the study design was not a comparison study to show the effectiveness of CC to replace GnRH antagonist agent. Many recent studies showed acceptable cancellation rate for this protocol using CC (8.57%) which is lower than the standard rate (7). Using CC with gonadotrophin without adding GnRH antagonist was promising (8, 9, 10) as it was claimed to be user friendly with less injection, less gonadotrophin requirement and less cost burden to patients.

AIM

CC has been used since last few decades in our center as ovarian stimulation agents in ICSI cycle. One of the advantage of using CC in ICSI is that it could reduce the usage and total cost of gonadotropin as we are movingly slowly towards minimal stimulation protocol. Recently we have extended it's used to 10 days and we hypothesize that CC is as effective as GnRH antagonist in preventing LH surge by looking at the laboratory outcome of the oocyte and embryo. Fertilization rate is our primary outcome and our secondary outcome were oocyte retrieval (OR) rate, mature oocyte rate and top quality embryo rate. Poor oocyte and embryo quality and less number of fertilized embryo is best reflecting the evidence of LH surge without the need of checking LH level in blood or urine. We do not study on pregnancy outcome as there are various confounding factors affecting the outcome particularly the causes if infertility.

MATERIALS AND METHODS

Patient selection

This was a retrospective study evaluating the efficacy of clomiphene citrate in comparison with the conventional GnRH antagonist protocol in preventing LH surge among patients aged 18-40 years old undergoing ICSI in our MAC clinic at University Kebangsaan Malaysia (partially funded hospital) between January 2016 and December 2017. The data was retrieved via medical records kept at MAC Fertility Clinic Registry. The exclusion criteria were 1) poor ovarian reserve (either FSH > 10 iu/L or AMH < 5.4 pmol/L); 2) azoospermia patients; 3) Large ovarian cyst > 3 cm, 4) previous failed IVF cycle; 5) BMI >30.0.

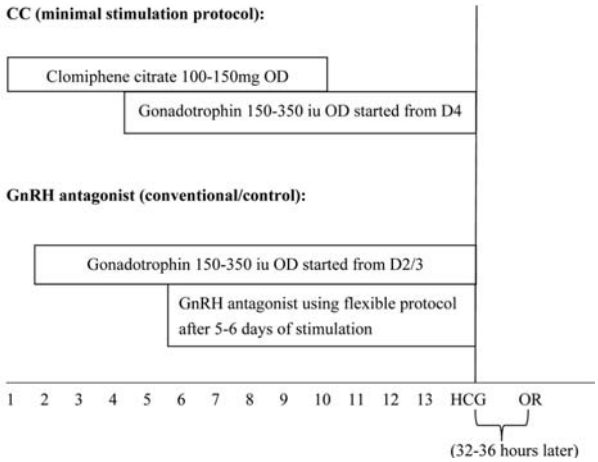

Figure 1. *Ovarian stimulation protocol*

The women were stimulated using mild stimulation protocol with CC 50mg two tablet a day commenced since day one of menses. Gonadotropin with recombinant FSH (Gonal-F, Puregon) or hMG (Menopur) were added from day four of menstrual cycle onwards. The starting dose of gonadotropin was dependent on the age and causes of infertility. CC would be extended till day 10 of menstrual cycle. Transvaginal scan would be performed on day 8 or day 10 and scan would be repeated every 2 to 3 days according to the

ovarian response. Gonadotropin would be continue beyond day 10 till the leading follicle reaching ≥18 mm. Final oocyte maturation was achieved by human chorionic gonadotropin (HCG) with either subcutaneous pregnyl 10,000 iu or Ovidrel 0.25 mg.

For conventional controlled ovarian stimulation using GnRH antagonist protocol, patients would be stimulated with recombinant FSH (Gonal-F, Puregon) or hMG (Menopur) from day 2 or day 3 of cycle. Transvaginal scan would be performed on day 8 of cycle and repeated every 2 to 3 days depending on the response. GnRH antagonist agent (Cetrotide or orgalutran 0.25 mg) was added when the leading follicle reaching 14 mm (flexible protocol) in diameter and continued till triggering day with single dose HCG as mentioned earlier.

TVOR was performed 32-36 hours following HCG administration under sedation at day care theatre. Patient would be excluded if unexpected poor ovarian response or premature ruptured of follicle during the TVOR day. Serum E2, P4 and LH were not routinely performed during the ovarian stimulation in our centre.

ICSI

We performed ICSI for all the cases as these were partially funded program to ensure higher fertilization rate than conventional IVF. Embryo transferred was performed under ultrasound guided. One to two day 5-6 blastocyst was transferred in CC group and two to three day 3 cleavage embryo was transferred in control group. Luteal phase support was given based on different clinician and cases.

Study outcomes

We extracted data from the medical records on patient demographic, type of protocol, stimulation parameter and the laboratory outcome. Oocyte atresia, poor quality of oocyte and failure of fertilization was a consequences of premature LH surge. The indirect method to measure the evidence of LH surge was by study on the laboratory outcome as we do not perform blood or urine LH test for patients as the blood test result would release by few days after the blood taking. The study primary outcome was on fertilization rate and the secondary outcome included oocyte retrieval rate, mature oocyte rate and top quality embryo rate. OHSS risk was studied if the number of follicle during TVOR reaching ≥ 15. Top quality was defined by Veeck's criteria for cleavage embryo day 3 and Gardner and Schoolcraft (1999) criteria for blastocyst day 5-6 blastocyst. The study was approved by ethical committee with NMMR-18-44-39835 S1 and FF-2018-164.

Statistical analysis

Descriptive analysis would be conducted for all variable. Appropriate cut off point would be obtained based on mean ± standard deviation (SD). Independent t test would be use to see the association between the demographic of the study group including age, baseline FSH level before starting treatment, total dosage and injectionof gonadotropin and total stimulation days. OR rate, mature oocyte rate, fertilization rate and top quality embryo rate were compared among the two stimulation protocol with independent t test. Chi square test would be use for comparison between the 2 groups with type of infertility and the risk of developing OHSS. Binary regression is conducted to analyze the causes of infertility for the dependent variable. Statistical significant would be set when p value is less than 0.05 using SPSS software 22 version.

RESULTS

In 2016-2017, there were total of 532 fresh stimulated ICSI cycle conducted in my centre. Among them, 382 (71.8%) cases using these two ovarian stimulation protocol. Fertilization rate was highly dependent on the quality of the oocyte and sperm, hence we decided to exclude patients with female age >40 years old, poor ovarian reserve (FSH > 10 iu/mL or AMH < 5.4 pmol/L) and azoospermia patients. We included total of 235 patients with complete data that underwent first and fresh stimulated ICSI cycle (no history of failed IVF) with CC plus gonadotropin protocol (n = 117) and GnRH antagonist protocol (n = 118).

The demographic and hormonal characteristic of study groups was shown in Table 1. There were no signif-

Table 1. *Demographic and hormonal characteristic of study groups*

	CC group (n = 117)	GnRH Antagonist group (control) (n = 118)	P value
Age (years)*	34.3 ± 3.2	34.6 ± 3.4	0.497
Type of infertility®			0.168
Primary	82 (70.1%)	92 (78.0%)	
Secondary	35 (29.9%)	26 (22.0%)	
Causes of infertility∞			
Male	22 (18.8%)	20 (16.9%)	0.711
Tubal	22 (18.8%)	14 (16.9%)	0.711
PCOS	10 (8.5%)	10 (8.5%)	0.984
Endometriosis	17 (14.5%)	18 (15.3%)	0.876
Others	15 (12.8%)	22 (18.6%)	0.220
Unexplained	31 (26.5%)	28 (23.7%)	0.625
Baseline FSH (miu/ml)*	5.65 ± 1.51	5.33 ± 1.52	0.103

FSH: follicle stimulating hormone

*expressed as mean + SD and p value was calculated by the student t test

∞ expresses in n (%) and p value was calculated by binary logistic regression

® expresses in n (%) and p value was calculated by chi square test

Table 2. *Stimulation protocol and complications*

	CC group (n = 117)	GnRH Antagonist group (control) (n = 118)	P value
Total Gonadotropins dosage, iu	1450.6 ± 545.2	2445.3 ± 569.0	< 0.001
Total days of gonadotropin injection, d	6.9 ± 2.1	11.3 ± 1.1	< 0.001
Total days of stimulation with medication, d	11.3 ± 1.9	11.3 ± 1.1	0.945
Endometrial thickness during HCG triggering day, mm	8.5 ± 1.0	9.4 ± 1.1	< 0.001
Risk of OHSS®	15 (12.8 %)	52 (44.1%)	< 0.001

® expresses in n (%) and p value was calculated by chi square test
*expressed as mean + SD and p value was calculated by the student t test

icant difference among both protocol in age, type of infertility, causes of infertility and the baseline FSH level.

Total gonadotropin dosage, total days of gonadotropin injection and OHSS risk were significant difference in CC group (Table 2). The total days of ovarian stimulation among two groups was not significant difference similar with standard protocol. Our study showed significant difference of endometrial thickness among both groups (8.5 mm ± 1.0 mm VS 9.4 mm ± 1.1 mm, $p < 0.001$).

Our primary outcome as in fertilization rate were significant difference with p value 0.003. Fertilization rate for CC group was 73.2% and GnRH-antagonist group was 64.2%. However the secondary outcome for our study for OR rate (78.4% ± 17.6% VS 80.3% ± 13.4%, $p = 0.368$), mature oocyte rate (85.2% ± 19.0% VS 81.7% ± 16.7%, $p = 0.130$) and top quality embryo rate (79.4% ± 24.2% VS 74.9% ± 22.9%, $p = 0.178$) were comparable among those two protocol.

DISCUSSION

CC is commonly used as an ovarian stimulation medication in intrauterine insemination (IUI) as the principle of management is targeting monofollicular stimulation unlike COS IVF-ICSI aiming for eight to twelve follicles in one cycle. The reason behind was not all follicles contain mature oocyte and the ultimate number of surviving embryo reaching cleavage or blastocyst stage would be lesser than the number of follicles seen during triggering day depending. Clinician would feel much comfortable getting more oocyte assuming that it is rather safe to keep the number of top quality embryo in optimum number to avoid cycle cancellation.

CC has been used in IVF-ICSI as minimal stimulation protocol particularly in high responder patients as these are the patients at risk of OHSS. The usage on CC in minimal stimulation has been commonly seen in patient with premature ovarian insufficiency with low anti-Mullerian hormone. Various studies have shown that natural or minimal stimulation protocol is more cost effective than COS as the ovarian reserved is markedly reduced. It is well known that increasing dosage of gonadotropin to maximum dose seems pointless to change the fact that only few number of primordial follicle that would be stimulated in each menstrual cycle. Unlike those normo-responsive patients in which 15-20 primordial follicle would be stimulated in each menstrual cycle. Gonadotropin is used to stimulate these follicles in order to get reasonable number of oocytes. COS has various complications include overstimulation leading to unwanted complications such as OHSS.

Our CC group were considered as minimal stimulation protocol as gonadotropin injection was started slightly later on day 4 of cycle where the peak of FSH was about to drop on this day. On the other hand, control group using GnRH antagonist as the pituitary down regulation agents had the gonadotropin injection started from day 2 to 3 onwards. This is consistent with other study(1-5, 11, 12) result in which the total gonadotropin dosage in ICSI cycle and total days of gonadotropin injection were significant lesser than the control group. This study protocol give various benefit to patients include less pain due to lesser subcutaneous injection of gonadotropin and acceptable cost for one IVF cycle. It is considered more user friendly particularly for those with needle phobic patients. Stimulated ICSI is costly due to expensive medications including injectable gonadotropin for ovarian stimulation, HCG or GnRH agonist trigger and prolonged luteal phase support with progesterone (intramuscular or vaginally) till 7-12 week of gestation if patient conceived. Replacing the GnRH antagonist by extending CC usage could further reduce the cost in ovarian stimulation agents as GnRH antagonist is rather costly compared to oral CC (Cetrorelix, Serono: $83.94 USD/vial; CC: $1.35 USD/tablet). In general, total dosage of GnRH antagonist is usually 5-6 vial per cycle (one vial 0.25 mg) and our total CC dosage is 1000 mg (one tablet 50 mg). It was very obvious that CC has added benefit as cheaper compared to GnRH antagonist agent (total amount of Cetrorelix: $419.7 USD-$ 503.6 USD VS CC: $27 USD). Our study has proved that it is more cost effective than conventional COS with antagonist protocol and we believed that it is suitable to be used particularly in financial constraint patients or funded hospital. This would give the opportunity to low or middle class patients to rear a genetically related child.

As CC group was minimal stimulation protocol as mentioned earlier, the number of follicle during TVOR, number of oocyte retrieved during TVOR, number of mature oocyte MII, number of fertilized embryo and number of top quality embryo were significant lesser compared to control group (Table 3). It sounded logical to obtain this findings as both groups were using two different approach where CC as in minimal stimulation protocol and control group was using COS protocol. This was not the aim of our study as we wanted to show that CC could be used safely as in GnRH antagonist in preventing premature LH surge without compromising the IVF outcome. We strongly believe that to start the gonadotrophin earlier as in antagonist group from day 2 or day 3 of menses would achieve the similar number of follicles, oocyte and embryos as in the control group.

The fertilization rate is very much dependent on the quality of oocyte and sperm. The result was 73.2% VS 64.2% in which the fertilization rate in CC group was significant higher than GnRH antagonist group. We concluded that CC could be safely use in preventing premature LH surge as it does not affect the quality of oocyte being fertilized by sperm but even better than conventional protocol with GnRH antagonist. We have proved that extensive ovarian stimulation with excessive number of follicles would certainly affect the quality of oocyte. It was consistent with the result by Bhandari S et al (7) with fertilization rate of 76.1%.

Our study results showed no significant difference in secondary outcome but the top quality embryo rate and mature oocyte rate were higher than control group (79.4% ± 24.2% VS 74.9% ± 22.9% and 85.2% ± 19.0% VS 81.7% ± 16.7% respectively). It has clearly shown that minimal stimulation with CC plus gonadotropin without adding GnRH antagonist is effective in preventing LH surge. The only benefit of maximal ovarian stimulation is to obtain more number of embryos which most of the clinician believe that by increasing the number of embryo could increase the pregnancy rate particularly cumulative pregnancy per cycle. But we would like to against this statement in why need to get more but moderate quality of embryo. Isn't it redundant by doing so with added complications and risk to our patients?

Table 3. *IVF parameters and outcome*

	CC group (n = 117)	GnRH Antagonist group (control) (n = 118)	P value
No. follicle before TVOR	8.3 ± 4.7	14.8 ± 8.5	< 0.001
No. oocyte retrieved	6.4 ± 3.9	11.8 ± 6.7	< 0.001
Ooctye retrieval rate, %	78.4 ± 17.6	80.3 ± 13.4	0.368
No. of mature oocyte MII	5.3 ± 3.3	9.4 ± 5.2	< 0.001
Mature oocyte rate, %	85.2 ± 19.0	81.7 + 16.7	0.130
No. fertilized embryo	3.6 ± 2.3	5.8 ± 3.8	< 0.001
Fertilization rate, %	73.2 ± 24.0	64.2 ± 21.7	0.003
No. top quality embryo	2.6 ± 1.6	4.1 ± 2.7	< 0.001
Top quality embryo rate, %	79.4 ± 24.2	74.9 ± 22.9	0.178

All expresses in mean ± SD and p value was calculated by student t test

Medroxyprogesterone has been used in preventing premature LH surge (13) by Kuang et al (13) however it does not has added role as in CC for ovarian stimulation and it does not has an extra benefit as CC in reducing the total dosage of gonadotropin and total days of stimulation (CC has positive effect on ovarian follicle development). This would provide an advantage in resources restricted ART centre (6) by providing an affordable assisted reproductive technique to patients (11, 12, 14, 15). Gonadotropin agents are potent ovarian stimulation compared to CC and close monitoring every 2 to 3 days is needed during the ovarian stimulation to allow clinician to adjust the dosage of gonadotropin depending on the response of the ovarian stimulation. As CC has added advantage in reducing the total days of gonadotropin injection in CC group, minimal stimulation protocol requires fewer clinic visits for monitoring (12, 14)compared to conventional GnRH antagonist protocol. Frequent clinic visits to clinic would add burden to patients in applying leave and indirectly affect their performance in work.

In recent years, we moved toward single day 5 top quality embryo transfer as this could reduce the likelihood of multiple birth and increase the chances of pregnancy rate. The transitional of practice in embryo transfer from cleavage embryo to day 5 blastocyst was adopted by most of the IVF centre in decadesto increase the implantation rate as clinician believe that only the top quality embryo could survive till day 5. The number of embryos left in day 5 compared to day 3 would definitely lesser possible facing the problem of cycle cancellation. This would explain the reason of reduction in the number of top quality day 5 blastocyst in CC group (mean ± SD: 2.6 ± 1.6) compared to day 3 embryo in GnRH antagonist group (mean ± SD 4.1 ± 2.7).

Conventional COH may offer a higher chance for embryo transfer with more surplus embryo compared to mild stimulation protocol (16). Cumulative pregnancy per one IVF cycle would be higher in conventional COH which make minimal stimulation less popular (5) and it is still a debatable issue regarding the long term cost effectiveness (1, 17) as patient need to restart a new stimulated IVF cycle if no surplus embryos left for the embryo transfer which is commonly seen in mild stimulation protocol.

Endometrial thickness on the day of HCG administration is prognostic of fecundity and continuing of pregnancy in cycle of ovulation induction and Richard et al, 1993 has proved that CC plus gonadotropin would affect the EM thickness (18) many years ago which is consistent with my study results. However with the advancement of vitrification system, freeze-thaw embryo transfer is the best option to solve this problem and in future we are slowly moved towards freeze all as reported to achieve higher success rate (19).

OHSS is a common complications following ovarian stimulation particularly when the number of follicle is more than 15 > during TVOR. Despite knowing that OHSS would not affect the pregnancy but Barbara et al has proved that it is associated with adverse pregnancy outcome (20). The OHSS risk for our CC group was significant low (12.8% VS 44.1%) which is safer to the over-respondent patients. We shall provide the best care and plan to patients without causing more harm to patients. The limitation of our study is retrospective and present of confounding factors such as various causes of infertility, different usage of HCG triggering agent and different type of gonadotropin that would affect the outcome. Our study is still ongoing on pregnancy outcome such as clinical pregnancy rate, ongoing pregnancy rate, live birth rate and cumulative pregnancy rate as most of the clinician concerned about. In future, we shall consider the usage of Letrozole (aromatase inhibitor) instead of CC that would not affect the endometrial thickness allowing fresh embryo transfer (21, 22).

Our study showed that by extending CC to 10 days without adding antagonist is safe without antagonist agent. In Herndon et al (11) study, no GnRH antagonist protocol and hormonal blood test was taken during COS IVF-ICSI. Their results has showed the fertilization rate in CC plus gonadotrophin (flare protocol) has comparable result ($p = 0.37$) as other protocol like CC/Letrozole alone, sequential protocol and gonadotrophin alone. It is feasible not to add GnRH antagonist agent if extending CC from 5 to 10 days as an agent to prevent premature ovarian surge.

CONCLUSION

It has always being a challenge for clinician using gonadotrophin stimulation in the occurrence of premature LH surge. The incidence of premature LH surge varies depending on different studies. Luteinization before ovarian follicle maturation give negative impact on the quality of oocyte and embryos.

Although the use of GnRH antagonist co-administration is advisable for mild stimulation to prevent LH surge but our study examine that not adding GnRH antagonist in mild stimulation protocol is viable option with comparable outcome with conventional COH –ICSI consistent with other studies (5, 7, 23). Minimal stimulation protocol with CC and gonadotropin may be the answer to many infertile couples in need of IVF and yet having financial situation deterring them in attempting IVF treatment. It may be a suitable protocol for over-respondent patients knowing the fact that the quality of the oocytes is inversely with the number of oocytes obtaining from transvaginal oocyte retrieval.

In conclusion, IVF cycle is a burdensome process and we as a clinician should provide a friendly COH protocol to our patients and the safety of the patients should be prioritized avoiding or minimizing the complications. We should rethink the aim of "successful IVF" by helping patient to conceive or to complete the family. Why need more and extensive ovarian stimulation like conventional GnRH antagonist protocol by risking the patients to unwanted complications? CC is effective in COH-ICSI particularly in low economy country and did not have negative influence on the treatment outcome. Management for ART protocol should always be individualized (24) as every patients are different.

Abbreviations

ICSI — Intracytoplasmic sperm injection
CC — Clomiphene citrate
OI — Ovulation induction
GnRH — Gonadotropin releasing hormone
FSH — Follicular stimulating hormone
LH — Luteinizing hormone
OHSS — Ovarian hyperstimulation syndrome
OR — Oocyte retrieval
MII — Metaphase II
COS — Controlled ovarian stimulation
FET — Frozen embryo transfer

Acknowledgment

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DECLARATION OF INTEREST

The investigators declare they have no conflict of interest.

