Clinical and Experimental Health Sciences

Contents

ORIGINAL ARTICLES

Contents

Clinical and Experimental Health Sciences

Management of Mechanical Problems of Totally Implantable Venous Catheters

Halil Kara[1], Akif Enes Arikan[2], Onur Dulgeroglu[1], Cihan Uras[2]

[1] Acibadem Mehmet Ali Aydinlar University, Vocational School of Health Sciences, Istanbul, Turkey.
[2] Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of General Surgery, Istanbul, Turkey.

Correspondence Author: Halil Kara
E-mail: halil.kara@acibadem.edu.tr

Received: 07.02.2020 **Accepted:** 11. 03. 2020

ABSTRACT

Objective: Although the most common complications of totally implantable venous catheters(TIVC) are infection and thrombosis, mechanical complications can also affect the treatment and cause catheter removal. This study aimed to investigate mechanical complications of TIVC and prevention methods.

Methods: Data of 983 procedures in 961patients who underwent TIVC implantation between 2010 and 2019 in AcibademMaslak, Bakirkoy, and Atakent Hospitals were retrospectively analyzed for mechanical complications.

Results: Mechanical complications were encountered in 33(3.3%) cases: 12(1.2%) were detachment of TIVC, 8(0.8%) occlusions, 5(0.5%) pneumothorax, 1(0.1%) hemothorax, 1(0.1%) malposition, 1(0.1%) extravasation, 2(0.2%) TIVC rotation, 3(0.3%) skin necrosis and extrusions.

Conclusion: The catheter tip should be placed in distal superior vena cava, reservoir pocket must be sufficient in size, reservoir should be fixed to pectoral muscle or fascia at least two points with nonabsorbable sutures. Subcutaneous fatty tissue resection from reservoir pocket should be performed in obese patients. The nature of the withdrawn blood form Seldinger needle should be checked visually whether venous or not. Risk of pneumothorax and detachment can be reduced by inserting the catheter from 1/3 outer part of the clavicle during percutaneous technique. While complication rate can be reduced by peroperative fluoroscopy use, control X-ray should be taken in symptomatic patients, not routinely. Malposition can be seen in the peroperative period and can usually be corrected by good manipulation. Percutaneous transcatheter retrieval in addition to surgery is the gold standard treatment for detachment of TIVC. The most important factors in preventing complications are surgical experience and good care.

Keywords: Totally implantable venous catheter, complication, detachment, malfunction, malposition.

1. INTRODUCTION

Totally implantable venous catheters (TIVC), which have become a part of oncologic therapies today, are being used especially for the administration of chemotherapy agents (1-3). They are more tolerable in daily life as there is no external unit like peripheral central catheters (2-7).

Although the most common complications are infection and thrombosis, mechanical complications such as catheter malposition, pneumothorax, catheter detachment, malfunction can also affect the treatment of the patient and cause catheter removal (7).

This study aimed to investigate the mechanical complications of TIVC and prevention methods.

This is a single-institution retrospective cohort study of oncologic patients who had TIVCs implanted by the same surgeon.

2. METHODS

Data of patients who underwent TIVC implantation between 2010 and 2019 in AcibademMaslak, Bakirkoy, and Atakent Hospitals were retrospectively analyzed. Non-oncologic indications were excluded.

TIVC implantations were performed in operating room under general anesthesia through subclavian vein(SV). In cases where the percutaneous technique failed, TIVCswereimplanted with open technique.

Catheter was inserted into the SVby Seldinger technique from 1/3 outer part of the clavicle with the catheter tip directed to suprasternal notch and a sufficient pocket for reservoir was prepared two cm caudally. Under guidance of fluoroscopic examination, length of the catheter was adjusted so that tip of the catheter remained in superior vena cava(SVC) near right atrium(RA). Catheter is attached to reservoir and secured with lock mechanism.Reservoir was fixed on pectoral

DOI: 10.33808/clinexphealthsci.685346

muscle fascia with 2/0 polypropylene sutures.After testing for catheter integrity, the system was filled with heparinized solution. Control chest X-ray was not performed if there were no complaints such asdyspnea, cough, arrhythmia or malfunction in postoperative period.

In open technique, cephalic vein was released from the incision made ondeltopectoral sulcus. Following the cut-down, the catheter was inserted into the vein and length of the catheter was adjusted similar as in percutaneous technique. The catheter was fixed to the vein by silk ligation. From the same incision, pocket for the reservoir was prepared and same procedures were applied as in percutaneous technique.

General TIVC care was performed every 40 days by experienced medical staff in chemotherapy units.

This study was approved by Ethical Review Board of Acibadem Mehmet Aydinlar University on 09 January 2020 with number of 2020/01.

3. RESULTS

Data of 983 procedures in 961 patients who underwent TIVC implantation were evaluated retrospectively. At the time of implantation, the median age was 48.6±12.3 years, body mass index (BMI) was 26.04±4.79 kg/m^2. Primary malignancy was breast in 789, gastrointestinal in 139, and miscellaneous in 33 cases.

Total complication rate was 6% (n=60). Venous thrombosis (n=12,1.2%) and detachment of TIVC (n=12,1.2%) were the most common complications. Mechanical complications were encountered in 33 (3.3%) cases: 12 (1.2%) were detachment of TIVC, 8(0.8%) occlusions, 5(0.5%) pneumothorax, 1 (0.1%) hemothorax, 1 (0.1%) late malposition, 1 (0.1%) extravasation, 2 (0.2%) TIVC rotation, 3 (0.3%) skin necrosis and port extrusions.

In patients with detachment of TIVC, reservoir and associated catheter was excised surgically, while embolizedfragment was removed percutaneously. Only in one patient, the catheter which was migrated to hearth wall could not be removed. The patient was followed-up with anticoagulant therapy and no complication was detected during 11 months' follow-up. TIVC explantation was required in patients with occlusion, extravasation, malposition and skin necrosis. Surgical correction was performed for port rotation. Thorax tube drainage was applied to three of five patients with symptomatic pneumothorax and to the patient with hemothorax. There was no mortality due to mechanical complications at mean 34±74 months follow-up period.

4. DISCUSSION

TIVC improves quality of life and has lower infection risk than other catheter types, however, mechanical complications still can be encountered (1-3, 5-7). Complications rate decreases as surgical experience increases (8). However, they still increase hospitalizationduration, treatment cost, and morbidity and may cause delay in treatment (9).

Tip of catheter

There is no clear consensus on where the catheter tip should be; distal SVC, RA or atriocaval junction (6, 7, 10-12). The important thing is that the catheter tip should be in a high flow vein not to constantly contact vessel wall or not be in heart leading to arrhythmia. Placing the catheter in small caliber vessels increases endothelial damage, risk of thrombosis, vascular stenosis, and perforation (11). While Mudan et al (6) placed mid-atrial, considering that there is less risk of thrombosis, Machat et al (7) and Zhang et al (12) placed at distal SVC. During implantations, the patient should be well monitored forarrhythmias. In our clinic, the catheter tip is placed in SVC near RA, and no complications associated with its location were detected.

Post-implantation X-ray

In many clinics, peroperative fluoroscopy is used to control the position of catheter, but post-implantation X-ray control can be performed in centers without fluoroscopy (13). Kim et al. (4) reported that surgical experience, asepsis, and fluoroscopy use were effective in reducing surgical complications. X-ray control after fluoroscopy-guided implantation is controversial. Some centers advocate X-ray necessity due to pneumothorax risk (6, 7). In minimal pneumothorax, patient can be followed without thorax drainage, however, drainage should be considered in patients with respiratory complaints. Chest X-ray should be performed selectively according to patient's complaints, not routinely. Mudan et al. (6) routinely performed X-rays. Considering the pneumothorax rate of 1.2% (n=12) in their series, unnecessary X-ray was taken in 978 patients. Similarly, Velioğluet al. (14) routinely performed X-ray at postoperative period, however, considering pneumothorax rate of 0.8%, X-ray was unnecessarily taken in 2038 patients. However, in another study, routine X-ray use was found not cost-effective due to low complication risk (7). In our clinic, routine X-raywas not used if patient was asymptomatic.

Subcutaneous thickness

Two problems may arise related with patient's BMI and local subcutaneous thickness in the implantation area. The first problem is cosmetic dissatisfaction especially in patients with low subcutaneous tissue or in petite patients. In TIVC with smaller reservoir, catheter diameter also decreases, which is not preferreddue to increased occlusion risk. Therefore, it is necessary to inform patient before implantation for cosmetic results.

Contrary, in patients with high BMI or subcutaneous tissue thicker than 2 cm, reservoir cannot be felt or reservoir remains too deep to place port access needle sufficiently. Because the reservoir is fixed to pectoral muscle fascia, tissue thickness over it gains importance. For this reason, subcutaneous tissue should be excised if needed. Fosh et al. (8) recommended to place port in subcutaneous pocket, rather than suturing to pectoral fascia and various authors suggested to prepare reservoir pocket superficially in fatty tissue in obese patients, however reservoir rotation risk increases, so we do not recommend (3, 6).

Arterial puncture

Arterial puncture during implantation has been reported up to 11% (7, 14, 15). It can be distinguished by observing withdrawn blood from Seldinger needle by the color and pulsation of the blood. Generally, arterial puncture alone does not cause any complication, however, if dilatator or catheter is inserted complications such as hemothorax, arterial dissection, embolism, neck hematoma,pseudoaneurysm, a-v fistula, and thrombosis can be seen (7). Clinically unrecognized arterial puncture can be detected by fluoroscopy or subsequent chest X-ray (7). Risk of arterial puncturing can be reduced with USG guidance (7). Although USG is recommended for this purpose, we believe that USG is not crucial in experienced centers because of no arterial catheterization and associated complications seen in our clinic.

Pneumothorax and Hemothorax

These are the most feared and fatal complications. Pneumothorax is not expected with appropriate open surgical technique, but it has been reported in 1.5-6% with percutaneous technique (7). Zerati et al.(3) reported thatpneumothorax rate was 0.1% and USG is not necessary to reduce pneumothorax risk. In contrast, Mudan et al. (6) detected pneumothorax as 1.2% under USG guidance and recommended routine use. In this study, rate of pneumothorax was 0.5% (n=5) and hemothorax was 0.1% (n=1), so we conclude that USG guidance is not crucial in experienced centers. BMI of three patients with symptomaticpneumothorax was 19.92, 19.48 and 19.13kg/m^2 and BMI of the patient with hemothorax was 16.8kg/m^2. Although statistical analysis cannot be performed due to low patient number, we think that more care should be taken in patients with low BMI, considering the mean BMI was 26.04±4.79kg/m^2 in this series.

Thorax drainage may not be necessary in all patients with pneumothorax. Li Ma et al. (10) observed pneumothorax in 9 (0.3%) patients and drained two of them. Velioğlu et al. (14) detected pneumothorax in 16 (0.8%) patients and 14 of these underwent thorax tube drainage. In this study, drainage was performed in three of five patients who had respiratory symptoms. Postoperative chest X-ray is recommended in case of suspicion. In some centers, chest X-ray is recommended routinely, but in our center, we recommend only if the patient has symptoms.

Skin erosion and extrusion

It has been reported in literature at 0.7-5% (4, 13, 15). Incision site tension, repeated needle puncture, extravasation of chemotherapeutics may lead to skin erosion especially in lean patient (16). The reservoir pocket must be sufficient in size in order to reduce incision site tension (4). In cachectic patients, TIVC with a smaller reservoir can be used to reduce tension. Kim et al. (4) found 6 (0.7%) erosions; TIVC was removed in two patients, debridement, irrigation and resuture in others. In this study, three patients had erosion, and two developed extrusions and TIVC removal was required in all patients. In series of Yanık et al. (15), decubitus developed in 121(3%) patients, even though reservoirs were placed behind pectoral muscle in patients with very thin subcutaneous tissue.

Malfunction

General definition is inability to infuse fluids and/or aspiration of blood (14, 17). Considering that the primary purpose of TIVC is infusion,not aspiration, we believe that definition of malfunction should be only inability of infusion. It may be encountered in cases where catheter tipwas not be placed in correct position, kinking of catheter especially at entrance to vein, clot formation in tip of catheter, andport rotation (3). As catheter enters vein at right angles, it may increase risk of complications as it will reduce infusion flow rate and require more forceful injection. Fibrin sheath occurs around catheter within the first 24 hours after implantation (11). This fibrin sheath is usually fragmented but may cause occlusion if complete.In situations such as prolonged infusion time, injection of saline cannot be done, arm swelling, neck pain, and inability to puncture reservoir or extravasation, mechanical complications with fluoroscopic examination have been reported in 4.3% (7). In cases while fluids can be infused but blood cannot be withdrawn, once the catheter has been checked with X-ray and deep venous thrombosis has been excluded, catheters can still be used.

By minimizing use of catheter in procedures (i.e. blood drawing) other than infusion of chemotherapeutics, risk of catheter occlusion may be reduced (14). In cases of catheter occlusion with clot, fibrinolytic agent can be a choice.

In open technique, the catheter should not be angled to prevent malfunction. Additionally, extremely tight knotting should be avoided during ligation and fixation of catheter to vein.

Port rotation

Port rotation isa rare complication where reservoir is reversed and cannot be punctured with a needle (10, 15). Diagnosis can be made by physical examination or by X-ray. Yanık et al. (15) reported 4 (0.12%) patients and Li Ma et al. (10) reported 3 (0.10%) patients with port rotation. Treatment is revision of the reservoir. To prevent this complication, reservoir should be fixed to pectoral muscle or fascia at least from two points with nonabsorbable sutures. Port rotation was detected in 2 patients during years 2016-2017 when absorbable sutures were used as fixation sutures in this series. Diagnosis was made by physical examination and corrected by simple surgical intervention

Malposition

When upper extremity veins were used, orientation of catheter tip to a vessel except SVC or RA is called malposition (14). Risk of malposition has been reported 0.1-4.5% (1, 2, 10, 13, 15). Matiotti-Neto et al. (2) found no difference between open and percutaneous techniques. Malpositions

usually occur at time of implantation, but may develop later in cases of increased intra-thoracic pressure (11).

Malposition of catheter inserted into the SV may occur in internal jugular vein (IJV) or vice versa, or in veins such as azygos/hemiazygos vein, internal mammary vein, or ipsilateral/contralateral SV (7, 11) (Fig. 1, 2). It can also form coil within vein or migrate into the subintimalzone (11). Such malpositions can be easily detected during fluoroscopy or postoperative chest X-ray. In case of suspicion, it is necessary to take two-way radiographies or evaluate with computed tomography (11).

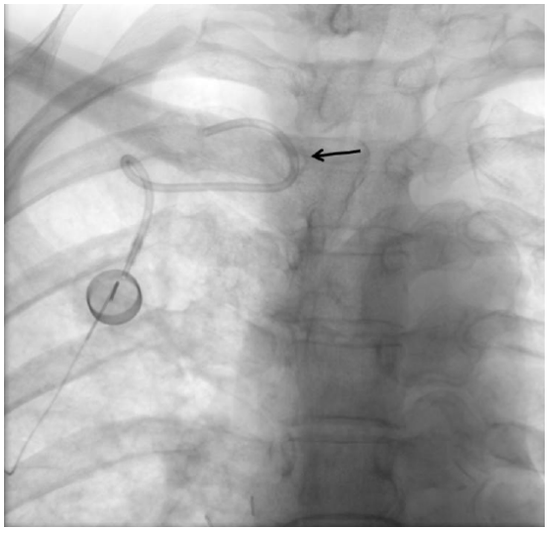


Figure 1. *Malpositionof catheter to ipsilateral subclavian veinis indicated by black arrow*

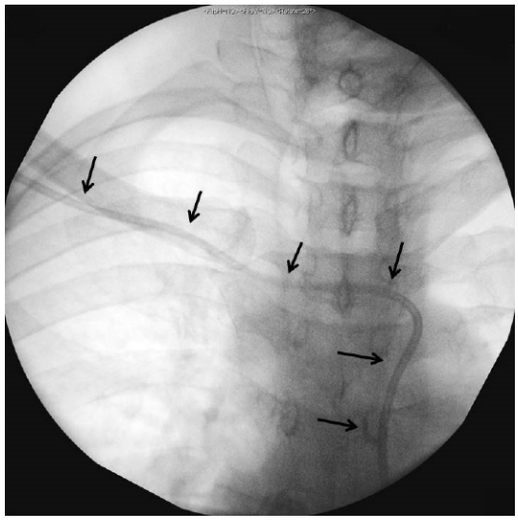


Figure 2. *Malposition of catheter to hemiazygos vein. The catheter is indicated by black arrows*

Venous thrombosis, erosion and perforation of the vessel wall, and catheter malfunction may be encountered due to malpositions.Various treatment methods can be tried (11). Catheter malposition can be corrected by forceful saline injection in small caliber catheters. If malposition is detected during implantation, inserting the guide wire into catheter and correcting malposition is an option.

Malposition of guide wire or catheter to contralateral SV during implantation is a condition we occasionally encounter (Fig. 3, 4). Correction of malpositions and orientation of catheter to SVC can be difficult. In such cases, we recommend to push forward the catheter rather than retracting and redirecting. Excess portion of catheter will be looped into the SVC,subsequently, when catheter is slowly retracted, tip will be placed in distal SVC by weight of the loop-shaped segment.

Malposition to IJVis rare in our clinic. To prevent this, patient is positioned to reverse Trendelenburg position while catheter is advanced.

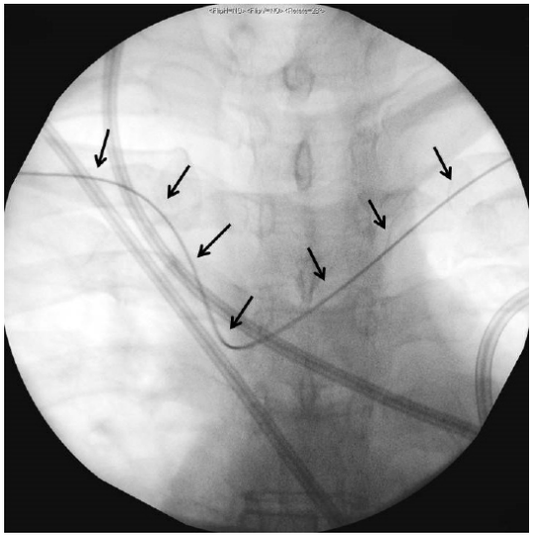


Figure 3. *Malposition of guide wire to contralateral subclavian vein is indicated by black arrows*

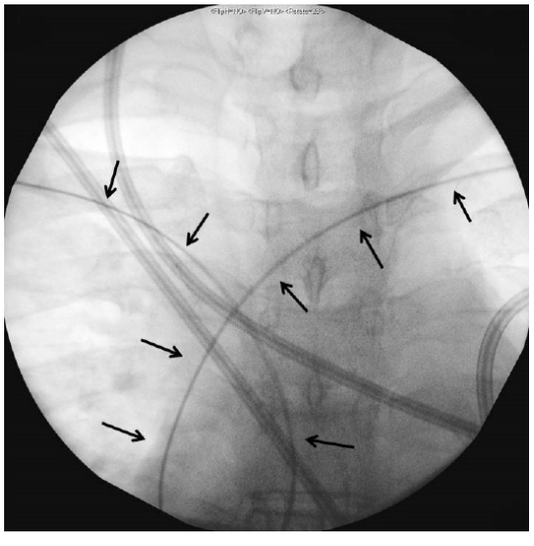


Figure 4. *Loop formation during correction of the malposition to contralateral subclavian vein. Black arrows indicate the looped guide wire*

Malposition into heart during implantation may be seen. Therefore, arrhythmias up to 41% have been reported in the literature (7). Catheter length should be well adjusted, should not be advanced too much and site should be checked by fluoroscopy during implantation. The presence of catheter on left mediastinum or aortic arch should suggest intraarterial malposition (11). Extravascular malposition should be suspected in cases where the catheter does not follow the expected venous trace, and care should be taken in this respect during follow-up, especially in post-traumatic radiographs (11).

The diagnosis of late malposition can be made on follow-up radiographs. Although routine chest radiography is not recommended as late malposition is not common, X-rays taken in the follow-up of primary disease should be looked at in this respect or should be taken if in doubt. Leaving the catheter tip short at the SVC may cause late malposition. We think that if catheter tip is left distal SVC or atrial, late malposition will not occur due to length and weight of catheter.Late malposition was detected in one patients and TIVC was excised because of catheter dysfunction (Figure 1).

Detachment

It is a rare complication reported requiring treatment due to potential complications (7, 18). In this series, we detected in 12 (1.2%) patients (Figure 5). It is usually seen after percutaneous SV catheterization, but it can also be seen when jugular vein is used or after open technique (19-22). Improper connection of catheter to reservoir, problems due to lock mechanism, incorrect manipulation, material fatigue, forced flushing, aggressive neck/extremity movements, trauma, orincreased intra-thoracic pressuremay lead todetachment of catheter. Pinch-off syndrome (POS) is the case where catheter is trapped and detached between 1st rib and clavicle (5, 9, 11, 23, 24).Surov et al. (21) reported POS (40.9%) as the most common cause of detachment, there was no etiologic factor in 19.1% of cases. In contrast, Chang et al.(24) reported fracture at the connection between reservoir and catheter (77 of 92 patient-84%) as the most common cause. It may be due to technical or production error (25). POS (66%) was the most common cause in this series.

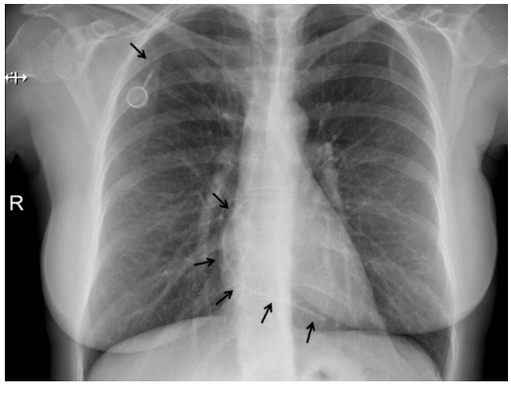


Figure 5. *Detachment and embolization of catheter to heart. The point of detachment and embolized catheter fragment is indicated by black arrows*

Most of the patients were asymptomatic and diagnosis was made by routine chest X-ray (3, 5). However, catheter malfunction, arrhythmia, pulmonary symptoms, septic syndromes, severe pain, inability to draw blood, extravasation, edema around the catheter and reservoir, and pain can also be seen (9, 21, 24, 26).

To prevent POS, insertion of catheter from lateral 1/3 of the clavicle is recommended (9, 23, 27). Two-stage treatment therapy is recommended (23). Percutaneous transcatheter retrieval of embolized fragment in addition to surgical removal of reservoir and attached catheter is the gold standard (5, 9, 21, 23, 24, 26, 28). If percutaneous removal is impossible, it can be removed by thoracotomy or followed by anticoagulant treatment for a long period (5, 21, 24, 29).

Experience and TIVC care

Experience of healthcare personnel is important in preventing complications. TIVC implantation following guidelines and with good care, complication rate can be reduced (6). Ertel et al. (30) reported that rate of complications was related to clinician implanting TIVC more than technique.Fosh et al. (8) draw attention to learning curve and report that complication rate is reduced with surgical experience. Particular care should be taken during port access needle entry toavoid accidental damage to catheter. Additionally, attention should be paid to infusion pressure range of implanted catheter.

It is recommended to wait for healing ofincision before use of TIVC, and flush catheter with heparinized solution after each use or at most 4-6 weeks (1, 5). However, Zhang et al. (12) stated that port can be used on implanted day. In our clinic, TIVCs are used by experienced nurses in the next day following implantation and we did not detect any complications associated with early use.

5. CONCLUSION

Diagnosis and treatment of mechanical complications of TIVCs are important to prevent delay in the treatment of the patient.The catheter tip should be placed in SVC near RA, the reservoir pocket must be sufficient in size, reservoir should be fixed to pectoral muscle or fascia at least from two points with nonabsorbable sutures. Subcutaneous fatty tissue resection should be performed in obese patients. The nature of the withdrawn blood form Seldinger needle should be checked visually whether venous or not. Risk of pneumothorax and detachment can be reduced by inserting the catheter from 1/3 outer part of the clavicle in percutaneous technique. While complication rate can be reduced by peroperative fluoroscopy use, control X-ray should be taken in symptomatic patients, not routinely. Malposition can be seen in the peroperative period and can usually be corrected by good manipulation. Percutaneous transcatheter retrieval in addition to surgery is the gold standard treatment for detachment of TIVC. The most important factors in preventing complications are surgical experience and good care.

Funding: No funding was received.

Conflicts of Interest: There is no conflict of interest regarding the publication of this article.

Informed consent: Informed consent was not needed due to being a retrospective study.

REFERENCES

[1] Kock HJ, Pietsch M, Krause U, Wilke H, Eigler FW. Implantable vascular access systems: experience in 1500 patients with totally implanted central venous port systems. World J Surg 1998; 22: 12-16.

[2] Matiotti-Neto M, Eskander MF, Tabatabaie O, Kasumova G, Bliss LA, Ng SC, Tawa NE, Jr., Murphy B, Critchlow JF, Tseng JF. Percutaneous versus Cut-Down Technique for Indwelling Port Placement. Am Surg 2017; 83: 1336-1342.

[3] Zerati AE, Wolosker N, de Luccia N, Puech-Leao P. Totally implantable venous catheters: history, implanttaion technique and complications. J Vasc Bras 2017; 16: 128-139.

[4] Kim DH, Ryu DY, Jung HJ, Lee SS. Evaluation of complications of totally implantable central venous port system insertion. Exp Ther Med 2019; 17: 2013-2018.

[5] Intagliata E, Basile F, Vecchio R. Totally implantable catheter migration and its percutaneous retrieval: case report and review of the literature. G Chir 2017; 37: 211-215.

[6] Mudan S, Giakoustidis A, Morrison D, Iosifidou S, Raobaikady R, Neofytou K, Stebbing J. 1000 Port-A-Cath (R) placements by subclavian vein approach: single surgeon experience. World J Surg 2015; 39: 328-334.

[7] Machat S, Eisenhuber E, Pfarl G, Stubler J, Koelblinger C, Zacherl J, Schima W. Complications of central venous port systems: a pictorial review. Insights Imaging 2019; 10: 86-97.

[8] Fosh B, Canepa M, Eaton M. Long-term venous access insertion: 'the learning curve'. ANZ J Surg 2016; 86: 1038-1041.

[9] Ilhan BM, Sormaz IC, Turkay R. Pinch-Off Syndrome, a Rare Complication of Totally Implantable Venous Access Device Implantation: A Case Series and Literature Review. Korean J Thorac Cardiovasc Surg 2018; 51: 333-337.

[10] Ma LI, Liu Y, Wang J, Chang Y, Yu L, Geng C. Totally implantable venous access port systems and associated complications: A single-institution retrospective analysis of 2,996 breast cancer patients. Mol Clin Oncol 2016; 4: 456-460.

[11] Kidney DD, Nguyen DT, Deutsch LS. Radiologic evaluation and management of malfunctioning long-term central vein catheters. AJR Am J Roentgenol 1998; 171: 1251-1257.