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DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

STUDIJA EFIKASNOSTI KLOMIFEN CITRATA U POREĐENJU SA GnRH ANTAGONISTIMA U PREVENCIJI SKOKA LH MEĐU PACIJENTKINJAMA KOJE SU NA STIMULACIJI JAJNIKA U PROCESU IVF-ICSI

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Cilj: Cilj ove studije bio je da se utvrdi efikasnost klomifen citrata (CC) u prevenciji skoka luteinizirajućeg hormona (LH) bez dodavanja antagonista gonadotropnog oslobađajućeg hormona (GnRH) i GnRH agoniste u prethodno stimulisanim ćelijama ICSI ciklusom, koristeći zrelost oocita i kvalitet embriona kao ishod. **Dizajn studije:** retrospektivna kohortna studija **Lokacija:** Klinika za fertilitet pri Univerzitetskoj bolnici Kebangsaan Malezija. **Pacijenti:** Ukupno 235 pacijenata je bilo uključeno u studiju, starosne dobi 18-40 godina, koji su stimulisani ICSI ciklusom pri čemu je u jednoj grupi korišćen klomifen citrat u kombinaciji sa gonadotropinom (n = 117), dok je u drugoj korišćen GnRH antagonista u kombinaciji a gonadotropinom (n = 118). **Intervencija:** Upoređivanje dva različita protokola stimulaciju jajnika. **Varijable:** socio-ekonomska demografija, odgovor na stimulaciju ovarijuma i laboratorijske analize. Stopa fertilizacije kao primarni ishod, a kao sekundarni ishod uključeni su stopa oocitnog povlačenja, stopa zrelosti oocita i stopa kvaliteta embriona. **Rezultati:** Nije bilo statističke značajnosti u poređenju hormonskih i demografskih karakteristika među grupama. Kada se posmatra primarni ishod, odnosno stopa fertilizacije, primećena je statistička značajnost i p vrednost je 0,003; 73,2% CC grupe i 64,2% GnRH antagonist grupe. Sekundarni ishod stope (78,4% ± 17,6% vs 80,3% ± 13,4%, p = 0,368), stopa zrelosti oocita (85,2% ± 19,0% vs 81,7% ± 1,7%, p = 0,130) i stopa kvaliteta embriona (79,4% ± 24,2% vs 74,9% ± 22,9%, p = 0,178) bile su upoređivane između grupa. Pokazana je statistička značajnost između debljine endometrijuma na dan okidanja i OHSS rizik među grupama (8,5 mm ± 1,0 mm vs 9,4 mm ± 1,1 mm, p < 0,001 i 12,8% vs 44,1% respektivno). **Diskusija:** Protokol minimalne stimulacije sa CC i gonadotropinom može biti rešenje za većinu parova koji su suočeni sa problemom neplodnosti i kojima se savetuje IVF, a koji nisu u situaciji da to finansiraju. GnRH antagonist može da zameni CC, produžaanjem još 10 dana jer ovako unapređen protokol pokazuje bolji ishod i komparabilni sekundarni ishod sa manjim OHSS rizikom. Zna se da CC smanjuje debljinu endometrijuma. S toga, može nepovoljno uticati na impantaciju embriona. Međutim, imajući u vidu unapređenje vitrifikacionog Sistema i veći broj uspešnih embriotransfera širom sveta, može se reći da je i ova metoda odlično rešenje neplodnosti.

Ključne reči: klomifen citrate, minimalna stimulacija, GnRh antagonisti, prematurni LH skok, niska cena IVF.

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SANAMED
2018; 13(2): 139–143
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.240
UDK: 615.477.3
ID: 266982156
Original article

FACTORS AFFECTING INSOLE USAGE IN PATIENTS WITH PES PLANUS

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Primljen/Received 05. 07. 2018. god. Prihvaćen/Accepted 25. 07. 2018. god.

Abstract: **Introduction:** Insoles and exercise programs are the main treatment methods for pes planus patients. Insole using may prevent the formation of pain in daily activities as well as increasing the quality of walking. The aim of this study was to investigate insole usage time and the factors affecting this situation in patient with pes planus. **Materials and Methods:** 136 patients with pes planus who were prescribed an insole, included in the study. We invited patients to participate in a telephone survey. Patients´ demographics, insole usage time, reasons to quit and the quitting time were questioned. Insole usage rates and the demographic data of patients were compared. **Results:** Of the 136 patients included in the present study, 80 were women (59%) and 56 were male (31%). 86 of these patients used an insole six months and over, 15 of them used shorter than six months and 35 of them bought the insoles but they didn't use it (63%, 11% and 26% respectively). The average age of patients who used insoles was 32.80 ± 18.14 and who did not use insoles was 40.77 ± 12.54 (p = 0.04). 33.8 percent of women and 14.3 percent of men did not use insoles. This difference is statistically significant (p = 0.04). We did not find any significant relationship between height, weight, body mass index values and usage of insoles (p > 0:05). **Conclusion:** The use of insoles in treating patients with pes planus is widely accepted and may be affected by the demographics such as gender and age. Besides wearing comfort, female sex and older age should be considered that may affect the use of insoles.

Key words: Pes Planus, Insole, Orthosis.

INTRODUCTION

Acquired pes planus is a progressive and symptomatic deformity of foot that occurs as a result of dysfunction of structures that support medial longitudinal arch dynamically and statically (1). It is separated into two sub-groups as rigid and supple (2). Literature still hasn't reached a common consensus about its etiology; however posterior tibial tendon failure is considered to be the most common cause of supple pes planus (3).

Pes planus deformity might be asymptomatic; but it can also cause mechanic back pain by causing a chain reaction of kinetic and kinematic changes on lower extremity (4). Asymptomatic supple pes planus can be kept under control with patient education, using appropriate footwear, weight loss, and patient follow-ups; however with symptomatic patient's activity modification, orthosis use, and stretching exercises are required. In cases of severe pain non-steroidal anti-inflammatory drugs may be prescribed and/or a physical therapy program may be initiated. In cases where there is a lack of adequate response to the conservative treatment surgical procedures that target bone structure and/or soft tissue may be considered (5-8).

Different types of orthosis can be used in accordance to severity of complaints and deformity in patients with pes planus. Pre-made insoles that are produced from materials like silicon, ethyl vinylacetate (EVA) are often preferred because of accessibility and cost. However there are also custom-made or partially custom-made insoles available commercially. In custom made insoles for pes planus deformity the aim is to correct foot composition by placing different combinations of forefoot medial wedge, heel medial wedge, and longitudinal and transverse arch supports in different heights according to specific requirements in a mold produced for foot measurements of the patient (9). Other methods such as using a negative model that is created by covering foot with cast bandage or pressing foot in pedilen foam or Cad/Cam method are also used in production of insoles (10).

Hardness of the material used in insole is also a very important factor for usability and effectiveness. To be able to show correcting effect the insole must be hard; and for it to be able to show balanced weight distribution the insole must have shock-absorbing properties. In studies moderate and high density EVA or polyurethane are mostly preferred (10, 11). Footwear used is closely related to effectiveness and patient's continued usage of the insole. Insole's capability of staying stable in footwear and its part covering around the heel being hard enough to resist bending are basic factors required for the control of the calcaneal movement. Velcro or lace sports shoes or trekking shoes produced for nature walks are preferred (12).

As discussed above patient's gain from the insole and the time it will be used is depend on factors such as production method, production materials and the footwear used in conjunction with the insole. In this study we aimed to study the insole usage time and factors that impact this in patients diagnosed with pes planus.

MATERIAL AND METHOD

Files of the patients who were diagnosed with pes planus and prescribed insoles were identified and scanned retrospectively. Patients whose plantar pressures were measured and, their custom-made EVA insoles produced in accordance to these measurements were included. Patient's demographics, whether they use the insole or not, and their insole usage times were recorded. Patients who didn't use or ceased using the insoles were questioned about the reasons and time. After the data was recorded, association between insole usage rates and demographic data, and patients who used insole were compared.

Statistical analyses were performed on Windows based SPSS 15.0 package software, p was ≤ 0.05. Variables acquired from measurements were described as mean standard deviation. Q-square and t-tests were used for statistical analyses.

RESULTS

136 patients were included in this study. 56 were male, 86 were female. Sociodemographic data of the patients were shown in Table 1. 86 (63%) patients used insole for 6 months or more, 15 (11%) used insole for less than 6 months. 35 patients (26%) never used the insole even though they acquired it (Figure 1).

Mean age for patients who used and didn't use the insole were 32.80 ± 18.64 and 40.77 ± 15.54 respectively. Patients who kept using insole were younger (p = 0.04). Rates of patients who didn't use insole were 14.3% and 33.8% for males and females respectively

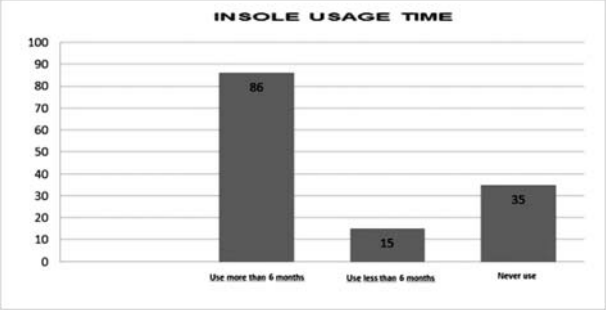

Figure 1. *Insole usage time and number of patient*

Table 1. *Patient sociodemoghraphics (n = 136)*

Variables		
Sex (n, %)	Female	80, %59
	Male	56, %41
Age (Mt ± SD)	Using insole	32.80 ± 18.14
	Not using insole	40.77 ± 12.54
Usage time (months) (M ± SD)	Female	6.70 ± 5.04
	Male	8.64 ± 5.94
VKI (Ort ± SS)	Female	24.5 ± 4.52
	Male	24.4 ± 3.89
Reason for stopping nsole usage n, (%)	Insole is hard	25, %18
	Can't use with footwear of choice	16, %12
	Increase in pain or discomfort	4, %3
Constant usage n, (%)		86, %63
Inconstant usage n, (%)		5, %4

(Table 1). Rate of not using insole in female patients compared to male patients was statistically significant (p = 0.04). No association between insole usage, and weight, height and BMI was found (p > 0.05).

45 patients haven't used their insoles. Most common reason presented was that the insole was too hard. Second most common reason presented was that the insole didn't fit the footwear of choice (Table 1).

DISCUSSION AND CONCLUSION

Insoles are frequently used inconservative treatment of pes planus for re-establishing the medial arch and increase foot stability. However questions such as "Are insoles therapeutically effective?", "Are patients happy with insole usage?", and "What are the reasons behind not using the insole?" are still unanswered. In this study we aimed to study the insole usage time and factors that impact this in our 136 patients who were diagnosed with pes planus and were prescribed with in-

soles. We found that patients who haven't used insoles were of more advanced aged and rates for not using was higher for women compared to men.

Some patients doesn't use insoles regardless of potential gains. In a study investigating causes of this assessments were made under three main categories: (1) usability, (2) communication with health care professionals, and (3) views of other people. In this study of 23 patients it was concluded the most important reason of not using insole was usability. It was concluded that if the insole was providing the requirements of patient, improve his/her mobility, reduce the pain, and provide comfort usability increases paralelly (13). In our study 29 patients felt their pain increased because the insole was too hard and ceased usage. In our study we found that most common reason for cease of usage was usability.

In a meta-analysis consisting of 11 different randomized controlled trials, in line with ISO standards, usability was assessed under 4 main categories (efficacy, effectiveness, satisfaction and status of use). In all these studies efficacy was evaluated but effectiveness, satisfaction and status of usage were evaluated in 1, 5 and 3 studies respectively. Since only few studies included all four of the categories under usability, no conclusion on which parameter is more impactful on orthosis usage was reached (14).

In another meta-analysis which focuses on patient compliance with orthosis and orthopedic shoes, 10 studies were included (1576 patients). It was recorded that 6-80% of the patients weren't using the prescribed orthosis. Rates for not using was highest for AFO and lowest for orthopedic shoes. In four studies the most important reason was that the device was aesthetically unacceptable. Other important reasons were difficulty of usage, pain during use, unsatisfactory rates of relief in walking, and discomfort. Rate for dissatisfaction in orthopedic shoes was higher for women. This was attributed to women's higher amount of aesthetic concerns (15). In our study rates for not using insole was higher for women. But since the insoles were invisible to outside eyes we attributed this to discomfort rather than aesthetic concerns.

There is a close relationship between patient's continued usage the orthotic device and acceptance of said device. Most important reason against acceptance of the device is that it is visible to the outside. Visible device reminds the patient and people around him about his condition. If the patient makes peace with his condition and comes to the terms of acceptance with the condition and the device, then it could be used properly (16, 17).

107 patients suffering from diabetic ulcers were prescribed with custom made insoles and their time of usage for insoles and their step counts while using insole were calculated. It was seen that patients preferred to use the insole more frequently while being outside compared to while being at home (18). In our study 14 patients stated that they stopped using insole because they were unable to use the footwear of their choice. If the patients is spending more time at home compared to outside insole must also be used at home in order to reduce the severity of symptoms. So making the insole appropriate for usage with a footwear suitable for home use or prescribing different insoles for home and outside usage will improve usability.

There are discrepancies between the results of papers which investigate the association between device usage and age. In his study Haworth and Hopkins (19) found that device compliance in older patients was better compared to younger ones. In contrast Geiger (20) found that compliance of age group 60-69 was lower. Other studies reported no association between device usage andage (21, 22). In our study we observed that patients who used insole were younger than patients who didn't use insoles. Most striking aspect about this situation is that most common complaint of the older patients who didn't use insoles was a lack of comfort stemming from hardness. In older patients insole usage times is lowercompared to younger patients due to time they spent at home is higher compared to younger patients.

Another important factor about device usage is doctor-patient communication. If the complaints are taken into account and the patient is allowed to express himself comfortably; confidence in the physician increases while also increasing patient compliance (23). Also only way to understand the complaints and expectations of the patient is establishing a strong communication.

In the end, every patient who uses insoles shares the same purpose: to be able to walk with comfort like before, and become free and independent again. If the insole does not meet these expectations of the patient and/or patient achieves his purpose by other means, the insole will be left to gather dust. This leads to an increase in health-care costs, patient problem left unsolved and a lack of professional satisfaction for health-care professionals who work in orthosis-prosthesis field. Performing a comprehensive patient evaluation, informing the patient about all available treatment choices, involving him in the process of treatment and making sure a device meets the patient expectations while being aesthetically acceptable, will increase the device compliance and usage time.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Licensing

Sažetak

FAKTORI KOJI UTIČU NA KORIŠĆENJE ULOŽAKA ZA OBUĆU KOD PACIJENATA SA PES PLANUS-om

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Uvod: Ulošci za obuću i program vežbi su glavni način lečenja kod pacijenata sa pes planusom. Ulošci mogu da preveniraju stvaranje bolova tokom dnevnih aktivnosti, kao i da povećaju kvalitet hoda. Cilj ove studije bio je da se napravi korelacija između vremena korišćenja kao i drugih faktora kod pacijenata sa pes planusom.

Material i metode: 136 pacijenata koji su dobili uloške je bilo uključeno u studiju. Pacijenti su uključeni u studiju preko telefonskog poziva. Demografske karakteristike pacijenata, vreme korišćenja uložaka, razlog i vreme nekorišćenja uložaka su uzeti u razmatranje. Stope korišćenja uložaka kao i demografski podaci pacijenata su upoređivani.

Rezultati: Od 136 pacijenata koji su bili uključeni u studiju, žena je bilo 80 (59%), a muškaraca 56 (31%). 86 ovih pacijenata je koristilo uloške preko šest meseci, 15 ih je koristilo kraće od 6 meseci, a 35toro je kupilo, ali nije koristilo (63%, 11% i 26% respektivno). Prosečni uzrast pacijenata koji su koristili uloške bio je 32,80 ± 18,14 a onih koji nisu koristili 40,77 ± 12,54 ($p = 0,04$). 33,8 % žena i 14,3 % muškaraca nije koristilo uloške. Ova razlika je bila statistički značajna ($p = 0,04$). Nismo našli statističku značajnost ni u jednom parametru od interesa, kao što je visina, težina, BMI i korišćenje uložaka ($p > 0,05$).

Zaključak: Korišćenje uložaka u lečenju pacijenata sa pes planusom je široko prihvaćena metoda lečenja na koju mogu da utiču demografski parametri kao što su pol i godine starosti. Pored udobnosti nošenja, ženski pol i starija životna dob trebaju se uzeti u razmatranje kao faktori koji utiču na korišćenje uložaka.

Ključne reči: pes planus, uložak, ortoza.

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SANAMED
2018; 13(2): 145–152
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.254
UDK: 616.61-074-056.24
ID: 267041804
Original article

MICROALBUMINURIA BESIDES TO URINARY ENZYMATIC PROTEIN LEVELS INCREASE IN DIABETIC KIDNEY DISEASE WITH TYPE II DIABETICS

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Primljen/Received 15. 07. 2018. god.

Prihvaćen/Accepted 10. 08. 2018. god.

Abstract: **Background:** Diabetic kidney disease (DKD) is a time progressive problem, give rise in uncontrolled Diabetics increasing risks for chronic kidney disease (CKD) and/or end-stage renal disease (ESRD). The vulnerability to renal dysfunction manifested with sudden glomerular hypofiltration associated with micro-to macroalbuminuria passing to renal failure. So that, screening of specific enzymes shifts, or urinary albumin may predict onset diabetic nephropathy. **Objective:** The assessment of urinary alkaline phosphatase (ALP), alanine aminopeptidase (AAP), acid phosphatase (ACP) and microalbuminuria (MAU) for type II diabetic patients. Patients and Methods: In this study,120 type II diabetic patients were compared to 90 healthy volunteers of matched age and sex in Al-Leith General Hospital, Al-Leith Kidney Unit (AKU), Al-Leith, Makkah area, KSA in which random urine samples were collected for testing of MAU, ALP, AAP, ACP and Cr. **Results:** Mean values of measured biomarkers in patient group for MAU, ALP, AAP, ACP and Cr were 51.92 mg/I, 41.55 U/L, 20.17 U/L, 570.10 U/L and 2.92 mg/dl VS in control group were 12.59 mg/I, 8.84 U/L, 6.94 U/L, 385.87U/L and 1.07 mg/dl respectively. Additionally, there were statistically positive correlation between AAP with MAU and ALP; ACP with MAU, ALP and AAP; Cr level with MAU, ALP, AAP and ACP; on the other hand, there were positive significant correlation between duration of diabetes with all studied markers. **Conclusion:** Using of MAU in addition to other urinary enzymes could be beneficial non-invasive indicators for renal deterioration in type II diabetics.

Key words: Alkaline phosphatase, Alanine aminopeptidase, Acid phosphatase, Microalbuminuria, Diabetic kidney disease, Diabetes mellitus type II.

INTRODUCTION

Diabetes mellitus (DM) is a major public health problem worldwide. Current global estimates indicate that this condition affects 415 million people and is set to escalate to 642 million by the year 2040. A further 193 million people with diabetes remain undiagnosed due to the often mild or asymptomatic nature of this condition especially in type 2 DM (T2DM) (1). DM is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (2). The most prevalent diabetes form in human is type-1 and type-2 DM, the latest accounts for more than 90% of patients (3). Many patients with type-2 diabetes are asymptomatic; there are no sharp clinical manifestations; and hence patients may remain undiagnosed for many years. Type-2 DM is characterized by two major defects, impaired insulin secretion or a decrease in its peripheral action (4, 5). The cause of Type-2 DM is multifactorial. Genetic susceptibility plays a crucial role in the etiology and manifestations of type-2 DM, with roots in the interaction of environmental factors; physical inactivity, obesity, ethnic, drugs and toxic agents, viral infection, and location; individual with a susceptible gene may become diabetic if environmental factors modify the expression of these genes (6). It is evident that environmental factors are playing a more increasing role in the cause of DM (7). Diabetic kidney disease (DKD) is a major cause of morbidity and mortality in diabetes. Indeed, the excess mortality of diabetes occurs mainly in individuals with diabetes and proteinuria and results not only from end-stage renal disease (ESRD) but also from cardiovascular disease, with the latter being par-

ticularly common in patients with type 2 diabetes (8, 9, 10).

Approximately 20% to 40% of patients with type 1 or type 2 diabetes mellitus develop DKD. This is a clinical syndrome characterized by persistent albuminuria (> 300 mg/24 h, or > 300 mg/g creatinine), a relentless decline in glomerular filtration rate (GFR), raised arterial blood pressure, and enhanced cardiovascular morbidity and mortality. In classical diabetic nephropathy, the first clinical sign is moderately increased urine albumin excretion (microalbuminuria: 30–300 mg/24 h, or 30–300 mg/g creatinine; albuminuria grade A2). Untreated microalbuminuria will gradually worsen, reaching clinical proteinuria or severely increased albuminuria (albuminuria grade A3) over 5 to 15 years. The GFR then begins to decline, and without treatment, end-stage renal failure is likely to result in 5 to 7 years (11).

Microalbuminuria (MAU) was defined as subclinical increasing of urinary albumin, mainly due to abnormality of urine excretion of albumin between 30 and 300ìg/ml (12). MAU can be considered as a biomarker of kidney damage, end-stage renal disease (ESRD). MAU has considered as a strong candidate in the prediction of renal risk in diabeticpatients and can referred to the presence of functional and / or structural renal abnormalities that precedes and envisage the onset of GFR deterioration. The pathophysiological mechanisms underlying the presence of MAU are still not clear, though intra renal hemodynamic changes that are brought about by increased systemic blood pressure, or capillary leakiness at the glomerular level have been implicated with the latter reflecting a more generalized atherosclerotic vascular damage (13).

Albuminuria is the first sign of diabetic nephropathy, the first symptom is usually peripheral edema, which occurs at a very late stage. Regular, systematic screening for diabetic kidney disease is needed in order to identify patients at risk of or with presymptomatic diabetic kidney disease. Annual monitoring of urinary albumin-to-creatinine ratio, estimated GFR, and blood pressure is recommended. Several new biomarkers or profiles of biomarkers have been investigated to improve prognostic and diagnostic precision, but none have yet been implemented in routine clinical care. In the future such techniques may pave the way for personalized treatment (11). So that our paper aimed for assessment of urinary alkaline phosphatase (ALP), alanine aminopeptidase (AAP), acid phosphatase (ACP) and microalbuminuria (MAU) for diabetic patients type II (11).

Alkaline phosphatase

Alkaline phosphatase, which is naturally expressed along the brush border of the proximal tubule, reduces renal inflammation via dephosphorylation of extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to adenosine, which has anti-inflammatory effects. Additionally, just as intestinal alkaline phosphatase detoxifies bacterial endotoxins via dephosphorylation, alkaline phosphatase in the kidney inhibits bacterial activation of proinflammatory toll-like receptor 4 (TLR4) via dephosphorylation of their lipopolysaccharide (LPS) cell membranes (14).

Alanine aminopeptidase

Alanine aminopeptidase activity of DPP-4, an enzyme found free and in epithelial cells in most tissues, especially in the intestinal mucosa, is to cleave the N-terminal of the incretins GLP-1 and GIP. DPP-4 is not only responsible for peptide degradation, but has also an effect on immunomodulation, cell adhesion and cell movement. The enzyme DPP-4 preferentially cleaves peptides with a proline oralanine residue in the second last aminoterminal position and removes the X-proline and X-alanine dipeptides from the N-terminal end of peptides and proteins, which may have relevance in inhibiting their degradation by unspecific proteases (15).

Acid phosphatases (ACPs)

Acid phosphatases (ACPs) (EC 3.1.3.2, orthophosphoric monoester phosphohydrolases) can catalyze a remarkable variety of challenging hydrolytic enzymes that occur in multiple molecular forms with lysosomes of cells from a variety of tissues. ACP belongs to a group of enzymes that hydrolyze phosphomonoesters at acidic pH. It is an allergen produced from bee venom component that can release histamine and induce wheal and flare reactions in sensitized humans. The general reaction mechanism catalyzed by ACP was the hydrolysis of ester phosphate linkages of organophosphate compounds, resulting in the release of inorganic phosphate (16).

PATIENTS AND METHODS

120 patients with type II diabetes were compared to 90 healthy participants of matched age and sex in Al-Leith General Hospital, Al-Leith Kidney Unit (AKU), Al-Leith, Makkah area, KSA. We declare that our study was initially approved from Alexandria Medical Research Institute ethical committee that matched with Helsinki declaration before getting any signed informed consents from both, patients and volunteers.

The criteria of Diabetes type 2 were Hemoglobin A1c level > 6.5 and fasting plasma glucose level > 126 mg/dl.

• ***Inclusion criteria:*** Patients who are already diagnosed as type-2 DM,who have type-2 DM for different periods and individuals who do not suffering of renal and liver abnormality.

• ***Exclusion criteria:*** Diabetics type I, diabetics with urinary tract infections and diabetics suffering from renal or liver disease and hypertensionand CVD patients.

For all the collected samples of the study population routine urine analysis (the macroscopic and microscopic test), MAU, Alanine Aminopeptidase (AAP), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), creatinine and fast blood sugar tests were estimated.

Urinary albumin concentration was measured using the Fitzgerald® Industries International (United States) ELISA Kit, a quantitative competitive immunoassay for measurement of Human albumin in urine. Renal functional test estimation [creatinine, urea and electrolytes (Sodium, Potassium and Chloride)] - were measured using the Beckman Coulter Olympus AU480 automated chemistry analyzer.

Patients with albumin levels less than 30 mg/g of creatinine were defined as having normoalbuminuria, those with albumin levels 30 - 300 mg/g as having MAU. Random collected serum and urine samples were tested for ALP, AAP, ACP and Cr using.BTS 350 Semi-Automatic Analyzer Biosystems spectrophotometer.

Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as mean ± SD (standard deviation) for quantitative parametric data, while it was done for qualitative data as number and percentage. Inferential analyses for independent variables were done using Chi square test for differences between proportion, while correlations were done using Pearson's correlation for numerical parametric data. Student t-test was used to compare between two groups if the mean and SD was used. The level of significance was taken at p value ≤ 0.05, otherwise is non-significant. The p-value is a statistical measure for the probability that the results observed in a study could have occurred by chance. The discriminative power of all studied biomarkers was evaluated by receiver operating characteristic (ROC) curve analysis.

RESULTS

Table (1) summarized all demographic and clinical data of patients and control group the mean of age was 53.98 ± 12.92 in patient group and 51.71 ± 12.35 in control group without any apparent statistical significance. This study included 59 (49.2%) and 48 (53.3%) males and 61 (50.8%) and 42 (46.7%) females for pati-

Table (1). Demographic and clinical data of patient and control group

	Patient		Control		p
Age (Year)	53.98 ± 12.92		51.71 ± 12.35		0.202
Sex					
Male	59	49.2%	48	53.3%	0.357
Female	61	50.8%	42	46.7%	
BMI (kg/m^2)					
Normal	20	16.7%	37	41.1%	0.0001*
Overweight	30	25.0%	30	33.3%	
Obese	52	43.3%	23	25.6%	
Morbid obese	18	15.0%	0	0.0%	
Systolic BP(mmHg)	130.58 ± 12.20		120.17 ± 5.85		0.0001*
Diastolic BP (mmHg)	82.00 ± 7.54		80.20 ± 6.55		0.072

* Significant at p value ≤ 0.05.

Table 2. *Comparison between different markers in patient and control*

Parameters	Patient Group	Control Group	p value
MAU mg/l	51.92 ± 23.78	12.59 ± 4.86	0.0001*
ALP U/L	41.55 ± 23.87	8.84 ± 4.50	0.001*
AAP U/L	20.17 ± 8.07	6.94 ± 1.54	0.0002*
ACP U/L	570.10 ± 254.52	385.87 ± 180.28	0.0001*
Cr mg/dL	2.92 ± 1.44	1.07 ± 0.30	0.013*

* Significant at p value ≤ 0.05.

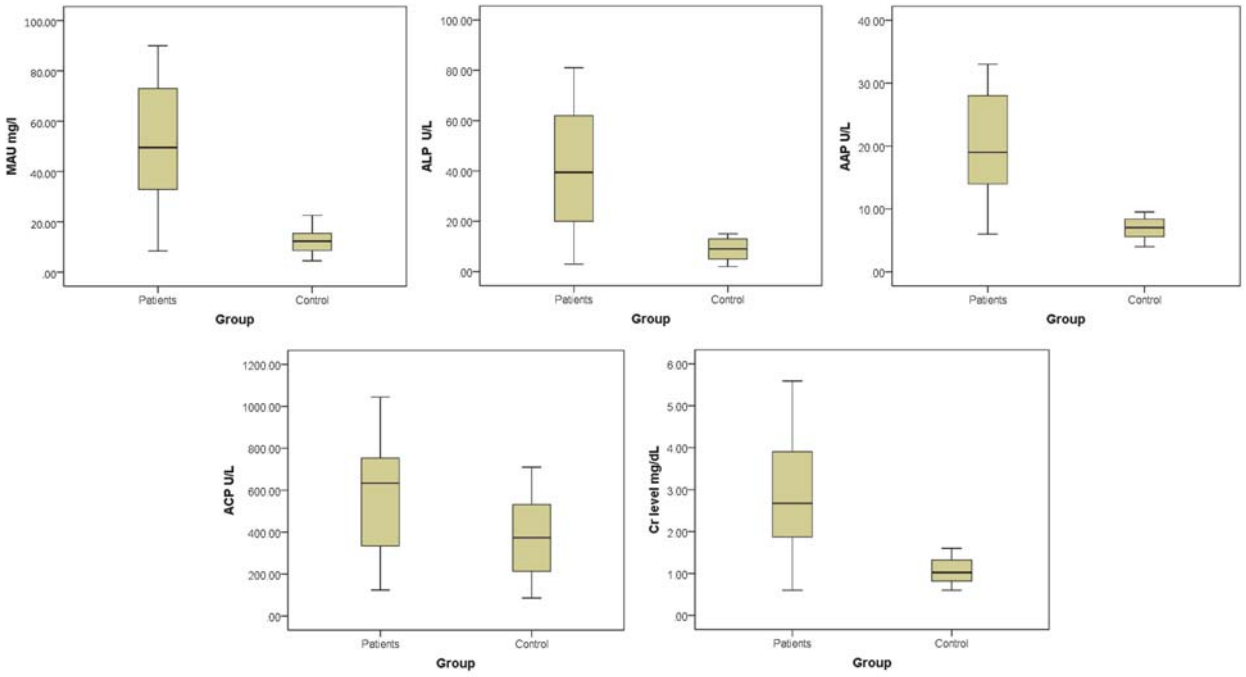

Figure 1. *Comparison between different biomarkers in patient and control groups*

ent and control groups respectively with p = 0.357. Dependent on BMI, patients were variably distributed as normal (17.6%), overweight (25.0%), obese (43.3%) and morbid obese (15.0 %) with p < 0.001. The systolic BP mean (mmHg) was (130.58 ± 12.20) in patient group overcoming that in control group (120.17 ± 5.85) with p < 0.001. While the mean of diastolic blood pressure (mmHg) was 82.00 ± 7.54 and 80.20 ± 6.55 for patient and control groups with no statistical significant differences.

Comparison between different studied parameters in patient and control groups were presented in Table 2 and Figure 1, showing significant higher values of microalbuminuria (MAU), alkaline phosphatase (ALP), alanine aminopeptidase (AAP), acid phosphatase (ACP) and creatinine levels (Cr)in patient group than in control group.

Table 3 epitomized the correlation between duration of disease and all different studied markers, illustrating that, there were statistically positive correlations between levels of AAP with MAU and ALP; ACP with MAU, ALP and AAP; Cr with MAU, ALP, AAP and ACP; on the other hand, there were positive significant correlations between the duration of diabetes with all studied biomarkers.

Relationships between levels of different biomarkers in patient group and type of medications were perfectly recapped as in Table 4, indicating that means of MAU, ALP, AAP, ACP, Cr levels were two fold in pati-

Table 3. *Correlation between duration of disease and different studied biomarkers*

		MAU mg/l	**ALP U/L**	**AAP U/L**	**ACP U/L**	**Cr level mg/dL**
ALP U/L	r	.998				
	p-value	0.001*				
AAP U/L	r	.998	.995			
	p-value	0.001*	0.001*			
ACP U/L	r	.986	.989	.983		
	p-value	0.001*	0.001*	0.001*		
Cr level mg/dL	r	.991	.991	.989	.977	
	p-value	0.001*	0.001*	0.001*	0.001*	
Duration of diabetes	r	.878	.880	.872	.873	.859
	p-value	0.001*	0.001*	0.001*	0.001*	0.001*

* Significant at p value ≤ 0.05.

Table 4. *Association between different biomarkers levels in patient group and type of medication*

	Type of medication		p
	Hypoglycemic agents' Tabs	Insulin injections	
MAU mg/l	39.45 ± 16.35	81.03 ± 6.65	0.0001*
ALP U/L	29.12 ± 16.56	70.56 ± 7.19	0.0002*
AAP U/L	15.89 ± 5.49	30.14 ± 1.99	0.0035*
ACP U/L	448.70 ± 198.00	853.36 ± 96.88	0.001*
Cr mg/dL	2.14 ± 0.88	4.72 ± 0.67	0.0021*

* Significant at p value ≤ 0.05.

Table 5. *Relation between different markers in patient group and the regularity of taking medication*

Parameters	Treatment regularity		p
	Regular	Irregular	
MAU mg/l	26.37 ± 8.69	66.72 ± 15.79	0.0041*
ALP U/L	15.50 ± 8.07	56.63 ± 15.44	0.0001*
AAP U/L	11.61 ± 3.30	25.12 ± 5.43	0.0021*
ACP U/L	283.68 ± 107.67	735.92 ± 141.95	0.0041*
Cr mg/dL	1.46 ± 0.55	3.76 ± 1.07	0.0021*

* Significant at p value ≤ 0.05.

Table 6. *Prediction values of different markers in DKD with type II diabetics*

Test Result Variable(s)	Area	P value	Cut off value	Sensitivity	Specificity	Accuracy	Asymptotic 95% C.I.	
							Lower	Upper
MAU mg/l	0.966	0.00012	32.0	98.0	95.4	96.0	.943	.988
ALP U/L	0.906	0.00011	28.0	90.5	88.9	92.0	.865	.948
AAP U/L	0.955	0.00025	14.2	91.3	92.6	91.0	.926	.985
ACP U/L	0.718	0.016	420.6	68.0	72.2	69.0	.651	.786
Cr mg/dL	0.888	0.0036	1.52	81.0	90.0	84.0	.840	.936

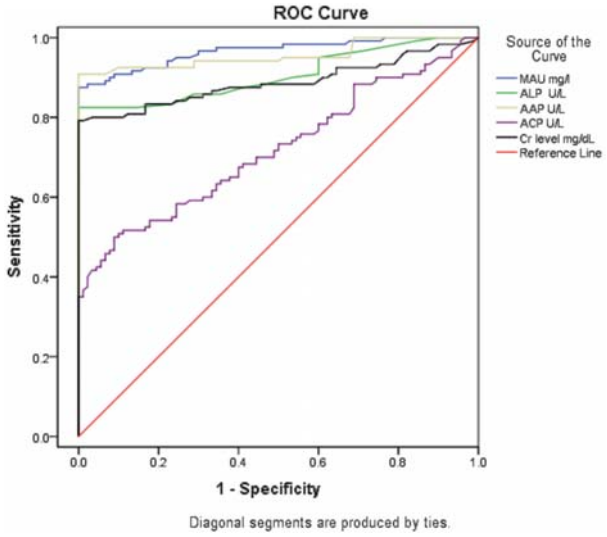

Figure 2. *ROC curve of different studied markers in predict the DKD*

ents using insulin injections than hypoglycemic agents' tabs approximately with significant p values.

Moreover, levels of different biomarkers in patients group and the regularity of use of medication were presented in Table 5, elucidating that means of MAU, ALP, AAP, ACP, Cr levels were significantly reduced upon regular ongoing treatment compared to irregular one.

Evaluation of the diagnostic performance of all studied biomarkers against DKD using ROC curve including; sensitivity, specificity and accuracy indicated that MAU was highly predictive followed by AAP as shown in Table 6 and Figure 2.

DISCUSSION

Previous studies showed shown that microalbuminuria (MAU) is an independent risk factor for kidney diseases in diabetics, hypertensive patients and in the gene-

ral population (17, 18). However, the correlation of MAU with the severity of kidney disease in diabetic patients had not been addressed in detail. This study aimed to assessment of urinary alkaline phosphatase (ALP), alanine aminopeptidase (AAP), acid phosphatase (ACP) and microalbuminuria (MAU) for type II diabetic patients. 100 patients type II diabetic compared to 51 healthy volunteers of matched age and sex.

In our study, most of patient's groups was over weight (25.0%) and obese (43.3%) with significant differences. The mean of systolic blood pressure was 130.58 ± 12.20 and 120.17 ± 5.85 for patients and control groups respectively, patients group have values statistically higher than control group.

In agreement with our study, Al-Houli NH et al (14) and GO AS et al. (15) reviewed aspects of the association of diabetes and obesity with renal disease, emphasizing that CKD and albuminuria are associated with increased rates of cardiovascular disease (CVD) and mortality, and should be considered part of the cardiovascular risk factors in persons with diabetes. Lorenzo (16), found that the development of glomerular filtration rate < 60 mL/min per 1.73 m^2 was associated with increased fasting insulin, triglycerides, free fatty acids, obesity, and uric acid and also with antihypertensive treatment, although not with waist circumference, controlling for age, sex, ethnicity, blood pressure, glucose, and C-reactive protein in nondiabetic persons. This points towards the association of CKD with the risk of development of diabetes (16).

In this study, there were statistically positive correlation between AAP with MAU and ALP; ACP with MAU, ALP and AAP; Cr level with MAU, ALP, AAP and ACP; on the other hand, there were positive significant correlation between duration of diabetes with all studied markers. Also, significant differences were found in a comparison between the means of the 5 markers in patient group.

These results indicated that these markers could be of interest to use in early detection of DKD in the preclinical stage. Otherwise, it was recommended for using such markers as early predictors of gradual deterioration of the transplanted kidney that perhaps lead eventually to graft rejection (19). Inverse correlation of Cr level was found between the DM and the control group (decreased in the patients compared to control group). This could be explained by the fact that glomerular hyperfiltration develops at initial stages of the disease (20). The same results were found in Lary SA study (21), who studied one hundred and fifty-five subjects, not under medication and without clinical evidence of renal disease, hypertension, or diabetes mellitus were used as controls. Two urinary enzymes, N-acetyl-beta-D-glucosaminidase (NAG) and AAP were measured in the urine, together with total protein and creatinine concentration. MAU, glucose and pH were measured using test strips. Increased levels of both NAG and AAP were found in the diabetic subjects. Increased excretion of both enzymes with MAU was found in hypertensive groups suggesting the more prevalent renal complications in the studied groups (21).

Currie Get al, (22) showed that assessment of urinary alkaline phosphatase, alanine aminopeptidase and micro/macro albuminuria for insulin and regular treatment patients very useful to predict kidney failure, the development of new technologies had led to exciting possibilities in the search for ideal biomarkers for DN but, despite of huge enormous number of studies, none of them demonstrated so far, the superiority to albuminuria. While biomarker research in the preclinical setting is advancing, none of those biomarkers described above was neither be validated nor available commercially for clinical use and any association with nonalbuminuric DN that might reflect a separate disease process still unexplained. All such potentially interesting markers require further large-scale validation in prospective clinical studies to determine whether they can make the transition from bench to bedside (22).

Although, all studied markers exhibited significant high sensitivity, specificity and accuracy but, the higher one was MAU followed by AAP. This result was the same as Lee M et al study indicated MAU sensitivity superfat if combined with serum creatinine (23). So that calculation of the microalbumin: creatinine ratio might increase the predictive value of MAU.

CONCLUSION

The findings of this study reflected the importance of microalbuminuria and urinary enzymes as non-invasive tests to find diabetic patients who are at risk to develop renal disease or kidney failure, also the combination of elevated MAU and AAP may indicate DKD with a sensitivity of 98%.

Abbreviations

DKD — Diabetic kidney disease
CKD — Chronic kidney disease
ESRD — End-stage renal disease
ALP — Alkaline phosphatase
AAP — Alanine aminopeptidase
ACP — Acid phosphatase
MAU — Microalbuminuria
Cr — Creatinine
DMII — Diabetes mellitus type II
DM — Diabetes mellitus
GFR — Glomerular filtration rate

ADP — Adenosine diphosphate
ATP — Adenosine triphosphate
TLR4 — Toll-like receptor-4
LPS — Lipopolysaccharides
DDP-4 — Dipeptidyl peptidase-4
GLP-1 — Glucagon-like peptide-1
GIP — Gastric inhibitory polypeptide
CVD — Cardiovascular disease
NAG — N-acetyl-beta-D-glucosaminidase

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

PORAST NIVOA MIKROALBUMINURIJE UZ URINARNE ENZIME KOD DIABETIČNE BOLESTI BUBREGA

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Uvod: Diabetična bolest bubrega (DKD) je progresivan problem posebno kod nekontrolisanih diabetičara koji povećava rizik za nastanak CKD i/ili ESRD. Renalna disfunkcija manifestovana iznenadnom glomerularnom hipofiltracijom sa mikro ka makroalbuminuriji prelazi u bubrežnu insuficijenciju. Prema tome, skriningom specifičnih enzima ili albumina u urinu može se predvideti diabetična nefropatija.

Cilj: Procena značaja urinarne alkalne fosfataze (ALP), alanin aminopeptidaze (AAP), acido fosfataze (ACP) i mikroalbuminurije (MAU) kod pacijenata sa diabetesom Tip II.

Pacijenti i Metode: U ovoj studiji je 120 pacijenata sa diabetesom Tip II poređeno sa 90 zdravih volontera uparenih godina i pola. Ispitivanje je izvršeno u Al-Leith Opštoj bolnici, Odeljenju nefrologije, Al-Leith, u Saudijskoj Arabiji, gde su slučajni uzorci urina sakupljani za određivanje MAU, ALP, AAP, ACP i Cr.

Rezultati: srednje vrednosti određivanih biomarkera u grupi pacijenata za MAU, ALP, AAP, ACPi Cr bile su: 51,92 mg/I, 41,55 U/L, 20,17 U/L, 570,10 U/Li 2,92 mg/dl, a u kontrolno jgrupi 12,59 mg/I, 8,84 U/L, 6,94 U/L, 385,87U/Li 1,07 mg/dl.

Zaključak: Ispitivanje MAU uz specifične urinarne enzime može biti koristan neinvazivni indikator za bubrežnu insuficijenciju kod pacijenata sa diabetesom Tip II.

Ključne reči: Alkalna fosfataza, Alanin aminopeptidaza, Kisela fosfataza, Mikroalbuminurija, Dijabetična bolest bubrega, Dijabetes melitus tip II.