[12] Zhang P, Du J, Fan C, Mo X, Dong J, Fan Z, Zhao Q. Utility of totally implantable venous access ports in patients with breast cancer. Breast J 2019; 26: 333-334.

[13] An H, Ryu CG, Jung EJ, Kang HJ, Paik JH, Yang JH, Hwang DY. Insertion of totally implantable central venous access devices by surgeons. Ann Coloproctol 2015; 31: 63-67.

[14] Velioğlu Y, Yüksel A, Sınmaz E. Complications and management strategies of totally implantable venous access port insertion through percutaneous subclavian vein. Turk Gogus Kalp Damar Cerrahisi Derg 2019; 27: 499-507.

[15] Yanik F, Karamustafaoglu Y, Karataş A, Yoruk Y. Experience in totally implantable venous port catheter: Analysis of 3,000 patients in 12 years. Turk Gogus Kalp Damar Cerrahisi Derg 2018; 26: 422-428.

[16] Zerati AE, Figueredo TR, de Moraes RD, da Cruz AM, da Motta-Leal Filho JM, Freire MP, Wolosker N, de Luccia N. Risk factors for infectious and noninfectious complications of totally implantable venous catheters in cancer patients. J Vasc Surg Venous Lymphat Disord 2016; 4: 200-205.

[17] Goossens GA, Stas M, Jerome M, Moons P. Systematic review: malfunction of totally implantable venous access devices in cancer patients. Support Care Cancer 2011; 19: 883-898.

[18] Turner DD, Sommers SC. Accidental passage of a polyethylene catheter from cubital vein to right atrium; report of a fatal case. N Engl J Med 1954; 251: 744-745.

[19] Pignataro BS, Nishinari K, Wolosker N, Bomfim GA. Fracture and migration into the coronary sinus of a totally implantable catheter introduced via the right internal jugular vein. BMJ Case Rep 2014; 2014:1-3.

[20] Mirza B, Vanek VW, Kupensky DT. Pinch-off syndrome: case report and collective review of the literature. Am Surg 2004; 70: 635-644.

[21] Surov A, Wienke A, Carter JM, Stoevesandt D, Behrmann C, Spielmann RP, Werdan K, Buerke M. Intravascular embolization of venous catheter—causes, clinical signs, and management: a systematic review. JPEN J Parenter Enteral Nutr 2009; 33: 677-685.

[22] Saijo F, Mutoh M, Tokumine J, Yoshinobu O, Hama H, Namima T, Shibahara M, Tokumura H. Late fracture of Groshong ports: A report of the three cases. J Vasc Access 2019; 20: 563-566.

[23] Motta Leal Filho JM, Carnevale FC, Nasser F, Santos AC, Sousa Junior Wde O, Zurstrassen CE, Affonso BB, Moreira AM. Endovascular techniques and procedures, methods for removal of intravascular foreign bodies. Rev Bras Cir Cardiovasc 2010; 25: 202-208.

[24] Cheng CC, Tsai TN, Yang CC, Han CL. Percutaneous retrieval of dislodged totally implantable central venous access system in 92 cases: experience in a single hospital. Eur J Radiol 2009; 69: 346-350.

[25] da Motta Leal Filho JM, Carnevale FC, Cerri GG. Subclavian vein an unusual access for the removal of intravascular foreign bodies. Ann Vasc Surg 2010; 24: 826 e821-824.

[26] Gowda MR, Gowda RM, Khan IA, Punukollu G, Chand SP, Bixon R, Reede DL. Positional ventricular tachycardia from a fractured mediport catheter with right ventricular migration—a case report. Angiology 2004; 55: 557-560.

[27] Aitken DR, Minton JP. The "pinch-off sign": a warning of impending problems with permanent subclavian catheters. Am J Surg 1984; 148: 633-636.

[28] Carroll MI, Ahanchi SS, Kim JH, Panneton JM. Endovascular foreign body retrieval. J Vasc Surg 2013; 57: 459-463.

[29] Tazzioli G, Gargaglia E, Vecchioni I, Papi S, Di Blasio P, Rossi R. Retained embolized fragment of totally implantable central venous catheter in right ventricle: it is really necessary to remove? J Vasc Access 2015; 16: 431-433.

[30] Ertel AE, McHenry ZD, Venkatesan VK, Hanseman DJ, Wima K, Hoehn RS, Shah SA, Abbott DE. Surgeon, not technique, defines outcomes after central venous port insertion. J Surg Res 2017; 209: 220-226.

How to cite this article: Kara H, Arikan AE, Dulgeroglu O, Uras C. Management of Mechanical Problems of Totally Implantable Venous Catheters. Clin Exp Health Sci 2020; 10: 81-86. DOI: 10.33808/clinexphealthsci.685346

Clinical and Experimental Health Sciences

Effect of a Short Nutritional Training Program on Nutrition Literacy and Food Habits in Adolescents

Indrani Kalkan, Fatma Feyzan Aydin

Istanbul Aydin University, Nutrition and Dietetics Department, Istanbul, Turkey.

Correspondence Author: Indrani Kalkan
E-mail: indranikalkan@aydin.edu.tr

Received: 26.11.2019 **Accepted:** 20.03.2020

ABSTRACT

Objective: Balanced nutrition is of special importance in adolescence however, eating behaviors may be negatively influenced during this stage due to several factors. The objective of this research was to investigate the effect of a nutrition training program on nutrition literacy level and food habits of a group of adolescents.

Methods: Adolescent Nutritional Literacy Scale (ANLS) and Adolescent Food Habits Checklist (AFHC) was used to determine the nutrition literacy level and food habits of 200 highschool students in Istanbul between September 2018 and April 2019. Effect of a face to face training, for 8 hrs over 4 weeks on nutrition related topics, were investigated on nutrition literacy level and food habits of the students.

Results: Mean age of the students was 15 ± 0,66 years and 57% comprised of females. ANLS and AFHC scores in general, were higher in females, however the difference was statistically significant ($p < 0,05$) only in pre-test scores. The average adolescent nutrition literacy (ANLS) score in males were 3,31 out of 5,0 and remained unchanged after training. In females however, it significantly decreased from 3,56 to 3,44 ($p < 0,05$). Out of 19, AFHC scores decreased significantly from 9,80 to 8,94 in males and 10,03 to 9,34 in females ($p < 0,05$) following training.

Conclusion: A short nutritional training was not sufficiently effective in reflecting the positive change in nutrition literacy level and food habits among adolescents. Possible reasons should be investigated in depth and more comprehensive and longer training should be provided to increase nutritional awareness.

Keywords: Adolescent, nutrition, nutrition literacy, food habits.

1. INTRODUCTION

Adolescence is described as a transitory period between childhood and adulthood during which growth and development are the fastest in an individual leading to an increasing need for energy, protein, vitamins and minerals in the diet (1). Studies conducted with adolescents indicate that although individuals are aware of the importance of adequate and balanced nutrition in health and prevention of diseases; however, they fail to apply the knowledge in their everyday life (2). Peer influences, unhealthy eating habits as skipping of meals, increased consumption of fast food and sugar added beverages, snacking as well as lack of physical activity lead to obesity and other health risk factors in this age group (3). Psychosomatic disorders as stress, depression, social anxiety disorders, faulty body image perception, self esteem etc. also lead to eating disorders and inadequate nourishment during this age (4,5).

In this context, there is a need for nutritional interventional programs to raise the awareness of youth enabling them to increase nutritional knowledge level and adopt healthy eating and lifestyle. The term nutrition literacy has gained popularity over the last decade and is defined as the degree to which people can process, and understand basic nutrition information (2).

Nutrition literacy was found to have a positive effect on food habits in a group of young Turkish adults and based on the findings the author suggested administration of nutritional training programs in order to raise nutrition awareness in adolescents (3). However, the content and length of the training program that would be adequate to bring about the desired effect is an issue that must be researched thoroughly.

The objective of this research was to study the nutrition literacy level and food habits of a group of high school students, as well as to investigate the effect of a short nutrition training program on both.

DOI: 10.33808/clinexphealthsci.651265

2. METHODS

2.1. Participants and Setting

The prospective cross sectional study was conducted on all students (n=200) who had voluntarily wished to participate in the study and were studying in grade 10 and 11 of a high school (Fahrettin Kerim Gökay Anatolian High School) in Istanbul randomly chosen in a neighbourhood having families of mixed economic and social status. The study was conducted between September 2018 and April 2019.

2.2. Instruments

A questionnaire with 18 general questions regarding demographic characteristics was directed to the participants followed by the validated Adolescent Nutrition Literacy Scale (ANLS) tool developed by Bari, 2012 (6) and validated in Turkey by Türkmen et al, 2017 (7).

The validated ANLS scale comprised of 22 questions listed under three sub-groups namely Functional Nutrition Literacy (FNL), Interactive Nutrition Literacy (INL) and Critical Nutrition Literacy (CNL) (2). FNL indicated the individual's knowledge and follow up of literature in the field of nutrition. INL indicated the individual's application of nutrition knowledge in practical life and CNL reflected his/her tendency to participate and take an active role in nutrition related topics. Every item was scored between 1–5 on a "5 point likert type scale" (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree) (6,7). A mean score out of 3.0 or more out 5.0 could be considered to be above average nutrition literacy level among the participants (7). Finally, Adolescent Food Habits Checklist (AFHC) developed by Johnson et al. 2002 (8) and validated in the Turkish language by Arikan et al. 2012, comprising of 19 items was aimed at measuring healthy eating behavior of adolescents was administered on the participants. A positive / negative (Yes/No) response format was selected to make the checklist easier to complete. Participants were granted one point for each 'healthy' response. The maximum score to be attained by the Turkish version of AFHC instrument was determined to be 19. Attaining a high score demonstrated healthy eating habits on the part of the individual (9). The questionnaires were filled by the students in the lecture hall in presence of the research team. The tools were applied twice, one prior to the training and afterwards, to study the effect of the training program on ANLS and AFHC scores.

2.3. Nutritional Training Program

After the three questionnaires were conducted, a short nutritional face to face training comprising of 8 lecture hours (2 hours each week for 4 weeks) was provided to the students. The lectures were given by the research team members, supported by visuals, videos, printed materials and interactive discussion. The nutritional training material comprised of topics as nutritional terminology, concept of nutrition literacy, healthy eating, diet related chronic diseases, food safety, healthy food selection, reading of food labels etc. The training material also included subjects as preparation of an adequate and balanced menu by choosing the right foods, nutrients, and food sources, normal body weight, body mass index etc. Some of the measurement techniques as height and weight as well as calculation of body mass index were applied in class. After 3 months, the questionnaires were repeated on the same participants to evaluate the effects of the training.

2.4. Ethical Aspects of the Study

Permission was sought from Istanbul Provincial Directorate for National Education and ethical approval was taken from Istanbul Aydin University Ethical Committee dated February 2018 prior to the study. All participants and families were informed about the research work and their consent was taken.

2.5. Statistical Analyses

Descriptive statistics (frequency, mean, standard deviation) were used to analyse the data. Differences between two independent groups (female and male) were analysed by Independent Sample T Test. Differences between two dependent numerical variables (for example, interactive nutrition literacy scores before and after education) were analyzed using the Dependent Sample T Test. The relationships between two independent numerical variables (ANLS and AFHC scores) were evaluated by Pearson Correlation Coefficient. Statistical significance was accepted as $p < 0.05$. Statistical analyses were carried out using the SPSS version 23.0 Statistical Package Program.

3. RESULTS

Number of students included in the study was 200 of which 57% were females. The demographic characteristics of the participants have been summarized in Table 1.

On enquiring about frequency of skipping meals among participants, 60,5% consumed three major meals, 29,5 % consumed 2 major meals in a day. Regarding snacking habits, 50,5% of the students skipped snacks, 34% stated that they snacked twice in a day, 15,5 % snacked more than twice in a day. Among the preferred beverages during snacks. The most popular was tea (50,5%) followed by butter milk (43,5%) and milk (39%). Most preferred snack item was chocolate bars (65%) followed by cookies (57,5%) and salty crackers (49%).

The amount of water/beverage consumption in day by the students has been summarized in Table 2. As per results, only 42% consumed 2 L or more of water in a day whereas, 47,5% consumed up to 200 ml and 9,5% consumed up to 500 ml of sugar added beverages in a day.

Table 1. *Demographic Characteristics of the participants (n=200)*

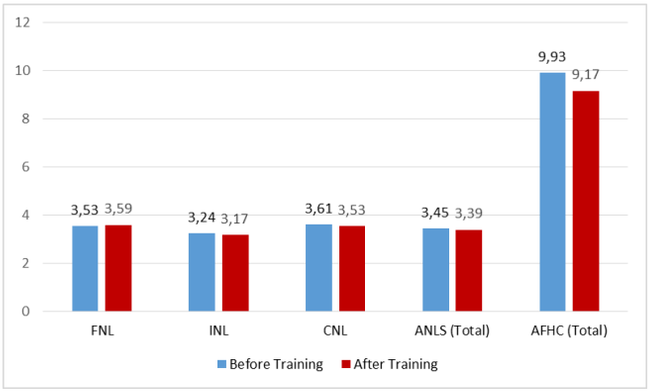
Gender	Frequency	Percent (%)
Female	114	57,0
Male	86	43,0
Age		
15 Years	107	53,5
16 Years	74	37,0
17 Years	18	9,0
Body Weight		
≤ 50 kg	35	17,5
51-60 kg	82	41,0
≥ 61 kg	83	41,5
Height		
≤ 165 cm	77	38,5
166-175 cm	75	37,5
≥ 176 cm	48	24,0
Monthly Allowance for Food		
≤ 100 TL	70	35,0
101-200 TL	74	37,0
≥ 201 TL	56	28,0
Performing Sports on a Regular Basis		
Yes	88	44,0
No	112	56,0

Table 2. *Consumption of Water/Beverages on a Daily Basis (n=200)*

Daily Consumption of Water	Frequency	Percent (%)
400 ml or less	5	2,5
401-1000 ml	57	28,5
1001-1500 ml	35	17,5
1501-2000 ml	19	9,5
2000 ml or more	84	42,0
Daily Consumption of Tea/Coffee		
Does not Consume	26	13,0
0-200 ml	116	58,0
201-500ml	49	24,5
501-1000 ml	9	4,5
Daily Consumption of Sugar Added Beverages		
Does not Consume	77	38,5
0-200 ml	95	47,5
201-500 ml	19	9,5
501-1000	8	4,0
1001 ml or more	1	0,5

FNL mean scores of students increased from 3,53 to 3,59 after training. However, INL mean score decreased from 3,24 to 3,17. However, these changes were not statistically significant ($p>0,05$). On the other hand, decrease in CNL score from 3,61 to 3,53 after training was found to be statistically significant ($p<0,05$). Also, general ANLS means score decreased from 3,45 to 3,39 after training. Similarly, AFHC mean score also decreased from 9,93 pre-training to 9,17 post-training. Decrease in these values were also found to be statistically significant ($p<0,05$). Effects of training on Mean ANLS Subgroups, and AFHC scores have been illustrated in Figure 1.

Figure 1. *Effects of Training on Mean ANLS Subgroup and AFHC Scores*



FNL: Functional Nutrition Literacy, INL: Interactive Nutrition Literacy, CNL: Critical Nutrition Literacy, ANLS: Adolescent Nutrition Literacy Scale, AFHC: Adolescent Food Habits Checklist

Effect of training was analysed for all items based on gender results of which have been summarized in Table 3. In general, ANLS scores and subgroup scores were higher in females as compared to males. However, ANLS pre-training and CNL pre and post training scores were found to be statistically higher in females (Table: 3).

Contrary to what was expected, effect of training exhibited negative results. Apart from FNL, other subgroups of ANLS tool and AFHC scores fell after the training and this decrease was found to be statistically significant as shown in Table 3.

Table 3. *Effects of Training on ANLS Subgroups and AFHC Scores Based on Gender(n=200)*

	Female (n=114)	Male (n=86)		
	Mean S.D.	Mean S.D.	t	p^a
FNL (BT)	3,57 0,516	3,47 0,735	1,047	0,297
FNL (AT)	3,60 0,643	3,57 0,829	0,301	0,764
	t=-0,585 p^b=0,559	**t=-1,017 p^b=0,312**		
INL (BT)	3,33 0,651	3,13 0,863	1,746	0,083
INL (AT)	3,18 0,666	3,16 0,844	0,247	0,805
	t=2,300 p^b=0,023*	**t=-0,277 p^b=0,782**		
CNL (BT)	3,80 0,694	3,36 1,095	3,250	0,001*
CNL (AT)	3,71 0,533	3,30 0,748	4,380	0,000*
	t=2,677 p^b=0,009*	**t=1,117 p^b=0,267**		
ANLS (BT)	3,56 0,421	3,31 0,552	3,692	0,000*
ANLS (AT)	3,44 0,4999	3,31 0,554	1,767	0,079
	t=3,085 p^b=0,003*	**t=-0,050 p^b=0,960**		
AFHC (BT)	10,03 3,571	9,80 3,467	0,445	0,657
AFHC (AT)	9,34 3,788	8,94 4,533	0,663	0,508
	t=2,365 p^b=0,020*	**t=2,127 p^b=0,036***		

p^a = Independent variable (between gender)
p^b = Dependent variable (within gender)
p<0,05 (statistically significant) BT= Before Training AT=After Training

FNL: Functional Nutrition Literacy INL: Interactive Nutrition Literacy CNL: Critical Nutrition Literacy BT: Before Training AT: After Trainig

Pearson Correlation test was conducted to study the correlation between ANLS (including sub-groups) and AFHC tool. As per findings, there is a significant positive correlation between ANLS (including sub-groups) pre-test and post-test scores with AFHC pre-test and post-test scores (p<0,05). The degree of correlation was weak in some cases and moderate in others. The results have been summarized in Table 4.

Table 4. *Correlation between ANLS and AFHC Tools (n=200)*

ANLS Sub-Groups		AFHC Pre-Test	AFHC Post-Test
FNL Pre-Test	r	,234**	,261**
	p	0,001	0,000
INL Pre-Test	r	,374**	,299**
	p	0,000	0,000
CNL Pre-Test	r	,347**	,244**
	p	0,000	0,001
ANLS Pre-Test	r	,437**	,340**
	p	0,000	0,000
FNL Post-Test	r	,248**	,321**
	p	0,000	0,000
INL Post-Test	r	,317**	,416**
	p	0,000	0,000
CNL Post-Test	r	,345**	,205**
	p	0,000	0,004
ANLS Post-Test	r	,446**	,507**
	p	0,000	0,000

*r: Pearson Korelasyon *:p<0,05 **:p<0,01*

r	*Degree of Correlation*	*Direction of Correlation*
0,00	*No Correation*	*r= – negative*
0,01 – 0,29	*Weak Correlation*	*r= + positive*
0,30 – 0,69	*Medium Correlation*	
0,70 – 0,99	*Strong Correlation*	
1,00	*Perfect Correlation*	

FNL: Functional Nutrition Literacy, INL: Interactive Nutrition Literacy, CNL: Critical Nutrition Literacy

4. DISCUSSION

This study evaluated the effect of a short nutritional training program on Adolescent Nutritional Literacy (ANLS) and Food Habits (AFHC) of a group of high school students.

In this study, 56% of the students stated that they could not spare time for regular sports or physical activity on a daily basis. Aksoydan et al. in their study with adolescents reported that only 15,7% of adolescents performed sports for 4 hours or more in a week, and the main reason suggested was the long hours spent by them in front of electronic devices such as smart phones and computers (10). According to the WHO

report, 77,1% of young Turkish men and 86,9% of young women maintained an inactive lifestyle (11). Regular physical activity has been suggested to increase bone density, lean tissue mass and rate of basal metabolism whereas decrease body fat and prevent the occurrence of chronic diseases such as obesity, hypertension, diabetes, coronary heart disease (12).

In this study, 0,5% of adolescents stated that they consumed three meals whereas 29,5% consumed two meals during the day. Meal skipping behavior is common during adolescence. In a study conducted on 411 university students in which nutritional habits, physical activity levels and body mass indexes of adolescents were evaluated, 59,5% of students consumed three meals a day, but 20,9% consumed two meals. It has been suggested that as the number of meals decreases, the availability of nitrogen in the body decreases. There is an increase in glucose absorption, glycogen synthesis and fat storage leading to metabolic disorders. On the other hand, increasing meal frequency in a day without increasing the amount of food consumption resulted in a significant decrease in blood LDL cholesterol (13).

The most popular beverage among students was found to be tea followed by the buttermilk. Consumed by 50,5% and most popular snack item was chocolate bars followed by cookies and salty crackers. Snacking item preference by students in this study was very similar to the study performed by Kalkan et al in Konya on 643 high school students (14). Students' desire to consume such snack items frequently is due to their easy accessibility in school canteens, markets, and advertising of such foods on screens and media. Similar eating habits and preference of snacking items and beverages have also been reported in other studies performed on Turkish adolescents (10,13,15).

As a result of the Dependent Sample T Test performed before and after education, it was observed that all three sub-groups of ANLS had different and variable results. Firstly, FNL (basic reading and writing skills necessary to follow and understand simple nutrition information) increased at the end of education because students could read and comprehend texts easily. On the other hand, INL (advanced nutritional literacy involving cognitive and interpersonal communication skills necessary to manage nutritional issues in collaboration with professionals) and CNL (ability to critically analyze nutritional information, the ability to overcome barriers and act in order to raise awareness) decreased after the training. This could be explained by the fact that students may not have followed advanced part of the training within the short period and may have responded to the questionnaires without comprehending and influenced by peers. Another reason may be that the compact nutritional information presented in a short time was unable to create the expected impact, the presentation techniques may not have been very emphatic and finally the timing of the post-test coinciding with the Final exams may have resulted in students responding to the questionnaires in a careless and haphazard manner. Moreover, nutritional habits in individuals develop over time reflecting culture and family traditions and does not change easily. Nevertheless, repeated trainings and education creating awareness in the society is bound to bring positive changes in the long run.

In this study, Adolescent Food Habits (AFHC) scores were found to decrease at the end of nutrition training. The short compact training failed to have an impact on the food habits of the participants. Studies performed on food habits of adolescents indicate that the young age groups are inadequately nourished and have an unbalanced diet (14,16,17). In a study evaluating Nutritional Habits Index (NHI) of adolescents, nearly all participants were found to be at risk in terms of nutritional habits and 64,5% were in the medium and 22,5% in the high-risk group (18).

In this study, in general females had a higher nutrition literacy level and better food habits scores as compared to males. The results were similar to the study performed by Matsumoto et al on 1165 Japanese adults between 18-24 years of age (19) and some other studies analysing the relationship between nutrition knowledge and genders (20, 21). The author also suggested that eating behaviors of individuals were affected by nutritional literacy (3). In the prevention of obesity-related chronic diseases, awareness of young adults about healthy nutrition will be of great importance. Adolescents are influenced by family, friends, peers, media, advertisements etc in food selection. Fast foods are preferred as they are cheap, easily accessible and savory due to high salt, sugar and fat content. Attention must be drawn towards food process owners who have added responsibility in the production and sale of reliable food. Apart from parents and family members, teachers must also supervise the nutrition habits of children right from the elementary school, raising awareness by organizing training for various age groups, stressing on the consequences of unhealthy nutrition. it is recommended to draw people's attention by creating public spots and often publishing them in news sources (22).

5. CONCLUSION

Although nutrition literacy issues have been addressed in several studies in Turkey, yet such a project as experimented in this study on a group of adolescents (investigating the effect of a training on nutritional awareness and food habits) has not been conducted in this region of the country. Contrary to the expectations, the study implemented here was not effective as per the results. As mentioned above there may have been many reasons not fully understood that may have led to these unexpected findings. Therefore, it is extremely important to consider these issues in the future studies and it is thought that the possible reasons leading to this result should be investigated in depth and a more comprehensive and longer education should be provided in schools to raise awareness of students and adolescents in the field of nutrition and health.

Conflict of Interest: None stated by the authors.

Funding: No funding was received for this study.

REFERENCES

[1] Altay M, Cabar HD, Altay B. Adolesan döneminde beslenme ve okul sağlığı. Sinop Üniversitesi Sosyal Bilimler Dergisi 2018;2(1):173-180.

[2] Ronto R, Ball L, Pendergast D, Harris N. Adolescents' perspectives on food literacy and its impact on their dietary behaviours. Appetite 2016;107:549-557.

[3] Kalkan I. The impact of nutrition literacy on the food habits among young adults in Turkey. Nutr Res Prac 2019;13(4):352-357.

[4] Bell MG, Zeiler M, Herrero R, Kuso S, Nitsch M, Etchemendy E, Baezae S, Olivere E, Adamcik T, Karwautzb A, Wagner G, Banos R, Botella C, Görlich D, Jacobii C, Waldherr K. Healthy teens school: Evaluating and disseminating transdiagnostic preventive interventions for eating disorders and obesity for adolescents in school settings. Internet Interventions 2019;16:65-75.

[5] [5] Arslan M, Aydemir I. Relationship between emotional appetite, eating attitudes, sleep quality and body mass index in healthcare workers: a multı-centre study. Psychiatr Clin Psychopharmacology 2019;29 (3):346-353.

[6] Bari NN. Nutrition Literacy Status Of Adolescent Students in Kampala District, Uganda", Master Thesis. Oslo and Akershus University College of Applied Sciences, Lillestrøm, Norway. 2012.

[7] Türkmen AS, Kalkan I. Filiz E. Adolesan Beslenme Okuryazarlığı Ölçeğinin Türkçe' ye uyarlanması: geçerlilik ve güvenilirlik çalışması. Uluslararası Hakemli Beslenme Araştırmaları Dergisi 2017;10:117-125.

[8] Johnson F, Wardle J, Griffith J. The adolescent food habits checklist: reliability and validity of a measure of healthy eating behaviour in adolescents. Eur J Clin Nutr 2002;56:644-649.

[9] Arikan I, Aksu AE, Metintas S, Kalyoncu C. The adaptation of the adolescent food habits checklist to Turkish young adults. TAF Rev Med Bull 2012;11:49-56.

[10] Aksoydan E, Çakır N. Adölesanların beslenme alışkanlıkları, fiziksel aktivite düzeyleri ve vücut kitle indekslerinin değerlendirilmesi. Gülhane Tıp Dergisi 2011;53:264-270.

[11] [11] WHO Global Health Observatory Data Repository [online database]. Geneva, World Health Organization, 2013 (http://apps.who.int/gho/data/view.main, accessed 21 May 2013)

[12] Şanlıer N. Gençlerde biyokimyasal bulgular, antropometrik ölçümler, vücut bileşimi, beslenme ve fiziksel aktivite durumlarının değerlendirilmesi. GÜ, Gazi Eğitim Fakültesi Dergisi 2005;25(3):47-73

[13] Sağlam F, Yürükçü S. Ankara Üniversitesi Eğitim Bilimleri Fakültesi Yüksekokul öğrencilerinin besin tüketim durumu, beslenme alışkanlıkları ve beslenme bilgi düzeylerinin saptanması. Beslenme ve Diyet Dergisi 1996;25(2):16-23.

[14] [14] Kalkan İ, Türkmen A, Filiz E. Dietary habits of Turkish adolescents in Konya, Turkey. Global Journal on Advances in Pure & Applied Sciences 2015;7:190-196.