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SANAMED
2018; 13(2): 153–158
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.226
UDK: 616.895.8
ID: 267005196
Original article

CORRELATION ANALYSIS BETWEEN THE MORPHOMETRIC CHARACTERISTICS OF THE HEAD OF NUCLEUS CAUDATUS AND THE INTENSITY OF PSYCHOTIC MANIFESTATION IN SCHIZOPHRENIA

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Primljen/Received 16. 04. 2018. god. Prihvaćen/Accepted 10. 06. 2018. god.

***Abstract*: Introduction:** One of the significant functional disorders of the central nervous system in patients with schizophrenia is the increased activity of the mesolimbic dopaminergic system. By the nigrostriatal pathway, the caudate nucleus is closely related to other dopaminergic systems of the brain. Since the function of caudate nucleus relies on the action of dopamine in the brain; the role of this anatomical structure in the pathogenesis of schizophrenia is not sufficiently clarified. The aim of this paper was to examine whether the caudate nucleus participates in the modulation of the intensity of psychotic manifestations in schizophrenia. **Patients and Methods:** The study included a total of thirty-one patients with schizophrenia. Diagnosis of the schizophrenia was based on the DSM-IVcriterion (Diagnostic and Statistical Manual of Mental Disorders, fourth edition), and the intensity of psychotic manifestations was evaluated by using Brief Psychiatric Rating Scale (BPRS). The size of the caudate nucleus was determined on axial non-contrast CT images on the surface of the largest cross-section using AutoCAD 2007 digital morphometry. The statistical data were processed by the SPSS 16.0 program package. The statistical conclusions are presented on the basis of two-tail $p < 0.05$. **Results:** In this study, we have observed a negative correlation between the area as well as the perimeter of the left caudate nucleus head section and the intensity of the psychotic manifestations (area: regression coefficient $B = –0.17$, $p = 0.050$, perimeter: regression coefficient $B = –0.010$, $p = 0.012$). On the right hemisphere of the brain we observed only a negative correlation of the intensity of the psychotic manifestations from the perimeter of the head section of caudate nucleus (regression coefficient $B = –0.013$, $p = 0.011$). **Conclusion:** In our research we found that the higher intensity of psychotic manifestations in schizophrenia was accompanied with the smaller area and the perimeter of left caudate head as well as the smaller perimeter of the head of right caudate nucleus. The finding of the dependence of the intensity of psychotic manifestations on the perimeter of the right caudate head and not on its area speaks in favor of the caudate head surface deformations as one of the markers of intensity of psychotic manifestations in patients with schizophrenia.

Key words: caudate nucleus, schizophrenia, analysis, correlation.

INTRODUCTION

One of the significant functional disorders of the central nervous system in patients with schizophrenia is the increased activity of the mesolimbic dopaminergic system (1, 2). Caudate nucleus belongs to the subcortical gray masses of the brain i.e.to basal ganglia. By the nigrostriatal pathway, the caudate nucleus is closely related to other dopaminergic systems of the brain. Since the function of caudate nucleus relies on the action of dopamine in the brain; the role of this anatomical structure in the pathogenesis of schizophrenia is not sufficiently clarified. The aim of this paper was to examine whether the caudate nucleus participates in the modulation of the intensity of psychotic manifestations in schizophrenia.

PATIENTS AND METHODS

The study encompassed a total of thirty-one persons with schizophrenia (F20): 15 females and 16 males. Schizophrenia diagnosis was based on the DSM-IV criterion (3), and the intensity of psychotic manifestations was evaluated using the Brief Psychiatric Rating Scale (BPRS) (4). The intensity of psychotic manifestations in this scale is scored by the values from 1 to 7 (1 = symptoms not present, 2 = very mild, 3 = mild, 4 = moderate, 5 = moderately strong, 6 = strong and 7 = extremely strong). More BPRS score values reflect higher intensity of the psychotic manifestations.

The size of the caudate nucleuswas determined on axial non-contrast CT images (layer thickness of 5 mm) on the surface of the largest cross section. The area and the perimeter of the caudate head were determined using the AutoCAD digital planimetry (Figure 1). AutoCAD 2007 for PC Windows (developed by Autodesk, Inc. San Rafael, California, U.S.) belongs to a group of software packages designed for drawing, designing and other aspects of computer application engineering practice. This software package can also be used to measure surfaces of irregular geometric figures, such as central nervous system structures (5).

Patients with severe comorbid states (cardiac decompensation, unstable angina pectoris, myocardial infarction in the previous and the year of the study, infectious diseases, malignant and chronic immune diseases) were excluded from the study because of the confounding effect. Also, the study did not include patients with other psychiatric disorders diagnoses, neurological disorders and patients with the history of stroke.

The statistic data are processed in the SPSS 16.0 program package. The hypotheses of the regression models used were tested. The statistical conclusions are presented on the basis of two-tail $p < 0.05$.

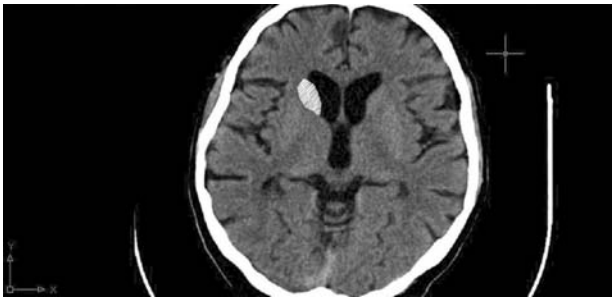

Figure 1. *Display of the AutoCAD digital morphometry of the largest cross-section of the right caudate nucleus head: area 141.18 mm2, perimeter 48.802 mm*

RESULTS

Examination of the correlationbetween intensity of psychotic manifestations in patients with schizophrenia comparing the gender and age of respondents is shown in Table 1.

Since the deviation of BPRS scores from the normal distribution was observed (Shapiro-Wilk test $p = 0.044$), in the correlation of psychotic manifestations intensityregarding the gender and age of respondents the generalized linear model of the subclass gamma with the log link (robust estimator) was used. Negative correlation i.e. higher intensity of symptoms of psychotic manifestations in younger personswas observed(regression coefficient $B = -0.00036$, $p < 0.001$) (Table 1). By incorporating only one parameter i.e. age of the respondents in the examined model, the aforementioned correlation was also confirmed (regression coefficient $B = -0.00040$, $p < 0.001$).

Examining the correlation of the intensity of psychotic manifestations (BPRS score) of patients with

Table 1. *Dependence of the intensity of psychotic manifestations of patients with schizophr enia regarding the gender and the age of respondents*

Parameter Estimates							
Parameter	B	Standard Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-square	df	p
(Intercept)	4.254	0.0530	4.150	4.358	6433.764	1	< 0.001
Years	-0.00036	0.00009	-0.0005	-0.0002	16.217	1	< 0.001
[Male]	0.075	0.0738	-0.069	0.220	1.042	1	0.307
[Female]	0[a]						
(Scale)	0.045[b]						

BPRS: Brief Psychiatric Rating Scale
Dependent Variable: BPRS score
a. This parameter is set to zero because it is redundant.
b. Calculation based on thePearson's Chi-Squared test.

Table 2. Examination of the dependency of the intensity of psychotic manifestations from the largest cross section area of the left caudate nucleus head

Parameter Estimates							
Parameter	B	Standard Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p
(Intercept)	6.631	1.2016	4.276	8.986	30.453	1	< 0.001
Years	-0.054	0.0291	-0.111	0.003	3.429	1	0.064
Cross-sectional area of the left caudate head (mm^2)	-0.017	0.0087	-0.034	-0.00004	3.858	1	0.050
Years *Cross sectional area of the left caudate head	0.00039	0.0002	-0.00003	0.001	3.370	1	0.066
(Scale)	0.042[a]						

BPRS: Brief Psychiatric Rating Scale
Dependent Variable: BPRS score
a. Calculation based on thePearson's Chi-Squared test.

Table 3. *Examination of the dependency of the intensity of psychotic manifestations from the largest cross section perimeter of the left caudate nucleus head*

Parameter Estimates							
Parameter	B	Standard Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p
(Intercept)	4.747	0.1950	4.365	5.129	592.721	1	< 0.001
Years	-0.00047	0.00008	-0.0006	-0.0003	37.224	1	< 0.001
Cross-sectional perimeter of the left caudate head (mm)	-0.010	0.0038	-0.017	-0.002	6.357	1	0.012
(Scale)	0.042[a]						

BPRS: Brief Psychiatric Rating Scale
Dependent Variable: BPRS score
a. Calculation based on thePearson's Chi-Squared test.

schizophrenia and the age and the area of the largest cross section of the left caudate nucleus head we found greater intensity of psychotic manifestations in persons with smaller caudate head area (regression coefficient B = -0.17, p = 0.050) (Table 2). For the examination of statistical significance, the generalized linear model of the subclass gamma with the log link (robust estimator) was used.

Examining the correlation between intensity of psychotic manifestations (BPRS score) of patients with schizophrenia and the age and the perimeter of the largest cross section of the left caudate nucleus head we found greater intensity of psychotic manifestations in younger persons (regression coefficient B = -0.00047, p < 0.001) with smaller caudate head perimeter (regression coefficient B = -0.010, p = 0.012) (Table 3). For the examination of statistical significance, the generalized linear model of the subclass gamma with the log link (robust estimator) was used.

Examining the correlation between the intensity of psychotic manifestations (BPRS score) of patients with schizophrenia and the age and the area of the largest cross section of the right caudate nucleus head we did not certain correlation between the intensity of psychotic manifestations and caudate head area (regression coefficient B = -0.002, p = 0.404) (Table 4). For the examination of statistical significance, the generalized linear model of the subclass gamma with the log link (robust estimator) was used.

Examining the correlation between the intensity of psychotic manifestations (BPRS score) of patients with schizophrenia and the age and the perimeter of

Table 4. *Examination of the dependency of the intensity of psychotic manifestations from the largest cross section area of the right caudate nucleus head*

Parameter Estimates							
Parameter	B	Standard Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p
(Intercept)	4.640	0.4095	3.837	5.443	128.398	1	< 0.001
Years	-0.0005	0.000084	-0.0006	-0.0003	29.959	1	< 0.001
Cross-sectional area of the right caudate head (mm^2)	-0.002	0.0028	-0.008	0.003	0.695	1	0.404
(Scale)	0.046a						

BPRS: Brief Psychiatric Rating Scale
Dependent Variable: BPRS score
a. Calculation based on thePearson's Chi-Squared test.

Table 5. *Examination of the dependency of the intensity of psychotic manifestations from the largest cross section perimeter of the right caudate nucleus head*

Parameter Estimates							
Parameter	B	Standard Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p
(Intercept)	4.906	0.2470	4.421	5.390	394.367	1	< 0.001
Years	-0.0005	0.00008	-0.0006	-0.0003	34.615	1	< 0.001
Cross-sectional perimeter of the right caudate head (mm)	-0.013	0.0052	-0.023	-0.003	6.448	1	0.011
(Scale)	0.040[a]						

BPRS: Brief Psychiatric Rating Scale
Dependent Variable: BPRS score
a. Calculation based on thePearson's Chi-Squared test.

the largest cross section of the right caudate nucleus head we found greater intensity of psychotic manifestations in younger persons(regression coefficient B = -0.0005, $p < 0.001$) with smaller caudate head perimeter (regression coefficient B = -0.013, $p = 0.011$) (Table 5). For the examination of statistical significance, the generalized linear model of the subclass gamma with the log link (robust estimator) was used.

DISCUSSION

In terms of morphology of caudate nucleus and its association with psychotic manifestations in schizophrenia results in the literature are different. Thus, for example, Rich AM et al. did not observe the morphometric caudate nucleus alterations in patients with chronic and early schizophrenia (6). Brugger SP et. Howes OD in meta-analysis also did not indicate the modified caudate nucleus in patients with the first episode of schizophrenia (7). Authors(8) came up with a similar conclusion. On the other hand, Ebdrup BH et al. indicated a reduction in volume and smaller caudate nucleus in patients with the first episode of schizophrenia without the use of antipsychotics (9). Also, Stegmayer K et al. in patients with schizophrenia and significant emotional dysregulation indicate the reduction of gray mass in ventral striatum and caudate nucleus (10). Li Y et al. in meta-analysis showed reduction of grey mass of the left caudate nucleus (11), and Brandt GN et Bonelli RM (12) emphasize, besides the reduction of caudate nucleus in the first episode of schizophrenia, to the increased caudate nucleus in chronic phase, as a consequence of the antipsychotic application.

The authors (13) also point out to the smaller caudate nucleus in early schizophrenia in comparison to a healthy population, with a subsequent increase in the

size of the caudate nucleus after ordering antipsychotic therapy. Zampieri E et al. emphasize the importance of antipsychotics to the increase of caudate nucleus too (14). However, in this case, different findings are also depicted in the literature. Emsley R et al. do not only challenge the smaller caudate nucleus in patients with early schizophrenia, pointing to their findings of a larger caudate nucleus of schizophrenic patients, but also negating the increase of the caudate nucleus after administration of antipsychotics such as risperidone and flupentixol (15). In support of the aforementioned larger caudate nucleus in patient with schizophrenia refer also the studies(16, 17). Regarding the increase of caudate nucleus after ordering antipsychotics, Mouchlianitis E et al. cited different findings in meta-analysis i.e. three studies where antipsychotic clozapine led to caudate nucleus reduction, not increment (18). However, in the later case, there are literature counterparts that point to the increased metabolism of caudate nucleus after the administration of clozapine (19). So, the issues are not completely settled yet.

In our study, we found a negative correlation between intensity psychotic manifestations from the size of the cross section of caudate nucleus head; on the left brain hemisphere from the both i.e. area and perimeter (Table 2 and 3), and from the perimeter on the right brain side (Table 5). Greater intensity of psychotic manifestations in patients with smaller head size of caudate nucleus was also noted by the authors (20). Our finding of the dependence of the intensity of psychotic manifestations on the perimeter of the right caudate head and not from its area speaks in favor of the deformations of the surface of caudate nucleus head in patients with schizophrenia, as indicated by the study (21).

CONCLUSION

In our research we found that the higher intensity of psychotic manifestations in schizophrenia was accompanied with the smaller area and the perimeter of left caudate head as well as the smaller perimeter of the head of right caudate nucleus. The finding of the correlation between intensity of psychotic manifestations on the perimeter of the right caudate head and not on its area speaks in favor of the caudate head surface deformations as one of the markers of intensity of psychotic manifestations in patients with schizophrenia.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

ANALIZA KORELACIJE MORFOMETRIJSKIH KARAKTERISTIKA GLAVE REPATOG JEDRA I INTENZITETA PSIHOTIČNIH MANIFESTACIJA KOD SHIZOFRENIJE

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Uvod: Jedan od značajnih funkcionalnih poremećaja centralnog nervnog sistema kod pacijenata sa shizofrenijom je povećana aktivnost mezolimbičkog dopaminergičkog sistema. Preko nigrostrijatnog puta repato jedro je usko funkcionalno povezano sa drugim dopaminergičkim sistemima mozga. Budući da se funkcija nc. Caudatusa naslanja na djelovanje dopamina u mozgu, uloga ove anatomske strukture u patogenezi shizofrenije nije dovoljno razjašnjena. Cilj ovog rada je bio da se ispita da li repato jedro učestvuje u modulaciji intenziteta psihotičnih manifestacija kod shizofrenije. **Ispitanici i metode:** Istraživanje je obuhvatilo ukupno trideset i jednu osobu obolelu od shizofrenije. Dijagnoza shizofrenije je postavljana na osnovu DSM-IV kriterijuma, a intenzitet psihotičnih manifestacija je procenjivan primenom skale za procenu psihotičnih poremećaja - BPRS (Brief Psychiatric Rating Scale). Veličina glave repatog jedra određivana je na aksijalnim nekontrasnim CT snimcima na površini najvećeg poprečnog preseka primenom AutoCAD 2007 digitalne morfometrije. Statistički podaci su obrađivani u SPSS 16.0 programskom paketu. Statistički zaključci izneseni su na osnovu dvosmernog $p < 0.05$. **Rezultati:** U ovom istraživanju uočili smo negativnu korelaciju površine i obima preseka leve glave repatog jedra i intenziteta psihotičnih manifestacija (površina: regresioni koeficijent B = -0.17, p = 0.050, obim: regresioni koeficijent B =

-0.010, p = 0.012). Na desnoj hemisferi mozga uočili smo negativnu korelaciju intenziteta psihotičnih manifestacija samo od obima preseka glave repatog jedra (regresioni koeficijent B = -0.013, p = 0.011). **Zaključak:** U našem istraživanju uočili smo da je veći intenzitet psihotičnih manifestacija kod pacijenata sa shizofrenijom bio praćen manjom površinom i obimom glave levog nc. Caudatusa i manjim obimom glave desnog kaudatusa. Nalaz zavisnosti intenziteta psihotičnih manifestacija od obima glave desnog kaudatusa, a ne i od površine govori u prilog prisutnih deformacija na površini glave repatog jedra kao jednog od korelata intenziteta psihotičnih manifestacija kod pacijenata sa shizofrenijom.

Ključne reči: nc. caudatus, shizofrenija, analiza, korelacija.

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SANAMED
2018; 13(2): 159–162
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.218
UDK: 617.751.6-02
ID: 266978316
Original article

COMPARISON BETWEEN AMBLYOPIC AND OTHER NON-AMBLYOPIC EYES IN TERMS OF MACULA AND RETINAL NERVE FIBER LAYER THICKNESS

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Primljen/Received 26. 03. 2018. god.

Prihvaćen/Accepted 04. 06. 2018. god.

***Abstract*: Statement of Significance:** The prospect of this study is that the macula thickness is reduced in amblyopic eyes and may be a potential diagnostic tool in the future screening of amblyopic eyes. We did the unilateral anisometropic amblyopic patient comparison between amblyopic and non-ambliopic eyes in terms of average macular thickness and average retinal nerve fiber layer thickness with the help of spectral optical coherens tomography.

Material and Methods: The study included 74 over 6 year old patients with unilateral anisometropic amblyopia. Comparison between 74 amblyopic and 74 non-amblyopic eye in terms of average macular thickness and average retinal nerve fiber layer thickness, with the help of spectral optical coherens tomography (OPKO Instrumentations, Miami, FL). The difference between healthy and amblyopic eyes, mean macular thickness and retinal nerve fiber layer thickness were evaluated by Mann-Whitney U test.

Results: The mean age of the patients was 9.46 ± 1.86 (7-14 years old) and 40 patients were female (54.05%), 34 patients were male (45.95%). Amblyopia group average macular thickness and mean retinal nerve fiber layer thickness respectively 235.31 ± 21.3 micron (μ) and 100.96 ± 11.45 μ while non-amblyopia group average macular thickness and mean retinal nerve fiber layer thickness respectively 258.59 ± 13.91 μ and 103.68 ± 14.55 μ. Although statistically significant difference (p = 0.001) was observed with Mann-Whitney U test in terms of macular thickness, while in terms of retinal nerve fiber layer the difference between two groups was not statistically significant (p = 0,34).

Conclusions: Average macular thickness measurements taken with OCT device varies in anisometropic amblyopic eyes is an important concept for future studies.

Key words: Amblyopia, spectral oct, macular thickness.

INTRODUCTION

Amblyopia is the most common cause of unilateral visual loss in children. This is defined as a decrease in unilateral or bilateral uncorrected visual acuity due to visual deprivation and / or abnormal binocular interaction without an organic pathology (1,2,3). The incidence varies from 0.5 to 3.5% (4). There are 3 main subgroups depending on strabismic, anisometropic and deprivation (5, 6). Functionally, amblyopic eye suppression results in loss of binocular functions of the binocular neurons in the visual cortex (5, 7, 8). Histopathological changes in lateral geniculate nuclei in amblyopic eyes have been demonstrated in human and animal experiments (9-13).

The issue of retinal involvement in amblyopic eyes is controversial. Electroretinography (ERG) was significantly lower in the amblyopic eye in one study (14), while another study did not achieve the same result (15).

Optical coherence tomography (OCT) is a non-invasive, non-contact method of evaluating retina with axial resolution less than 10 microns using interferometry technique with near infrared laser beams. Retinal nerve fiber layer (RNFL) thickness measurements measured by OCT were similar to histological studies (16). It has been found that spectral OCT (S-OCT) measurements provide more detailed and accurate results than time-dependent OCT measurements (17).

The aim of this study was to compare the mean macular thickness and mean retinal nerve fiber thickness of the healthy and amblyopic eyes in cases of unilateral anisometropic amblyopia using S-OCT.

METHODS

This cross-sectional observational study was performed between April 2017 and March 2018 in Ahi Evran University Education and Research Hospital, Department of Ophthalmology. The research was carried out in accordance with Helsinki declaration rules and with informed consent forms of patients.

In this study, patients over 6 years of age, diagnosed with unilateral anisometropic amblyopia, were evaluated for amblyopic and non amblyopic eyes. A comprehensive ophthalmologic examination was performed on all cases. Refractions of the patients before and after cycloplegic instillation with autorefractometer were determined.

Uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) were obtained with Snellen's sphere at a distance of 6 m without cycloplegic effect. In all cases, a goldmann applanation tonometer was used to measure intraocular pressure, a biomicroscopic evaluation to assess anterior segment and a dilate fundus examination. In addition, strabismus examination was performed to all cases.

One eye had a visual acuity of at least 20/20, and the other eye's visual acuity less then non-amblyopic eye at least two lines and an eye pathology that could affect the eye other than anisometropia could not be determined were accepted as amblyopia. Two diopters (D) spherical equivalents and one diopter (D) refraction difference between the two eyes were accepted as anisometropia.

Macula and optic disc in ambliyopic and intact eyes were photographed with OCT (OPKO Instrumentations, Miami, FL,USA). Spectral OCT / SLO (OPKO / OTI Instru- mentation, Miami, FL, USA) was used. The device provides spectral OCT and SLO images of the tissues at the same time, at the time of scanning approximately 27,000A-scan per second, using "super luminescent diode" light at 830-840 nm wavelength. The axial resolution is 5-6 μm, the posterior resolution is 15 μm, and the scan depth is 2.3 mm (18). In all cases, a 3.4 mm diameter peripapillary RLL thickness measurement was performed in an RSLT mode of the device by an experienced OCT user. The device takes averages of 3 peripapillary measurements as standard. These three measurements were also taken from us and were not taken into account if there are cross-sections with RSLT boundary detection error or signal-to-noise ratio less than 50%. In the RSLT mode, the device receives thickness measurement; mean RNFL thickness, superior-nasal-inferior-temporal, and 8-hour quadrant thicknesses. The mean macular thickness was adjusted to 512 × 64 maculer cubes with the instrument set to OCT mode.

The obtained data was recorded in the computer "SPSS" (statistical package for social sciences) for Windows "15". The Kolmogorov-Smirnov test was used to show that the data of the groups were not homogeneously distributed ($p = 0.001$). The difference between the 2 independent groups was assessed by the Mann-Whitney U test, a nonparametric test. When the p-value was less than 0.05, the results were considered significant.

RESULTS

In 74 patients with unilateral anisometropic amblyopia, 74 amblyopic and 74 non-ambliyopic eyes were evaluated. The mean age of the patients was 9,46 ± 1,86 (7-14 years); 40 patients were female (54,05%) and 34 patients were male (45,95%).

The average macula thickness in the amblyopic group was 235.31 ± 21.33 and the mean retinal nerve fiber thickness was 100.96 ± 11.45; mean macular thickness was 258.59 ± 13.91 and mean retinal nerve fiber thickness was 103.68 ± 14.55 in the non-amblyopic group. The Mann-Whitney U test was used to compare two independent groups after demonstration with the help of the Kolmogorov-Smirnov test ($p = 0.001$ and 0.0001).

Mean macular thickness was significantly lower in amblyopic eye group ($p = 0.001$), but there was no statistically significant difference between two groups in terms of mean RNFL thickness ($p = 0,34$).

DISCUSSION AND CONCLUSION

Different results have been reported in studies in which retinal thicknesses of the amblyopic and intact eyes were evaluated. When we looked at the literature, Yen (19) and Yoon (4) Peripapillary RSLT analyzes revealed a statistically significant height in amblyopic group; Altintas (7), Kee (5) and Repka (20) did not show any statistically significant difference between these two groups. Yoon et al. reported that there was no difference in macular thickness between the unilateral anisometropic amblyopia and amblyopic eyes and that the RNFL thickness was thicker in amblyopic eyes (4). Dickmann et al. anisometropic amblyopia, and strabismus amblyopia, there was no statistically significant difference between the macula and RNFL thickness of the amblyopic and non-amblyopic eyes (21). As a result of our study, statistically significant decrease in average macular thickness was observed ($p = 0,001$), unlike previous studies, and no statistically significant difference was found in terms of mean RNFL thickness as similar to the previous studies ($p = 0,34$).

Amblyopia studies have shown that atrophy occurs in lateral geniculate cells (22). In animal studies, reduction of optic nerve dimensions in amblyopic

eyes, ganglion cytoplasm and thinning in internal plexiform layer were detected (23).

It is important to be diagnosed as early as possible. Because the earlier the diagnosis is made, the greater the likelihood of treatment. We think that pathologic findings detected in the brain in histological studies can be detected with OCT also in RSLT, which may be useful in the detection of amblyopia. In some studies amblyopia was observed with macular thickness in the eyes and no difference was observed in some studies as we showed in our study with RNFL thicknesses.

There are many factors that affect the reproducibility and manufacturability of OCT images. These include patient co-operation, fixation, and refraction (24). In addition, it has been reported that retinal thickness values obtained with different brand and model OCT devices can not be used in place of each other due to the different measurement algorithms used in the devices, but the reproducibility and manufacturability of measurements taken in the same way are quite high (24). In addition to device factors, patient characteristics can also affect the outcome of OCT. When the level of vision is low, the fixation of the cases becomes difficult and the quality of the shot is reduced and the reliability of the results of OCT to be used for follow-up of the cases is reduced.

As a result, there was a statistically significant difference between the macular thicknesses measured by spectral OCT in children with unilateral anisometropic amblyopia. It is clear that this result we obtained should be supported by extensive participatory randomized studies, but we still think it may be important to follow up with this imaging method which is likely to become a valuable follow-up tool in the future of amblyopia cases.

Abbreviations

ERG — Electroretinography
OCT — Optical coherence tomography
RNFL — Retinal nerve fiber layer
UCVA — Uncorrected visual acuity
BCVA — best corrected visual acuity

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Licensing

Sažetak

POREĐENJE IZMEĐU AMBLIOPNIH I NEAMBLIOPNIH OČIJU U POGLEDU DEBLJINE MAKULE I DEBLJINE SLOJEVA NERVNIH VLAKANA RETINE

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Uvod: Prospekt ove studije je to da je debljina makule manja kod ambliopnih očiju i može predstavljati potencijalno dijagnostičko sredstvo u budućem skriningu ambliopije. Izveli smo poređenje kod unilateralne anizometropijske ambliopije između ambliopnog i drugog neambliopnog oka u pogledu prosečne debljine makule i prosečne debljine retinalnih nervnih slojeva, uz pomoć spektralne optičke koherentne tomografije.

Materijal i metode: Studija je uključila 74 pacijenta preko 6 godina sa unilateralnom anizometrijskom ambliopijom. Poređenje između 74 ambliopna i 74 neambliopna oka u pogledu prosečne debljine makule i prosečne debljine nervnih slojeva retine uz pomoć spektralne optičke koherentne tomografije (OPKO Instrumentations, Miami, FL). Razlika između zdravog i ambliopnog oka, prosečna vrednost debljine makule i prosečna debljina slojeva nervnih vlakana retine su bili procenjeni korišćenjem Mann-Whitney U testa.

Rezultati: Prosečna vrednost godina pacijenata bila je 9.46 ± 1.86 (7-14 godina života) i 40 pacijenata su bile žene (54,05%), 34 pacijenta su bili muškarci (45,95%). U grupi ambliopnih očiju srednja makularna debljina i srednja debljina slojeva nervnih vlakana retine bila je 235,31 ± 21.3 mikrona (μ) i 100,96 ± 11,45 μ, dok je u grupi neambliopnih očiju prosečna makluarna debljina i prosečna debljina slojeva nervnih vlakana retine respektivno 258,59 ± 13,91 μ i 103,68 ± 14,55 μ. Iako je statistički značajna razlika bila viđena koristeći Mann-Whitney U test u pogledu makularne debljine, kod srednje debljine slojeva nervnih vlakana retine nije bila nađena statistički značajna razlika između grupa (p = 0,34).

Zaključak: Prosečna debljina makule određivana pomoću OCT-a razlikuje se u anizometrijskom ambliopnom oku u odnosu na drugo neambliopno oko i može se posmatrati kao zanačajna osnova za buduće studije.

Ključne reči: ambliopija, spektralna optička koherentna tomografija, makularna debljina.

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SANAMED
2018; 13(2): 163–169
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.215
UDK: 613.2-053.9
ID: 267003660
Original article

RELATION TO FUNCTIONAL AND NUTRITIONAL STATUS AMONG HOSPITALIZED ELDERLIES

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Primljen/Received 19. 03. 2018. god.

Prihvaćen/Accepted 10. 06. 2018. god.

Abstract: **Introduction:** Adding years to life is a great achievement when this is accompanied by a good level of health and well-being and independence. Major indicators for prediction mortality risk in older adults are the nutrition status and physical functional ability. The aim of this study is to present the nutritive and functional status among institutionalized elderlies and the relatedness with certain risk factors. **Material and methods:** Cross-sectional study has been conducted at certain nursing homes with participation of individuals over 60 years old. As for the research Scale of Daily Living Activities (ADL) has been used to present the functional capacity of the elderly and MNA has been used to detect the nutrition status. **Results:** The results from the research conducted among elderlies have shown the following socio-demographic characteristics: out of 127 participants, most of them were females- 77% and 69% at the age between 75 and 84. Most of the elderlies (in 68%) have completed secondary education. The results from MNA have shown that 69,2% are well- nourished, 27,6% are at risk for malnutrition and 3,2% are malnourished. There is a relatedness of the nutrition status with the gender ($p < 0,001$) and the level of education ($p < 0,001$).ADL scale among 127 elderly participants has shown that 37% are independent, 45% are with a moderate impairment and 18% are with severe functional impairment. In comparison with the females, the males show higher level of functional ability in all ADL components except the continence. **Conclusion:** The results from the research have shown that the nutrition status among elderlies is satisfactory, emphasizing the factors such as gender, education level and functional ability as key points for the level of nutrition status at the elderlies.

Key words: malnutrition, functional capacity, elderly.

INTRODUCTION

Aging among population worldwide is quite present regardless of its level of development. Even though the progression is higher in the developed countries yet the population aging includes those countries where the young people is present as well (1). In Macedonia the presence of people over 60 years in 2012 expressed in percentage is 17,5% and those over the age of 80 is 2,3% from the total number of population.

Adding years to life is a great achievement when this is accompanied by a good level of health and well-being and independence. Nevertheless ageing increases the dependence of other people due to reduction of the level of functional independence (2).
Major indicators for prediction mortality risk in older adults are the nutrition status and physical functional ability (3). A decline in functional status is a profound predictor of mortality (4). The death rate increases from 8 % in individuals with no disability, to 15% with one or more Instrumental Activities of Daily Living (IADL) disabilities, to 21% in persons with one or two ADL dependencies, and up to 37% in those with five or six ADL dependencies during a 2-year period (5). Many studies have shown that malnutrition among elderly increases the death rate opposite to elderly with good nutrition status (5-10).

Nursing homes are the ones offering proper care for elderly of 80 years and above (11). The inability to live at home is intensifying the necessity of institutional care of elderly offered by the nursing homes (12). Nursing homes across Europe provide care for 2 to 10% of the elderly (13, 14). Several studies have shown that the institutionalized female elderly register higher prevalence of malnutrition in terms of elderly people in general (15-18).

Mini Nutritional Assessment (MNA) is s method used for identification of malnutrition risk among elderly. The MNA is a simple, low cost and noninvasive method that can be done at bedside (19). Added MNA scores allow one to screen the elderly who have an adequate nutritional status, those who are at risk of malnutrition and those who are malnourished.

The aim of this study is to present the nutritive and functional status among institutionalized elderlies and the relatedness with certain risk factors.

MATERIAL AND METHODS

Cross-sectional study has been conducted at certain nursing homes in R. Macedonia with participation of individuals over 60 years old. Before the start of the research, the author has provided consent from the relevant authorities of the institutions. Each participant was explained in details about the aim of the research as well as the anonymity and the voluntary aspect of this study. Before the beginning of the anonymous questionnaire a verbal and official /sighed consent has been provided by each participant (addition 1). Out of 148 elderlies the research has been conducted to 127 as of the absence or inability to participate due to mental disorder.

Nutritional Status

MNA (addition 2) has been used to detect the nutrition status, which is composed of 18 items such as: anthropometry: body mass index (weight in kilograms / Height in Meters x Height in Meters), calf circumference (measuring the calf at the widest part) and arm circumference (the distance between the acromial surface of the scapula (bony protrusion surface of upper shoulder) and the olecranon process of the elbow (bony point of the elbow) on the back of the arm.), dietary (number of meals, autonomy to feed, water and food ingestion), and global assessment (medicines, residence, mobility, dementia, stress, how does the patient consider his/her health status and nutritional status). The interpretation of the results is done based on the total score such as: 24 and above are considered as adequate nourished, 17 to 23,5 are considered as individuals at risk of malnutrition and 17 and below are considered to be malnourished (7).

Addition 1

Consent form for participants

1. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.

2. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications

_____ _____

Patients name Signature

Table 1. *Variable associated with functional activates, gender and BMI*

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	253.5	2	126.75	6.019789	**0.021899**	4.256495
Within Groups	189.5	9	21.05556			
Total	443	11				

Table 2. *Factors linked with the nutrition status among hospitalized elderly in R. Macedonia*

	N	Risks with malnutrition and with malnutrition	Well nourished	P value
Registered illnesses less than 3 Registered illnesses more than 3	121	31 (35%) 46 (65%)	14 (32%) 30 (68%)	0,216
Moderate physical activity Physical inactivity	105	38 (54,2%) 32 (45,8)	21 (60%) 14 (40%)	0,689
Dependent and partly dependent Independent	127	45 (52,9%) 40 (47,1%)	5 (11,9%) 32 (88,1%)	**< 0,0001**

Mini Nutritional Assessment MNA®

Nestlé Nutrition Institute

Last name: First name:

Sex: Age: Weight, kg: Height, cm: Date:

Complete the screen by filling in the boxes with the appropriate numbers.
Add the numbers for the screen. If score is 11 or less, continue with the assessment to gain a Malnutrition Indicator Score.

Screening

A Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?
0 = severe decrease in food intake
1 = moderate decrease in food intake
2 = no decrease in food intake ☐

B Weight loss during the last 3 months
0 = weight loss greater than 3kg (6.6lbs)
1 = does not know
2 = weight loss between 1 and 3kg (2.2 and 6.6 lbs)
3 = no weight loss ☐

C Mobility
0 = bed or chair bound
1 = able to get out of bed / chair but does not go out
2 = goes out ☐

D Has suffered psychological stress or acute disease in the past 3 months?
0 = yes 2 = no ☐

E Neuropsychological problems
0 = severe dementia or depression
1 = mild dementia
2 = no psychological problems ☐

F Body Mass Index (BMI) (weight in kg) / (height in m²)
0 = BMI less than 19
1 = BMI 19 to less than 21
2 = BMI 21 to less than 23
3 = BMI 23 or greater ☐

Screening score (subtotal max. 14 points) ☐☐
12-14 points: Normal nutritional status
8-11 points: At risk of malnutrition
0-7 points: Malnourished

For a more in-depth assessment, continue with questions G-R

Assessment

G Lives independently (not in nursing home or hospital)
1 = yes 0 = no ☐

H Takes more than 3 prescription drugs per day
0 = yes 1 = no ☐

I Pressure sores or skin ulcers
0 = yes 1 = no ☐

J How many full meals does the patient eat daily?
0 = 1 meal
1 = 2 meals
2 = 3 meals ☐

K Selected consumption markers for protein intake
- At least one serving of dairy products (milk, cheese, yoghurt) per day yes ☐ no ☐
- Two or more servings of legumes or eggs per week yes ☐ no ☐
- Meat, fish or poultry every day yes ☐ no ☐

0.0 = if 0 or 1 yes
0.5 = if 2 yes
1.0 = if 3 yes ☐.☐

L Consumes two or more servings of fruit or vegetables per day?
0 = no 1 = yes ☐

M How much fluid (water, juice, coffee, tea, milk...) is consumed per day?
0.0 = less than 3 cups
0.5 = 3 to 5 cups
1.0 = more than 5 cups ☐.☐

N Mode of feeding
0 = unable to eat without assistance
1 = self-fed with some difficulty
2 = self-fed without any problem ☐

O Self view of nutritional status
0 = views self as being malnourished
1 = is uncertain of nutritional state
2 = views self as having no nutritional problem ☐

P In comparison with other people of the same age, how does the patient consider his / her health status?
0.0 = not as good
0.5 = does not know
1.0 = as good
2.0 = better ☐.☐

Q Mid-arm circumference (MAC) in cm
0.0 = MAC less than 21
0.5 = MAC 21 to 22
1.0 = MAC 22 or greater ☐.☐

R Calf circumference (CC) in cm
0 = CC less than 31
1 = CC 31 or greater ☐

Assessment (max. 16 points) ☐☐.☐
Screening score ☐☐.☐
Total Assessment (max. 30 points) ☐☐.☐

Malnutrition Indicator Score

24 to 30 points	☐	Normal nutritional status
17 to 23.5 points	☐	At risk of malnutrition
Less than 17 points	☐	Malnourished

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For more information: www.mna-elderly.com

Addition 2

Functional capacity

As for the research Scale of Daily Living Activities (ADL) has been used to present the functional capacity of the elderly. It is consisted of activities demonstrating daily living independency. The scale is assessed with values 1- for independent and 0- for dependence. The score of 6 indicated complete independence, score 4 indicated moderate impairment and 2 and below indicate severe functional imparement (20, 21).

Data were analyzed with the Statistical Package for Social Science (SPSS), version 19.0. The analysis involved elderly with malnutrition and risk of it, using Pearson correlation coefficient with significance level of 5%. Poisson regression has been performed, presenting the reasons for raw and adjusted prevalence and their respective confidence intervals at 95% (CI 95%). Variables that were associated with significance level ($p < 0.10$) were included in the model.

RESULTS

The results from the research conducted among elderlies have shown the following socio-demographic characteristics: out of 127 participants, most of them were females- 77% and 69% at the age between 75 and 84. Most of the elderlies (in 68%) have completed secondary education.

As for the estimation of their own health condition, most of the participants (in 47%) have stated that their health is good and 41% of the elderlies estimated their health condition as poor. 76% of them use more than 3 medications a day and 17% of them use less than 3 medications a day. As of the results at 61% of the participants less than 3 diseases has been registered as presents and 34% reported more than 3 diseases.

According to BMI 19% are with low wight, 33% are with a normal weight, 31% are overweight and 17% are obese. Mid value of BMI is 22,3 with statistical significance between males and females ($p < 0.001$).

The results from MNA have shown that 69, 2 % are well- nourished, 27,6% are at risk for malnutrition and 3,2% are malnourished. There is a relatedness of the nutrition status with the gender ($p < 0,001$) and the level of education ($p < 0,001$).

ADL scale among 127 elderly participants has shown that 37% are independent, 45% are with a moderate impairment and 18% are with severe functional impairment. In comparison with the females, the males show higher level of functional ability in all ADL components except the continence. From the results of the chosen items (functional activates, BMI and gender) represented in the table no 1 we have: independent (37%), moderate impairment (45%) and severe functional impairment (23%). According to the represented results we have significant differences between the groups ($p < 0,05$).

Regarding the variables related to elderly health, it has been found that presence of more than 3 diseases, physical inactivity and functional capacity for ADLs was associated to nutritional status. Table 2 presents PR (Prevalence Rations) of independent variables and their confidence intervals. It was observe as a result of the multivariate analysis, that only the variable functional capacity for ADL was statistically significant.

DISCUSSION

This research presents the socio- demographic characteristics, health, nutritive status, activity daily living among institutionalized elderlies. As for the study most of the participants expressed in percentage were females at the age between 75 and 84. Most of the participants have completed secondary education. The participants that were mostly included in this research estimate their own health condition as good and most of them use more than 3 medications a day, and for most of them less than 3 diseases are registered.

Self-evaluating research among elderlies in Korea has shown that 78% of the people have registered an illness that is related with the estimation of their own health (22).

The morbidity in this research can be explained with the fact that the institutionalizing of the elderlies is mostly a result of the presence of illness and inability for different types of care to this population.

According MNA scores 27,6% from the participants were at risk of malnutrition and 3.2% were registered as malnourished. Those results were with a lower range than most of the international studies, whereas the results from 32 multinational studies including 6,821 institutionalized elders indicated that the prevalence of malnutrition and its risk were 5-71% and 27-70% respectively (17). A study conducted at a nursing homes in Spain presented a prevalence of 2,8% of participants are with malnutrition and 37,3% are at risk of malnutrition. (23).

The nutrition status among participants is most probably registered as good due to the exclusion of the severely ill and elderlies suffering with dementia, during the conduct of this research.

In this study the nutrition status has shown relatedness also with the gender and the level of education among hospitalized elderlies.

A research conducted in Brazil has shown that the nutritional status was associated with gender, education, where ålderly men had a greater potential for malnutrition and malnutrition risk (24).

High proportion of elderly with low weight mostly man was found in the research done by Menezes and Marucci (25). As for the education and nutritional status a relatedness was indentified in a other studies conducted in Brazil (26). As for this study along with the aforementioned studies conducted in Brazil most of the examined are registered with a low level of education. This result may be related to low socioeconomic status and poor access to information. Barreto et al.also suggests that low education is a risk factor for low weight, explained by lower income in old age (27).

The results of ADL scale have shown that most of the participants are able to perform their basic daily activities, comparing that males show higher percentage of independence in all components of ADL except the continence, than females.

Similar results were shown with the study conducted at nursing homes in Lebanon where the males have shown higher level of functional ability compared to the females (28).

This study has also shown relatedness of the nutrition status and presence of more than 3 diseases among participants which is confirmed with the results of the research of Stratton RJ whereas the results shown that Somatic diseases may also increase the risk of malnutrition (29).

Participants included in this research show moderate physical activity and physical inactivity in the hospitalized elderly. Study conducted by Walid Kamal M. Abdelbasset approved beneficial effects of physical activity on depression status and pain in elderly people (30).

The results have shown a relation with the nutrition status and the functional capacity of ADL same as the research conducted in Brazil where it has been observed that dependent or partially dependent individuals for performing ADLs are approximately 1.6 times more malnourished or at risk of malnutrition than independent individuals (24).