[15] Tözün M, Sözmen MK, Babaolu AB. Türkiye'nin batısında bir üniversite'nin sağlık ile ilişkili okullarında beslenme alışkanlıkları ve bunun obezite, fizik aktivite ve yaşam kalitesi ile ilişkisi. Türk Dünyası Uygulama ve Araş Merkezi Halk Sağlığı Derg 2017;2(1):1-16.

[16] Sevil J, Garcia Gonzalez L, Abos A.,Generelo E, Aibar A. Can high schools be an Effective Setting to Promote Healthy Lifestyles? Effects of a Multiple Behavior Change Interventionin Adolescents. J Adolescent Health 2019;64:478-486.

[17] Addressing nutritional disorders in adolescents. Position paper /J Adolescent Health 63, 2018;120-123.

[18] Altundağ S, Ceylan SS, Çetinkaya B, Turan T. Meslek lisesi öğrencilerinin obesite sıklığının ve beslenme alışkanlıklarının incelenmesi. TAF Prev Med Bull 2009;8(1):5-12.

[19] Matsumoto M, Ishige N, Sakamoto A, Saito A, Ikemoto S. Nutrition knowledge related to breakfast skipping among Japanese adults aged 18–64 years: a cross-sectional study. Pub Health Nutr 22(6), 1029–1036.

[20] Hendrie GA, Coveney J, Cox D. Exploring nutrition knowledge and the demographic variation in knowledge levels in an Australian community sample. Pub Health Nutr 2008; 11, 1365–1371.

[21] Parmenter K, Waller J, Wardle J. Demographic variation in nutrition knowledge in England. Health Educ Res 2000; 15, 163–174.

[22] Sinir G, İncedayı B, Çopur ÖU, Kaplan E, Bekaroğlu M. Uludağ Üniversitesi'nde eğitim gören öğrencilerin beslenme alışkanlıklarının araştırılması U. Ü. Ziraat Fakültesi Derg 2014; 28(2):37-47.

How to cite this article: Kalkan I, Aydin FF. Effect of a Short Nutritional Training Program on Nutrition Literacy and Food Habits in Adolescents. Clin Exp Health Sci 2020; 10: 87-92. DOI: 10.33808/clinexphealthsci.651265

Clinical and Experimental Health Sciences

Camellia Sinensis Leaves Hydroalcoholic Extract Improves the Alzheimer's Disease-Like Alterations Induced by Type 2 Diabetes in Rats

Muhammet Emin Cam[1,2,3], Turgut Taskin[4]

[1] Marmara University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey.
[2] Marmara University, Center for Nanotechnology and Biomaterials Research, Istanbul, Turkey.
[3] University College London, Department of Mechanical Engineering, London, UK.
[4] Marmara University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey.

Correspondence Author: Muhammet Emin Cam
E-mail: muhammet.cam@marmara.edu.tr
Received: 06.02.2020 **Accepted:** 21. 03. 2020

ABSTRACT

Objective: Novel investigations have confirmed that hyperglycemia is strictly associated with the development of cognitive impairment and dementia. Sodium-dependent glucose transporter (SGLT) inhibitors, which are oral antidiabetic drugs, are currently being investigated as the medication in Alzheimer's disease (AD). In our study, *Camellia sinensis* (green tea), which inhibits sodium-dependent glucose transporter-1 (SGLT-1), was used in the treatment of type 2 diabetes mellitus (T2DM)-induced AD-like alterations via its antidiabetic effects.

Methods: High-fat diet/streptozotocin-treated rat model was chosen to provide T2DM-induced AD-like alterations. Antidiabetic effects were evaluated with the measurement of blood glucose level (BGL), oral glucose tolerance test (OGTT), and insulin tolerance test (ITT). On the other hand, novel object recognition test (NORT), open field test (OFT), passive avoidance test (PAT), and Morris's water maze (MWM) test were performed to investigate the anti-Alzheimer's effects of *C. Sinensis.*

Results: *C. sinensis* tolerated BGL for a short time but metformin, the first medication prescribed for T2DM, tolerated BGL during the test for 120 min. *C. sinensis* increased the number of square crosses and the frequency of grooming activity in a similar manner to metformin in OFT. *C. sinensis* treatment improved exploratory behavior and memory retention components in NORT. The step-through latency decreased in HFD/STZ-treated rat model but it improved with metformin and *C. sinensis* treatment in PAT. According to the results obtained by the MWM test, *C. sinensis* treatment slightly improved learning.

Conclusion: *C. sinensis* improved short-term memory and increased the locomotor activity in rats according to the results obtained by NORT, OFT, and PA.

Keywords: *Camellia sinensis*, sodium-dependent glucose transporter, diabetes mellitus, Alzheimer's disease, memory

1. INTRODUCTION

Alzheimer's disease (AD), a progressive brain disorder, is the most common form of dementia among older adults. (1) An estimated 6 million Americans of all ages are living with AD in 2019. Out of the total USA population, one in 10 people at the age of 65 and older has AD. This disease is becoming a menace to aging population all over the world and it is expected to has 13–20 million AD cases in the next three decades. (2-4)

According to studies, AD is characterized with the occurrence of loss of synapses, changes in neurotransmitter expression, reduced neutrophil numbers, synaptotoxicity, extracellular amyloid-β (Aβ) plaques and neurofibrillary tangles in the intracellular environment, neuronal death and neural atrophy in the final phase. Beta-amyloid plaques may contribute to cell death by interfering with neuron-to-neuron communication at synapses, while tau tangles block the transport of nutrients and other essential molecules inside neurons. Actually this differances that occurs with damaging and destroying of neuron cells in the brain are thought to began 20 years or more before symptoms arise with small changes that are unnoticeable to the person affected. (3, 5-8)

When the first stage of the onset of Alzheimer's appearance, the brain can initially compensate it, but as the damage to nerve cells continues, the brain can no longer compensate for the changes and AD causes to loss intellectual and social skills of people by affecting their ability such as thinking, speaking, learning, memory, walking, swallowing, resuming daily activities, planning family events or participating in sports. When patients reach the final stages of Alzheimer's disease, they become bed-bound and require 24-hour care. Alzheimer's disease is ultimately fatal.(3, 9)

In some studies AD is found to be associated with multiple risk factors such as aging, psychosocial (e.g., low educational level, lack of social engagement, and poor social

DOI: 10.33808/clinexphealthsci.685280

networking), genetic (family history), genetic mutations (the extra copy of chromosome 21 that characterizes Down syndrome), traumatic brain injury, carrying the e4 form of the apolipoprotein E (APOE) gene, vascular parameters (e.g., obesity, tobacco use, and blood cholesterol) and vascular diseases (e.g., diabetes mellitus, hypertension, and stroke). (5, 8, 10, 11)

Type 2 Diabetes Mellitus (T2DM), characterized by hyperglycemia, insulin resistance, and impaired insulin sensitivity. T2DM accounts for 90–95% of all diabetes by affecting over 300 million people worldwide. T2DM causes various serious complications of heart, eyes, nerves, liver and kidneys and is associated with decrements in cognitive function and changes in brain structure. Normal brain function is further compromised by the decreased ability of the brain to metabolize glucose that is main fuel of brain. (12-14)

In in vitro, in vivo and human clinical studies, after the appearance of T2DM, T2DM has been found to be effective in the initiation and progression of AD with common biological mechanisms directly or as cofactor. These biological mechanisms are insulin resistance, impaired glucose metabolism, β-amyloid formation, oxidative stress and the presence of advanced glycation and products. (15) It is thought that AD occurs when a similar cellular insulin resistance and insulin deficiency process occurs in the brain. (16)

In literature, it has been shown that high-fat diet (HFD) is a common risk factor for T2DM and AD. Therefore, HFD/streptozotocin (STZ)-treated rat models were used to provide T2DM-induced AD-like alterations in some studies and also in our study. (17-19)

AD treatment is divided into pharmacological and non-pharmacological, but both treatments do not slow or stop the damage and destruction of neurons that cause AD symptoms. The pharmacological treatment of rivastigmine, galantamine, donepezil, memantine, donepezil and tacrin combined with memantine treatment is approved by the US Food and Drug Administration (FDA) and this treatment includes vitamins C, D and E, omega-3 fatty acids and ginkgo biloba for use as complementary therapy. Also, some plant extracts are used and found effective in the treatment of AD. (20-23) Non-pharmacological treatment includes computerized memory training, listening to your favorite music and special lighting to reduce sleep disturbances. (11, 24)

Moreover, a sufficient medical treatment targeting AD in T2DM patients has not yet been proved. To use antidiabetic drugs such as metformin in order to treat AD is an alternative treatment strategy and has been given more importance in the last decade. (25) The possible mechanisms of green tea for decreasing blood glucose level are including the inhibition of α-glucosidase activity, intestinal sodium-glucose co-transporter-1 (SGLT-1) and glucose transport-2. In our study, a plant extract prepared by *Camellia sinensis* (green tea) was used in the treatment of T2DM-induced AD-like alterations via its SGLT-1 inhibitor effects. (26, 27, 28)

Camellia sinensis that is a member of the Theaceae family, is the second most consumed beverage in the world, after water. This plant native to China and Southeast Asia. (29) The leaves of *C. sinensis* are dark green, alternate and oval, with serrated edges, and the blossoms are white, fragrant, and appear in clusters or singly. This plant can reach to the heights of 30 feet, but they are usually pruned to 2–5 feet for growing. (30) It has 3 different varieties: Green tea, black tea, and oolong tea. These are made from the same plant but are processed differently, depending on their degree of fermentation. (31)

Chemical components of 70% ethanol extract of *C. sinensis* are polyphenols, caffeine, flavonoids, catechins such as catechin, gallocatechin, epigallocatechin, epicatechin, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate, and catechin gallate. (32)

These chemical compounds show some medicinal effects such as hepatoprotective, cardioprotective, neuroprotective, anticancer, antiobesity, antidiabetic, antibacterial, and antiviral. (33) Furthermore in some studies, *C. sinensis* is thought to be effective in the treatment of AD with its anti-neurodegenerative effects. (31, 34-36)

In this study, we aimed to investigate anti-Alzheimer's effect of *C. sinensis* in T2DM-induced AD model via its antidiabetic effects. Antidiabetic effects were evaluated with the measurement of blood glucose level (BGL), oral glucose tolerance test (OGTT), and insulin tolerance test (ITT). On the other hand, novel object recognition test (NORT), open field test (OFT), passive avoidance test (PAT), and Morris's water maze (MWM) test were performed to investigate the anti-Alzheimer's effects of *C. Sinensis.*

2. MATERIALS AND METHOD

2.1. Materials

Streptozotocin (STZ) and glucose were purchased from Santa Cruz Biotechnology, Inc (Dallas, TX, USA). High-fat diet (HFD) was obtained from MFD Company and it contains as percentage of calories 58% fat. Insulin glarjin (Lantus®) was bought from Sanofi Aventis.

2.2. Plant material and extraction

The methanolic extract of *C. sinensis* leaves were given to the rats in this study. *C. sinensis* was obtained from local market and identified by Assist. Prof. Dr. İsmail Şenkardeş, Marmara University, Faculty of Pharmacy, Pharmaceutical Botany Department. The leaves of *C. sinensis* was extracted using methanol: water (70:30, v/v) solvent by maceration for 7 days. After extraction, the sample was filtered using filter paper, then solvent was evaporated using rotary evaporator. The crude extract was stored in 4 °C to use in the experiment.

2.2. In vivo animal test

In vivo animal test were perfomed with the permission of Marmara University Animal Experiments Local Ethics Committee (permission number: 77.2018.mar). Adult female and male Sprague dawley rats at the age of 3-4 month and weighting 250 to 300 g were obtained from Marmara University Experimental Animal Implementation and Research Center (DEHAMER). The rats were maintained in controlled temperature (20±2 °C), humidity (40-60 %) and 12 h dark/light cycle)-regulated rooms. All experiments were performed at the fixed hours between 09:00-12:00. Water were provided ad libitum to all groups. However, HFD was given to all groups except of control group. All necessary precautions were taken before the experiment and the factors that would adversely affect the parameters were minimized during the study. All rats were kept in their cages for a week to adapt conditions before starting the behavioral tests.

2.2.1. Experimental scheme

There are 4 groups in the animal test and 12 animals in each test group. 1st group is control group (C) and distilled water (5 ml/kg) was given intragastrically to healty rats. 2nd group is Alzheimer's disease group (AD) and T2DM-induced AD model was applied to the rats such as 3rd and 4th groups. However, only distilled water was given to 2nd group but *C. Sinensis* (625 mg/kg) (37) and metformin (400 mg/kg) (13) treatments were given intragastrically for 22 days to the 3rd (AD+CS) and 4th groups (AD+M, positive control group), respectively. Metformin and *C. Sinensis* were given in distilled water (5 ml/kg).

2.2.2. Induction of T2DM-induced AD

Rats were administered a HFD (58% kcal fat) for 8 weeks. Rats fed HFD were injected once at week 4 with a low dose of STZ (40 mg/kg, solved in 0.1 M citrate buffer, pH:4.5) to shorten the time taken for the animal model to be established by inducing partial insulin deficiency. One week after the injection, BGLs were measured using a glucometer (Contour Plus, Bayer Diagnostics) and the rats with BGL over 200 mg/dL were classified as T2DM (13). All treatments were started after STZ injection.

2.3. Determination of body weight and BGL

Body weight (b.w.) and BGL of all groups were measured weekly and blood samples were taken from the tail vein to monitore BGL using a glucometer.

2.4. Oral glucose tolerance test (OGTT)

Glucose tolerance test is a commonly used clinical test to diagnose glucose intolerance and T2DM. After 12 h of fasting, treatments were applied and a single dose of glucose (2g/kg, b.w.) was given by gavage 30 minutes later. Glucose concentrations were monitored in the blood collected from the tail vein at 0, 30, 60, and 120 min following the glucose injection. (14)

2.5. Insulin tolerance test (ITT)

Rats were fasted for 12 h before the test and they were injected with insulin (1 U/kg, i.p.). BGLs from blood samples taken from the tail vein using glucometer at 0, 30, 60, and 120 min following the insulin injection. (38)

2.6. Behavioral tests

2.6.1. Open field test (OFT)

OFT is carried out to evaluate animal locomotor activity and anxiety. (39) The rats were taken inside the apparatus of OFT on the 13th day of the study. OFT apparatus in which rats have previously unknown, was constructed of square based Plexiglas box (50 x 50 cm with 25 cm walls). The ground is divided into twenty-five evenly spaced squares, each 10 x 10 cm and consisted of two parts: the peripheral part (10 cm from each wall in the area) and central part. Each animal was separetly placed in the bottom right corner of the OFT apparatus and evaluated. The behavior of animals was recorded for 10 minutes using a video camera. Some parameters were evaluated such as the time spent in the central area, the number of squares passed, and the number of grooming and rearing of rats by two researchers as double-blind. The number of squares crossed by rats was considered as a measure of locomotor activity. The frequency of rearing was used to indicate discovery behavior. The time spent in the central area and the number of grooming was used to indicate the anxiety behavior of rats. The surface of apparatus were cleaned with 70% alcohol in consecutive trials to eliminate any bias that might have occurred due to odor of the previous rat.(40)

2.6.2. Novel object recognition test (NORT)

NORT is a widely used, relatively simple, and straightforward behavioral test for the examination of various aspects of learning and memory in rats. NORT is on the strength of spontaneous behavior of rodents to explore novelty with a naturally stimulating stimulus. When spontaneous behavior is examined, artificial stimulus, food deprivation, reinforcement and/or prior training are not required. NORT apparatus was constructed of black plexiglass open area (50 x 50 cm with 40 cm walls) in the dim light on day 14 (habituation) and 15 (test) of the study. Rats were habituated to an empty test area for 30 minutes to acclimate to the environment 24 hours before testing. NORT consisted of two three-minute test stage separated by an intertrial interval of one hour. Two identical objects were located in opposite corners for the first stage and these objects are now called familiar (F). During the second stage, one of the objects was replaced with a novel one (N). The objects used in NORT differed by color, texture, and shape. NORT apparatus was cleaned after each rat with 70% alcohol to remove any urine or scent cues.

Object placement was randomized for each test. Exploration of identical and novel objects was defined if rats licked, sniffed or touched the object. Based on the exploration time of each object, the discrimination index [(N-F)/(N+F)] and preferential index [N/(N+F)] was calculated. Exploration time was scored by the treatments blind researcher. (41)

2.6.3. Passive avoidance test (PAT)

PAT is a classic fear-motivated test to examine long and short-term memories in a relational manner. The test requires the animals to act opposite to their natural tendencies for the preference of dark areas and avoidance of bright ones. The test was carried out using an apparatus that comprises two compartments isolated with a retractable lid. One of the compartments is illuminated by a bright light, the other compartment is covered by dark opaque walls. Dark and light chambers are seperated by a guillotine door. The ground in both compartments is made of metal shocking grids. On the dark compartment, the ground is wired for 3 second to receive an electric shock of 0.5 mA.

The acquisition and test stages of PAT were applied on day 16 and 17. On acquisition day, rats were placed in the bright compartment when the guillotine door was closed. At the end of 10-second period, the door was opened. After the rats moved into the dark compartment, the door was closed and electric shock of 0.5 mA was delivered via metal shocking grids. The first passage time of rat to the dark compartment was recorded. On day 17, the test stage was performed 24 h after the acquisition stage and it was similar to the previous stage with an exception that no electric shock was applied. The time spent for moving into the dark compartment was measured. In the test, a cut-off time was determined as 300 seconds if the animal did not enter the dark compartment. The experiment is terminated in this stiuation. Both compartments were cleaned with 70% ethanol after each rat tested.(40)

2.6.4. Morris's water maze (MWM) test

MWM was carried out to evaluate the spatial performance of the rats. MWM is consisting of two stages. MWM training was performed between day 18 and 21 and MWM prob test was performed on day 22. MWM apparatus comprised a round stainless steel tank (160 cm in diameter) with 40 cm walls filled with water (23±1°C). The tank is divided into 4 quarters with 4 fixed points around it and there are different shapes and color 4 cues attached to the opposite of each direction. During all escape platform trials, a platform was submerged 1~1.5 cm under the water surface and this platform is the same color as the rest of the maze (to eliminate any false positives due to vision) in the middle of one of quarters of the area. Each trial commenced with the rats being released in the pool at one of the four main compass positions around the perimeter of the pool according to a pseudo-random sequence and allowed 75 s for finding the platform. If the rat could not find the escape platform within 75 seconds, it was gently directed to the platform and allowed to stay on it for 20 seconds. After a minute rats were placed in water to another direction. Thus, all rats were trained 16 times in 4 days, 4 different directions each day. The direction order was changed every day. Rats were subjected to learning, which was evaluated for reaching the platform. On day 5, 24 hours after the previous training session, the probe test in which the platform was removed from the tank and the rats were allowed to swim freely for 60 seconds was performed. The time to reach the target quadrant in the probe trial and the time spent in the target quadrant in both training and prob trials were recorded. Behaviors in the maze were monitored by a digital camera and the parameters were evaluated by two separate researchers.(42)

2.7. Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). The results of the tests were analyzed with ANOVA followed by Tukey post-hoc test and represented as mean ± S.E.M. P values <0.05 were considered significant. Data analysis was performed using GraphPad Prism 6.5 software (San Diego, USA).

3. RESULTS

3.1. Body weight

There was no significant difference in b.w. between test groups for the first 4 weeks. After the injection of STZ, the difference in b.w. between the groups began to change significantly. However, the b.w. of AD and AD+CS group decreased significantly ($p<0.05$) compared to the control group after 6 weeks. There was no significant difference between C and AD+M groups for 9 weeks.

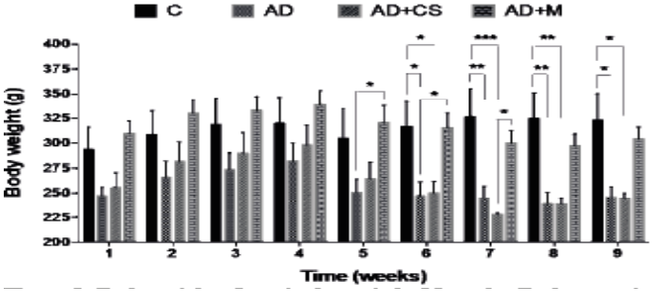


Figure 1. *Body weight of rats in the period of 9 weeks. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. Values are represented statistically when *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

3.2. Blood Glucose Level

The glucose level of the control group was almost 100 mg/dl for the last 5 weeks of the experiment. Metformin treatment started to decrease BGL ($p < 0.05$) before *C. Sinensis* treatment compared to the control group as shown in Figure 2 on week 5. The blood glucose lowering effect of metformin continued until the end of the experiment. *C. Sinensis* treatment started

significantly lowering BGL ($p < 0.01$) compared to the control group on week 8 and similar results were observed also on week 9. Metformin treatment tolerated BGL better than *C. Sinensis* treatment ($p < 0.05$) according to the results shown on weeks 8 and 9.

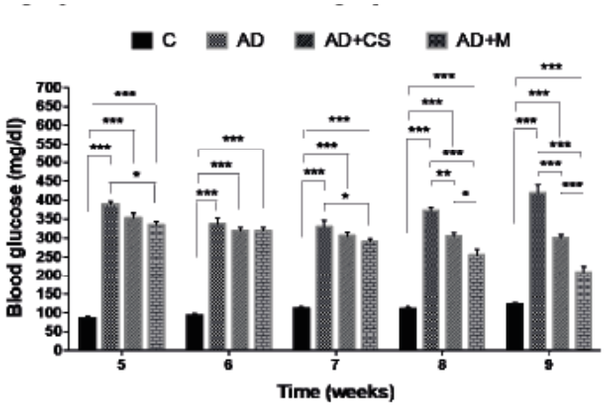


Figure 2. *Blood glucose level of rats after starting the treatment. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. Values are represented statistically when *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

3.3. Oral glucose tolerance test (OGTT)

To determine the effect of *C. Sinensis* on glucose tolerance in rats, OGTT was applied (Figure 3). The slope of the curve of blood glucose was significantly higher in the non-treated rats than treated rats during the first 30 min. The slope of the curve was lower in the AD+M group than the AD+CS group due to the ability of Metformin to better tolerate glucose. As shown in Figure 3, metformin better tolerated blood glucose levels from 30 min to 90 min compared to *C. Sinensis* ($p < 0.01$) and control group ($p < 0.001$). Whereas, *C. Sinensis* treatment demonstrated better glucose tolerance only at 60 min compared to the control group ($p < 0.05$).

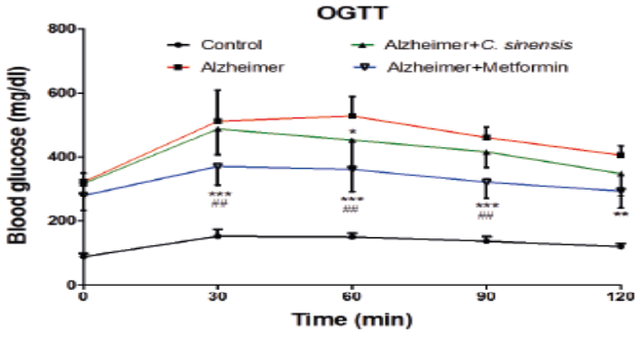


Figure 3. *Oral glucose tolerance test in rats. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 represent statistical differences of metformin and C. Sinensis groups compared to Alzheimer group and #p < 0.05, ##p < 0.01, ###p < 0.001 represent statistical differences of metformin group compared to C. Sinensis group.*

3.4. Insulin tolerance test (ITT)

As shown in Figure 4, an ideal insulin tolerance was observed in the control group. BGL decreased significantly for 60 min in healthy rats in a similar manner to other groups but BGL was well tolerated by the healthy body. Whereas, BGL was not tolerated by the non-treated Alzheimer group and was not well tolerated with the AD+CS group. The slope of the curve belongs to control and AD+CS groups were similar until 90 min and the slope decreased for the AD+CS group after 90 min but there is no significant difference between AD and AD+CS group in 120 min. When we compared the AD+CS group with the AD+M group, it is clearly seen that Metformin tolerated BGL significantly better than *C. Sinensis* according to 120 min ($p < 0.05$).

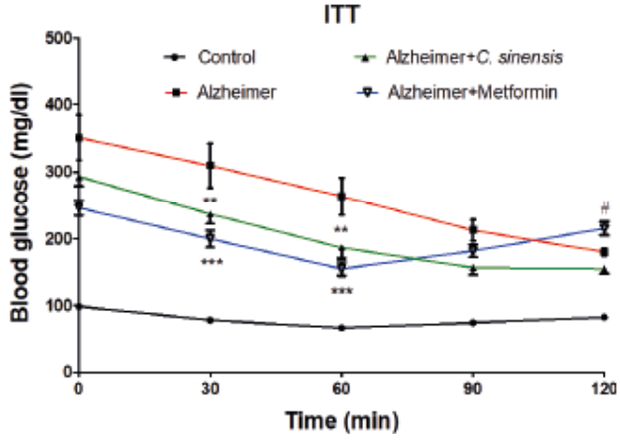


Figure 4. *Insulin tolerance test in rats. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 represent statistical differences of metformin and C. Sinensis groups compared to Alzheimer group and #p < 0.05, ##p < 0.01, ###p < 0.001 represent statistical differences of metformin group compared to C. Sinensis group.*

3.6. Behavioral tests

3.6.1. Open field test

3.6.1.1. Crossing activity

As shown in Figure 5A, the number of square crosses of rats were measured to evaluate locomotor activity. There is no significant difference between treatment groups on squares crossed in OFT. The number of square crosses significantly decreased in the AD group compared to the control group ($p < 0.05$) and increased in the treatment groups compared to the AD group but there is no significant difference.

3.6.1.2. Rearing activity

The number of rearings of rats were measured to evaluate exploratory behavior. The number of rearings significantly decreased in the AD, AD+CS, and AD+M groups compared

to the control group ($p < 0.05$) and there is no significant difference between treatment gorups and the AD group (Figure 5B).

3.6.1.3. Latency to enter center

The latency to enter center was measured in OFT. Latency to enter the center zone of the open-field was not found to be altered significantly between treatment groups. It considerably decreased in AD and AD+CS groups compared to the control group ($p < 0.05$) but it was similar to the control group in the AD+M group (Figure 5C).

3.6.1.4. Grooming activity

The number of grooming of rats was counted as a measure of anxiety (Figure 5D). The number of rearings significantly decreased in the non-treated Alzheimer group compared to the control group ($p < 0.05$) and increased in the treatment groups compared to the AD group but there is no significant difference.

3.6.1.5. Time spent in the central zone

The time spent in the central zone was also evaluated as a measure of anxiety. There is no significant difference between groups (Figure 5E).