Nutrition status among institutionalized elderly affects the impaired functional capacity, appearing whether as a cause of a consequence. It is possible that nutritional status is an important factor in maintaining functional capacity, due to some aspects such as the lowest level of physical activity and muscle atrophy (31).

CONCLUSION

The results from the research have shown that the nutrition status among elderlies is satisfactory, emphasizing the factors such as gender, education level and functional ability as key points for the level of nutrition status at the elderlies.

MNA and ADL are both simple and noninvasive scales that can easily be administered among hospitalized elderlies. Simple applying can be effective and economic manner of identification of persons that are in need of intervention.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

ODNOS FUNKCIONALNOG I NUTRITIVNOG STATUSA KOD STARIH

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Uvod: Starenje je izrazito uspešno kada je praćeno dobrim nivoom zdravlja, blagostanja i nezavisnosti. Glavni indikator za predviđanje rizika mortaliteteta kod starijih ljudi je nutritivni status i funkcionalna fizička sposobnost. **Cilj** ove studije bio je da se predstavi nutritivni i funkcionalni status među institucionalizovanih starih osoba u korelaciji sa određenim faktorima rizika. **Materijal i metode:** studija slučajeva i kontrola je sprovedena u određenim staračkim domovima sa učešćem pojedinaca koji su imali preko 60 godina. Za analizu je uzeta Merna skala dnevnih aktivnosti (ADL) koja je prezentovala funkcionalni kapacitet starih lica kao i MNA koja je određivala nutritivni status. **Rezultati:** Rezultati ove studije pokazali su sledeće demografske karakteristike: od 127 paciejanta, većina je bila ženskog pola – 77% i 69% između 75-84 godine. Većina starih (68%) je imala srednju stručnu spremu. Rezultati MNA pokazali su da je 69,2% ispitanika bilo dobro hranjeno, 27,6% je bilo pod rizikom od malnutricije i 3,2% je bilo pothranjeno. Postoji povezanost između nutritivnog statusa i pola ($p < 0,001$) i nivoa obrazovanja ($p < 0,001$). ADL skala je od 127 ispitanika pokazala da je 37% nezavisno, 45% sa srednjim oštećenjima, kao i 18% sa ozbiljnim funkcionalnim

oštećenjima. U poređenju sa ženama, muškarci su pokazali viši nivo funkcionalne sposobnnosti u svim ADL komponentama, osim kontinencije.

Zaključak: Rezultati ove studje pokazali su da je nutritivni status među starim licima zadovoljavajući i da skreće pažnju na pol, nivo obrazovanja i funkcionalne sposobnosti kao ključne tačke u nutritivnom statusu starih.

Ključne reči: malnutricija, funkcionalni kapacitet, stari.

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SANAMED
2018; 13(2): 171–174
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.243
UDK: 616.35-072.1-06
ID: 266977036
Case report

A RARE PHENOMENON CREATING DILEMMA FOR THE SURGEON: PNEUMOPERITONEUM AFTER COLONOSCOPY

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Primljen/Received 23. 07. 2018. god.

Prihvaćen/Accepted 05. 08. 2018. god.

Abstract: Pneumoperitoneum is free air existence in abdomen and usually caused by the perforation in gastrointestinal system. Peptic ulcer disease is the most common cause of perforation and pneumoperitoneum. The presence of free air in abdomen usually indicates emergency surgery. However, surgical approach is not required in some cases of pneumoperitoneum if there is no evidence of perforation and no sign of peritoneal irritation. Herein, we present an 82-year–old male patient who had pneumoperitoneum after colonoscopy and treated non-surgically.

Key words: colonoscopy, complication, pneumoperitoneum.

INTRODUCTION

Colonoscopy is known as a safe procedure, but complications may occur. The most common complications are perforation and massive haemorrhage (1). The incidence of these serious complications is low but, despite increased experience with the procedure, it remains unchanged over time. The risk for adverse events has been also associated with comorbidity: diabetes mellitus, history of previous stroke or cardiovascular disease, chronic obstructive pulmonary disease. But pneumoperitoneum without sings of peritonitis after colonoscopy is a rare phenomenon which creates a management dilemma for the surgeon (2). Herein, we present an 82-year–old male patient who had pneumoperitoneum after colonoscopy and treated non-surgically.

CASE PRESENTATION

An 82-year–old, Jordanian male with a history of hypertension and oesophagus carcinoma surgery 14 months ago was undergoing routine upper gastrointestinal system endoscopy and colonoscopy with sedoanalgesia. Both procedures complicated uneventfully and no pathologies were found. Two hours after endoscopic procedures, he complained about abdominal and shoulder pain. On examination, the patient's blood pressure was 155/90 mm Hg, heart rate was 72 bpm and body temperature was 37.2 °C. His abdominal examination did not reveal any signs of rigidity or rebound but abdomen was distended and tympanic by percussion. Complete blood count revealed a white blood count of 4550 cells/mm^3 (4600–10200 cells/mm^3) and haematocrit level of 36% (40%–54%). His electrolytes, liver function tests, blood urea nitrogen and creatinine were normal. Intravenous and oral contrast-enhanced computed tomography scan revealed only pneumoperitoneum (Figure 1). Non-surgical treatment decision was taken due to the fact that the patient has no sign of acute abdomen, no fever, his leukocyte value was normal, and there was no free fluid in the computed tomography. His oral diet was stopped, intravenous fluid and 2nd generation cephalosporin antibiotics started as a precaution. On the third day of his follow up, his complains about abdominal and shoulder pain decreased also body temperature was 36.8 °C. on his examination, his abdominal distension and

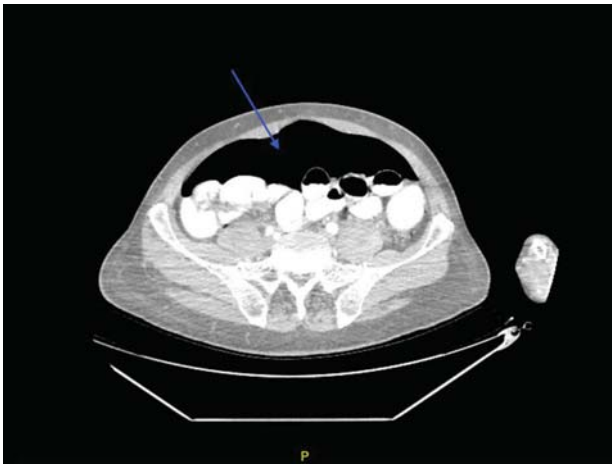

Figure 1. *Computed tomography image of pneumoperitoneum (Blue arrow)*

tympanic sound by percussion decreased. His bowel sounds were normoactive and he had defecation. Complete blood count revealed a white blood count of 6500 cells/mm^3. He was started on an oral liquid diet on the third day and given a normal salt-free diet on the fourth day. During the period of hospitalisation, his vital signs were normal. The patient stayed in hospital for 7 days. The patient was discharged without any problem.

DISCUSSION

Colonoscopy is a safe and effective examination technique for visualization of colonic lumen and it is commonly used to diagnose colonic pathologies and nowadays, it is used for treating some pathologies. The literature reports a complication rate of up to 0.5% (1). Most common complications of colonoscopy including perforation and massive haemorrhage are rare but have potential to cause very serious consequences even lead to fatal outcome. Direct mechanical trauma of colonoscopy, thermal trauma from electrocautery of laser device and barotrauma from excessive air insufflation are mechanisms of colonoscopy related perforation (2) which has prevalence ranges from 0.016% to 0.2% following diagnostic examination and up to 5% after therapeutic colonoscopy (3). Polypectomy, pneumatic di-

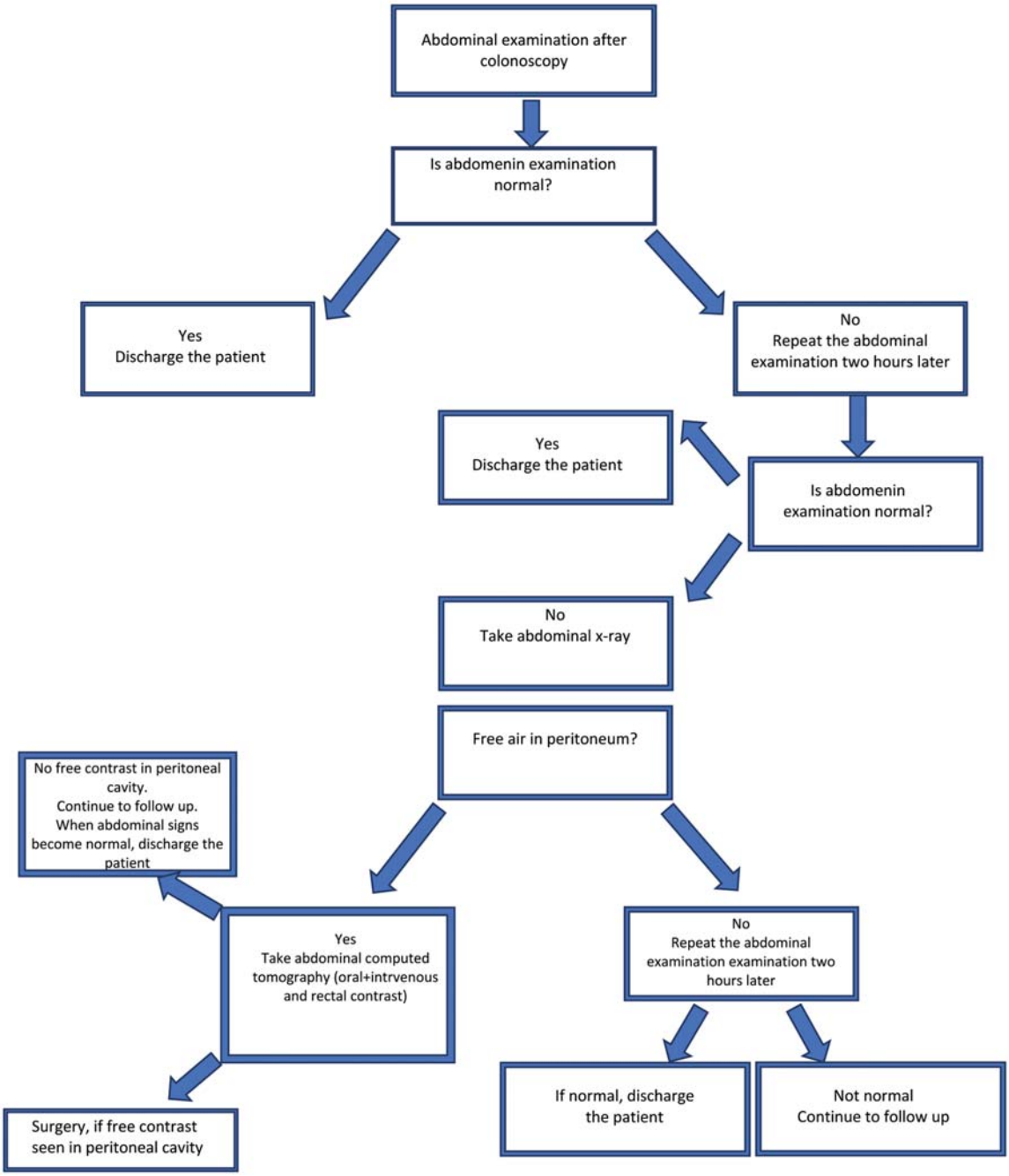

Figure 2. *Algorthym of management free peritoneal air after colonoscopy*

latation for Chron stricture, mucosal resection of lesions during colonoscopy are high risk procedures. Patients older than 75 years or those with comorbid diseases also have increased risk of perforation (4). After colonoscopy, patients usually describe abdominal and shoulder pain from referred diaphragmatic irritation. Physical examination may reveal a distended, rigid abdomen which is tympanic by percussion. Intestinal sounds may be hypoactive or absent. Abdominal tenderness varies and is present due to peritoneal irritation. Subcutaneous air and crepitation in the abdominal wall may be present. Increase of abdominal pressure may causes upward distension of diaphragm muscle which leads to respiratory problems (3). Pneumoperitoneum without sings of peritonitis after colonoscopy is a rare phenomennon which creates a management dilemma for the surgeon. Pneumoperitoneum may occur without perforation. Colonic mucosa may become air permeable if mucosa is herniated via muscular layer and serosa (5). In contrast with this hypothesis, 2n the study of Pearl JP et al, 100 patients underwent colonoscopy and then radiography of the chest and abdomen to detect free air. They revealed, no cases of benign pneumoperitoneum were detected and they argued pneumoperitoneum after colonoscopy is possibly non-existent and advocated to treat all cases of free intraabdominal air after colonoscopy as perforations (6). Other causes of pneumoperitoneum are emphysematous cholecystitis (7), spontaneous bacterial peritonitis (8), intestinal cystic pneumatosis (9), and liver abscess (10). Most common gynecological cause is rupture of pyometra (11). As a result of this rare condition and management dilemma, some of these patients may underwent unnecessary laparotomy (Figure 2).

CONCLUSION

Pneumoperitoneum after colonoscopy is a rare and unusual situation which creates a dilemma for the surgeon. We think that patients can be followed without surgery if there is no free fluid and no evidence of acute abdomen despite free air.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

REDAK FENOMEN KAO DILEMA ZA HIRURGA: PNEUMOPERITONEUM NAKON KOLONOSKOPIJE

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Pneumoperitoneum je prisustvo slobodnog vazduha u trbuhu i obično je uzrokovan perforacijom u gastrointestinalnom sistemu. Peptički ulkus je najčešći uzrok perforacije i pneumoperitoneuma. Prisustvo slobodnog vazduha u trbuhu je obično indikacija za hitnu operaciju. Ipak, hirurški tretman nije neophodan u nekim slučajevima pneumoperitoneuma, i to ukoliko ne postoji dokaz o perforaciji i ukoliko nema znakova iritacije peritoneuma. Predstavljamo pacijenta muškog pola, starog 82 godine, koji je imao pneumoperitoneum nakon kolonoskopije i nije lečen hirurški.

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SANAMED
2018; 13(2): 175–181
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.236
UDK: 616.31:616.5-002
ID: 266974988
Review article

THE ROLE OF CANDIDA ALBICANS ON THE DEVELOPMENT OF STOMATITIS IN PATIENTS WEARING DENTURES

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Primljen/Received 22. 06. 2018. god. Prihvaćen/Accepted 28. 07. 2018. god.

Abstract: Denture stomatitis is the most common inflammatory reaction that occurs in people who wear dentures. It is believed that in 60-65% of cases the cause of this inflammation is infections by yeasts from the genus *Candida* (*C.*), primarily *Candida albicans* infection. *C. albicans* is a part of the normal microflora of the respiratory and digestive tract. This yeast has the ability to adhere to the oral mucosa and to the base of the denture, as well as to form a biofilm. Its virulence is especially supported by the state of weakened resistance of the organism, when *C. albicans* expresses its pathological effect. This paper presents the pathogenesis of *C. albicans*-associated denture stomatitis, as well as the most common diagnostic and therapeutic procedures used to diagnose and successful therapy.

Key words: dentures, stomatitis, Candida albicans, biofilm, oral hygiene.

INTRODUCTION

Denture stomatitis is a very common inflammatory reaction occurring in the carriers of the dentures and involves the mucous membrane underneath the base of the denture (1, 2). The etiology of this process is multifactorial, but in 89% of cases, the occurrence of denture stomatitis is associated with infection of yeast of the genus *Candida* (*C.*) (3). *Candida* species are microorganisms that form part of the normal microflora of the oral cavity. However, if the condition of the normal flora is disturbed or the general resistance of the organism is reduced, *Candida* species cause inflammation on the surface of mucosa, which is most often manifest in the form of denture stomatitis in the denture carriers. For the first time *Candida* species were described by Cahn in 1936 as possible causes of this inflammatory process (4). *Candida albicans* is thought to be most responsible for the development of inflammation, although there are studies proving the participation of other yeasts of this strain in the development of oral candidiasis and, therefore, denture stomatitis. Among them there are *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* and *C. glabrata* (5, 6). Their primary localization is on the dorsal side of the tongue and on the oral mucosa, while the biofilm covering the surface of the teeth colonizes secondary (7). Increased use of antifungal drugs in the treatment of candidiasis has lead to that non-albicans *Candida* species can also contribute to the development of this infection (8).

Therapy of denture stomatitis involves the maintenance of adequate oral hygiene, removal of dentures during the night, the use of various disinfectants such as sodium hypochlorite and chlorhexidine, as well as the use of local and systemic antifungal drugs (1, 9). In addition to antifungal agents, in the treatment of *Candida*-associated denture stomatitis, extracts of various plants that have antifungal, anti-iflamatory and antioxidant effect are increasingly applied.

PATHOGENESIS OF DENTURE STOMATITIS ASSOCIATED WITH CANDIDA ALBICANS

C. albicans is a part of the normal microflora of the respiratory and digestive tract. However, in certain

situations, especially in the case of immunodeficiency, it can exhibit pathological effects and cause candidiasis. Factors that contribute to its pathological effect are the ability of this yeast to adhere to mucosal cells, to convert from single cell to filamentous form, to secret enzymes such as aspartyl proteinase and phospholipase, as well as to build biofilm (10).

The role of *C. albicans* in the development of denture stomatitis is associated with the fact that this yeast has the ability to colonize the oral mucosa and the surface of the dentures and build aggregates with oral bacteria (11). What also increases the incidence of denture stomatitis associated with Candida is the fact that the presence of denture reduces the flow of saliva and oxygen to the tissues below the denture base, which increases the local acidity and anaerobic conditions and thus contributing to the growth of yeasts (12).

On the other hand, *C. albicans* has the affinity to adhere to the acrylic from which denture is made, and the acrylic resin possesses certain characteristics, such as hydrophobicity, which accelerates adhesion as a major step associated with the formation of biofilm (12, 13).

Formation of *C. albicans* biofilm goes through three phases. In the first phase, which lasts 1-11 hours, the *C. albicans* cells adhere to the rough surface of the denture, due to the action of hydrophobic and electrostatic forces between the cells and the surface of the denture. In a second phase that lasts 12-30 hours, the biofilm consists of a non-cellular layer or a so-called extracellular polymer substance that covers microcolonies of yeasts, while the third phase is the maturation phase of biofilm occurring within the 38-72 hours (9, 14).

Factors that favoring the adherence and formation of biofilm are the roughness of the inner surface of the dentures, the influence of salivary pelicla, as well as hydrophobic and electrostatic interactions (9).

The rough surface of the acrylate promotes increased retention of microorganisms and protects them from the forces that tend to remove, leaving the microorganisms trapped by the irregular surface of the prosthesis, even after cleansing (15). Also, the traumatic factors such as mechanical trauma caused by loose prosthesis can increase the risk of tissue penetration and colonization of *Candida* species (16). The age of the prosthesis is also an important factor, because it is more difficult to maintain hygiene if prosthesis are worn for a long time and there is a tendency towards to porosity of denture base, which favors the occurrence of infection (17).

As for the effect of saliva on *C. albicans* adhesion on the prosthesis surface, this effect is still controversial (7, 16, 18). Saliva plays an important role in mechanical cleansing and contains certain innate immune molecules such as lysosomes, lactoferrin, calprotectin, and IgA immunoglobulin, which reduce adhesion of *C. albicans* for oral surfaces (2, 19). Increased saliva flow allows the elimination of carbohydrates that are metabolized by bacteria that produce acidic products, and also the buffer saliva system neutralizes these acidic products, as well as the acids introduced through food and drink (20).

On the other hand, saliva proteins such as mucin and staterine act as adhesion receptors for manoproteins present in *Candida* species (12). Reduction or complete absence of saliva in individuals with xerostomia causes a disbalance in the normal microbiological composition, favoring the proliferation of *Staphylococcus aureus* which inhibits the adaptation of the commensal (21).

A particularly important factor that promotes the adhesion and proliferation of *C. albicans* for oral mucosa and therefore the base plate of the dentures is a low pH in the oral cavity. It is believed that pH 3 is critical, because it provides enzymatic activity of proteinases and phospholipases, which are the main factors of *Candida* virulence, due to their cytotoxic and cytolytic effects (12, 16). It is also observed that the pH value of oral mucosa in the elderly and edentulous patients are significantly lower than in younger and in patients with teeth, because the function of the salivary glands is reduced in the elderly, and this is in favor of the development of denture stomatitis in these patients because they are the most frequent carriers of dental prostheses (22, 23).

Hydrophobicity and electrostatic interactions influence the development of denture stomatitis caused by *C. albicans* by the fact that hydrophobic surfaces reduce cell adhesion, while yeasts whose surface has a positive charge are more adherent unlike the repulsive forces which exist between the negatively charged yeast and polymer surfaces (16).

In addition to numerous local factors responsible for the adhesion and colonization of *C. albicans* for oral mucosa and the base of the denture, there are also certain systemic factors that can accelerate the development of this infection. Particularly important is the state of immunodeficiency, in which *Candida* becomes more virulent and leads to the formation of oral candidiasis, including one or more places in the oral cavity (7). The most significant immunodeficiency are HIV infection, hereditary deficiency of myeloperoxidase and some syndromes, such as DiGeorge's syndrome (12).

Some of systemic factors that can increase a risk of denture stomatitis are diabetes mellitus, long-term use of antibiotics and corticosteroids, as well as kidney diseases (16, 24).

Particularly diabetes mellitus is associated with the onset of oral candidiasis, and consequently in pati-

ents who wear dental prostheses is associated with denture stomatitis (25).

Some studies find that it is not important only the presence of disease how much is important to control glycemic in diabetes, because poorly controlled glycemic will reduce salivary flow and pH in the oral cavity, increasing salivary glucose levels (24). Moreover, besides the presence of a high concentration of salivary glucose combined, low salivary secretion may enhance growth of yeasts and their adherence in epithelial oral cells (26).

Different kidney diseases requiring repeated and prolonged antibiotic therapy and sulphonamide therapy indirectly lead to the occurrence of oral candidiasis and denture stomatitis because these drugs change normal microflora and promote the growth of yeasts (16).

DIAGNOSIS AND TREATMENT OF DENTURE STOMATITIS ASSOCIATED WITH CANDIDA ALBICANS

Denture stomatitis is usually the asymptomatic inflammatory process and in this case it is usually revealed on routine dental examinations in the form of erythema or edema of the mucosa that is in contact with the prosthesis. Symptoms that the patients may complain are halitosis, slight bleeding, and swelling in inflamed area, or burning sensation, xerostomia or any alterations of taste (27). Denture stomatitis can be also associated with some other oral diseases such as medial rhomboid glossitis, atrophic glossitis and angular cheilitis, and these states have been recognized as *Candida*-associated lesions (28).

In general, to establish the diagnosis of oral candidiasis, there are several methods that can be used, such as taking a swab, target tissue biopsy (eg. palate), examining a saliva sample, so which method will be used depends on the nature of the lesion being examined (12). Seeding of samples on the nutrients the presence of yeasts is demonstrated. By increasing the colonies of yeasts on different nutrient media, a diagnosis of the species within the genus *Candida* (*C.*) can be given, however, the detection of hifa (an important factor for invasiveness, adhesion and virulence) by microscopy is for now a "golden" standard for the exact diagnosis of *Candida*-associated denture stomatitis (29).

Altough the culture-based identification is the gold standard for the diagnosis of fungal infection, detection and identification of fungal DNA by PCR is one of the most powerful and popular tools for the early detection and identification of pathogenic fungi, including *Candida* species (30). The PCR is the most sensitive of the existing rapid methods to detect microbial pathogens in clinical specimens. The PCR include several critical steps such as, DNA extraction, PCR amplification and the detection of amplicons (31). On the other hand, conventional PCR has a big impact with very good results in fungal identification, but researchers have difficulties due to the post-PCR steps for amplicon evaluation such as agarose gel electrophoresis (32). Due to it is suggested to use real-time PCR or real-time quantitative PCR (qPCR) assays, because of these methodes allows the researchers to actually view the increase in the amount of DNA as it is amplified and post-PCR manipulation of the amplicon is not required (32, 33).

Although *Candida*-associated denture stomatitis is often asymptomatic, this inflammatory process should be treated properly, because it can act as a reservoir for the development of severe inflammation which consequently leads to bone resorption (12). Therapy approach is complex due to multifactorial etiology, but some basic postulates in the therapy of these changes involve the use of local and systemic antifungal drugs, the use of disinfectants, the maintenance of adequate oral hygiene and hygiene of the prosthesis (7, 9, 16).

The use of antifungal drugs depends on the oral symptoms and the history of the patient's disease. In the case of an uncomplicated inflammatory process, the use of local antifungal drugs, available in the form of gel, lozenge, cream and oral suspensions is recommended (2).

The most commonly drugs used locally are nystatin and amphotericin b, while fluconazole oral suspension is proving to be a very effective drug in the treatment of oral candidiasis. Another topic drugs widely used are miconazole and clotrimazole (34). Mostly all drugs lead to the cessation of symptoms after administration of 12 to 14 days.

Nystatin is the most commonly used drug in oral candidiasis therapy, which is administered four times a day for two weeks in the form of oral pastille and suspension. The oral rinse contains sucrose, so it is is useful in edentulous patients and those with oral dryness such as patients receiving radiotherapy and those with HIV infection (26). In some studies, nystatin has been shown to be ineffective for *Candida* lesions in cancer patients (35). In addition, nystatin, myconazole cream (2%) and clotrimazole cream (1%) are also used in the treatment of denture stomatitis associated with angular cheilitis (23). Fluconazole oral suspension is administered in a dosage of 10 mg/ml aqueous suspension by administering 5 ml daily for 7 or 14 days (34).

There have been several studies comparing topical and systemic drugs. Isham N et al. compared the use of ketoconazole tablets (200 mg daily) with topical ketoconazole (2% twice daily) and miconazole muco-

adhesive tablets in the treatment of denture stomatitis (36). Due to the adverse effects of ketoconazole like nausea, vomiting and gastrointestinal problems it has been suggested the use of other drugs when treating candidiasis associated with wearing of dentures (37, 38). Thus the use of miconazole mucoadhesive tablet was established as the drug of first line of defense for this type of candidiasis (34).

The use of systemic antifungal drugs is recommended for severe forms of *Candida*-associated denture stomatitis, when local therapy has not been effective, as well as in immunodeficiency patients. Among systemic antifungal drugs, fluconazole is particularly useful due to low toxicity and good tolerability. The appropriate dose is between 50-100 mg daily (26, 34). On the other hand itraconazole is applied in case of resistance to fluconazole. Ketoconazole is also effective as fluconazole, but its use is not recommended in the elderly due to interactions with macrolide antibiotics and antihistamines, and because of adverse effects such as hepatotoxicity (26). Amphotericin B has previously been administered intravenously in the treatment of *Candida*-associated denture stomatitis, but today its application was reduced due to nephrotoxicity (39).

Antifungal drugs of new generations used in the treatment of oral candidiasis and severe forms of denture stomatitis associated with *Candida* are posaconazole, ravuconazole and echinocandin such as caspofungin, micafungin and anidulafungin. They represent less toxic alternatives than amphotericin B (26).

The aim of the disinfectants used in denture stomatitis is to reduce the formation of biofilm on the prostheses and inhibit the growth of yeasts (12). Especially applied disinfectants are chlorhexidine gluconate and sodium hypochlorite (16). Chlorhexidine gluconate is effective against numerous bacteria, viruses, bacterial spores and against yeasts (12). It can be applied as a 0.2% solution for rinsing the oral cavity, however, much better results are obtained when it is used in the form of a 2% solution for immersing of the prosthesis. However, it is good to have in mind that chlorhexidine reduces the effectiveness of nystatin when it is administered at the same time, so the advice is to use nystatin at least 30 minutes after rinsing the mouth with chlorhexidine (40). Another very effective disinfectant that reduces the formation of *C. albicans* biofilms is sodium hypochlorite, which is used in the form of a 0.2% solution, and it is also advised to immerse the dentures into this solution. However, the use of sodium hypochlorite must be restricted to a shorter period of time because it has the ability to damage the dentures (12, 16).

All therapeutic methods in the treatment of denture stomatitis would not produce results without adequate oral hygiene. Maintenance of adequate oral hygiene and local application of antifungal drugs in most cases are sufficient for the treatment of uncomplicated oral candidiasis. Oral hygiene includes daily cleaning of teeth, tongue and dental prostheses. Dentures should be removed at night or at least six hours during the day (26).

ALTERNATIVE AGENTS IN THE TREATMENT OF DENTURE STOMATITIS ASSOCIATED WITH CANDIDA ALBICANS

In addition to the classic therapy of denture stomatitis associated with *C. albicans*, lately it is increasingly resorting to alternative agents that have less side effects and at the same time exhibit antibacterial and anti-inflammatory activity. There are a large number of preparations based on plants and their essential oils that have an anti-inflammatory effect and as natural antiseptics express antifungal and antibacterial activity (9). Natural compounds from essential oils: eugenol, farnesol, geraniol, linalool, menthol, menthone, terpinen- 4-ol, α-terpineol and tyrosol, carvacrol, expressed strong antifungal *in vitro* activity (12).

One of the investigated essential oils exhibiting a remarkable antioxidative and antifungal activity is the thyme oil. Thyme oil is very rich in phenols and is classified as antifungicide due to its strong effect on *Candida* species (41).

Some authors, have shown that timolol as a component of thyme oil possessed pretty strong antifungal activity against *C. albicans in vitro*. In view of its broad activity, thyme essential oil may be used as a natural disinfectant for the prevention of denture stomatitis (42). Omran SM et al. in their study have examined the effect of essential oils on *Candida* yeasts, they found that thyme oil had the highest inhibitory effect (43).

The essential oil of tea tree has a significant effect on *C. albicans*. Pachava et al. have shown that tea tree oil incorporation to denture soft liner decreased *C. albicans* growth significantly (44). Similarly, Amornvit et al. combined lemongrass essential oil to tissue conditioner and demonstrated the anti-*Candida* activity (45).

On forty isolates of *C. albicans* from the oral cavity obtained from twenty-five patients using orthodontic appliance, eight essential oils were applied, the largest antifungal effect was shown by the essential oil of cinnamon, laurel, lemon and mint (46).

It is also considered that garlic in combination with nystatin may be highly effective in the treatment of oral candidiasis, in particular those associated with denture stomatitis (9, 47). Other essential oils that that have a positive antifungal effect are the oil of the following plants: *Pelargonium graveolens*, *Satureja hortensis*, *Zataria multiflora*, *Punica granatum*, *Salvia of-*

ficinalis, *Streblus asper*, *Boesenbergia pandurate*, *Phyllanthus emblica*, *Scutellaria baicalensis*, *Azadirachta indica*, *Melaleuca alternifolia* (9, 48).

CONCLUSION

Denture stomatitis is one of the most common inflammatory reactions affecting carriers of dental prostheses, and in most cases it is associated with infection of yeast of the genus *Candida*, primarily with *C. albicans*. Although this is the most common asymptomatic tissue reaction below the prosthesis base, it is important to diagnose the disease as early as possible, otherwise *Candida* would act as a reservoir for more extensive infections that would eventually lead to bone resorption. Treatment of *Candida*-associated denture stomatitis requires the use of antifungal drugs and adequate oral hygiene.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

ULOGA CANDIDE ALBICANS U RAZVOJU STOMATITISA KOD PACIJENATA KOJI NOSE ZUBNE PROTEZE

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Protezni stomatitis je najčešća zapaljenska reakcija koja se javlja kod osoba koje nose zubne proteze i obično zahvata palatinalnu sluzokožu. Smatra se da je u 60-65% slučajeva uzrok ovog zapaljenja infekcija gljivicama iz roda *Candida* (*C.*), a prevashodno *Candida albicans*. Ova gljivica ima sposobnost da adherira za oralnu sluzokožu i za bazu protezne ploče, i da formira biofilm. Njena virulencija je naročito potpomognuta stanjima oslabljene otpornosti organizma, kada *C. albicans* ispoljava svoje patološko dejstvo. U ovom radu prikazana je patogeneza proteznog stomatitisa udruženog sa *C. albicans*, kao i najčešće dijagnostičke i terapijske procedure koje se koriste za postavljanje dijagnoze i sprovođenje uspešne terapije.

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SANAMED
2018; 13(2): 183–190
ISSN-1452-662X

DOI: 10.24125/sanamed.v13i2.225
UDK: 618.39-021.3-084; 616-005.6-085
ID: 266976012
Review article

THERAPEUTIC OPTIONS IN PREVENTING UTEROPLACENTAL UNIT HYPOXEMIA CAUSED BY TROMBOPHILIAS

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Primljen/Received 04. 04. 2018. god.
Prihvaćen/Accepted 10. 05. 2018. god.

Abstract: A miscarriage is primarily defined as an unintentional loss of pregnancy before the 20th week of gestation. Both the practice and theory show that pregnancy losses up to the 10th week of gestation may, besides many other gynecological and microbiological causes, also be brought down to the existence of some of the acquired thrombophilia such as antiphospholipid syndrome. Numerous studies have been conducted to examine the efficiency of therapy administration to prevent adverse outcomes of pregnancy. Hereditary thrombophilia are more connected with adverce pregnancy outcomes.

Key words: pregnancy; thrombophilias, LMWH, therapy, outcomes.

INTRODUCTION

A miscarriage is primarily defined as an unintentional loss of pregnancy before the 20th week of gestation, observed both from the aspect of pathophysiology and anatomy. The clinical practice standard, in most developed and medium developed countries, allows the interpretation of premature delivery from the 24th week of gestation, namely the moment when type 2 pneumocytes are embryologically and anatomically formed. Moreover, some of the exceptionally economically developed countries define premature deliveries starting from the 20th week of gestation. Both the practice and theory show that pregnancy losses up to the 10th week of gestation may, besides many other gynecological and microbiological causes, also be brought down to the existence of some of the acquired thrombophilia such as antiphospholipid syndrome. However, even in the cases which should be further investigated, there should be a more detailed examination of other causes including chromosome aberrations, microbiological causes (incidence of ureaplasma, mycoplasma, chlamydia, as well as vaginosis or severely manifested human papilloma virus infections). Moreover, the part of cause is lying in the anatomic variations of the uterus, more frequently called anomalies (anomalies of the uterine body, existence of septum and sub-septum, polyps, myoma located in the area of chorionnidation or pathological vascularizations). The part of cause of early pregnancy losses may also be of hormonal nature, either by hormones related to primarily gynecological aspect or more frequently by hormonalaxes of the whole body, starting from hypophysis activities, hyperprolactinemia incidence, as well as inadequate function of thyroid and adrenal glands. The loss of pregnancy is partly related to complete organism state, chemodynamically as well as metabolically, adequate glucoregulation, the existence of inherited or acquired problems at the level of functioning of other organs, such as late detection of nephrological or urological problems. It should not be forgotten that hypoproteinemia, or hypovitaminosis and avitaminosis are also stated as potential causes of early pregnancy (1-6).

Studies concluded - not enough effect of antiaggregative or anticoagulative therapy

Several studies have been conducted to examine the efficiency of therapy administration (1). In the study based on adequate methodology, approved by Ethic Committee for disease protection Brest University hospital, the patients were followed in the period from 4th April 2007 to 31st October 2012. Results from 13 hospital centers in France were summarized. Criteria for inclusion were: pregnant patients aged between 18 and 45 with unexplained causes of miscarriage. Re-

current, habitual miscarriage was defined as more than two pregnancy losses before the 15th week of gestation in the patient with same paternal genomes and without offspring. Both partners' karyograms were done for all the included cases. There was no anatomic anomaly of the uterine body found. The absence of antiphospholipid syndrome, factor V Leiden disturbance and prothrombin G20210A mutation was established in all of the cases, as well as disturbances related to absence of protein S or C or antithrombin 3 deficiency. Cases with established indication for aspirin or anticoagulation therapy administration were excluded, namely all the conditions with high risk for deep venous thromboembolism or conditions of cardiovascular nature where the named therapy is necessary. Moreover, all standard contraindications for administration of 40 mg enoxaparin injections, such as anemia with less than 10g/dL or thrombocytes level below 150 x 10.3, or creatinine clearance level below 30 ml/h. After well-established terms for study following, all the women with confirmed pregnancies were randomized and, at the same time educated for therapy self-administration. All the patients were advised to take folic acid. The administration of therapy or placebo was blinded for researchers during the course of study. Enoxaparin Sanofi Aventis (branch ROVI for placebo enoxaparin syringes, Madrid, Spain) was used. All the participants were regularly followed by their clinicians, during the course of pregnancy and two months after delivery. Besides ultrasonography aspects, complete clinical state of the patient was analyzed, as well as laboratory tests, while all side-effects were noted in the treatment adherence notebook. Two subgroups were formed in the group of possible complications. Primary outcomes, obtaining healthy live offspring, as well as premature deliveries and low birth weight children. As secondary outcomes, the incidence of miscarriages, intrauterine fetal loss up to the 20th week of gestation, as well as the incidence of preeclampsia, low birth weight babies delivery, placental abruption and premature deliveries were analyzed. The laboratory evidence of maternal thrombocytopenia (defined as a number of thrombocytes under 0.6 in relation to basal level of thrombocytes which was under 100,000/mm^3) was followed, together with all skin reactions or bleeding episodes. During the analysis of 314 women results, 258 cases were followed, with 138 cases in the enoxaparin group and 120 cases in the placebo group. None of the 258 examined patients was lost during the study; however, one patient refused the treatment while in 5 cases the treatment had to be discontinued due to danger of bleeding and the fact that those patients did not want to further participate in the study. In 3 cases the therapy was stopped because of changes in the throat, skin reaction and the incidence of thrombocytopenia which was later confirmed to be heparin induced thrombocytopenia. The percentage of delivered babies was 66.6% in the enoxaparin group comparing to 72.9% in placebo group. Thrombocytopenia was found in 7 patients, out of which number 4 patients were in the therapy group while 3 patients were in placebo group.

Since the beginning of this study in 2006, only one placebo controlled study was performed, called ALIFE study, which did not prove efficiency of antiaggregation aspirin therapy administration (2).

The randomized SPIN STUDY showed no thrombophilia in over 85% of the cases. Moreover, the effect of improvement by administration of both aspirin and LMWH was not found (1, 2, 3).

In the HABENOX study, the efficiency of enoxaparin administration in relation to aspirin administration was not proven (4). This study concluded that the administration of enoxaparin in the dose of 40 mg per day did not increase the number of live birth in conditions when patients did not have thrombophilia or when reasons for pregnancy losses were undetermined. It is concluded that the administration of LMWH did not increase the chance for live birth in the conditions without thrombophilia and is considered that routine administration of this type of therapy may not be useful in patients without established thrombophilia. The authors state the limitation of their study to be the fact that pathohistological and physiological changes in angiogenesis of patients with congenital and acquired thrombophilia are expected, however caused by different mechanisms and expressed in different ways. Moreover, this study provides the information that even authors themselves find insufficiently well stated in relation to the existence or nonexistence of congenital or acquired hematologic or immune disturbance (5).

Studies concluded – moreeffect of antiaggregative or anticoagulative therapy

Within TREATS study, the incidence of complications in the first and second trimester was followed (6). From sudden miscarriages, over incidence of preeclampsia, the pregnant patients with established disturbances in factor V Leiden as well as prothrombin G20210A defect were followed (6).

The results of Nimes Obstetricians and hematologists Antiphospholipid Syndrome study (NOH-APS) were also analyzed, where a group of pregnant patients without diagnosed thrombophilia was primarily observed but where the incidence of antiphospholipid syndrome was also found. Study methodology included 6.318 patients with spontaneous abortion before the

10th week of gestation. The study exclusion criteria included thrombotic events (existence of at least one element of thrombosis of venous, arterial or small blood vessels type), except in conditions where the placenta was analyzed. All the patients who used antithrombotic, immunosuppressive or immuno modulating therapy were excluded. The study did not include patients whose determined cause of pregnancy loss was infection, metabolic, anatomic or hormonal pathology. Also, the women with HIV infection as well as hepatitis type B or C infection were excluded. Out of stated 6.318 patients, the study included 4.801 patients. All the patients had to fulfill two inclusion criteria. Their anamnesis data had to include 3 pregnancy losses before the 10th week of gestation, without any of the stated other possible causes of miscarriage, as well as both partners' karyotype. The other inclusion criterion was one pregnancy loss after the 10th week of gestation, confirmed by ultrasound, where the pathology analysis found no fetal developmental anomaly. All the patients included in the study were examined for: complete hemogram, fibrinogen, antithrombin 3, protein C, protein S, factor V Leiden (polymorphism F5 rs 6025) as well as disturbance in prothrombin gene G20210A (F2 rs 1799963). Besides, JAK 2 V 617 F mutation was followed as well as the analysis for the existence of antiphospholipid antibodies (aPLAbs). Further investigation did not include 152 patients with established incomplete form of thrombophilia and those with antithrombin 3, protein C and protein S deficiency, and patients with abnormal fibrinogen finding (JAK 2 V617F mutation). In the control group, which was formed, included persons completely free of thrombophilia. The group included 152 persons. Out of total number of negative thrombophilia persons, 3.604 agreed to participate in the study. The persons included in the study as a positive group had isolated Leiden disturbance as well as isolated prothrombin G20210A disturbance. There were totally 301 persons in the Leiden positive group and 279 persons in the G20210A positive group. The other positive group included 517 persons with existing elements for the diagnosis of antiphospholipid syndrome. At the end of summary, 796 persons with thrombophilia and 279 persons without thrombophilia were analyzed. Women with determined antiphospholipid syndrome were not followed in the course of this study. The obtained results show different effects in patients with positive Leiden or positive prothrombin G20210A disturbance. Patients with thrombophilia and previous habitual miscarriages, who did not use antithrombotic therapy in the new pregnancy, had an increased risk of sudden fetal death incidence in relation to the group of women who had negative finding to thrombophilia. There were less pregnancy losses and sudden fetal deaths in patients with established thrombophilia and administered prophylactic doses of LMWH. Moreover, they also had less hypertensive complications, such as preeclampsia, and less sudden preeclampsia developments. They had better results in delivering live newborns, capable for extrauterine life, comparing to the group of patients with thrombophilia and poor outcomes in patient history data. However, it is necessary to emphasize that patients with established thrombophilia and new pregnancy but without therapy were not examined. The study took this position due to the fact that in vitro data in experimental conditions show that LMWH may be considered a promoter of extravilloustrophoblast development and thus may stimulate their invasive processes (7, 8). In the analyses of late spontaneous miscarriages, by serial placental biopsies, in embryos with normal karyotype, the existence of decreased, completely reduced, trophoblast invasion was proven (9). This led to early embryo damage as a secondary complication of pathological trophoblast invasion (10). One of the studies proved the existence of heparin administration positive effects comparing to heparin binding epidermal growth factor on endometrial stromal cells (11).