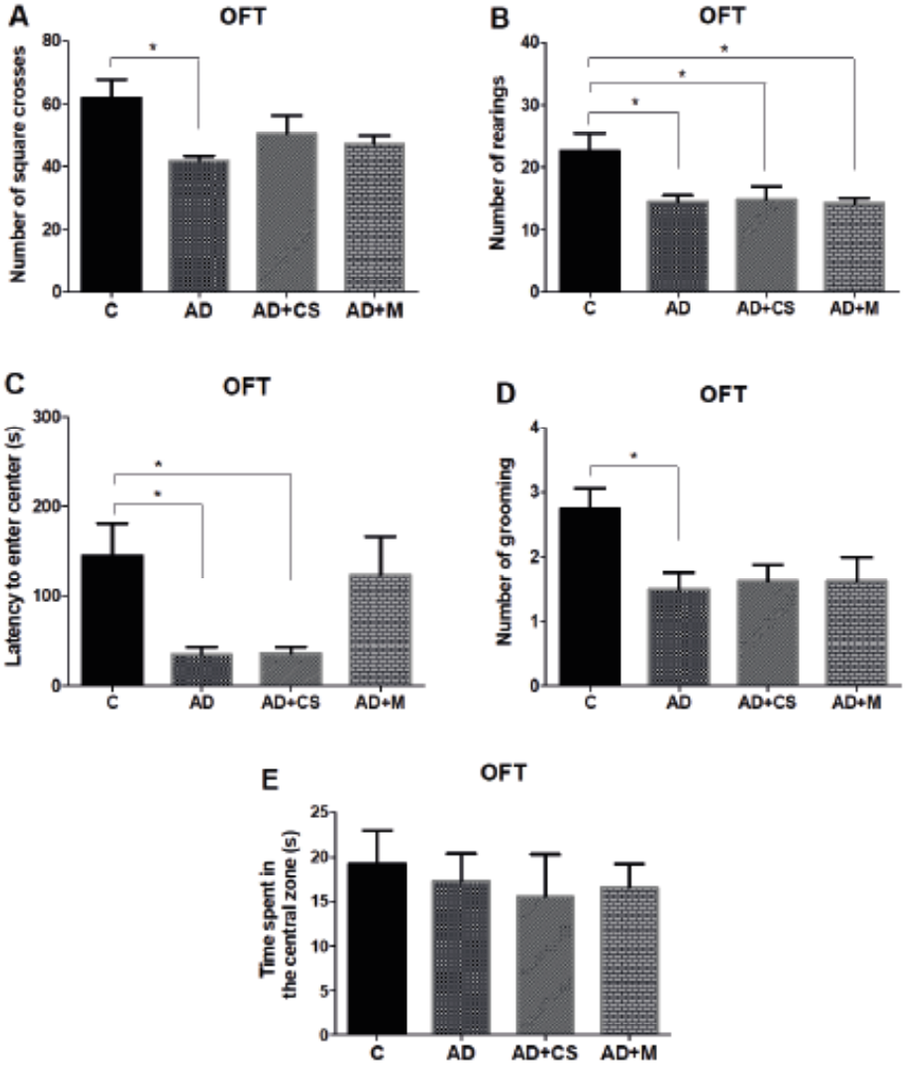


Figure 5. *(A) The numer of square crosses, (B) the number of rearings, (C) latency to enter center, (D) the number of grooming, and (E) time spent in the central zone of rats in OFT. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

3.6.2. Novel object recognition test

NORT was used for evaluating short-term memory. In NORT, the object recognition was performed between the training phase and the test phase at 1-hour intervals. There are significant differences between the exploration time of familiar and novel object in control ($p < 0.001$), AD+CS ($p < 0.05$), and AD+M groups ($p < 0.05$) but there is no significant difference in AD group (Figure 6).

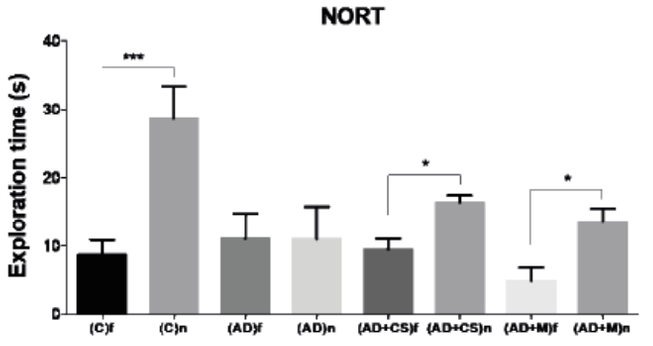


Figure 6. *Exploration time of rats in NORT. Each group (n = 12) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. ((C)f: Control group with familiar object, (C)n: Control group with familiar object, (AD)f: Alzheimer's disease group with famailiar object, (AD)n: Alzheimer's disease group with novel object, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

Discrimination and preferential index were also evaluated in NORT and it is clearly seen that all groups are significantly higher ($p < 0.05$) than AD group in discrimination index (Figure 7A). C and AD+M groups had significantly higher preferential index compared AD group (Figure 7B). Short-term memory impairment was improved with metformin and *C. Sinensis* treatments.

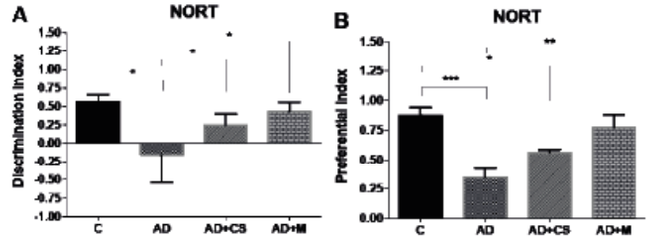


Figure 7. *(A) Discrimination index and (B) preferential index of rats in NORT. Each group (n = 6) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

3.6.3. Passive avoidance test

The step-through latency (STL) of rats was measured to evaluate long and short-term memories in a relational manner. The STL time decreased in AD group compared to C group. The treatments increased STL time compared to AD group but there was no significant difference between them.

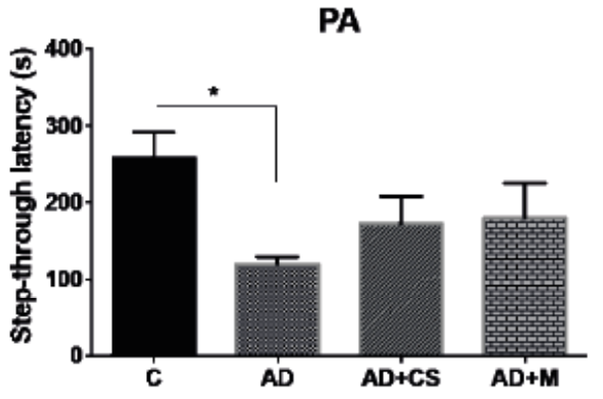


Figure 8. *Step-through latency of rats in PA test. Each group (n = 6) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with eachother. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. Sinensis treatment group, AD+M: Metformin treatment group)*

3.6.4. Morris's Water Maze Test

Probe test was performed 24 hours after the last training day. In this test, the total time spent and the time to reach the target quadrant where the platform is located was observed. AD group reached the target quadrant later than control group ($p < 0.05$) in all days and the total time spent in the quadrant decreased. When the treatment groups were examined, it was observed that AD+M group significantly improved it compared to AD+CS group on day 4 ($p < 0.01$).

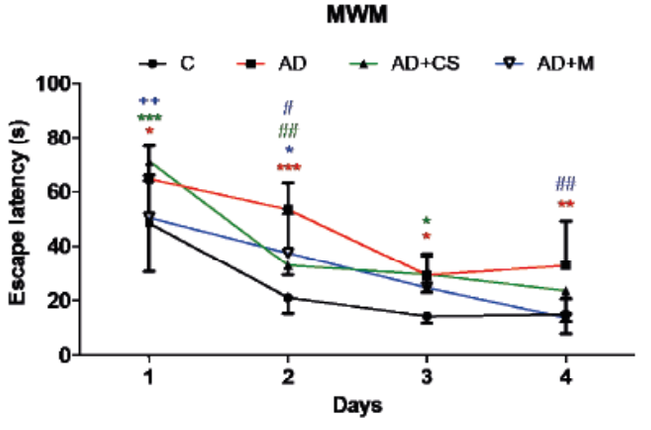


Figure 9. *Latency to platform of rats in MWM test. Each group (n = 6) represents Mean ± SEM. ANOVA was performed followed by Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 represent statistical differences compared to C group and #p < 0.05, ##p < 0.01, ###p < 0.001 represent statistical differences compared to AD group. +p < 0.05, ++p < 0.01, +++p < 0.001 represent statistical differences compared to AD+CS group. (C: Control group, AD: Alzheimer's disease group, AD+CS: C. sinensis treatment group, AD+M: Metformin treatment group)*

4. DISCUSSION

AD is an irreversible disorder destroys memory and thinking skills and it is estimated that at least 50 million are living with AD or other dementias (43). T2DM is a chronic disease characterized by high levels of glucose in the blood and affecting over 300 million people worldwide (44). When diabetes is not controlled well, BGLs rise and stay high for days to weeks. Within this period, most organs including the brain will be damaged and researchers have found many pieces of evidence that T2DM could link to AD, which is the most common form of dementia. Moreover, epidemiological studies have shown that there are pathophysiological connections between T2DM and AD. Insulin resistance majorly affects the disturbance of various biological processes and signaling pathways according to the recent evidences (45). Therefore, HFD/STZ-treated rat models were used to provide T2DM-induced AD-like alterations in our study. HFD is one of the most common risk factor for T2DM and AD, thus it has been chosen for inducing T2DM. HFD is also mimicking a real human diet, which causes insulin resistance and then type 2 diabetes to occur (46). It has been stated that diet-induced diabetes models represent a more true mechanism of DM pathogenesis rather than genetic factors (47). In literature, this model has been used in many studies in the evaluation of treatments for the most type of dementia (48).

Current pharmacological treatment for AD with antidementia drugs, which are cholinesterase inhibitors and memantine, have limited properties such as temporary and symptomatic support to cognitive functions. Substantial funding has been dedicated to the development of new pharmaceutical compounds with disease-modifying properties over the past decade (49). Besides, traditional medicine presents different plant-derived lead molecules that may be beneficial for further medical research. Traditional medicines are consumed as a memory enhancer for centuries and they have been used in the treatment of memory deficits such as dementia, amnesia, and AD (50). Green tea (*C. sinensis*) is one of these traditional medicines which are used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease (51).

SGLT inhibitors are currently being investigated as the medication in AD (52). In our study, *C. sinensis* was chosen due to its SGLT-1 inhibitor effects and T2DM-induced AD model was chosen to observe its anti-Alzheimer effects. The antidiabetic potential of *C. sinensis* with OGTT and ITT; and mainly its anti-Alzheimer effect with behavioral tests such as open field, novel object recognition, passive avoidance, and Morris's water maze test was investigated.

OGTT is currently the gold standard for the diagnosis of diabetes. Our results are in accordance with what showed that *C. sinensis* hydroalcoholic extract slighlty decreased the BGL of diabetic rats in 60 min. It is clearly seen that *C. sinensis* tolerated BGL for a short time but metformin, the first medication prescribed for T2DM, tolerated BGL during the test for 120 min.

In the present study, HFD/STZ-treated rat model occurred a significant memory deficit according to the learning and memory tests, which are OFT, NORT, PAT, and MWM. The cognition-enhancing activity of drugs is assessed by NORT and PAT, which are a relatively high-throughput, robust, and sensitive tests. MWM test is used to analyze spatial memory by measuring the escape latency time to reach a hidden platform. Locomotor activity, exploratory behavior, and anxiety was measured by OFT (53-55).

Animal behaviors such as the number of square crosses, frequency of rearing activity, latency to enter center, frequency of grooming activity, and time spent in the central zone are used as measures of locomotor activity and exploration. An increase in these parameters indicates a rise in locomotion and exploration (56). According to the results, *C. sinensis* increased the number of square crosses and the frequency of grooming activity in a similar manner to metformin.

The NORT evaluates the natural propensity of a rat to explore a novel versus a familiar object. This test provides to gain information about two different components, which are exploratory behavior and memory retention. Hence, the rats should sufficiently explore the familiar object during the pretest phase and then distinguish it with the new one in the test phase (57). The exploration of the novel object was reduced in HFD/STZ-treated rat model. It was also shown in the discrimination index in the NORT and HFD/STZ-treated rats demonstrated decreased exploratory behavior. In contrast, metformin and *C. sinensis* treatments improved these parameters.

The PAT is connected with the amygdala and evaluates emotional memory. PAT is associated with long-term or reference memory and it is for studying learning and memory after a stressful stimulus (58). The step-through latency decreased in HFD/STZ-treated rat model but it improved with metformin and *C. sinensis* treatment.

MWM test was performed to rats for evaluating spatial learning (59). There was a decrease in latency to find the platform underwater in all groups during the training phase. These results show that all rats learned the platform. However, it was clearly seen that AD group needs more time for training. These results show that there is a deterioration in the coding and remembering of the spatial memory. According to the results obtained by the MWM test, metformin treatment improved learning.

5. CONCLUSIONS

C. sinensis was used in the treatment of T2DM-induced AD-like alterations via its antidiabetic effects. OGTT and ITT were performed to evaulate its antidiabetic effects and also OFT, NORT, PAT, and MWM were performed to investigate its anti-Alzheimer effects. *C. sinensis* improved short-term memory and increased the locomotor activity in rats according to the results obtained by NORT, OFT, and PAT.

Conflict of Interest: The authors have no conflict of interest to declare.

Acknowledgements: This study was supported by Marmara University, Scientific Research Projects Committee (MU-BAPKO; SAG-K-170.118.0001).

REFERENCES

[1] Swerdlow RH. Pathogenesis of Alzheimer's disease. Clin Interv Aging 2007;2:347-359.

[2] Butterfield DA. Phosphoproteomics of Alzheimer disease brain: Insights into altered brain protein regulation of critical neuronal functions and their contributions to subsequent cognitive loss. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease 2019;1865:2031-2039.

[3] Association As. 2019 Alzheimer's disease facts and figures. Alzheimer's & Dementia 2019;15:321-387.

[4] Liang D, Lu H. Salivary biological biomarkers for Alzheimer's disease. Archives of Oral Biology 2019;105:5-12.

[5] Kocahan S, Doğan Z. Mechanisms of Alzheimer's Disease Pathogenesis and Prevention: The Brain, Neural Pathology, N-methyl-D-aspartate Receptors, Tau Protein and Other Risk Factors. Clin Psychopharmacol Neurosci 2017;15:1-8.

[6] Calderon-Garcidueñas AL, Duyckaerts C. Chapter 23 – Alzheimer disease. In: Kovacs GG, Alafuzoff I, editors. Handbook of Clinical Neurology: Elsevier; 2018. p. 325-337.

[7] Beyer L, Schnabel J, Kazmierczak P, Ewers M, Schönecker S, Prix C, Meyer-Wilmes J, Unterrainer M, Catak C, Pogarell O, Perneczky R, Albert NL, Bartenstein P, Danek A, Buerger K, Levin J, Rominger A, Brendel M. Neuronal injury biomarkers for assessment of the individual cognitive reserve in clinically suspected Alzheimer's disease. NeuroImage: Clinical 2019;24:101949.

[8] Albensi BC. Chapter Two – Dysfunction of mitochondria: Implications for Alzheimer's disease. In: Fernyhough P, Calcutt NA, editors. International Review of Neurobiology: Academic Press; 2019. p. 13-27.

[9] Veljkovic E, Xia W, Phillips B, Wong ET, Ho J, Oviedo A, Hoeng J, Peitsch M. Chapter 2 – Alzheimer's Disease. In: Veljkovic E, Xia W, Phillips B, Wong ET, Ho J, Oviedo A, Hoeng J, Peitsch M, editors. Nicotine and Other Tobacco Compounds in Neurodegenerative and Psychiatric Diseases: Academic Press; 2018. p. 13-23.

[10] Green RC, Cupples LA, Go R, Benke KS, Edeki T, Griffith PA, Williams M, Hipps Y, Graff-Radford N, Bachman D, Farrer LA. Risk of dementia among white and African American relatives of patients with Alzheimer disease. Jama 2002;287:329-336.

[11] Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018;141:1917-1733.

[12] Ding F, Yao J, Rettberg JR, Chen S, Brinton RD. Early Decline in Glucose Transport and Metabolism Precedes Shift to Ketogenic System in Female Aging and Alzheimer's Mouse Brain: Implication for Bioenergetic Intervention. PLOS ONE 2013;8:e79977.

[13] Cam ME, Hazar-Yavuz AN, Yildiz S, Ertas B, Ayaz Adakul B, Taskin T, Alan S, Kabasakal L. The methanolic extract of Thymus praecox subsp. skorpilii var. skorpilii restores glucose homeostasis, ameliorates insulin resistance and improves pancreatic β-cell function on streptozotocin/nicotinamide-induced type 2 diabetic rats. Journal of Ethnopharmacology 2019;231:29-38.

[14] Cam ME, Yildiz S, Ertas B, Acar AE, Taskin T, Kabasakal L. Antidiabetic effects of Salvia triloba and Thymus praecox subsp. skorpilii var. skorpilii in a rat model of streptozotocin/nicotinamide-induced diabetes. Marmara Pharmaceutical Journal 2017;21:818-827.

[15] Mushtaq G, Khan JA, Kamal MA. Biological mechanisms linking Alzheimer's disease and type-2 diabetes mellitus. CNS & neurological disorders drug targets 2014;13:1192-1201.

[16] Yarchoan M, Arnold SE. Repurposing Diabetes Drugs for Brain Insulin Resistance in Alzheimer Disease. Diabetes 2014;63:2253.

[17] Kang S, Kim CH, Jung H, Kim E, Song HT, Lee JE. Agmatine ameliorates type 2 diabetes induced-Alzheimer's disease-like alterations in high-fat diet-fed mice via reactivation of blunted insulin signalling. Neuropharmacology 2017;113:467-479.

[18] Magalhaes DA, Kume WT, Correia FS, Queiroz TS, Allebrandt Neto EW, Santos MPD, Kawashita NH, Franca SA. High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. Anais da Academia Brasileira de Ciencias 2019;91:e20180314.

[19] Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. J Diabetes Investig 2014;5:349-358.

[20] Chauhdary Z, Saleem U, Ahmad B, Shah S, Shah MA. Neuroprotective evaluation of Tribulus terrestris L. in aluminum chloride induced Alzheimer's disease. Pakistan journal of pharmaceutical sciences 2019;32:805-816.

[21] Zhang X, Wang X, Hu X, Chu X, Li X, Han F. Neuroprotective effects of a Rhodiola crenulata extract on amyloid-beta peptides (Abeta1-42) – induced cognitive deficits in rat models of Alzheimer's disease. Phytomedicine : international journal of phytotherapy and phytopharmacology 2019;57:331-338.

[22] Raheja S, Girdhar A, Kamboj A, Lather V, Pandita D. Aegle marmelos leaf extract ameliorates the cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin in male rats. Life sciences 2019;221:196-203.

[23] Wagle A, Seong SH, Shrestha S, Jung HA, Choi JS. Korean Thistle (Cirsium japonicum var. maackii (Maxim.) Matsum.): A Potential Dietary Supplement against Diabetes and Alzheimer's Disease. Molecules (Basel, Switzerland) 2019;24.

[24] Berg-Weger M, Stewart DB. Non-Pharmacologic Interventions for Persons with Dementia. Mo Med 2017;114:116-119.

[25] Koenig AM, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Xie L, Detre JA, Wolk DA, Arnold SE. Effects of the Insulin Sensitizer Metformin in Alzheimer Disease: Pilot Data From a Randomized Placebo-controlled Crossover Study. Alzheimer Dis Assoc Disord 2017;31:107-113.

[26] Suraphad P, Suklaew PO, Ngamukote S, Adisakwattana S, Mäkynen K. The Effect of Isomaltulose Together with Green Tea on Glycemic Response and Antioxidant Capacity: A Single-Blind, Crossover Study in Healthy Subjects. Nutrients 2017;9:464.

[27] Li H, Wu X, Wu Q, Gong D, Shi M, Guan L, Zhang J, Liu J, Yuan B, Han G, Zou Y. Green tea polyphenols protect against okadaic acid-induced acute learning and memory impairments in rats. Nutrition (Burbank, Los Angeles County, Calif) 2014;30:337-342.

[28] Snoussi C, Ducroc R, Hamdaoui MH, Dhaouadi K, Abaidi H, Cluzeaud F, Nazaret C, Le Gall M, Bado A. Green tea decoction improves glucose tolerance and reduces weight gain of rats fed normal and high-fat diet. The Journal of nutritional biochemistry 2014;25:557-564.

[29] Shivashankara AR, Kumar A, Ravi R, Simon P, Rai P, Francis A, Baliga MS. Chapter 10 – Use of Tea (Camellia sinensis [L.] Kuntze) as a Hepatoprotective Agent in Geriatric Conditions. In: Watson RR, editor. Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults. San Diego: Academic Press; 2015. p. 99-104.

[30] Al-Attar AM, Zari TA. Influences of crude extract of tea leaves, Camellia sinensis, on streptozotocin diabetic male albino mice. Saudi J Biol Sci 2010;17:295-301.

[31] Pervin M, Unno K, Ohishi T, Tanabe H, Miyoshi N, Nakamura Y. Beneficial Effects of Green Tea Catechins on Neurodegenerative Diseases. Molecules (Basel, Switzerland) 2018;23:1297.

[32] Yang Z, Xu Y, Jie G, He P, Tu Y. Study on the antioxidant activity of tea flowers (Camellia sinensis). Asia Pacific journal of clinical nutrition 2007;16 Suppl 1:148-152.

[33] Bedrood Z, Rameshrad M, Hosseinzadeh H. Toxicological effects of Camellia sinensis (green tea): A review. Phytotherapy research : PTR 2018;32:1163-1180.

[34] Prasanth MI, Sivamaruthi BS, Chaiyasut C, Tencomnao T. A Review of the Role of Green Tea (Camellia sinensis) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. Nutrients 2019;11.

[35] Ide K, Matsuoka N, Yamada H, Furushima D, Kawakami K. Effects of Tea Catechins on Alzheimer's Disease: Recent Updates and Perspectives. Molecules (Basel, Switzerland) 2018;23:2357.

[36] Sharangi AB. Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review. Food Research International 2009;42:529-535.

[37] Asadbegi M, Yaghmaei P, Salehi I, Ebrahim-Habibi A, Komaki A. Neuroprotective effects of metformin against Aβ-mediated inhibition of long-term potentiation in rats fed a high-fat diet. Brain Research Bulletin 2016;121:178-185.

[38] Boland B, Mumphrey MB, Hao Z, Gill B, Townsend RL, Yu S, Münzberg H, Morrison CD, Trevaskis JL, Berthoud H-R. The PYY/Y2R-Deficient Mouse Responds Normally to High-Fat Diet and Gastric Bypass Surgery. Nutrients 2019;11:585.

[39] Zimcikova E, Simko J, Karesova I, Kremlacek J, Malakova J. Behavioral effects of antiepileptic drugs in rats: Are the effects on mood and behavior detectable in open-field test? Seizure 2017;52:35-40.

[40] Abdel-Aal RA, Assi AA, Kostandy BB. Rivastigmine reverses aluminum-induced behavioral changes in rats. European journal of pharmacology 2011;659:169-176.

[41] Smith BM, Yao X, Chen KS, Kirby ED. A Larger Social Network Enhances Novel Object Location Memory and Reduces Hippocampal Microgliosis in Aged Mice. Frontiers in aging neuroscience 2018;10:142.

[42] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006;1:848-858.

[43] Browne D, McGuinness B, Woodside JV, McKay GJ. Vitamin E and Alzheimer's disease: what do we know so far? Clin Interv Aging 2019;14:1303-1317.

[44] Sami W, Ansari T, Butt NS, Hamid MRA. Effect of diet on type 2 diabetes mellitus: A review. Int J Health Sci (Qassim) 2017;11:65-71.

[45] Mittal K, Katare DP. Shared links between type 2 diabetes mellitus and Alzheimer's disease: A review. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2016;10:S144-S9.

[46] Moreno-Fernández S, Garcés-Rimón M, Vera G, Astier J, Landrier JF, Miguel M. High Fat/High Glucose Diet Induces Metabolic Syndrome in an Experimental Rat Model. Nutrients 2018;10:1502.

[47] Zhuo J, Zeng Q, Cai D, Zeng X, Chen Y, Gan H, Huang X, Yao N, Huang D, Zhang C. Evaluation of type 2 diabetic mellitus animal models via interactions between insulin and mitogen-activated protein kinase signaling pathways induced by a high fat and sugar diet and streptozotocin. Mol Med Rep 2018;17:5132-5142.

[48] Kang S, Kim C-H, Jung H, Kim E, Song H-T, Lee JE. Agmatine ameliorates type 2 diabetes induced-Alzheimer's disease-like alterations in high-fat diet-fed mice via reactivation of blunted insulin signalling. Neuropharmacology 2017;113:467-479.

[49] Aprahamian I, Stella F, Forlenza OV. New treatment strategies for Alzheimer's disease: is there a hope? Indian J Med Res 2013;138:449-460.

[50] Akram M, Nawaz A. Effects of medicinal plants on Alzheimer's disease and memory deficits. Neural Regeneration Research 2017;12:660-670.

[51] Prasanth MI, Sivamaruthi BS, Chaiyasut C, Tencomnao T. A Review of the Role of Green Tea (Camellia sinensis) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. Nutrients 2019;11:474.

[52] Simes BC, MacGregor GG. Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors: A Clinician's Guide. Diabetes Metab Syndr Obes 2019;12:2125-2136.

[53] Rinwa P, Jaggi AS, Singh N. Pharmacological investigation of memory restorative effect of riluzole in mice. Indian J Pharmacol 2012;44:366-371.

[54] Mathiasen JR, DiCamillo A. Novel object recognition in the rat: a facile assay for cognitive function. Curr Protoc Pharmacol 2010;Chapter 5:5.59.

[55] Terry AV. Spatial Navigation (Water Maze) Tasks. 2nd ed: CRC Press/Taylor & Francis, Boca Raton (FL); 2009.

[56] Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Effects of DCM Leaf Extract of Gnidia glauca (Fresen) on Locomotor Activity, Anxiety, and Exploration-Like Behaviors in High-Fat Diet-Induced Obese Rats. Behav Neurol 2019;2019:7359235.

[57] Foyet HS, Abaïssou HHN, Wado E, Acha EA, Alin C. Emilia coccinae (SIMS) G Extract improves memory impairment, cholinergic dysfunction, and oxidative stress damage in scopolamine-treated rats. BMC Complement Altern Med 2015;15:333.

[58] Wang J, Wang X, Lv B, Yuan W, Feng Z, Mi W, Zhang H. Effects of Fructus Akebiae on learning and memory impairment in a scopolamine-induced animal model of dementia. Exp Ther Med 2014;8:671-675.

[59] Yildiz S, Cam ME, Keles R, Hazar-Yavuz AN, Kabasakal L. A PPAR-alpha agonist gemfibrozil ameliorates cognitive and memory impairments in a sporadic Alzheimer's disease rat model. European Neuropsychopharmacology 2019;29:S263-S4.