The results obtained by examining therapy in the conditions of Leiden and G20210A mutations in the Italian study (5) confirm the benefits of therapy administration. These results are in favor of better pregnancy outcomes if LMWH is administered. Also, the obtained values of statistical data are consistent with the multicentric study conducted in Italy where it is confirmed that LMWH prophylaxis may decrease the risk of miscarriage in women with established Leiden mutation or prothrombin G20210A pathology (12). In relation to the incidence of preeclampsia, the clinical trials confirmed less frequent incidence of preeclampsia with Leiden and G20210A disturbances of the patients were administered LMWH. The same results on the frequency of preeclampsia are obtained in the similar study done in 2012 (13). The American College for Chest Physicians still holds the position that women with inherited thrombophilia and history of pregnancy complications should not use antithrombotic therapy (14, 15).

There are data on the usefulness of LMWH administration during pregnancy in women with standard type of thrombophilia (factor V Leiden and G20210A) as well as in newly established thrombophilia (MTHFR, PAI-1 and ACE) (15). In this study, "conventional" thrombophilia such as disturbance in factor V Leiden, prothrombin G20210A mutation, antithrombin 3, protein S and protein C deficiency were analyzed. Also, a "novel" group was analyzed which included disturbances in methylene tetrahydrofolate reductase (MTHFR), plasminogen activator inhibitor

(PAI-1) as well as angiotensin converting enzyme polymorphism (ACE). First trimester disturbances were followed, such as early abortion, then second trimester disturbances such as premature delivery, as well as late pregnancy disturbances such as premature delivery, intrauterine fetal growth restriction, intrauterine fetal death, preeclampsia, placental abruption and deep venous thrombosis. The study concluded that the administration of LMWH had no influence on decrease of earlyspontaneous abortions in conditions of thrombophilia. However, it is also stated that the frequency of intrauterine fetal death in treated patients in "conventional" group was decreased ($p = 0.011$). Comparing to the "novel" group, the incidence of fetal growth restriction, intrauterine fetal demise as well as premature deliveries was decreased. The study concluded that in conditions of "novel" thrombophilia, namely MTHFR, PAI and ACE polymorphism, the administration of LMWH was largely justified and needed further investigation.

In the cohort study, 50 women were followed in the period from July 2008 to September 2012 at the Department of Obstetrics and Gynecology of the University Hospital in Split, a tertiary referral center with about 4,500 deliveries per year. The methodology was based on establishing criteria for adverse pregnancy outcomes: the loss of embryo up to the 12th week of gestation, as the first trimester loss, but with previously established viability of the same embryo by ultrasonography; second trimester pregnancy loss between 12th and 21st week of gestation (plus 6 days); fetal growth restriction but with body weight under 5th percentile for gestational age and adapted to the population not standards; severe forms of preeclampsia defined as an arterial blood pressure over 160 systole and over 110 mmHg diastole together with proteinuria above 5g/24h; the incidence of HELLP syndrome (erythrocyte hemolysis, elevated liver enzymes and low platelets, under 100,000/ml); intrauterine fetal demise as well as fetal demise and death above 22nd week of gestation; premature delivery defined as delivery before the 37th week of gestation; each and every thromboembolic event immediately before or during the pregnancy; second trimester spontaneous abortion; intrauterine fetal death. Exclusion criteria were: patients under 42 years of age; the presence of acquired thrombophilia; uterine body congenital anomaly; perinatal infections (TORCH – toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus); diabetes mellitus, chronic hypertension, endocrine disturbances, kidney transplantation, use of medications; multiple pregnancies, twins, triplets; abnormal first trimester screening tests, such as double and triple tests; pathological karyotype; established fetal anomalies. After the established pregnancy and proven embryo viability, LMWH therapy was administered. Inclusion period was from the 5th to the 9th week of gestation. Out of 50 women, 47 received dalteparin while only 3 received enoxaparin. Dalteparin doses were 2,500 IU/day and enoxaparin doses were 40 mg/day. In the period between 25th and 28th week of gestation, the doses were doubled, being 5,000 IU/day for dalteparin and 80 mg/day for enoxaparin. Both type LMWH therapies were administered up to 6 weeks postpartum. In the analyzed sample of 50 women there were 13 cases (26%) of factor V Leiden mutations, 5 cases (10%) of PT G20210A mutations, 6 cases (12%) of protein C mutations and 6 cases (12%) of protein S deficiency mutations while 25 women had MTHFR mutations. It is interesting to mention that there were 23 conditions of heterozygous MTHFR and 2 cases of homozygous MTHFR (50% of analyzed cases); in 33 cases (66%) PAI-1 polymorphism was found while 25 women (50% of the cases) had ACE polymorphism. Out of this number, the mutations were followed in terms of incidence of one, two or all three mutated genes for ACE polymorphism. Monogenic mutation was found in 7 patients (14%), two gene mutations in 25 patients (50%), three gene mutations in 16 cases (32%) and mutation of all four genes in 2 cases (4%) (15). Within the discussion part of the study, authors state several important comparisons. They primarily emphasized that it was the first time they had made the difference between congenital thrombophilia and divided them into segments, which was not done before. A rather small number of studies had observed "novel" thrombophilia and with conflicting conclusions. Ten papers had been stated confirming the data, i.e. having contradictory conclusions on the significance of LMWH administration in relation to adverse perinatal outcomes (16-26). They also found that there was no difference between the "conventional" and "novel" group in relation to the number of live born children or number of spontaneous abortions; a significant conclusion was the fact that there was no difference between the groups in the number of first and second trimester miscarriages. This was explained at the level of basic medicine, embryology, histology, as well as immunology. It is well-known that placentation takes place in two phases. The first phase is between the 8th and 12th week of gestation, while the placentation second phase is between the 16th and 18th week of gestation. This leads to the conclusion that the loss of pregnancy, whether in the first or second trimester, basically occurs due to inadequate placentation and that the moment when a woman would lose pregnancy does not depend on individual or other pregnancy characteristics, as well as individual response and woman's organism and placentation disturbance. The authors have concluded that the administration of LMWH contributed to the success of

pregnancy management. Out of 50 cases, there were 48 deliveries and 2 spontaneous abortions (30). An impressive conclusion is the fact that different clinical complications of thrombophilia may be observed through different categories of disease. A unique position, confirmed by basic medicine, is that in conditions of thrombophilia trophoblast invasion disturbances are created during angiogenesis at the level of placental blood vessels. In the process of embryology, independently of thrombophilia, an intensive change occurs at the level of trophoblast cells, new blood vessels, together with the incidence of trophoblastapoptosis and coagulation in the intervillous space. Each type of thrombophilia is characterized by these disturbances. However, even though each subtype of thrombophilia has identical system of origin, the degree of different level in the process may be explained by the fact that other form of thrombophilia is attached to the existing thrombophilia, thus influencing one target base, by vector system, they basically express a stronger effect.

Another conclusion is related to the incidence of intrauterine fetal death as well as fetal growth restriction. Thus, the administration of LMWH in conditions with conventional thrombophilia reduced only the incidence of intrauterine fetal death, while in the group with novel thrombophilia both the incidence of IUGR and intrauterine fetal death was reduced, but also the number of premature deliveries. Regarding the incidence of preeclampsia, the number of patients with incidence of preeclampsia was reduced by administration of LMWH with statistical significance, especially in the conventional group. This study has proven that the administration of LMWH in the group with novel thrombophilia has decreased the incidence of IUGR, intrauterine fetal death, as well as preeclampsia. Not one of the patients had sudden fetal death, out of 50 cases in both conventional and novel groups. In the group of patients who did not receive LMWH, there were 5 cases of sudden intrauterine fetal death in the group with conventional thrombophilia while there were 9 cases of intrauterine fetal death in the novel thrombophilia group without LMWH therapy. The lack of this study is the fact that there were no patients that were continuously followed and they were not given therapy, since it would be unethical. Also, the analysis was performed only on congenital, inherited thrombophilia and not the acquired thrombophilia. The significance of LMWH administration was emphasized in both groups.

Studies about separate and combined antiaggregative and anticoagulative therapy

The significance of LMWH administration, as well as unfractionated heparin, was mentioned for the first time in the Langer studies in 1980 (27). The studies were performed on different bases and methodologies. The incidence of early pregnancy loss and late pregnancy loss was analyzed. Also, maternal complications during pregnancy were observed.

The fact that trophoblast cell differentiation, analyzed in vitro, is obviously promoted by heparin administration, has been shown in 2004. The study also observed the difference in the effects of unfractionated heparin and LMWH. By administration of LMWH, the effect of antiphospholipid antibodies is decreased. During in vitro studies, it has been proven that the bonding of antiphospholipid antibodies on trophoblast cells (28).

Aspirin, as the thrombocytes aggregation inhibitor, may reduce the coagulation activity within placenta. Since coagulation is a part of immune set of phenomena and activation of complement system, it is considered that the effect of aspirin should be primarily interpreted through immune prism of complement activity. In relation to preventing thrombocytes aggregation, the aspirin has a role by acting on preventing the activity of complements leading to increased coagulation (29). Clinical studies on the effect of antithrombotic therapy to pregnancy losses have been analyzed in relation to antiphospholipid syndrome (by administration of aspirin or heparin), as well as conditions of thrombophilia by use of different therapy. Regarding the loss of one fetus and the analysis of antiphospholipid syndrome, there are no valid and available data. In relation to multiple fetal losses, namely the incidence of recurrent or habitual miscarriages, studies have been done and were summarized in Cochran review in 2005 (30). The efficiency of heparin administration together with aspirin for these conditions has been confirmed by Ziakas study (31).

Inefficiency of aspirin alone administration has been favored in several studies. Some studies, done on limited number of patients, showed that aspirin had no effect in preventing spontaneous abortion. The analysis has been done in three separate studies and a total number of 71 patients were analyzed. The conclusion was that aspirin had no benefits, however comparing to the group without any treatment but had fetal loss. Pregnancy loss relative risk was 1.05 with 95% CI. The results of all three studies have been summarized (32).

Regarding the efficiency of administration of heparin alone, the studies - which were later accepted as quasi random because of the fact that most patients were subjected to heparin administration without the use of aspirin – conclude that the administration of heparin was much more efficient and contributed to better pregnancy outcomes. The examined cases were patients with antiphospholipid syndrome and history of at least two spontaneous abortions before the 20th week of

gestation. The patients were given LMWH (Bemiparin in the dose of 2,500 IU/day). 80 cases were analyzed. The study conducted in 2012 by Alalaf et al emphasizes higher effects of LMWH administration. Statistics given in the conclusion states RR for healthy live children in women who were given LMWH to be 1.20 (95% CI 1.00-1.43). It is interesting that the authors emphasize that all the patients were given the dose of 2,500 IU without mentioning BMI of the patients. Also the fact of inadequate randomization limits the contribution of these conclusions (33).

As for the combined administration of heparin and aspirin, the performed meta-analysis (34) concluded that in conditions of antiphospholipid syndrome there was higher impact on positive pregnancy outcomes if the administered therapy combined aspirin and LMWH. In 103 patients who received a combined therapy, with previous two or more miscarriages, there was a significant reduction in first trimester abortions, with high statistical significance, in relation to the results given by Ziakas in 2010 where only aspirin was administered. Ziakas study analyzed 109 patients (RR 0.26, 95% CI 0.14-0.48) (31).

In relation to the pregnancy loss analysis, there was no statistical significance in sample processing in combined LMWH and aspirin treatment comparing to the group with aspirin alone. Two groups and 96 women with combined therapy were observed, and compared with a group of 90 cases with aspirin alone therapy. Summarized pregnancy loss RR was 0.70 and without statistical significance (95% CI, 0.34-1.45) (31).

The same result is obtained in the analysis of any type of heparin (unfractionated or LMWH) comparing to the use of aspirin alone. In fact, the benefits of heparin administration in relation to first trimester fetal loss and aspirin alone therapy is undisputable, however without statistical significance (RR 0.39, 95% CI 0.24-0.65)(31).

The effects of aspirin alone administration as well as combination of aspirin and heparin were analyzed. However, if the cause of habitual miscarriage is unknown, none of the studies explicitly suggests the use of antithrombotic therapy. All the analyzed studies, even though they prove the positive effects of the therapy, conclude that it is necessary to continue further investigations.

CONCLUSION

We have a confirmation by basic medicine that in conditions of thrombophilia disturbances of trophoblast invasion is primarily created during angiogenesis.

That leads us to the conclusion that the loss of pregnancy, whether in the first or second trimester, basically occurs due to inadequate placentation, in cases when a woman would lose pregnancy does not depend on other more dominant individual or other reproductive characteristics.

Considering the fact that authors of all studies insiston further investigations, we have to agree that these findings make way for further, more detailed studies about this condition.

DECLARATION OF INTEREST

The autors declare that there are no conflicts of interests.

Sažetak

TERAPIJSKE MOGUĆNOSTI PREVENCIJE NASTANKA HIPOKSEMIJE UTEROPLACENTARNE JEDINICE IZAZVANE TROMBOFILIJOM

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Spontani pobačaj je primarno definisan kao nenamerni gubitak trudnoće pre 20. nedelje gestacije. I teorija i praksa pokazuju da gubitak trudnoće do 10. nedelje gestacije može, pored mnogih drugih ginekoloških i mikrobioloških faktora, da se svede na postojanje neke stečene trombofilije kao što je antifosfolipidni sindrom. Brojne studije su sprovedene da se ispita efikasnost primene terapije kako bi se sprečili negativnii ishodi trudnoće. Urođene trombofilije, više se povezuju sa kasnijim gubicima trudnoće.

Ključne reči: trudnoća; trombofilija; LMWH;terapija, ishodi.

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Treba otkucati listu svih skraćenica upotrebljenih u tekstu. Lista mora biti uređena po abecednom redu pri čemu svaku skraćenicu sledi objašnjenje. Uopšte, skraćenice treba izbegavati, ako nisu neophodne.

U donjem desnom uglu naslovne strane treba otkucati ime i prezime, telefonski broj, broj faksa i tačnu adresu autora sa kojim ce se obavljati korespodencija.

Stranica sa sažetkom. Sažetak mora imati do 350 reči. Treba koncizno da iskaže cilj, rezultate i zaključak rada koji je opisan u rukopisu. Sažetak ne može sadržati skraćenice, fusnote i reference.

Ključne reči. Ispod sažetka treba navesti 3 do 8 ključnih reči koje su potrebne za indeksiranje rada. U

izboru ključnih reči koristiti Medical Subject Headings — MeSH.

Stranica sa sažetkom na engleskom jeziku. Treba da sadrži pun naslov rada na engleskom jeziku, kratak naslov rada na engleskom jeziku, naziv institucije gde je rad urađen na engleskom jeziku, tekst sažetka na engleskom jeziku i ključne reči na engleskom jeziku.

Struktura rada. Svi podnaslovi se pišu velikim slovima i boldovano.

Originalni rad treba da ima sledeće podnaslove: uvod, cilj rada, metod rada, rezultati, diskusija, zaključak, literatura.

Prikaz bolesnika čine: uvod, prikaz bolesnika, diskusija, literatura.

Pregled iz literature čine: uvod, odgovarajući podnaslovi, zaključak, literatura.

Bolesnici i metode/materijal i metode. Treba opisati izbor bolesnika ili eksperimentalnih životinja, uključujući kontrolu. Imena bolesnika i brojeve istorija ne treba koristiti.

Metode rada treba opisati sa dovoljno detalja kako bi drugi istraživači mogli proceniti i ponoviti rad.

Kada se piše o eksperimentima na ljudima, treba priložiti pismenu izjavu u kojoj se tvrdi da su eksperimenti obavljeni u skladu sa moralnim standardima Komiteta za eksperimente na ljudima institucije u kojoj su autori radili, kao i prema uslovima Helsinške deklaracije. Rizične procedure ili hemikalije koje su upotrebljene se moraju opisati do detalja, uključujući sve mere predostrožnosti. Takođe, ako je rađeno na životinjama, treba priložiti izjavu da se sa njima postupalo u skladu sa prihvaćenim standardima.

Treba navesti statističke metode koje su korišćene u obradi rezultata.

Rezultati. Rezultati treba da budu jasni i sažeti, sa minimalnim brojem tabela i slika neophodnih za dobru prezentaciju.

Diskusija. Ne treba činiti obiman pregled literature. Treba diskutovati glavne rezultate u vezi sa rezultatima objavljenim u drugim radovima. Pokušati da se objasne razlike između dobijenih rezultata i rezultata drugih autora. Hipoteze i spekulativne zaključke treba jasno izdvojiti. Diskusija ne treba da bude ponovo iznošenje zaključaka.

Literatura. Reference numerisati rednim arapskim brojevima prema redosledu navođenja u tekstu. Broj referenci ne bi trebalo da bude veći od 30, osim u pregledu literature, u kojem je dozvoljeno da ih bude do 50.

Izbegavati korišćenje apstrakta kao reference, a apstrakte starije od dve godine ne citirati.

Reference se citiraju prema tzv. Vankuverskim pravilima, koja su zasnovana na formatima koja koriste *National Library of Medicine* i *Index Medicus*.

Primeri:

1. **Članak:** (svi autori se navode ako ih je šest i manje, ako ih je više navode se samo prvih šest i dodaje se *"et al."*)

Spates ST, Mellette JR, Fitzpatrick J. Metastatic basal cell carcinoma. J Dermatol Surg. 2003; 29(2): 650–652.

2. **Knjiga:**

Sherlock S. Disease of the liver and biliary system. 8th ed. Oxford: Blackwell Sc Publ, 1989.

3. **Poglavlje ili članak u knjizi:**

Latković Z. Tumori očnih kapaka. U: Litričin O i sar. Tumori oka. 1. izd. Beograd: Zavod za udžbenike i nastavna sredstva, 1998: 18–23.

Tabele. Tabele se označavaju arapskim brojevima po redosledu navođenja u tekstu, sa nazivom tabele iznad.

Slike. Sve ilustracije (fotografije, grafici, crteži) se smatraju slikama i označavaju se arapskim brojevima u tekstu i na legendama, prema redosledu pojavljivanja. Treba koristiti minimalni broj slika koje su zaista neophodne za razumevanje rada. Slova, brojevi i simboli moraju biti jasni, proporcionalni, i dovoljno veliki da se mogu reprodukovati. Pri izboru veličine grafika treba voditi računa da prilikom njihovog smanjivanja na širinu jednog stupca teksta neće doći do gubitka čitljivosti. Legende za slike se moraju dati na posebnim listovima, nikako na samoj slici.

Ako je uveličanje značajno (fotomikrografije) ono treba da bude naznačeno kalibracionom linijom na samoj slici. Dužina kalibracione linije se unosi u legendu slike.

Uz fotografije na kojima se bolesnici mogu prepoznati treba poslati pismenu saglasnost bolesnika da se one objave.

Za slike koje su ranije već objavljivane treba navesti tačan izvor, treba se zahvaliti autoru, i treba priložiti pismeni pristanak nosioca izdavačkog prava da se slike ponovo objave.

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The manuscript should be arranged as following: title page, abstract, key words, introduction, patients and methods/material and methods, results, discussion, conclusion, references, tables, figure legends and figures.

Each manuscript component (title page, etc.) begins on a separate page. All pages are numbered consecutively beginning with the title page.

References in the text are designated with Arabic numerals in parentheses, and the order in which they appear in the text.

Manuscript volume. The complete manuscript, which includes title page, short abstract, text of the article, literature, all figures and permisions for them and legends (tables, images, graphs, diagrams, drawings), title page and abstract in English, can have the length up to 5000 words for original paper, report, paper on the history of medicine and literature overview, while for patient presentation, practice paper, educative article it can be up to 3000 words, and other papers can be up to 1500 words.

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A short title of less than 50 spaces, for use as a running head, is included.

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A list of abbreviations used in the paper, if any, is included. List abbreviations alphabetically followed by an explanation of what they stand for. In general, the use of abbreviations is discouraged unless they are essential for improving the readabillity of the text.

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References. References are identified in the text by Arabic numerals in parentheses. They are numbered consecutively in the order in which they appear in the text. Number of references should not exceed 30, except in the literature review, which is allowed to be to 50.

Avoid using abstracts as references and abstract older than two years are not cited.

References are cited by the so-called Vancouver rules, which are based on formats that use the National Library of Medicine and Index Medicus. The following are examples:

1. **Article:** (all authors are listed if there are six or fewer, otherwise only the first six are listed followed by *"et al."*)

Spates ST, Mellette JR, Fitzpatrick J. Metastatic basal cell carcinoma. J Dermatol Surg. 2003; 29(2): 650–652.

2. **Book:**

Sherlock S. Disease of the liver and biliary system. 8th ed. Oxford: Blackwell Sc Publ, 1989.

3. **Chapter or article in a book:**

Trier JJ. Celiac sprue. In: Sleisenger MH, Fordtran J5, eds. Gastro-intestinal disease. 4 th ed. Philadelphia: WB Saunders Co, 1989: 1134–52.

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SANAMED / glavni i odgovorni urednik Avdo Ćeranić. — God. 1, br. 1 (2006)– . — Novi Pazar : Udruženje lekara Sanamed, 2006– (Novi Pazar : ProGraphico). — 30 cm

Tri puta godišnje. — Drugo izdanje na drugom medijumu: Sanamed (Online) = ISSN 2217-8171

ISSN 1452-662X = Sanamed

COBISS.SR-ID 135154444

ISSN 1452-662X