How to cite this article: Cam ME, Taskin T. Camellia sinensis leaves hydroalcoholic extract improves the Alzheimer's disease-like alterations induced by type 2 diabetes in Rats. Clin Exp Health Sci 2020; 10: 93-103. DOI: 10.33808/clinexphealthsci.685280

Clinical and Experimental Health Sciences

Effect of Dietary N-3 Series Fatty Acids on Sperm Motility Duration of Rainbow Trout (*Oncorhynchus Mykiss* Walbaum, 1792)

Seyma Ozer Kaya[1], Seyfettin Gur[1], Kenan Koprucu[2], Gaffari Turk[1], Mustafa Sonmez[1], Sinan Ozcan[2]

[1] Firat University, Faculty of Fisheries, Department of Aquaculture, Elazig, Turkey
[2] Firat University, Faculty of Fisheries, Department of Aquaculture, Elazig, Turkey

Correspondence Author: Seyma Ozer Kaya
E-mail: sozer@firat.edu.tr

Received: 10.02.2020 **Accepted:** 28. 02. 2020

ABSTRACT

Objective: This study was performed to determine the effect of different ratios of n-3 series fatty acids added to the diets of rainbow trout (*Oncorhynchus mykiss*) on sperm motility duration.

Methods: A total of 48 male rainbow trouts about 3 years of age were used in the study and 4 groups were formed. The one that n-3 series fatty acids were not added to the diet was used as control group. n-3 series fatty acids were added to the experimental groups diets at the ratios of 1% (E1), 2% (E2) and 3% (E3), respectively. Semen was collected by abdominal massage. The time-dependent sperm motility change was detected under the microscope at x400 magnification.

Results: A significant decrease was detected in the motility only at 360th hour in E3 group compared to the control group ($p<0.05$).

Conclusion: As a result, it was concluded that feeding with diets containing different ratios of n-3 series fatty acids had no effect on the motility of rainbow trout semen under in vitro condition.

Keywords: Diet, duration of motility, fatty acid, rainbow trout, sperm motility.

1.INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*), which is a fresh-water fish, farming increases year by year in our country depending on its existing potential. Rainbow trout belongs to the Salmonidae family. It matures in 2-3 years and spawns between December and May (1).

The fast growth of the world population leads to a rapid decline in animal protein sources. Therefore, the production of animal proteins should be accelerated. The current biotechnological research contributes to the increase in fish farming worldwide. Recently, studies focused on the yield increased significantly. As in the hatcheries in our country, farmers aim to increase the number of tiddlers, knowledge about the evaluation criteria of the yield characteristics in the brood fishes, which will be used in farming, is critical. Sperm motility is one of the most important sperm parameters. To increase the yield, the use of sperm with good sperm quality is essential (2-4). The duration spermatogenesis is usually shorter in fish than in mammals. In principle, spermatogenesis can be divided, from a morpho-functional point of view, in three different phases: the mitotic or spermatogonial phase with the different generations of spermatogonia, the meiotic phase with the primary and secondary spermatocytes, and the spermiogenic phase with the haploid spermatids emerging from meiosis and differentiating, without further proliferation, into flagellated spermatozoa (5). Like other living creatures, also fishes need energy for growth, breeding, living, and their physiological activities. Lipids are their main source of energy. Lipids consist of fatty acids and are not water-soluble. Fatty acids are divided into two groups depending on the number of bonds. Fatty acids with one bond are called saturated and with double bonds are called unsaturated fatty acids. In addition, fatty acids with carbon atom number between 18-20 and double bonds between 2-4 are called polyunsaturated fatty acids (PUFA) and fatty acids with more than 20 carbon atom number and more than 4 double bonds are called highly unsaturated fatty acids (HUFA) (6). Like in other fishes, the rainbow trout needs also n-3 and n-6 fatty acids (7). Furthermore, PUFAs like linolenic, linoleic and α-linolenic acid are essential fatty acids and disorders related to growth, development, and proliferation may emerge if their need for these fatty acids is not met (6).

DOI: 10.33808/clinexphealthsci.687305

In this study, our objective was to investigate the effect of n-3 series fatty acids, which were added to the compound feed of rainbow trout in different proportions, on the duration of the sperm motility.

2. METHODS

This study was conducted in the Cip Fish Breeding Farming of the Firat University Aquaculture Faculty. A total of 48 male rainbow trouts at age 3 were included in the study. The study was conducted in 4 cement ponds (dimensions: 2x1x0.8m). The study sample was divided into 4 groups. 12 male broodstocks were placed in each pond. The fishes were adapted to the experimental conditions for one month. They were fed with control feed during this period. Then broodstocks were fed for three months three times daily according to the free feeding technique. They fastened for 24 hours before the sperm yield. They were anesthetized before the stripping process (5 mL phenoxyethanol/L) (8). The abdomen of the dried male broodstocks was manually massaged from front to the back and sperm was yielded (9).

2.1. Organization and preparation of the study feed

Soybean meal, corn gluten, anchovy meal, wheat starch, n-3 fatty acid concentrated from the anchovy oil, unrefined sunflower oil, antioxidants, vitamin mixture, mineral mixture, and wheat bran were purchased from a commercial company for the preparation of the study feed.

In the control group, fishes received feed with no added n-3 fatty acids. The experiment groups (E) E1, E2, and E3 received feed with 45% concentrated raw protein, 3619 kcal/kg metabolized energy and anchovy oil with an n-3 fatty acid concentration of 1%, 2%, and 3% respectively. Besides, we used corn gluten, soybean meal, anchovy meal, and fatty acids as the protein source and unrefined sunflower oil (as a source of n-6 fatty acids) n-3 fatty acids as the energy source (Table 1) (6, 10).

Table 1. *Formulation of the experimental diets*

Feed items (%)	Experimental Diets			
	Control	Experiment 1	Experiment 2	Experiment 3
Anchovy flour (%56,9)	35	35	35	35
Soybean meal (%42,2)	30	30	30	30
Corn gluten (%52,6)	5	5	5	5
Wheat starch (%10,2)	5	5	5	5
N-3 series fatty acid [1]	-	1	2	3
Sunflower oil	15.2	14.2	13.2	12.2
Antioxidant [2]	0.1	0.1	0.1	0.1
Vitamin Mix [3]	1	1	1	1
Mineral mix [4]	1	1	1	1
Wheat bran	7.7	7.7	7.7	7.7

[1] n-3 series fatty acid (Solgar OMEGA-3 700) was concentrated from anchovy oil; and containing 54.3% eicosapentaenoic acid (EPA), 37.1% docosahexaenoic acid (DHA) and 8.6% other n-3 series fatty acids (docosapentaenoic, linolenic, stearidonic acid).

[2] Butylene Hydroxy Toluene (BHT); 125.000 mg/kg.

[3] Vitamin Mix (as active ingredient per 1 kg Rovimix 107); Vitamin A 250.000 IU, vitamin D3 240.000 IU, vitamin E 10.000 IU, vitamin K 3.000 mg, vitamin B1 1.000 mg, vitamin B2 3.000 mg, vitamin B6 2.000 mg, vitamin B12 4 mg, choline chloride 100.000 mg, vitamin C 6.000 mg, niacin 30.000 mg, calcium d-pantothenate 10.000 mg, folic acid 600 mg, d-biotin 200 mg.

[4] Mineral Mix (mg/kg); Manganese 1.300, zinc 3.000, iron 6.000, copper 300, iodine 110, potassium 70, phosphorus 60, selenium 30, cobalt 20, magnesium 5.

2.2. Determination of the sperm motility and sperm duration

After semen collection, semen was stored in the cooling cabinet at 4 °C. Sperm motility determination was done at certain intervals until motility was exhausted. In order to determine the motility of the semen samples obtained from the fishes, 2 ml of the 119 mmol NaCl solution was poured in a tube. Then 1 drop semen was added and mixed. One drop of this mixture put on a slide glass and covered with a cover glass. The motility rate (%) was determined under a mirror microscope with a 400x magnification. The time-dependent change in this rate was investigated.

The study data were expressed in mean values and standard error of means (±SEM) after the statistical analysis. The software package SPSS (22.0, Chicago, IL, USA) was used for the comparative statistical analysis. For all analyses, $p<0.05$ was considered statistically significant.

Regarding the motility, non-parametric Kruskal-Wallis variance analysis was used for the intergroup comparisons and non-parametric Mann-Whitney U test for the paired comparisons.

3. RESULTS

The mean motility rate (%) according to time (min) in rainbow trout in the control and experimental groups, which were fed with diets containing different n-3 series fatty acid concentrations, were summarized in Table 2.

The sperm motility was numerically higher in the experimental groups than the control group during first 30th minute. At the 60th minute, while the motility value in the E3 group decreased numerically compared to the other groups, E1, E2 remained the same with the control. The numerical decrease in sperm motility of the experimental groups continued after 120th minute compared to the control group.

A significant decrease was detected in the motility only at 360th hour in E3 group compared to the control group ($p<0.05$).

Table 2. *Average (±SEM) motility (%) values of control and experimental groups according to time (minute)*

	Begining	5th min	10th min	15th min	20th min	25th min	30th min	60th min	120th min	180th min	240th min	300th min	360th min	420th min
Control	80.00±5.77	76.66±3.33	76.66±3.33	73.33±3.33	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	60.00±0.00	60.00±0.00	53.33±3.33	40.00±5.77	26.66±3.33^{a}	3.33±3.33
E1	88.75±3.14	80.00±0.00	77.50±2.50	75.00±2.88	72.50±2.50	72.50±2.50	70.00±0.00	70.00±0.00	65.00±2.88	55.00±2.88	55.00±2.88	40.00±4.08	20.00±4.08^{a}	0.0±0.00
E2	88.33±4.40	86.66±3.33	86.66±3.33	86.66±3.33	80.00±5.77	80.00±5.77	73.33±12.0	70.00±10.0	56.66±8.81	53.33±6.66	53.33±6.66	33.33±6.66	20.00±5.77^{a}	3.33±3.33
E3	88.33±4.40	80.00±5.77	80.00±5.77	76.66±6.66	76.66±6.66	76.66±6.66	73.33±3.33	63.33±3.33	53.33±3.33	43.33±8.81	43.33±8.81	26.66±6.66	3.33±1.66^{b}	0.0±0.00

a and b : Differents letters within a column showed significant differences between groups ($p<0.05$).

4. DISCUSSION

The yield of good quality sperm is one of the main goals of fish farming. Several biotic and abiotic factors affect the yield and quality of the sperm. The nutrition of the broodstocks has a direct effect on sperm quality. Although nutrition has an important effect on reproductive physiology, there is only limited evidence that these changes can affect sperm quality. Fatty acids especially PUFAs, n-3 series fatty acids and their derivatives can affect the number of eggs (11, 12).

In bony fish species, which are reproduced via external fertilization, the spermatozoon activity is short and reaches the maximum speed after the dilution. Besides, their speed declines during motion. The very short duration of sperm motility is the main reason for the low fertilization rate in salmonids (13). The mean duration of the sperm motility is 20-25 seconds in trouts and more than 1 minute in carps (14). The duration of spermatozoon motility in the active rainbow trouts is 30-35 seconds during the spawning season. The duration of motility drops up to 15 seconds at the end of the season (15).

In our study, the duration of motility was very long compared to the previous studies and the vitality of the rainbow trout spermatozoon was preserved up to 360 minutes. We believe that the high extracellular K^+ concentration inhibited the spermatozoon motility in the previous studies. Therefore, we believe that the spermatozoon motility can be activated with the extracellular K^+ diluent. This diluent causes membrane hyperplasia, which triggers the activation (16). Besides, osmotic pressure is one of the factors determining sperm activation and is commonly considered as a triggering factor for the initiation of sperm motility. Motility starts at a higher osmotic pressure in saltwater fishes compared to the freshwater species (17). Furthermore, as the duration of motility is short in the trout spermatozoon, the motility measurement should be performed quickly. The difference in the motile lifespans between the studies may depend in general on the environmental factors and the temperature in the laboratory.

The lipid and fatty acid composition of the feed are defined as the main factor for successful reproduction of fishes and their survival (18). As the lipid composition is closely related to the spermatozoon quality, its quantity in the feed is important. Lahnsteiner et al. (11) investigated the compositions of the total fatty acids in the seminal plasma and sperm in trouts and found saturated fatty acids such as myristic acid, palmitic acid, and stearic acid and unsaturated fatty acids like oleic acid, vaccenic acid, and linolenic acid. Another study reported that free fatty acids and sterols were the main lipid components in the seminal fluid and stated that the fatty acid composition of the sperm was affected by the nutrition (19). The investigators found out that the n-3 PUFA deficiency in the feed affected the sperm motility and the motility was lower compared to the controls. In our study, we determined that in the group, in which 3% of fatty acid was added to the diet, the sperm motility impaired the sperm lifespan only at 360th minute.

In salmonids, lipids are the main energy source for sperms and therefore they are important for the preservation of sperm vitality (11). Fatty acid oxidation occurs as a result of adenosine triphosphate production, which is stored in the sperm cell membrane, mitochondria, middle segment, and tail of the sperms. Therefore, the increase of the fatty acids in the testicular cells stimulates the sperm production capacity in testicles and thus the rate of the sperm survival and sperm motility increase (20). In our study, a significant decrease was detected in the motility from only at 360th hour in E3 group compared to the control group. Along with several factors affecting sperm survival, a high concentration of fatty acids increases the metabolism in sperms and leads to an early decline of the motility, which may be considered as an additional factor.

In a study conducted with the European seabass (*Dicentrarchus labrax*), Asturiano et al. (21) observed that diets containing PUFA had a positive effect on the reproduction parameters including sperm volume and density. In another study

conducted with *Barbus barbus* species, Alavi et al. (22) fed the fishes with PUFA-containing diet during the breeding season. However, their investigation on the sperm quality demonstrated that different diets did not affect sperm volume, total sperm quantity, concentration, and motility. In addition, in another study focused on *Carassius auratus*, it was determined that in vitro essential fatty acids stimulated the testicular testosterone production and affected theprostaglandins (18). Thus, the production of the steroid hormones declines in fatty acid deficiency, spermiation time is delayed and consequently, the fertilization rate may decline Lahnsteiner et al. (11) investigated the fatty acids during the short time preservation of sperm in rainbow trout and reported that the sperm survival prolonged and had a positive effect on sperm motility and consequently on fertility.

5. CONCLUSION

As a result, it was concluded that feeding with diet including different concentrations of n-3 series fatty acids has no effect on motility in rainbow trout semen under in vitro conditions.

REFERENCES

[1] Çelikkale MS. İçsu Balıkları ve Yetiştiriciliği. Karadeniz Teknik Üniversitesi, Sürmene Deniz Bilimleri ve Teknolojisi Yüksekokulu, Fakülte Yayın No: 2, Trabzon; 1988.

[2] Dreanno C, Cosson J, Suquet M, Seguin F, Dorange G, Billard R. Nucleotide content, oxidative phosphorylation, morphology and fertilizing capacity of turbot (psetta maxima) spermatozoa during the motility period. Mol Reprod Dev 1999; 53:230-243.

[3] Okumuş I. Rainbow trout broodstock management and seed production in Turkey: present practices, constraints and the future. Turkish J Fish Aqua Sci 2003; 2:41-56.

[4] Yavuz H. Farklı kromozomlara (XX ve XY) sahip erkek gökkuşağı alabalığı (*Oncorhynchus mykiss*) spermlerinin dölleme kalitesi. Yüksek Lisans Tezi. Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Elazığ. 2012.

[5] Cosson J. Fish sperm physiology: Structure, factors regulating motility, and motility evaluation. Borkurt Y, editor. Biological Research in Aquatic Science. IntechOpen; 2019.p.1-26.

[6] Sargent JR, Toche DR, Bell JG. The Lipids. Halver JE, Hardy RW, editors. Fish Nutrition. USA: San Diego; 2002.p.182-257.

[7] Goddard S. Feed Management in Intensive Aquaculture, 1st ed. New York: Chapman & Hall Press; 1996.

[8] Mattson NS, Riple TH. Metomidate, a better anaesthetic for cod (*Gadus morhua*) in comparison with benzocain, MS-222, choloro butanol and phenoxyethanol, Aquaculture 1989; 83:89-94.

[9] Munkittrick KR, Moccia RD. Seasonal changes in the quality of rainbow trout (*Salmo gairdneri*) semen: Effect of a delay in stripping on sprmatocrit, motility, volume and seminal plasma constituents. Aquaculture 1987; 64:147-156.

[10] National Research Council (NRC). Nutrient Requirements of Fish, National Academy Press, Washington DC. 1999.

[11] Lahnsteiner F, Mansour N, McNiven M A, Richrdson GF. Fatty acids of rainbow trout (*Oncorhynchus mykiss*) semen: Composition and effects on sperm functionality. Aquaculture 2009; 298:118-124.

[12] Huang X, Yin Y, Shi Z, Li W, Zhou H, Lv W. Lipid content and fatty acid composition in wild-caught silver pomfret (*Pampus argenteus*) broodstocks: effects on gonad development. Aquaculture 2010; 310:192-199.

[13] Aas GH, Refstie T, Gjerde B. Evaluation of milt quality of atlantic salmon. Aquaculture 1991; 95:125-132.

[14] Bromage NR, Roberts RJ. Broodstock Management and Egg and Larval Quality. 1st ed. Cambridge: Wiley-Blackwell Science Ltd.; 1995.

[15] Benau D, Terner C. Initiation prolongation and reactivation of the motility of salmonid spermatozoa. Gamete Res 1980; 3:247-257.

[16] Boitano S, Omoto CK. Membrane hyperpolarization activates trout sperm without an increase in intracellular pH. J Cell Sci 1991; 98:343-349.

[17] Chambeyron F, Zohar Y. A diluent for sperm cryopreservation of gilthead seabream, *Sparus aurata*. Aquaculture 1990; 90:345-352.

[18] Izquierdo MS, Fernandez-Palacios H, Tacon AGJ. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 2001; 197:25-42.

[19] Vassallo-Agius R, Watanabe Takeshi Yoshizaki G, Satoh S, Takeuchi Y. Quality of eggs and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and its effects on the lipid and fatty acid components of eggs, semen and livers. Fisheries Sci 2001; 67:818-827.

[20] Frøyland L, Lie Ø, Berge RK. Mitochondrial and peroxisomal β-oxidation capacities in various tissues from atlantic salmon salmo salar. Aquacult Nutr 2000; 6:85-89.

[21] Asturiano JF, Sorbera LA, Carrillo M, Zanuy S, Ramos J, Navarro JC, Bromage N. Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed two PUFAenriched experimental diets: a comparison with males fed a wet diet. Aquaculture 2001; 194:173–190.

[22] Alavi SMH, Psenicka M, Polica T, Rodina M, Hamackova J, Kozak P, Linhart O. Sperm quality in male *Barbus barbus* L. fed different diets during the spawning season. Fish Physiol Biochem 2009; 35:683-693.

How to cite this article: Ozer Kaya S, Gur S, Koprucu K, Turk G, Sonmez M, Ozcan S. Effect of Dietary N-3 Series Fatty Acids on Sperm Motility Duration of Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792). Clin Exp Health Sci 2020; 10: 104-107. DOI: 10.33808/clinexphealthsci.687305

Clinical and Experimental Health Sciences

In-vitro anticandidial efficacy of tick egg wax from *Hyalomma marginatum, Rhipicephalus bursa* and *Dermacentor marginatus*

Nazli Bilgin[1], Mayram Hacioglu[2], Cagla Bozkurt Guzel[2], Berna Erdal[1], Sirri Kar[3,4]

[1] Tekirdag Namik Kemal University, School of Medicine, Department of Medical Microbiology, Tekirdag, Turkey.
[2] Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul, Turkey.
[3] Tekirdag Namik Kemal University, Department of Biology, Tekirdag, Turkey.
[4] University of Texas Medical Branch, Department of Microbiology and Immunology, Galveston National Laboratory, Galveston, TX, USA.

Correspondence Author: Sirri Kar
E-mail: sirrikar@yahoo.com
Received: 23.02.2020 **Accepted:** 12.03.2020

ABSTRACT

Objective: In the previous studies, the antibacterial, antifungal, and antiviral efficacy of the tick egg wax-coating of certain tick species were examined and some significant results were obtained. However, related researches and studied tick species are limited. There are hundreds of tick species, and it is well known that the antimicrobial efficacy of the wax is closely related to the species. The aim of this study was to investigate the in-vitro anticandidial efficacy of the egg waxes belonging to three tick species, which have not been studied before and have quite different biological and ecological differences.

Methods: In the study, the egg waxes of the tick species, *Hyalomma marginatum, Rhipicephalus bursa,* and *Dermacentor marginatus*, were used on *Candida albicans* ATCC10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750. Antimycotic susceptibility test was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations using the M27-A3 microdilution method.

Results: It was determined that the wax of *Rhipicephalus bursa* has inhibitory effect on *Candida tropicalis* ATCC 750 in a particular concentration, and no significant effects were observed in other trials.

Conclusion: Anticandidial effect obtained from the egg wax of *R. bursa* can be associated with some distinctive biological characteristics, and it was concluded that the detailed studies with different tick species might yield significant results for the discovery of new generation antifungals.

Keywords: Antifungals, tick egg wax, *Rhipicephalus bursa, Candida*

1. INTRODUCTION

It has been reported that there are about 20 *Candida* species among the clinically important fungi seen worldwide, and of those, *Candida albicans*, the most common fungal pathogen in humans, has been reported to cause 250,000-400,000 deaths annually (1). Both cutaneous and systemic forms of candidiasis can be encountered, and the mortality can reach up to 40% in systemic *C. albicans* infections (2). Although *C. albicans* is the main agent of candidiasis, other pathogenic species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis, C. krusei,* and *C. lusitaniae* (non-albicans candida/ NAC) are frequently isolated from the cases. Furthermore, NAC species are more common in some regions in the world, and a steady increase has been observed in NAC-related cases in the last decades (e.g., 2-10 times in the cases of systemic candidiasis). This increase in the prevalence has been reported to be associated with high antifungal resistance (e.g., specific azole resistance in *C. glabrata* and *C. krusei*) seen in this group, and this problem has been indicated to be exacerbated by the irregular antifungal use (3-5). In *C. albicans* and NAC species, as well as many other fungi, significant antimycotic resistance, which has intrinsic or microbiological character, has been well documented (6, 7). For example, single or multiple resistances in various *Candida* species against different antifungals, such as fluconazole, voriconazole, amphotericin B, and caspofungin, have been reported from different parts of the world (6-10). The antifungal diversity in the clinical use is reported to be relatively low compared to the causative agent and disease variety in fungal infections. The widespread, repetitive, and irregular use of antifungal drugs are possibly the main reasons of the resistances. It was anticipated that this situation requires the discovery of new drugs, and moreover, this need will increase over time (6-7). At this point, due to the problems in the medical area such as the resistances and side-effects of the current chemical therapies, the researches on natural substances, which may ensure to overcome these problems, have particular

DOI: 10.33808/clinexphealthsci.707924

importance. The current status of the researches in this area is expressed as "a New Golden Age of natural products drug discovery is dawning" (11). On the other hand, most of the related studies are at the beginning level. The applications are mostly at the stage of using total extracts or selected fractions and the results are given as effective or non-effective (12, 13).

In order to determine the natural alternatives that can be used in the treatment of fungal infections, various substances from different origins have been investigated such as microorganismal substances (14), sea sponge (15), the poison of honey bee (*Apis mellifera*) (16), the larval secrets of a lepidopteran parasitoid, *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) (17), and the total isolates or some specific fractions of many kind of plant species (13, 18).

Tick egg wax is one of the sources investigated in the process of determining natural antimycotics. In ixodid ticks, the biological process includes eggs, larvae, nymphs, and adults (male and female). After detachment from the host, engorged adult female ticks hide in a suitable area on the ground and then they deposit up to thousands of eggs in a period that can last for several days or weeks before they die. The eggs, each 50-100 µg in weight and 0.5-1 mm, are laid as a batch. The surfaces of the eggs were covered with the wax, which consists of the secretions of the epithelial cells and accessory glands of the reproductive tract, the porose area, and principally the Gené organ which is a female-specific gland (19, 20). It has been reported that the wax, which thickness varies between 0.5-2.0 µm (21), has an average amount of 24 mg per gram eggs mainly according to the tick species (22). The wax is known to have some vital missions for the eggs such as holding the egg mass together, protecting the eggs against drying, environmental physical, chemical or microbiological factors, and ensuring the proper gas exchange between eggs and air (20, 21, 23, 24).

In the previous studies, the egg waxes of *Amblyomma habreum* (25, 26) *A. cajennense* (27-29), *A. aureolatum* (29), *Boophilus* (*Rhipicephalus*) *microplus* (29-32), *Rhipicephalus sanguineus* (29), *Haemaphysalis longicornis*, *H. doenitzi*, *Dermacentor silvarum* and *Hyalomma asiaticum* (22) have been tested against various bacteria, fungi, and viruses, and some considerable results were obtained. However, these studies have also revealed that the antimicrobial efficacy of the wax varies dramatically related to the tick species. In this study, the egg waxes of three tick species (*H. marginatum*, *R. bursa,* and *D. marginatus*), which have not been studied before and have quite different biological and ecological features, were investigated in terms of in-vitro anticandidial efficacy on three *Candida* species.

2. METHODS

2.1. Tick eggs

Non-infected laboratory colonies of *H. marginatum*, *D. marginatus* and *R. bursa* were used in the study. The maintenance of the colonies was carried out at Tekirdag Namik Kemal University Experimental Animal Application and Research Center, within the framework of the permissions received from the Tekirdag Namik Kemal University Animal Experiments Local Ethics Committee (2014/08-04). New Zealand rabbits (*Oryctolagus cuniculus*) were used to feed all of the developmental stages (larvae, nymphs, and adults). After the detachment from the host, the engorged females were washed with distilled water, dried, taken into sterile tubes individually and placed in the incubator. The conditions of the incubator were set at 25-27 °C and 70-80% humidity for H. *marginatum* and *R. bursa*, and at 20-22 °C and 80-90% humidity for *D. marginatus.* The process of the egg laying was regularly monitored, and the eggs were examined under a stereomicroscope. Incubation was continued until the first laid eggs reached the advanced stages (just before the larva began to hatch) of embryogenesis. Thus the wax extract was intended to include some possible changes which can occur in the egg wax during and after the laying, e.g., hardening in the texture, increase in the adhesive properties, and reduction in the levels of unsaturated fatty acids caused by oxidation (21, 23, 33). After this incubation egg batches were taken from the tubes and weighed, taken into sterile tubes and kept at – 80°C until the extraction of the wax.

2.2. Extraction of the tick egg wax

In the wax extraction process, previously described method was used (25). The following procedure has been carried out to include all the fractions of the wax to the extract as much as possible: just before the procedure, a chloroform: methanol solution (≥99.8%, LiChrosolv®, Merck, Germany / 24216-2-2.5L-R, 99-99.4%, 1% ethanol, Sigma-Aldrich, Germany) was prepared in a 2:1 (v/v) ratio. Of this solution, 40 ml was added to each of the 50 ml tubes containing 4 g eggs. The tubes were gently shaken for 1 minute and put on hold for 1 minute, and then vortexed for 1 minute. The vortexed suspension was separated from the eggs by filtration, and the filtrate was centrifuged at 1,000 rpm for 10 minutes. Subsequently, the supernatant transferred to a new tube was lyophilized, and the lyophilisate was kept at – 20 °C until antimycotic susceptibility assay.

2.3. Determination of antifungal activity

In order to prepare Roswell Park Memorial Institute-1640 (RPMI-1640) medium, 10.4 g RPMI-1640 (with L-glutamine, without sodium bicarbonate, Sigma, USA) and 0.165 M 34.53 g MOPS (3-N-morpholinopropanesulfonic acid, Sigma, USA) were dissolved in dH_2O. 18g glucose was weighed and added with a glucose concentration of 2%. The pH was adjusted to 6.9-7.0 with 1N NaOH. After the final volume was completed

to 1,000 ml, it was sterilized under aseptic conditions by filtering with a 0.22 µm pore-diameter filter (Millipore). The prepared medium was used in experiments after keeping overnight in a 37 ° C and the sterilization controls were performed.

In antifungal susceptibility test, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 standard strains were used. For the preparation of inoculums, 24-hour fresh cultures of all isolates in the Sabauroud Dextrose Agar (SDA) medium were used in the preparation of yeast suspensions. Five colonies with a ≥1 mm diameter size were taken and suspended in 5 ml of 0.85% sterile saline. The yeast suspensions were homogenized by vortexing them for 15 seconds and then adjusted to 0.5 McFarland turbidity. With this process, stock yeasts containing 1-5x10^6 cells per mililiter were obtained. Stock yeast suspensions were diluted with RPMI-1640 medium first at the rate of 1/50 and later at 1/20, and 1-5x10 ^{3} cell/ml concentration to be used in the study was reached. The appropriate dilutions were performed using these suspensions and planted in SDA, and the correct concentration was determined by counting the colonies formed at the next day (CLSI M27-A3) (34).

In order to prepare antifungal stock solutions, the tubes containing two-fold drug concentrations between 32-0.06 µg/ml for amphotericin B were prepared using a pre-prepared antifungal stock suspension and diluted to the degree that was twice as much as the final antifungal concentration. To prepare the egg wax solutions, stock solutions prepared from the waxes dissolved in 10 ml Dimethyl sulfoxide (DMSO) arranged at a concentration of 10,000 µg/ml.

In the study, the determination process of the minimal inhibitory concentration (MIC) value was performed as follows: In accordance with antifungal susceptibility test CLSI M27-A3 standards (34), the micro dilution method was employed. Amphotericin B (32-0.06 µg/ml) was used as a control group, and DMSO was used as the negative control in the study. Serial dilutions (5,000-9.76 µg/ml) of the wax stock solutions were performed. The first well of the microplate in the horizontal row was determined as the sterility control, and the last well (12th well) as the reproduction control well. 100 µl RPM-1640 and 50 µl RPMI-1640 was added to the sterility well and reproduction control well, respectively. 50 µl of the dilutions were distributed to each column of the microplate as one waxy cover and drug concentration per column. It was dispensed with a pipette, keeping the highest concentration in the second well and the lowest concentration in the 11th well. After the preparation of the microplates, 50 µl of yeast suspensions arranged as one isolate per row was added. Yeast suspension was not added into the sterility control well. At the end of this process, both yeast and drug concentrations were diluted by ½, and study concentrations were reached. After the microplates were covered and incubated at 35 °C for 48 hours, the susceptibility results were visually evaluated. The lowest concentration without reproduction after incubation was determined as MIC (CLSI M27-A3) (34). The procedures were repeated 3 times using waxy cover obtained from different egg groups of the same species.

3. RESULTS

In this study, it was seen that the amount of egg batches obtained from 10 fully satiated females of each tick species would be sufficient to determine the repeated MIC value to be tested against at least three agents. Due to the solvents benefited, it was understood that the use of glass material was ideal at almost all steps of the study. One of the biggest problems that may be encountered in the process is the calculation of the amount of obtained lyophilisate, and at this point, it may be more accurate to try to determine the weight of the waxy cover, which is resistant to repeated dissolution, by dissolving it in a certain amount of solvent after lyophilization. At least, with such an approach, it was seen that it is necessary to confirm the calculations made.

In the trials, no antifungal effect was observed in any of the serial waxy cover concentrations (5.000-9.76 µg/ml) belonging to *H. marginatum* and *D. marginatus*. This result did not change in repetitions. On the other hand, in the serial waxy cover concentrations (5.000-9.76 µg/ml) belonging to *R. bursa*, an inhibition against *C. tropicalis* was identified, the MIC value was found to be 625 µg/ml, and the same results were obtained in repeated trials (Table 1).

Table 1. *Minimum inhibitory concentration (MIC) values of the tick egg waxes and reference substance, Amphotericin B (µg/ml).*

	Microorganisms		
Substances	*Candida albicans* ATCC 10231	*Candida tropicalis* ATCC 750	*Candida parapsilosis* ATCC 22019
Hyalomma marginatum	-	-	-
Dermacentor marginatus	-	-	-
Rhipicephalus bursa	-	625	-
Amphotericin B	1	2	1

(-) No antimycotic effects were observed at the concentrations of ≤5000 µg/ml.

4. DISCUSSION

Within the life cycle in nature, one of the most critical problems of the ticks is to protect the eggs until the larval hatching and maintain their vitality. The engorged female ticks that detached from the host prefer shady and humid areas such as clefts or cracks on the ground for laying eggs. However, such sites are the places where many pathogens, saprophyte bacteria and fungi are also found. This coexistence

especially possesses a potential problem for the tick species which have a long period of larval hatching (23). It is known that the egg wax is essential in terms of protecting the eggs from the microbiological, chemical, or physical factors (25, 26). In the studies carried out on some tick species, different type of molecules have been determined in the wax such as long-chain hydrocarbons, branched and unbranched alkanes, fatty acids, cholesterol, and some other steroid types, alcohols (20, 24, 26), and some specific proteins and lipoproteins (23, 25). It was stated that the wax is resistant to some physical and chemical factors such as cold, heat, proteinase K, and pronase (25). However detailed data have not yet been obtained at the point of the determination of the wax content, especially its bioactive substances (20, 26).

In the studies carried out to determine the antimicrobial activities of the wax, successful results have been obtained in various degrees against different agents such as *Escherichia coli* (22, 29), *Staphylococcus epidermidis* (25-27, 32), *Staphylococcus aureus* (22, 26, 29), *Serratia marcescens* (25), *Bacillus cereus* (26), *Bacillus subtilis* (25-27), *Micrococcus luteus* (27, 29, 30), *Pseudomonas aeruginosa* (22, 31), *Enterecoccus faecalis, Enterecoccus faecium, Nocardia asteroides, Salmonella enteritidis, Klebsiella pneumonia* (22), picornavirus (28), and influenza virus (28, 29). Furthermore, the wax of *Boophilus microplus* was reported to be effective on *C. albicans* to some extent (30), and the wax of *Rhipicephalus sanguineus* was found to be effective on the same agent (29). In our study, no effect was observed related to the wax of *H. marginatum* and *D. marginatus* on examined *Candida* species. Although similar results were detected from the wax of *R. bursa* against *C. albicans* and *C. parapsilosis*, some concentration of the wax of this tick species inhibited *C. tropicalis* (MIC: 625 µg/ml).

Related studies have obviously shown that the antimicrobial efficacy of tick egg wax is directly correlated to some factors such as the extraction methods and application dose of the wax, and the species of the ticks and microorganisms (22, 25, 29). The feature of the wax is well known to vary significantly according to the tick species, and it was indicated that this is an expected result, since the sites of egg-laying and habitat preferences of tick species are more or less different from each other (21, 33, 35). It was also clearly observed in our study, that the differences in the species of ticks and *Candida* directly affect the results. In accordance with the related information mentioned by the researchers (20, 36), our laboratory and field studies conducted in Thrace, Turkey, have revealed that *H. marginatum* and *R. bursa* lay eggs in hot months and in the relatively dry sites and *D. marginatus* during colder months and in more humid area. This difference creates the expectation that the physical and chemical properties of the egg wax of *D. marginatus* can differ from those of other tick species. However, our related studies have shown that the laying and larval hatching procedures of *R. bursa* exhibit significant differences compared to *H. marginatum* and *D. marginatus.* While the whole process of the egg-laying, larval hatching, and larval activation usually take a couple of weeks in the last two species, it can take months in *R. bursa*. As a result of this delayed process, although the adult stages of this species feed on the host mostly in and around June, the larvae are found on the host in the late autumn or winter (detailed data not presented). In this context, it seems like a natural consequence that the eggs of *R. bursa* are supported by a more featured wax, which ensure adequate protection to the eggs during the relatively long hatching period.

5. CONCLUSION

The results obtained from the egg wax of *R. bursa* was interpreted as it could be associated with its some distinctive biological characteristics, and it was concluded that the detailed studies with this and some other tick species that share similar biological and ecological features might yield significant results for the discovery of the new antifungals.

CONFLICT OF INTEREST

Authors declare no scientific and financial interest.

REFERENCES

[1] da Silva Dantas A, Lee KK, Raziunaite I, Schaefer K, Wagener J, Yadav B, Gow NAR. Cell biology of *Candida albicans*–host interactions. Curr Opin Microbiol 2016; 34: 111-118.

[2] Vengurlekar S, Sharma R, Trivedi P. Efficacy of some natural compounds as antifungal agents. Pharmacogn Rev 2012; 6: 91-99.

[3] Miceli MH, Díaz JA, Lee SA. Emerging opportunistic yeast infections. Lancet Infect Dis 2011; 11: 142-151.

[4] Papon N, Courdavault V, Clastre M, Bennett RJ. Emerging and emerged pathogenic *Candida* species: beyond the *Candida albicans* paradigm. PLoS Pathog 2013; 9: e1003550.

[5] Jahagirdar VL, Davane MS, Aradhye SC, Nagoba BS. *Candida* species as potential nosocomial pathogens – A review. Electron J Gen Med 2018; 15: em05.

[6] Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS and Rogers PD. Azole antifungal resistance in *Candida albicans* and emerging non-albicans *Candida* species. Front Microbiol 2017; 7: 2173.

[7] Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. Infect Drug Resist 2017; 10: 249-259.

[8] Jensen RH, Astvad KMT, Silva LV, Sanglard D, Jørgensen R, Nielsen KF, Mathiasen EG, Doroudian G, Perlin DS, Arendrup MC. Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. J Antimicrob Chemother 2015; 70: 2551-2555.

[9] Wang H, Xu YC, Hsueh PR. Epidemiology of candidemia and antifungal susceptibility in invasive *Candida* species in the Asia-Pacific region. Future Microbiol 2016; 11:11.

[10] Sarma S, Upadhyay S. Current perspective on emergence, diagnosis and drug resistance in *Candida auris.* Infect Drug Resist 2017; 10: 155-165.

[11] Shen B. A new golden age of natural products drug discovery. Cell 2015; 163: 1297-1300.

[12] Abad MJ, Ansuategui M, Bermejo P. Active antifungal substances from natural sources. Arkivoc 2007; 7: 116-145.

[13] Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013; 62: 10-24.

[14] Wang S, Wang Q, Yang E, Yan L, Li T, Zhuang H. Antimicrobial compounds produced by vaginal *Lactobacillus crispatus* are able to strongly inhibit *Candida albicans* growth, hyphal formation and regulate virulence-related gene expressions. Front Microbiol 2017; 8: 564.

[15] Wright AE, Bothelo JC, Guzman E, Harmody D, Linley P, McCarthy PJ, Pitts TP, Pomponi SA, Reed JK. Neopeltolide a macrolide from a Lithistid sponge of the family Neopeltidate. J Nat Prod 2007; 70: 412-416.

[16] Park J, Kwon O, An HJ, Park KK. Antifungal effects of bee venom components on *Trichophyton rubrum*: A novel approach of bee venom study for possible emerging antifungal agent. Ann Dermatol 2018; 30: 202-210.

[17] Führer E Willers D. The anal secretion of the endoparasitic larva *Pimpla turionellea:* sites of production and effects. J Insect Physiol 1986; 32: 361-367.

[18] Badr AN, Nada F, Shehata MG, Amra HA. Anti-mycotic and anti-mycotoxigenic properties of Egyptian dill. J Applied Sci 2017; 17: 184-195.

[19] Labruna MB, Leite RC, de Oliveira PR. Study of the weight of eggs from six ixodid species from Brazil. Mem Inst Oswaldo Cruz, Rio de Janeiro 1997; 92: 205-207.

[20] Sonenshine DE, Roe RM. Biology of Ticks. Vol. 1, 2nd ed. New York, Oxford University Press; 2014.

[21] Lees AD, Beament JWL. An egg-waxing organ in ticks. Q J Microsc Sci 1948; 89: 291-322.

[22] Yang X, Jia Q, Chen J, Hou Y, Zhai S, Yu Z, Liu J. Antibacterial activity of eggs and egg wax covering of selected Ixodid (Acari: Ixodidae) ticks. J Entomol Sci 2017; 52: 387-394.

[23] Booth TF. Observation on the composition and biosynthesis of egg wax lipids in the cattle tick, *Boophilus microplus*. Exp Appl Acarol 1992; 14: 137-149.

[24] Matsuo T, Okura N, Kakuda H, Yano Y. Reproduction in a metastriata tick, *Haemaphysalis longicornis*(Acari: Ixodidae). J Acarol Soc Jpn 2013; 22: 1-23.

[25] Arrieta MC, Leskin BK, Kaufman WR. Antimicrobial activity in the egg wax of the African cattle tick *Amblyomma hebraeum* (Acari: Ixodidae). Exp Appl Acarol 2006; 39: 297-313.

[26] Yu Z, Thomson ELS, Liu J, Dennis JJ, Jacobs RL, Kaufman WR. Antimicrobial activity in the egg wax of the tick *Amblyomma hebraeum* (Acari: Ixodidae) is associated with free fatty acids C16:1 and C18:2. Exp Appl Acarol 2012; 58: 453-470.

[27] Lima-Netto S, Mendonca RZ, Franzolin MR, Utescher CL, Orozco S, Máximo-Espindola C, Labruna M, Barros-Battesti DM. An interesting antimicrobial activity of egg wax from *Amblyomma cajennense* (Acari: Ixodidae). Syst Appl Acarol 2011; 16: 3-6.

[28] Lima-Netto S, Pinheiro A, Nakano E, Mendonca RMZ, Barros-Battesti DM, Mendonca RZ. Antiviral effect of the egg wax of *Amblyommacajennense* (Acari: Ixodidae). Cytotechnology 2012; 64: 601-606.

[29] Alduini N, Silva M, Franzolin M, Mendonça R, Lima-Netto S. Antimicrobial activity from ticks eggs waxes. BMC Proceedings 2014; 8: 156.

[30] Esteves E, Fogaca AC, Maldonado R, Silva FD, Manso PPA, Pelajo-Machado M, Valle D, Daffre S. Antimicrobial activity in the tick *Rhipicephalus* (*Boophilus*) *microplus* eggs: Cellular localization and temporal expression of microplusin during oogenesis and embryogenesis. Dev Comp Immunol 2009; 33: 913-919.

[31] Zimmer KR, Macedo AJ, Nicastro GG, Baldini RL, Termignoni C. Egg wax from the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* inhibits *Pseudomonas aeruginosa* biofilm. Ticks and Tick-borne Dis 2013a; 4: 366-376.

[32] Zimmer KR, Macedo AJ, Giordani RB, Conceição JM, Nicastro GG, Boechat AL, Baldini RL, Abraham WR, Termignoni C. A steroidal molecule present in the egg wax of the tick *Rhipicephalus* (*Boophilus*) *microplus* inhibits bacterial biofilms. Environmental Microbiol 2013b; 15: 2008-2018.

[33] Sonenshine DE, Tigner JA. Oviposition and hatching in two species of ticks in relation to moisture deficit. Ann Entomol Soc Am 1969; 62: 628-640.

[34] Clinical and Laboratory Standard Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts-third edition: approved standard M27–A3. Wayne: CLSI; 2008.

[35] Teel PD. Effect of Saturation Deficit on Eggs of *Boophilus annulatus* and *B. microplus* (Acari: Ixodidae). Ann Entomol Soc Am 1984; 77: 65-68.

[36] Estrada-Peña A, Mihalca AD, Petney TN. Ticks of Europe and North Africa: A Guide to Species Identification. 1st ed. Switzerland, Springer International Publishing; 2017.

How to cite this article: Bilgin N, Hacioglu M, Bozkurt Guzel C, Erdal B, Kar S. In-vitro anticandidial efficacy of tick egg wax from Hyalomma marginatum, Rhipicephalus bursa and Dermacentor marginatus. Clin Exp Health Sci 2020; 10: 108-112. DOI: 10.33808/clinexphealthsci.707924

Clinical and Experimental Health Sciences

Transition Experiences of Newly Graduated Nurses

Cennet Ciris Yildiz, Yasemin Ergun

Marmara University, Faculty of Health Sciences, Department of Nursing, Istanbul, Turkey.

Correspondence Author: Cennet Ciris Yildiz
E-mail: cennet_ciris@hotmail.com

Received: 08.08.2019 **Accepted:** 22.11.2019

ABSTRACT

Objective: This is a qualitative study based on content analysis in order to reveal the transition experiences of nurses in the first year of their profession.

Methods: The study was carried out between January 2018 and March 2018 with 30 newly graduated nurses, who work in three Training and Research Hospitals, two University Hospitals and three Private Hospitals with JCI Quality Certificate and have a maximum of one year of professional experience. Data were collected through semi-structured in-depth individual interviews using face-to-face interviews.

Results: Themes defined in the transition process of newly graduated nurses were emotional, sociocultural and developmental, physical and intellectual. In general, transition experiences of individuals were greatly influenced by the support that is given to the new graduate, the experience, the predictability, sincerity and consistency of the relationships with the individuals they were in contact with, and the expectations of the institution or experienced employees. In the first year of their professional experience, graduates do not feel themselves qualified, comfortable, safe and secure, and experience emotional and physical exhaustion in a short time. In addition, changes in life patterns and routines have emerged as distracting developments or unexpected burdens, particularly for new graduates experiencing adaptation problems.

Conclusion: This study focused on the experiences of nurses during their first-year practice which is incredibly challenging for the new graduated nurses. It has been determined that formal and informal support programs are necessary for the new graduates to successfully start the profession.

Keywords: Nurse, Nursing Staff, Nurse's Scope of Practice, Transition

1. INTRODUCTION

The transition from professional education program to the profession was considered as a stressful period involving adaptation difficulties (1, 2). This is because new graduates join the work environment without feeling ready. Especially nurses are required to adapt to the working conditions as soon as possible due to reasons such as lack of nurse workforce and to have the necessary knowledge, skills and confidence (3). The newly graduated nurses undergo a number of developmental stages depending on the difficulties they face during the transition process. Duchsher (3) found that newly graduated nurses were selfish and independent in the first few months of their professional experience; that nurses felt safer and more self-confident between the 2nd and 3rd months; and that nurses developed critical thinking skills and started to work independently in the 5th month. Mckenna and Green (4) found that nurses tried to adapt to the profession and focused on themselves in the first 6 months of the transition period. They stated that the nurses focused on the larger picture such as patient care, relationships and their continuing development in the second 6 months of the transition period (4).

Initially, the newly graduated nurse tries to accept it when she encounters a new workplace culture. But in this process the nurse is likely to be uncomfortable and may experience a feeling of inadequacy. The new graduate experiences the shock of reality created by a clash of ideal and value when she moves to a culture that is often different from the culture, she comes from by gaining certain values and ideals. In some cases, reactions to inequality between expectations and reality are so great that individuals cannot cope with this situation (1). The entry of the new graduate into the work area with such an attitude and behavior is a clear indication that she is poorly prepared for the work area. It is inevitable that individuals who are equipped with non-realistic skills, technical knowledge, non-role-specific behaviors and who do

DOI: 10.33808/clinexphealthsci.604266

not have real values related to work will experience serious role conflict (1, 5). Duchsher (6) states that newly graduated nurses experience "awareness of the truth", "frustration" and "disassociation" because they encounter inconsistency between theory and practice environment. In addition, some studies have shown that newly graduated nurses experience different problems such as anxiety, tension, fear, panic and burnout in the first years of transition to the profession and these problems endanger their physical and mental health, accelerate the employee turnover rate (6).

The high turnover rate of nurses brings additional financial burden to both the hospital and the national economy. Kelly and Ahern (7) stated that in the first year of employment, the cost of turnover rate of newly graduated nurses represented a loss of approximately $ 40,000, including hiring and orientation costs. It was found that medical errors increased, and the quality of care decreased in the newly graduated nurses due to the problems experienced during the transition process.

Present study was carried out in order to reveal the transition experiences of nurses in the first year of their profession, because of the importance of the subject in terms of nursing services, health institutions and vocational education institutions, and due to the limited number of studies on transitional experience at national level.

2. METHODS

2.1. Design and participants

The participants of the study consisted of the nurses with bachelor's degree, who work in three Training and Research Hospitals, two University Hospitals and three Private Hospitals with JCI Quality Certificate in Istanbul's Anatolian side and have a maximum of one year of professional experience.

In the study, maximum diversity sampling method, which is one of the purposeful sampling methods that maximally reflect the diversity of individuals who can be a party to the study, was used. In addition, for sampling, attention was paid to select the participants that differ in terms of the aspects such as school that was graduated from, the hospital/unit that was worked for, and gender. As a result of repeated data, data saturation was achieved, and data collection was terminated in thirty interviews.

2.2. Data collection

In order to reveal the transition emotions of the nurses in the study, a 40-item open-ended "Semi-Structured Interview Form" was prepared by the researcher based on the "Transition Shock" model developed by Duchsher (6). Data were collected through semi-structured in-depth interviews. The interview form was drawn up on the basis of a review of the literature and contains questions on the participants' reasons for choosing nursing, their expectations after graduation and whether or not these expectations have been met, the difficulties they faced when first hired, the support they have, the ability to apply their training, differences between training and actual practice, satisfaction with work and their intent to leave the profession (1-4). The created questions were then assessed by a faculty member who an expert in the area of qualitative research was. A pilot interview was conducted with 2 nurses with the same characteristics as the interview form sampling group before starting the study. After the pilot application, final arrangements were made regarding the interview form. The answers to the study questions were recorded during the interview. Recorded responses were transferred to computer environment. The interviews were held over the period December 2017 – March 2018 following receipt of the needed approvals for the study. An appropriate setting was created for the interviews, which took place either in the department meeting rooms or in nurses' offices. A researcher took part in the interviews. Interviews of approximately 45-150 minutes were conducted with the interviewees. Although some of the participants were promised that their personal details would remain confidential, they were not comfortable during their assessment of their institution. During the interviews, length of some interview was long in order to make participants feel comfortable and to obtain detailed information.

2.3. Ethical considerations

Prior to the study, written and verbal permissions were obtained from the Clinical Research Ethics Committee (Marmara University Faculty of Medicine Clinical Research Committee – Date: December 03, 2017 – No: 09.2017.668) and hospital management. Each participant was informed about the purpose and method of the study, and an informed consent form was signed indicating that they voluntarily participated.

2.4. Statistical analysis

Content analysis and the MAXQDA program was used in the analysis of the data. The data recorded with the device were transferred to the computer, after which the researcher listened to the recordings once again. The report was then revised to ensure a correct rendition of what the participants meant to say. Responses that were meaningful were assigned names/codes. Similar codes appearing in different parts were brought together to form a code list. Examining the similarities and differences between codes led to the designation of themes/subthemes. The themes and subthemes were evaluated by 3 experts who had training in qualitative research and their final construct was created. The data collected in the study were categorized based on Duchscher's (6) "Transition Shock Model" where the categories were delineated as emotional, sociocultural and developmental, physical and intellectual. In this study, consistency formula was used to determine reliability in content analysis. In this study, the agreement percentage was determined as 76%. Descriptive content of the newly graduated nurses was coded by giving numbers like K1, K2,…. K30, and the findings related to the participants' characteristics are given in Table 1.

Table 1. *Findings on the Identifying Characteristics of the Participants*

Interview Number	Age	Gender	Institution worked	Department worked	Tenure	Having orientation training	Another department/ institution worked	Intention to Quit
K1	23	F	Private Hospital	Mixed Surgery	6 months	1 month	2 Months in Emergency	Considering
K2	24	F	Private Hospital	Cardiology	8 months	3 weeks	-	Considering
K3	23	F	Private Hospital	Intensive Care	1 year	1.5 months	-	Considering
K4	23	F	Private Hospital	Urology	1 year	2 months	-	Considering
K5	23	F	Private Hospital	Operation Room	6 months	1 month	4 months at Pharmacy	Not Considering
K6	24	F	Private Hospital	Cardiology	8 months	20 days	-	Considering
K7	25	F	Private Hospital	Anesthesia Intensive Care	7 months	3 days	8 months in Gynecology	Considering
K8	24	F	Private Hospital	Pediatric	11 months	2 months	-	Not Considering
K9	24	F	Private Hospital	Mixed Surgery	6 months	1 week	3.5 months in Gynecology	Not Considering
K10	25	M	Private Hospital	Emergency	3 months	1 month	-	Not Considering
K11	25	M	Training and Research Hospital	Intensive Care	7 months	1 month	3 months in Internal Medicines Unit	Considering
K12	24	F	Training and Research Hospital	Newborn Intensive Care	6 months	15 days	-	Considering
K13	23	F	Training and Research Hospital	Palliative Care	11 months	10 days	7 months at LOSEV	Considering
K14	23	M	Training and Research Hospital	Ophthalmology	7 months	2 weeks	-	Not Considering
K15	25	F	Training and Research Hospital	Urology	3 months	2 weeks	-	Considering
K16	24	M	Training and Research Hospital	Pediatric Emergency	6 months	1.5 months	-	Considering
K17	24	F	Training and Research Hospital	Newborn Intensive Care	6 months	2 months	-	Not Considering
K18	22	F	Training and Research Hospital	Hematology	6 months	1 week	-	Considering
K19	22	F	Training and Research Hospital	Internal Medicines	6 months	20 days	-	Considering
K20	23	F	Training and Research Hospital	Orthopedics	7 months	3 weeks	-	Not Considering
K21	23	F	Training and Research Hospital	Brain Surgery	4 months	2 months	-	Not Considering
K22	23	F	Training and Research Hospital	Intensive Care	1 year	1 month	2 months in Intensive Care	Not Considering
K23	23	F	University Hospital	Perinatology	1 year	1 month	-	Considering
K24	24	M	University Hospital	Emergency Surgery	10 months	1 month	-	Not Considering
K25	24	F	University Hospital	Otorhinolaryngology	10 months	2 months	4 months in Intensive Care	Not Considering
K26	24	F	University Hospital	Gynecology-Oncology	10 months	1 month	-	Considering
K27	25	F	University Hospital	Pediatric Emergency	11 months	2 months	2 months in Intensive Care	Not Considering
K28	23	F	University Hospital	Pediatric Endocrinology	7 months	1 month	3 months in Surgery	Not Considering
K29	23	F	University Hospital	Urology	11 months	1 month	-	Considering
K30	24	F	University Hospital	Private	10 months	2 months	8 months in Intensive Care	Considering

F: Female, M: Male

2.5. Research limitations

This study reflects the opinions of newly graduated nurses who participated in the study. Therefore, the results cannot be generalized.

3. RESULTS

As a result of the content analysis of the data obtained from the individual in-depth interviews with the newly graduated nurses, the transition experiences of the newly graduated nurses were collected under four main themes. The main themes obtained were: 1 – Emotional, 2 – Physical, 3 – Sociocultural and Developmental, 4 – Intellectual.

3.1. Emotional

Participants mentioned that they experienced intense emotional fluctuations in the first phase of the transition. Most of the nurses stated that they experienced boredom, stress and occasional physical and psychological weakness in the first year of the transition. The participants expressed their concerns by using the words "I was scared" and "I am sorry".

"... It's been 2 months since I moved to level 2 intensive care. I always monitor the care of the intubated patient… Different nurses performed the same practice in different ways in the unit. I was having trouble with what form of application I should adopt …" (K17)

"... I have come to understand that there is nothing as bad as for the newly graduated nurse to start in the intensive care unit… On the first day of my employment, patient care responsibility was given, and my patient suffered a cardiac arrest. I could not participate in the heart-lung resuscitation process, I cried, so much so I nearly walked away. I was saying "Find someone else, I cannot do it"... my other friends... they supported me as well... if they hadn't, I probably would have left the profession (K1).

"When I first saw the cardiac arrest, I thought if it happened because of me. In my first shift, I was looking at four elderly patients in their ninety years old.... I had difficulty getting used to. I was very upset, and I was crying constantly when I got home." (K3).

During the transition, the nurses are experiencing intense anxiety about taking the sole responsibility for the entire unit. In particular, it was determined that they spent the time required to sleep and relax by thinking about what might happen in shifts. This causes early burnout in nurses.

"In the past, for example, before I came to the shift, I would lose sleep... When communicating with doctors, for example, I did not know how to communicate with them, nor how to talk with them. I didn't know, for example, how to give blue code, etc., I didn't know where to get it… I was constantly asking at first, I was always ashamed, as if I didn't know anything" (K19).

3.2. Physical

The physical responses of the newly graduated nurses during the transition process are emerged due to heavy of the responsibilities.

*"... I thought I was very tired during the internships... I was taking home with me someone's responsibility... However, my working life ruined me in 8 months... I came here and there was an intensive tempo; there are days when I come to work every day, sometimes I keep watch and ward in a row, you are getting both mentally and physically tired" (*9K).

The nurses stated that their energies are high and willing at the beginning of the adaptation process. However, over time, this excitement gradually decreased, and even some nurses mentioned that they were exhausted in a short time.

"It was very difficult... When I came here, I had a fear inside, I was saying myself 'God, how will I do it?. I found myself in the tempo of working all of a sudden. Go to work in the morning, go out in the evening, get a salary at the end of the month... I have done something, I have achieved something, I was very excited at first and then I got used to the routine… (3K).

Changes in life patterns and routines have emerged as distracting developments or unexpected burdens, particularly for new graduates experiencing adaptation problems.

"Normally when you are in school, you hang out as you wish, you do not go to class if you do not want. No one is expecting too much from you. But once you are graduating, you are laid burdens on you, which are, for example, taking a patient's responsibility, financial support to family members, etc. Is not there a gradual transition to it?" (28K).

During the interviews, it was observed that the new graduates were given excessive role and responsibility without adaptation process. The nurses stated that they experienced physical complaints in a short time depending on the severity of the working conditions. The most common physical disorders are; low back and back pain, getting ill frequently, varicose veins.

"... So the workload is too much… Then constant twenty-four hours of shifts… For example, at eight o'clock in the evening I'll hand over the shift and leave, someone calls saying the service suffers from the lack of personnel, so you will continue the shift, or you are assigned to that service, I was constantly…. I was catching a severe flu thrice within two months…." (18K).

3.3. Sociocultural and developmental

Being accepted by the team, balancing work and private life and reflecting the education received into practice have been the main socio-cultural and developmental tasks that new graduates perform.

"… Senior nurses want us to learn and practice what they teach at work immediately, when we cannot, they blame us with inadequacy. Although I know everything, including

diseases, applications, etc., they consider new graduates as novice, and disdain.... " (1K).

"*My husband says I constantly work every weekend. He is grumbling about why it is always me who is going in the shifts, asking if it is because I am new? … In the process of adaptation to work life, negative situations may arise in private life. You have to leave your spouse or your mum at home when they are sick. I can't stay with them while they need me… You hate, you can't breathe, but you have to go to work anyway…*" (24K).

Professional identity problems, disapproval from the patients or relatives and insecure attitudes caused the new graduate to feel inadequate.

"I was so scared, I didn't want to be seen as a beginner. This time, patients and their relatives were saying 'You are new, you cannot do it, please send someone with experience', when they realized that I was a new graduate. And I had no choice but lying to them, saying that I had already been working for this hospital for 5 years, etc…" (6K).

Developmentally it was important to get feedback from colleagues and managers.

"I was very curious about the evaluations about my work after my shift and how I was, etc... I was just worrying to be not good enough and that they did not tell it to me. After the shift, when my nurse colleagues told me that I was pretty good, I felt relieved..." (27K)

On the other hand, nurses who have started to work in some institutions have adopted a work-centered approach instead of providing quality patient care.

Our teachers would tell us that you would approach the patient holistically at school. *They would say that, in the postoperative period, we should check their vital signs at regular intervals... However, the vital signs should be checked every 15 mins., but it is not possible as there are 13 patients, which is too much for us. You are trying to do it in the beginning, but then you lose your motivation and stop taking care of. As the result of such negative experiences, you quit being patient-oriented and take a work-oriented approach…" (24K).*

Negative relationships with other professionals in the clinical setting are factors that consume energy and hinder development.

"When I started to work, a few people made me feel like I was new. Initially I felt as if I was working for them… They were harsh like a boss, ordering me to bring this, do that, etc. When I experienced something like that, I held myself back…" (3K).

3.4. Intellectual

Some of the participants felt themselves inadequate as a nurse while others stated that they could not transfer the knowledge they learned during their education to practice.

"I have experienced the difficulty of lack of information deeply… Since I did not know anything, I felt inadequate to meet the care needs of the patients. I could not care for all four patients. From the patient's perspective, there could have been a nurse in my place who could give them better care. I am suffering now." (2K).

"I think that I am lack of self-confidence, I'm still nervous while attempting to care for the patient... If I have been unsuccessful in the patient I have undertaken before, I worry that I will fail again.... (26K).

Some of the difficulties that these graduates face in their transition to the profession stem from the approach of senior nurses, clinical trainers and nurse managers towards orientation. As a result, the new graduate is expected to undertake the workload of an experienced practitioner within a few weeks.

In the first month, you attend the orientation training, nobody tells you anything, in the 2nd month, they are trying to teach you, but in the 3rd month, you are now all alone, you are also afraid of asking something, you cannot ask because of the psychology of being considered inadequate, so I did not ask questions unless I am pushed for it…" (18K)

4. DISCUSSION

Duchscher (3) has demonstrated that the transition to the profession affects new graduated nurses emotionally. In the study, nurses also stated that their energies were high, and they were eager to work early in the adaptation process. However, over time, this excitement and eagerness have been replaced by physical and psychological burnout. Similarly, Hezaveh et al. (8) found that the stress experienced during the transition to the profession affected nurses physically, emotionally and mentally. Kumaran and Carney (9) found that during the transition period, newly graduated nurses initially felt themselves "excited", "happy", "relaxed", "proud" and "grown up", however, they found that these initial emotions were later replaced by "anger" and "frustration".

One of the main findings of this study was that the needs of newly graduated nurses are different from those of experienced nurses. For example, some newly graduated nurses expect feedback and support from colleagues and managers. Rush et al. (10) suggested that nurses needed formal and informal support in the first 6 to 9 months of professional experience. After starting work, the new graduates are expected to take responsibility of many patients in a short time and to be able to perform unit tasks. Duchscher (3) found that nurses had a "confidence crisis" in the first 5 to 7 months of their professional experience. Gregg et al. (11) revealed that nurses experienced frustration and intense anxiety during the transition period. The reasons for this can be listed as the lack of knowledge and skills, providing unsafe care, and the fear of giving harm to the patient and not being able to cope with responsibilities (3, 10, 11). In addition, lack of support and negative experiences

in the work environment can be added to the causes of the anxiety in the study.

Development of the professional identity, being accepted by the team, balancing work and private life and reflecting the education received into practice have been the main socio-cultural and developmental tasks that new graduates perform. Gordon et al. (12) suggested that the new graduate's taking on all roles and responsibilities as a nurse is the main source of stress during the transition period. They demonstrated that consistent support in the clinical setting is important in relieving this stress. Negative relationships with other professionals in the clinical setting are factors that consume energy and hinder development. It takes time for the new graduated to establish professional relationships, learn the norms of the working environment, reflect the education they receive to the practice, and make clinical decisions. In particular, experienced nurses should give time and opportunity to the new graduates and gradually give responsibility (13).

The adaptation of the new graduate to the professional role is achieved through observation within the social networks in the workplace. In particular, as nurses gain confidence in their new roles, they assume more responsibility and manage increasingly complex clinical situations. However, the new graduate's ability to effectively manage the workload and gain the necessary technical skills (13). Gordon et al. (12) emphasized that a new graduated nurse should be taken to the adaptation training in order to work like an experienced nurse and to develop her/his skills. In the study, it was determined that the majority of the nurses received orientation training in the institutions, but some of the difficulties faced by the new graduate in the transition to the profession were caused by the approach of the senior nurses, clinical trainers and nurse managers to the orientation. As a result, the new graduate is expected to undertake the workload of an experienced practitioner within a few weeks. Gordon et al. (12) suggested that new graduate adaptation trainings should be developed and reviewed in order to ensure adaptation of the new graduate to the work environment. Goode et al. (13) stated in their study that the new graduates who started to work in a healthy working environment adapted more quickly and had a lower turnover rate.

The transition from studentship to profession is considered to be stressful not only physically and emotionally, but also socio-culturally and developmentally. Goode et al. (13) found that newly graduated nurses had difficulties in areas such as delegation of authority, setting priorities, managing care, collaborating with other disciplines, conflict resolution and benefiting from feedback. In the studies, it is emphasized that the practice-based education and hospital adaptation programs realized after professional education should be reviewed in terms of content (12-13). These regulations not only reduce the turnover rate of new graduates, but also ensure cost-effectiveness (14). Transition programs that include patient-centered care, quality improvement, evidence-based practice, communication and teamwork, informatics, security, clinical expertise, feedback, reflection, and information have been reported to provide more support to new graduates (15, 16).

5. CONCLUSION

It was observed that nurses had emotional, physical, socio-cultural, developmental and intellectual problems during the transition process. Also, it was determined that while the support provided during the transition process facilitated the transition process of the nurses, however, this support was not sufficient. In particular, the concerns of new graduates need to be paid attention to facilitate the transition. Assessment of nurses' experiences and meeting of their needs in the first years of their professional life will increase the self-confidence and competence of new graduates. In that regard, nurse managers, trainers and researchers have important duties.

Acknowledgment: This article was based on a part of PhD thesis of the first author

REFERENCES

[1] Kramer M. Reality Shock: Why Nurses Leave Nursing. 1st ed. St Louis: Mosby Company; 1974. pp.3-4.

[2] Oermann MH, Garvin MF. Stresses and challenges for new graduates in hospitals. Nurse Educ Today 2002; 22:225–230.

[3] Duchscher JEB. A process of becoming: The stages of new nursing graduate professional role transition J Contin Educ Nurs 2008; 39(10): 441–450.

[4] McKenna LG, Green C. Experiences and learning during a graduate nurse program: An examination using a focus group approach. Nurse Educ Pract 2004; 4(4):258-263.

[5] Öztürk H, Kurt Ş, Serin GM, Bayrak B, Balık T, Demirbağ BC. Hastanelerde işe yeni başlayan hemşirelerin sorunları. ACU Sağlık Bil Derg 2016; 4:189-201 (in Turkish).

[6] Duchscher JEB. Transition shock: The initial stage of role adaptation for newly graduated Registered Nurses. J Adv Nurs 2009; 65(5):1103-1113.

[7] Kelly J, Ahern K. Preparing nurses for practice: A phenomenological study of the new graduate in Australlia. J Clin Nurs 2009; 18(6):910-918.

[8] Hezaveh MS, Rafil F, Khosravi S, Seyedfatemi N. The experience of stress among new clinical nurses. Nurs Pract Today 2014; 4: 199–206.

[9] Kumaran S, Carney M. Role transition from student nurse to staff nurse: Facilitating the transition period. Nurse Educ Pract 2014; 14(6): 605-611.

[10] Rush KL, Adamack M, Gordon J, Lilly M, Janke R. Best practices of formal new graduate nurse transition programs: An integrative review. Int J Nurs Stud. 2013; 50(3):345-356.

[11] Gregg MF, Wakisaka T, Hayashi C. Nurse managers' strategies for the integration of newly graduated nurses into clinical units in Japan: A qualitative exploratory study. Open Nurs J 2013; 7: 157-164.

[12] Gordon CJ, Aggar C, Williams AM, Walker L, Willcock SM, Bloomfield J. A transition program to primary health care for new graduate nurses: A strategy towards building a

sustainable primary health care nurse workforce? BMC Nurs 2014; 13(34):2-7.

[13] Goode CJ, Glassman KS, Ponte PR, Krugman M, Peterma T. Requiring a nurse residency for newly licensed registered nurses. Nurs Outlook 2018; 329–332.

[14] Letourneau RM, Fater KH. Nurse Residency Programs: An integrative Review of the Literature. Nurs Educ Perspect 2015; 36(2): 96-101.

[15] Spector N, Blegen MA, Silvestre J, Barnsteiner J, Lynn MR, Ulrich B, Alexander M. Transition to practice study in hospital settings. JNR 2015; 5(4), 24–38.

[16] Silvestre J, Ulrich J, Johnson T, Spector N, Blegen M. A multisite study on a new graduate Registered Nurse transition to practice program: Return on investment. Nurs Econ 2017; 35(3): 110-118.

How to cite this article: Ciris Yildiz C, Ergun Y. Transition Experiences of Newly Graduated Nurses. Clin Exp Health Sci 2020; 10: 113-119. DOI: 10.33808/clinexphealthsci.604266

Clinical and Experimental Health Sciences

Can *Juniperus communis* L. oil improve nephropathy in diabetic rats

Tahir Kahraman[1], Mehmet Berkoz[2], Oruc Allahverdiyev[3], Evan Abdulkarim Mahmood[2], Metin Yildirim[4], Serap Yalin[4]

[1] Karabuk University, Faculty of Medicine, Department of Medical Biochemistry, Karabuk, Turkey.
[2] Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Biochemistry, Van, Turkey.
[3] Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology, Van, Turkey.
[4] Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey.

Correspondence Author: Mehmet Berkoz
E-mail: mehmet_berkoz@yahoo.com

Received: 22.03.2019 **Accepted:** 07.10.2019

ABSTRACT

Objective: *Juniperus communis* L. (*J. communis*) is a shrub belonging to family Cupressaceae L. mainly growth in Eurasia. The antioxidant and antidiabetic activity of aqueous extract of *J. communis* L. berries indicated benefits as a potent antidiabetic in streptozotocin induced diabetic albino rats. This study was carried out to determine whether *J. communis* L. oil supplement will effectively manage renal dysfunction in diabetic rats.

Methods: Twenty eight rats were divided into 4 equal groups as follows; control group, diabetic group (45 mg/kg, i.p. streptozotocin) , *J. communis* L. oil (200 mg/kg) treated group, and diabetic+*J. communis* L. oil (200 mg/kg) treated group. At the end of the experimental period, all rats were sacrificed and renal function parameters such as kidney antioxidant and lipid peroxidation markers and serum glucose, HbA1c, creatinine, serum urea, blood urea nitrogen (BUN), and serum total protein levels were measured in all groups.

Results: HbA1c, serum glucose, urea, creatinine, BUN and, kidney lipid peroxidation levels increased ($p<0.05$), but serum total protein and antioxidant levels decreased in diabetic group comparing with control group ($p<0.05$). Furthermore, HbA1c, serum glucose, urea, creatinine and BUN and, kidney lipid peroxidation levels decreased and also, serum total protein and antioxidant levels increased in diabetic group treated with *J. communis* L. oil comparing with diabetic group ($p<0.05$).

Conclusion: This study has provided that *J. communis* L. oil provide a protective effect on the kidney as evidenced by an improvement of the renal function tests as well as reduction in oxidative stress parameters in experimental diabetic nephropathy model.

Keywords: *Juniperus communis* L., diabetes, nephropathy, antioxidant, lipid peroxidation, oxidative stress

1. INTRODUCTION

Diabetes, characterized by hyperglycemia and metabolic disturbance on lipids, carbohydrates, and proteins, affect the life quality of patients by bringing huge pressure to society and public health (1). Nearly 2.2% of total death in the world is caused by diabetes (2). Type II diabetes, considered as the common form of diabetes, will affect the health of 8 billion people in the world till 2025 (3). Persistent hyperglycemia in diabetes mellitus (DM) leads to the development of secondary complications including neuropathy, nephropathy, and retinopathy (2). Diabetic nephropathy is the major cause of end-stage renal disease with high mortality and morbidity (4). A major clinical manifestation of diabetic nephropathy is that microalbuminuria follows macroalbuminuria and further leads to renal dysfunction which is the reduced capacity of the kidney to excrete metabolic products which accumulate in the body system and can be detected via renal function test (5). Therefore, due to numerous degenerative effects of untreated DM on human system, numerous researches have been carried out and still ongoing for the management and treatment of DM. Management of DM usually involves adjustment of the diet of the individual, exercise at regular interval, health education, measurement of blood glucose level on a regular level, and, in case of insulin dependent DM, supplementary therapy with insulin (6,7).

Juniperus communis L. is a shrub belonging to family Cupressaceae L. mainly growth in Eurasia, North Africa and North America at an altitude of 1500–4000. Fruit is subspherical, purplish-black and seed contained 2-3 layers of thin-walled cells. The seeds and fruits of the plant contain camphene, d-α-pinene, formic acid, acetic acid, wax, gum, cyclohexinol, terpene, ascorbic acid, dihydrojunene, cadinene, juniper, and camphor (8,9). *J. communis* L. berry oil mainly contained monoterpene hydrocarbons such as α-pinene (51.4%), β-pinene (5.0%), sabinene (5.8%), and myrcene (8.3%) (10). *J. communis* L. can be used (traditionally) for renal suppression, acute

DOI: 10.33808/clinexphealthsci.543272

and chronic cystitis, catarrh of the bladder, albuminuria, leucorrhoea, and amenorrhoea. *J. communis* L. fruit can be used as being antiseptic, stimulant, and styptic. It can also be used in the treatment of migraine, infantile tuberculosis, rheumatic and painful swellings, chronic Bright's disease, piles, and nephrotic dropsy of children (11). The plant was reported to have analgesic, antibacterial, hepatoprotective, antihypercholesterolemic, antiinflammatory, antioxidant, antidiabetic, antihyperlipidemic, anticataleptic, and antimicrobial activities (9).

The antioxidant and antidiabetic activity of aqueous extract of *J. communis* L. berries indicated potential benefits as a potent antidiabetic in streptozotocin (STZ) induced diabetic albino rats (12). *J. communis* L. berries was also a good scavenger for nitric oxide radicals and has a potential source of natural antioxidant. *J. communis* L. berry has also nutraceutical uses and is used in treatment of hypercholesterolemia and hyperglycemia, and also, as a nutritional supplementation, it can be prescribed as food appendage for coronary artery disease patients along with their regular medicines (9).

Therefore, since renal dysfunction has been on increase in diabetic patients without a promising remedy, this study was carried out to determine whether dietary intake of *J. communis* L. oil will protectively or effectively manage renal dysfunction via examination of renal function parameters such as kidney antioxidant and oxidative stress markers and serum creatinine, serum urea, blood urea nitrogen (BUN), and serum total protein estimation in diabetic rats.

2. METHODS

2.1. Animals

All adult male Wistar albino rats were purchased from the Animal Experimental Center of Van Yuzuncu Yil University, aged 3-4 months with weights ranging from 300 to 350 g. Ethical approval of the study was obtained from Van Yuzuncu Yil University Ethical Commission for Animal Experiments (Decision number: 2015/14, Date: 24.12.2015). All animals were housed in a comfortable environment (12 h light-dark cycle, 20–25 °C temperature, 40-60 % humidity) and fed with rodent chow and water *ad libitum*.

2.2. Reagents

All the chemicals and reagents were supplied by Sigma-Aldrich, Merck or other standard suppliers.

2.3. Preparation of J. communis L. oil

The fresh air dried *J. communis* L. berries (300-400 g) were subjected to water-distillation boiling (>100 °C) for 3 h by using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and after filtration through Whatman filter paper (No. 1) stored at 4 °C until tested.

2.4. Experimental Procedure

Type 1 DM was induced by a single intraperitoneal (i.p.) injection of 45 mg/kg STZ to overnight fasted rats. STZ solution was freshly prepared by dissolving in 0.1 M cold citrate buffer (pH 4.5). In spite of the possibility of sudden hyperglycaemic shock following STZ injection in animals, this dose was considered appropriate. Diabetes was confirmed through the determination of blood glucose levels at 72 hr using OneTouch UltraMini glucometer (LifeScan, Inc., California, USA). Rats with blood glucose levels higher than 300 mg/dL were considered diabetic and selected for further experiments. Diabetes was further verified by measuring blood glucose levels 7 days after STZ injection. Normal control rats received a single i.p. dose of physiological saline. Twenty eight rats (14 diabetic and 14 normal) were divided into 4 equal groups (n=7) as follows; Group I (Control group): Nondiabetic rats received physiological saline orally for 21 days, Group II (Diabetic group): Diabetic rats received physiological saline by oral gavage for 21 days, Group III (*J. communis* L. oil group): Nondiabetic rats received *J. communis* L. oil (200 mg/kg) dissolved in 5% Na-CMC by oral gavage for 21 days, and Group IV (Diabetic+*J. communis* L. oil group): Diabetic rats received *J. communis* L. oil (200 mg/kg) dissolved in 5% Na-CMC by oral gavage for 21 days. Ethical approval of the study was obtained from Ethical approval of the study was obtained from Van Yuzuncu Yil University Ethical Commission for Animal Experiments (Decision number: 2015/14, Date: 24.12.2015).

2.5. Preparation of Serum and Kidney Tissue

At the end of the experimental period, all rats were fasted for 12 hours before being sacrificed by intraperitoneal injection of ketamine hydrochloride (15 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Kidney tissues were immediately excised from the surrounding tissues and were subsequently weighed. Immediately following collection, kidney tissues were washed with ice-cold phosphate-buffered saline (PBS). The samples were homogenized in phosphate buffer (25 mM, pH 7.4) to make approximately 10% w/v homogenates. The homogenates were centrifuged at 1700 rpm for 10 min, and the supernatant was collected and stored at −70°C for further biochemical analysis. The protein concentration in the supernatant was estimated by Lowry et al's method.

2.6. Serum Preparation and Biochemical Measurements in Serum

Blood samples were placed in dry test tubes and were allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 3500 rpm for 15 min. Enzymatic colorimetric kits (Bioscience, Cambridge, UK) were used to measure serum glucose, total protein, creatinine, urea, and blood urea nitrogen (BUN) levels.

2.7. Measurement of serum glycosylated hemoglobin (HbA1c) Level

Serum HbA1c levels were measured in whole blood using an automated chemistry analyzer (Roche Cobas Integra 800 Chemistry analyzer, Roche Diagnostics, Mannheim, Germany).

2.8. Measurement of lipid peroxidation and antioxidant enzyme activities in kidney

The supernatants obtained after this procedure was used for the analyses of MDA, NO, and GSH levels and SOD and catalase activities.

MDA Level: MDA reacts with thiobarbituric acid (TBA), giving a spectroscopically readable final product at 532 nm. MDA levels were expressed as nmol/mg protein using the extinction coefficient value of $1.56x10^5$ $M^{-1}.cm^{-1}$ (13).

Nitrite/Nitrate levels: Tissue nitric oxide is rapidly converted to nitrate and nitrite in aqueous solutions. Hence, for accurate assay of the total nitric oxide, both nitrate and nitrite levels must be determined. Tissue nitrate is chemically reduced to nitrite by granulated cadmium. Griess reagent reacts with total nitrite, and forms a coloured complex. The intensity of the colour is proportional to the concentration of the nitrite in the sample, which can be measured spectrophotometrically (14).

GSH level: GSH analysis was performed according to the method reported by Beutler et al (15). In this method, all proteins that do not carry the sulfhydryl in the tissue homogenates are precipitated. In the obtained clear liquid, the yellow complex formed by 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) and sulfhydryl groups is measured colorimetrically at a wavelength of 412 nm.

SOD Activity: The principle of measurement of SOD enzyme activity, which accelerates the aquatic and molecular oxygen dismutation of endogenous and exogenous sources of toxic superoxide radicals generated during the production of oxidative pathway energy, is based on the spectrophotometric measurement of superoxide radicals which are released by xanthine oxidase in the presence of xanthine in the presence of nitroblue tetrazolium (NBT) at 560 nm according to Sun et al (16).

Catalase Activity: The activity of the enzyme catalase was analysed according to Aebi method (17), measuring the initial rate of H2O2 decomposition at 240 nm. Catalase activity was expressed as U/mg protein.

Tissue Protein Content: In the alkaline solution, a copper-protein complex is formed. This complex reduces the phosphomolybdate phosphotungstate reagent (Folin-Ciocalteus-Phenol Reagent) and forms a dark blue color. The resulting darkness is directly proportional to the protein concentration in the medium according to Lowry el al (18).

2.9. Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) plus Tukey post-hoc analysis. Statistical analyses were performed using the SPSS software version 15.0 (SPSS Inc., Illinois, USA). All data are indicated as means ± SD. In addition, increases or decreases between the groups are indicated as percentages. $p<0.05$ was considered statistically significant.

3. RESULTS

Compared with Group I, the enhanced serum glucose, HbA1c, creatinine, urea, and BUN levels were observed after STZ injection in Group II ($p<0.05$). There was a significant decrease in serum total protein level of diabetic rats when compared to control group ($p<0.05$). *J. communis* L. oil at 200 mg/kg resulted in 12.7% reduction of serum HbA1c level, 73.6% reduction of serum glucose level, 19.2% reduction of serum creatinine level, 28.5% reduction of serum urea level, 28.5% reduction of BUN level, on the other hand, 16.4% augmentation of serum total protein level compared with the diabetic group. All parameters showed significant differences between Group II and Group III ($p<0.05$). Results on serum glucose, HbA1c, total protein, creatinine, urea, and BUN levels were shown in Table 1.

Table 1. *Effect of administration of J. communis L. oil for 21 days on serum glucose, serum HbA1c, serum creatinine, serum urea, BUN, and serum total protein levels in diabetic rats.*

	Group I	Group II	Group III	Group IV
Serum glucose level (mg/dL)	62.54±7.94	397.77±47.2[a]	70.51±8.93	105.08±12.85[a,b]
Serum HbA1c level (%)	5.8±0.47	7.1±0.43[a]	5.6±0.33	6.2±0.38[b]
Serum creatinine level (mg/dL)	0.57±0.04	0.73±0.06[a]	0.61±0.08	0.59±0.07[b]
Serum urea level (mg/dL)	36.7±4.59	49.84±4.76[a]	38.09±4.07	35.64±4.15[b]
BUN level (mg/dL)	17.14±1.35	23.26±1.68[a]	17.77±2.39	16.63±2.24[b]
Serum total protein level (g/dL)	8.19±0.64	6.75±0.46[c]	8.47±0.73	7.86±0.55[d]

[a] Significantly higher than Group I ($p<0.05$), [b] Significantly lower than Group II ($p<0.05$), [c] Significantly lower than Group I ($p<0.05$), [d] Significantly higher than Group II ($p<0.05$)

Oxidative stress has been implicated in inflammation which is directly related to the level of MDA, NO and GSH and activities of SOD and catalase. Low tissue concentrations of SOD, GSH, and catalase and high tissue concentrations of MDA and NO were noted in Group II compared with Group I ($p<0.05$). *J.*

communis L. oil at dose of 200 mg/kg enhanced 73.7% of SOD activity ($p<0.05$), 52.7% of catalase activity ($p<0.05$) and 38.8% of GSH level ($p<0.05$), besides this, decreased 31.4% of MDA level ($p<0.05$) and 29.8% of NO level ($p<0.05$) in kidney tissue of diabetic rats. Results on SOD, and catalase activities and GSH, MDA, and NO levels in kidney tissue were shown in Table 2.

Table 2. *Effect of administration of J. communis L. oil for 21 days on MDA, NO, and GSH levels and SOD and catalase activities in diabetic rats.*

	Group I	Group II	Group III	Group IV
MDA level (nmol/mg protein)	28.73±3.97	46.51±6.84[a]	30.71±4.87	31.92±4.5[b]
NO level (nmol/mg protein)	14.62±1.57	22.05±2.47[a]	15.18±2.93	15.47±2.19[b]
GSH level (nmol/mg protein)	69.81±5.35	51.64±4.43[c]	70.39±8.65	71.68±6.03[d]
SOD activity (U/mg protein)	3.91±0.38	2.13±0.27[c]	3.76±0.49	3.7±0.42[d]
Catalase activity (U/mg protein)	395.62±37.61	264.44±31.95[c]	388.52±40.17	403.86±37.51[d]

[a] Significantly higher than Group I ($p<0.05$), [b] Significantly lower than Group II ($p<0.05$), [c] Significantly lower than Group I ($p<0.05$), [d] Significantly higher than Group II ($p<0.05$)

4. DISCUSSION

Diabetic nephropathy like any other chronic diabetic complications is caused by various reasons, including poor glycemic control, high blood pressure, and high cholesterol (especially hypertriglyceridemia) (19). In this study, it was found that *J. communis* L. oil treatment could decrease the level of serum HbA1c and glucose levels, therefore *J. communis* L. oil could inhibit the development of diabetic nephropathy.

Renal dysfunction as a result of DM can be assessed by serum creatinine, urea, BUN, and total protein. Therefore, this suggested that there is strong relationship between these parameters and renal dysfunction. Thus, an increase in creatinine, urea, and BUN occurs when there is renal dysfunction or damage. The increment in serum creatinine, urea, and BUN observed in this study clearly indicated that DM causes damage or dysfunction of the kidney in diabetics. Hence, the results of this study were in accordance with various studies which showed raised serum creatinine, urea, and BUN levels in diabetic patients (20). Also, in this study, there were increased levels of these kidney function parameters (except serum total protein level) in diabetic control group when compared to normal control and diabetic test group. The increment observed in diabetic control group revealed that untreated DM caused severe dysfunction of the kidney compared to treated DM through dietary consumption of *J. communis* L. oil.

According to previous research, *J. communis* L. oil were reported to have renoprotective and regenerative effects on the kidney of hypercholesterolemic rats (21). Therefore, it is not unreasonable to suggest that *J. communis* L. oil has remedial and protective effects on the kidneys of diabetic rats.

However, the remedial effect of *J. communis* L. oil may probably be due to the earlier reported antioxidant and anti-inflammatory properties of *J. communis* L. oil as a result of its chemical components especially medium chain fatty acids (22,23).

In addition, in this study, it was observed that there was a decrease in serum total protein of diabetic group compared to control and diabetic group treated with *J. communis* L. oil. This was probably because of the damaging effects DM has on the kidney tissues of diabetic control group which was minimized in diabetic test group as a result of dietary consumption of *J. communis* L. oil.

Kidney is abnormally sensitive to oxidative stress; under the condition of high glucose, reactive oxygen species (ROS) can induce renal cell apoptosis and drop from basement membrane, causing damaged glomerular filtration membrane integrity and even proteinuria; moreover, thereby promoting the development of diabetic nephropathy (24). There are mainly several kinds of antioxidant defense system, including glutathione (GSH), catalase, and superoxide dismutase (SOD) (25). Unfortunately, in some cases antioxidant defense system mentioned cannot overcome the elevation of oxidative stress. Therefore, an excess oxidative stress can attack the cell components especially the polyunsaturated fatty acids leading to the increase level of MDA, a lipid peroxidation product (26). The role of nitric oxide in diabetic nephropathy is a rather controversial issue. Some researchers have reported that nitric oxide increased kidney injury through its reactions with a superoxide radical and generation of a cytotoxic peroxynitrite (27,28). The impairment in the oxidant/antioxidant equilibrium also induces tissue damage and diabetic complications (24). In this study, the level of GSH and activities of SOD and CAT and the content of MDA in serum and kidney tissue for analyzing systemic and local ability to clean the free radicals were detected. Compared with Group I, the level of GSH and activities of catalase and SOD in kidney tissue was decreased and level of MDA and NO was increased in diabetic rats; therefore the ability of scavenging free radicals was destroyed in diabetic nephropathy model. After *J. communis* L. oil treatment, the level of GSH and activities of catalase and SOD in kidney tissue were obviously increased, and the levels of MDA and NO were obviously decreased, therefore *J. communis* L. oil treatment was promoted to scavenge free radicals in kidney through regulating enzymatic and non-enzymatic antioxidants.

5. CONCLUSION

It could be concluded that *J. communis* L. oil succeeded in controlling hyperglycemia in rats with STZ induced diabetes. Furthermore, this study has provided direct evidence of a link between DM and diabetic nephropathy and demonstrated that *J. communis* L. oil provide a protective effect on the kidney as evidenced by an improvement of the renal function tests as well as reduction in oxidative stress parameters.

REFERENCES

[1] Ramachandran S, Rajasekaran A, Adhirajan N. In vivo and in vitro antidiabetic activity of *Terminalia paniculata* bark: An evaluation of possible phytoconstituents and mechanisms for blood glucose control in diabetes. ISRN Pharmacol 2013; 2013:484675.

[2] Sheikh BA, Pari L, Rathinam A, Chandramohan R. Trans-anethole, a terpenoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. Biochimie 2015; 112:57-65.

[3] Dyson T. World food trends and prospects to 2025. Proc Natl Acad Sci U S A. 1999; 96:5929-5936.

[4] Zhu K, Kakehi T, Matsumoto M, Iwata K, Ibi M, Ohshima Y, Zhang J, Liu J, Wen X, Taye A, Fan C, Katsuyama M, Sharma K, Yabe-Nishimura C. NADPH oxidase NOX1 is involved in activation of protein kinase C and premature senescence in early stage diabetic kidney. Free Radic Biol Med 2015; 83:21-30.

[5] Dey N, Bera A, Das F, Ghosh-Choudhury N, Kasinath BS, Choudhury GG. High glucose enhances microRNA-26a to activate mTORC1 for mesangial cell hypertrophy and matrix protein expression. Cell Signal 2015; 27:1276-1285.

[6] Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc 2018; 117:662-675.

[7] Yakush Williams JK. Management strategies for patients with diabetic kidney disease and chronic kidney disease in diabetes. Nurs Clin North Am 2017; 52:575-587.

[8] Clifton SJ, Ward LK, Ranner DS. The status of juniper *Juniperus communis* L. in North-East England. Biol Conserv 1997; 79:67-77.

[9] Bais S, Gill NS, Rana N, Shandil S. A phytopharmacological review on a medicinal plant: Juniperus communis. Int Sch Res Notices 2014; 2014:634723.

[10] Höferl M, Stoilova I, Schmidt E, Wanner J, Jirovetz L, Trifonova D, Krastev L, Krastanov A. Chemical composition and antioxidant properties of Juniper Berry (*Juniperus communis* L.) essential oil. action of the essential oil on the antioxidant protection of Saccharomyces cerevisiae model organism. Antioxidants (Basel) 2014; 3:81-98.

[11] Tavares WR, Seca AML. The current status of the pharmaceutical potential of Juniperus L. metabolites. Medicines (Basel) 2018; 5:81.

[12] Orhan N, Hoçbaç S, Orhan DD, Asian M, Ergun F. Enzyme inhibitory and radical scavenging effects of some antidiabetic plants of Turkey. Iran J Basic Med Sci 2014; 17:426-432.

[13] Yagi K. Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. Methods Mol Biol 1998; 108:107-110.

[14] Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5:62-71.

[15] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-888.

[16] Sun Y, Oberley LW, Ying L. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34:497-500.

[17] Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121-126.

[18] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1961; 193:265-275.

[19] Misra A, Sattar N, Tandon N, Shrivastava U, Vikram NK, Khunti K, Hills AP. Clinical management of type 2 diabetes in south Asia. Lancet Diabetes Endocrinol 2018; 6:979-991.

[20] Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003; 63:225-232.

[21] Akdogan M, Koyu A, Ciris M, Yildiz K. Anti-hypercholesterolemic activity of J. communis oil in rats: a biochemical and histopathological investigation. BioMed Res 2012; 23:321-328.

[22] Jones SM, Zhong Z, Enomoto N, Schemmer P, Thurman RG. Dietary juniper berry oil minimizes hepatic reperfusion injury in the rat. Hepatology 1998; 28:1042-1050.

[23] Han X, Parker TL, Benavente C. Anti-inflammatory activity of Juniper (Juniperus communis) berry essential oil in human dermal fibroblasts. Cogent Medicine 2017; 4:1-7.

[24] Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 2006; 55:225-233.

[25] Gupta V, Lahiri SS, Sultana S, Kumar R. Mechanism of action of Rhodiola imbricata Edgew during exposure to cold, hypoxia and restraint (C-H-R) stress induced hypothermia and post stress recovery in rats. Food Chem Toxicol 2009; 47:1239-1245.

[26] Buldanlioglu S, Turkmen S, Ayabakan HB, Yenice N, Vardar M, Dogan S. Nitric oxide, lipid peroxidation and antioxidant defence system in patients with active or inactive Behçet's disease. Br J Dermatol 2005; 153:526-530.

[27] Di Marco E, Jha JC, Sharma A, Wilkinson-Berka JL, Jandeleit-Dahm KA, de Haan JB. Are reactive oxygen species still the basis for diabetic complications? Clin Sci (Lond) 2015; 129:199-216.

[28] Tessari P. Nitric oxide in the normal kidney and in patients with diabetic nephropathy. J Nephrol 2015; 28:257-268.

How to cite this article: Kahraman T, Berkoz M, Allahverdiyev O, Mahmood EA, Yildirim M, Yalin S. Can Juniperus communis L. oil improve nephropathy in diabetic rats. Clin Exp Health Sci 2020; 10: 120-124. DOI: 10.33808/clinexphealthsci.543272

Clinical and Experimental Health Sciences

Relationship Between Marital Adjustment and Infant Attachment in Turkish Fathers

Birsel Canan Demirbag[1], Meltem Kuytuncu[2], Sumeyra Yasar Kivik[3]

[1] Karadeniz Technical University, Health Sciences Faculty, Nursing Department, Trabzon, Turkey.
[2] Bulent Ecevit University, Faculty of Health Sciences, Nursing Department, Zonguldak, Turkey.
[3] Obstetrics and Gynecology Hospital, Zonguldak, Turkey.

Correspondence Author: Birsel Canan Demirbag
E-mail: cdemirbag@gmail.com
Received: 22.01.2019 **Accepted:** 25.04.2020

ABSTRACT

Objective: This study aims to assess the relationship between marital adjustment and paternal-infant attachment.

Methods: This descriptive-cross sectional study was conducted between March and April 2017. No sampling was performed, the study was conducted with 110 fathers who could be accessed between the aforementioned dates and who had babies aged between 0 to 12 months old. Questionnaires were asked fathers by researchers.The outcome measurements: Socio-demographic Information Form, Marital Adjustment Test, and PIAQ. Statistical analysis was performed using SPSS 13.0 program.

Results: Fathers' MAT mean score was found 44.182±5.17, and PIAQ mean score was found 77.935±8.08. There was a positive, weak, but significant relationship between MAT and PIAQ (r=0.25; p=0.009). Marital adjustment scores were found to increase with the increase in "patience", "tolerance", and "pleasure in interaction" subscale scores. MATsubscales were found to affect each other positively, and this effect was found to have a significant relationship with paternal-infant attachment level (p<0.05). Good relationship with the spouse was found have statistically significant relationship with paternal-infant attachment (MWU=165.500; p=0.000).

Conclusion: This study revealed that marital adjustment has effects on paternal-infant attachment.

Keywords: Father, attachment, infant

1. INTRODUCTION

New-born attachment is initiated with the sensual connection with the mother, sucking, and seeking. This attachment is formed with the mother's presence next to the infant (1,2). Father, on the other hand, does not experience this attachment as strongly as mother does, but the sensual connection, eye contact and tone of voiceof the father have positive effects on this attachment (2,3). Infant's confidence is improved and the attachment is maintained through establishing close relationships and behaviours among mother, father and infant and being with the infant particularly in stressful situations (2). On the other hand, this attachment becomes stronger after birth, with father's giving his love and embracing his baby by acknowledging his roles as a father (1,2). Fulfilling paternal roles is affected by such factors as father's physical/psychological competence, a positive environment, maintenance of father-infant touch, and the nature/adjustment of mother-father relationships (2,4).

A well-adjusted marriageis defined as a marriage in which the couple makes a consensus about an issue related to the family and solves problems by talking and reaching an agreement (5). A well-adjusted marriage both increases quality of life of families and decreases stress in relation to responsibilities (6,7). Deterioration inmarital adjustment due to various reasons causes to either end the marriage or experience continuous domestic stress (8). To decrease stress, couples should regulate their relationship and identify expectations (5). When the adjustment between the couples start to deteriorate, negative effects are observed both in the family and psychological and physical health of the family members (9). Negative relationships between the parents cause weakness in attachment. Maintenance of the attachment between mother and father throughout the marriage also affects the strength of the love between parents and children (5,9). Some studies highlight the positive aspect of establishing mother and father attachment in infant development (4). The literature also involves a number of studies indicating that marital adjustment affects children's psychological health (9). However, given the healthy development of the individual, this can be achieved in a loving warm family environment, so

DOI: 10.33808/clinexphealthsci.516309

the communication of the fathers with their babies should not be ignored (10). In a study; father candidates; reported that they experienced paternal fetal attachment in three areas. These; differentiation of self is listed as acting and giving. In addition, a significant positive relationship was found between paternal fetal attachment in men with strong relationships with their husbands (11,12).

However, studies about therelationship between parental-infant attachment and marital adjustment is limited in number.Particularly in developing countries like ours, lack of studies about the relationship between paternal-infant attachment and marital adjustment is remarkable. In this regard, the present study aims to raise awareness and draw attention to the issue by analysing the relationship between marital adjustment andpaternal-infant attachment.

2. METHODS

This study, which aimed to identify the relationship between paternal-infant attachment and marital adjustment, is descriptive and cross-sectional. Target population of the study was the fathers of 0 to 12 months old babies who were treated in a state hospital which is the established in center of city and the most popular hospital for delivery. The research was conducted between 15th of March and 15th of April, 2017 in Trabzon, Turkey. The hospital which is the largest Women and Child Diseases Hospital of the city where the study was performed, would reflect the characteristics of the country as a whole. No sampling calculation or selection was performed; the study was conducted with fathers who volunteered to participate in the study. The study was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from each human subjects.In the time interval of the study determined by the researchers total number of 0 to 12 months old infants in the a for mentioned hospital was 160, and the number of fathers who volunteered to participate in the study was 110 between 15th of March and 15th of April, 2017. Data were collected through the 15-item Socio-demographic Information Form that aimed to collect information about fathers' age and marital status, education level of the parents, family type, health insurance, and baby's gender; MAT (MAT) (15 items), and PIAQ (PIAQ) (19 items) (2,7).

MAT was developed by Locke and Wallace to measure the quality of marriage. This scale is composed of most fundamental and distinguishing questions and was used in various studies in last 30 years as a valid and reliable scale. In Turkey, validity and reliability of the scale was verified by Tutarel-Kıslak in 1999 (13). Scale may be scored between 1 and 60. The lowest adjustment score is 1, the highest adjustment score is 60. The cut-off score for the scale have been assigned as 53.

PIAQ was improved by Condon et al. in 2008 to evaluate father–infant attachment after birth. The scale consists of 19 articles and three subscales. Each article of the scale has values from 1 to 5. The lowest score achievable on the scale is 19, and the highest is 95. A high score shows that attachment is high. In the sixth month, Cronbach's alpha was found to be 0.81, and in the 12th month, it was 0.78 (14). A Turkish validity and reliability test has been developed, and its Cronbach alpha was found to be 0.76 (2). For this study, Cronbach's alpha was found to be 0.86.

Data were collected through face-to face interviews conducted by the researchers by visiting the clinics during day shifts and explaining the purpose of the study. Questionnaires were asked fathers by researchers because of using time effectively. Data collection through interviews took about 15 to 20 minutes.

Demographic features of the fathers were described using frequencies, means, and standard deviation tests in SPSS statistical package programming. The data were tested for suitability for normal distribution by histogram and One-Sample Kolmogorov Smirnov Test. Comparison of the fathers' demographic features and scale scores were identified using Kruskal Wallis and Mann Whitney U tests. The relationship between the scales were identified using Spearman Correlation analysis. 95% confidence interval and $p<0.05$ significance level were used for the evaluation of the results.

Prior to data collection, ethical approval was obtained from the hospital where the study was conducted (04.03.2017, Number:201). Participation was on voluntary basis, and the fathers' verbal consent was obtained for data collection. That the study data were based on self-reporting is an important limitation in the research.

3. RESULTS

This section demonstrates findings obtained from the analysis of the data collected from the fathers through the scales used as data collection tools.The mean age of fathers was found between 20-49 years, (Mean: 33.79 ± 5.72; Min:20; Max:45; Median:). 52.7% of the fathers had high school degree; 54.5% was worker; 53.6% had middle income level; 84.5% of father had social security; 39.1% of father had 1 to 5 years marriage; 30% of father didn't have child before; 63.6% of babies was a girl; %67.3 ofwife were housewife. The fathers' MAT mean score was found 44.182 ± 5,174 (Mean: 44.182 ± 5.174; Min: 30, Max: 57; Median: 44), and PIAQ mean score was 77.935 ± 8,080 (Mean: 77.93 ± 0.77; Min: 48.5, Max:92.6; Median:78.44). As to the mean scores according to subscales, they were found 33.083±3.740 for "patience and tolerance", 26,388 ± 3,712 for "pleasure in interaction", and 18.464±1.974 for "affection and pride" (Table 1). Correlation analysis was conducted in order to find out the relationship between paternal-infant attachment total score and "patience and tolerance" subscale; result showed that there was a 91.3% positive, significant relationship between the scores ($r=0.913$; $p=0.000<0.05$). Accordingly, "patience and tolerance" subscale score increasesasthe paternal–infant attachment total score increases.

Table 1. *MAT and PIAQ means*

	n	Median	Mean	SD	Min.	Max.
Marital Adjustment	110	44.00	44.182	5.174	30.000	57.000
Patience and Tolerance Subscale	110	33.40	33.083	3.740	21.100	40.000
Pleasure in Interaction Subscale	110	26.75	26.388	3.712	13.000	34.000
Affection and Pride Subscale	110	19.00	18.464	1.974	8.300	20.000
PIAQ	110	78.45	77.935	8.080	48.500	92.600

Kruskal Wallis H-Test was performed in order to identify whether MAT and PIAQ subscale mean scores demonstrated any significant differences in terms of the definition of fatherhood variable; significant differenceswere detected in all subscale mean scores. Mann Whitney U test was performed for the identification of the group that created the difference (Table 2).

Correlation analysis was conducted in order to find out the relationship between paternal-infant attachment total score and "pleasure in interaction" subscale; results indicated a 85.7% positive, significant relationship between the scores (r=0.857; p=0.000<0.05).

Table 2. *MAT and PIAQ scores according to definitions of fatherhood*

		Mean Rank	Median (Min-Max)	Test scores KW p
Marital Adjustment		44.10	78.451(48.50-92.60)	
	I am not ready to become a father, therefore I cannot support my wife	33.00	34.000	KW:6.278
	I am not ready to become a father, I support my wife about the baby care	41.81	42.211	p: 0.959
	I take care of my baby willingly, I support my wife about this	45.40	44.031	
	I am always ready for fatherhood, but I cannot help my wife much because of my job	43.68	42.813	
Patience and Tolerance Subscale	I am not ready to become a father, therefore I cannot support my wife	27.20	27.303	
	I am not ready to become a father, I support my wife about the baby care	30.88	30.123	KW:20.875
	I take care of my baby willingly, I support my wife about this	34.77	34.876	**p: 0.001**
	I am always ready for fatherhood, but I cannot help my wife much because of my job	31.94	31.329	
Pleasure in Interaction Subscale	I am not ready to become a father, therefore I cannot support my wife	13.00	13.014	
	I am not ready to become a father, I support my wife about the baby care	25.10	25.823	KW:14.443
	I take care of my baby willingly, I support my wife about this	27.56	27.874	**p: 0.002**
	I am always ready for fatherhood, but I cannot help my wife much because of my job	25.73	26.001	
Affection and Pride Subscale	I am not ready to become a father, therefore I cannot support my wife	8.30	8.302	
	I am not ready to become a father, I support my wife about the baby care	17.01	17.001	KW:11.113
	I take care of my baby willingly, I support my wife about this	18.91	18.231	**p: 0.011**
	I am always ready for fatherhood, but I cannot help my wife much because of my job	18.54	18.320	
PIAQ		27.00	44.00(30-57)	
	I am not ready to become a father, therefore I cannot support my wife	48.50	48.632	KW:22.672
	I am not ready to become a father, I support my wife about the baby care	73.00	74.021	**p: 0.001**
	I take care of my baby willingly, I support my wife about this	81.25	82.342	
	I am always ready for fatherhood, but I cannot help my wife much because of my job	76.22	77.013	

Hence, "pleasure in interaction" subscale score increases as paternal-infant attachment total score increases. Correlation analysis was conducted in order to find out the relationship between paternal-infant attachment total score and "affection and pride" subscale; results indicated a 64.7% positive, significant relationship between the scores (r=0.647; p=0.000<0.05). Hence, "affection and pride" subscale score increases as the paternal-infant attachment total score increases. As a result of the correlation analysis conducted to determine the relationship between the father-baby attachment scale total and marital adjustment, a 25.0% positive relationship was found between the scores (r=0.250; p=0.009<0.05). Accordingly, as the total score of the father-baby attachment scale increases, the marital adjustment score also increases (Table 3)

While the correlation analysis between patience and tolerance subscale (r = 0.232; p = 0.015 <0.05), interaction interaction subscale (r=0.223; p=0.012 <0.05) and marital adjustment were positively significant, There was no significant relationship between love and pride subscale (r=0.121; p=0.209>0.05) and marital adjustment. (Table 3).

Table 3. *The relationship between the MATand PIAQ scores*

		Marital Adjustment	Patience and Tolerance Subscale	Pleasure in Interaction Subscale	Affection and Pride Subscale	PIAQ
Marital Adjustment	r	1,000	0,232	0,238	0,121	0,250
	p	**0,000**	**0,015**	**0,012**	0,209	**0,009**
Patience and Tolerance Subscale	r	0,232	1,000	0,672	0,455	0,913
	p	**0,015**	**0,000**	**0,000**	**0,000**	**0,000**
Pleasure in Interaction Subscale	r	0,238	0,672	1,000	0,429	0,857
	p	**0,012**	**0,000**	**0,000**	**0,000**	**0,000**
Affection and Pride Subscale	r	0,121	0,455	0,429	1,000	0,647
	p	0,209	**0,000**	**0,000**	**0,000**	**0,000**
PIAQ	r	0,250	0,913	0,857	0,647	1,000
	p	**0,009**	**0,000**	**0,000**	**0,000**	**0,000**

Comparison of father's demographic characteristics between MAT and PIAQT of total scores, we didn't find any statistically significant difference family type, health insurance, and baby's gender. Comparison of fathers' MAT total mean scores with the relationship with the spouse indicated statistically significant difference (Mann Whitney U=165.500; p<0,0001). Fathers who reportedly had good relationships with their wife were found to have high MAT scores (38.400±4.69) (Table 4). Comparison of fathers' total MAT mean scores with duration of marriage variable indicated significant differences between the group mean scores (KW=8.128; p<0.05) (Table 4). Comparison of fathers' education level and PIAQ total scores revealed statistically significant differences (KW=10.832; p<0.05). Mann Whitney U test results revealed that this difference resulted from the fact that university scores (Mean =81.300±6.432) were higher than secondary (Mean=75.863±6.702) and primary (Mean=74.729±6.819) school scores (Table 4).

Table 4. *Analysis of MAT and PIAQ scores according to relationships with the spouse, education, and marriage duration*

Characteristics	MAT Mean Rank (Min-Max)	Test scores MWU p	PAQT Mean Rank (Min-Max)	Test scores MWU p
Relationships with the spouse				
Good	44.231 (43.081-49.634)	MWU: 165.500 **p: 0.001**	79.654 (78.987-86.388)	MWU: 318.000 p: 0.058
Medium	38.934 (39.01-43.095)		74.080 (73.989-81.435)	
Marriage Duration				
1 to 5 years	45.765 (45.00 – 50.773)	MWU : 8.128 **p: 0.017**	79.982 (79.054-87.003)	MWU: 2.439 p: 0.295
6 to 10 years	44.987 (45.010-48.728)		78.185 (78.000-87.207)	
11 years and more	42.540 (43.000-47.342)		77.430 (76.980-82.015)	
Education Level				
Primary school	40.239 (39.289-47.357)	MWU: 4.045 p: 0.257	75.980 (75.100-81.548)	MWU : 10.832 p: **0.013**
Secondary School	43.875 (42.987-47.678)		76.962 (76.324-82.565)	
High school	44.687 (44.001-49.770)		78.094 (77.985-86.411)	
University	44.672 (44.027-49.585)		82.500 (81.989-87.730)	

According to the results of Kruskal Wallis H-Test conducted to determine whether the mean scores of the marital adjustment scores of the fathers participating in the study show a significant difference in terms of the marriage duration variable; The difference between group averages was found to be significant (KW= 8.128; p = 0.017 <0.05). Mann Whitney U test was used to determine which group the difference originated from. According to this, fathers' marriage compliance scores (Mean=45.861 ± 4.912) with a marriage duration of 1-5 years were higher than those of fathers with a marriage duration of 6-10 years (43.585±5.143). (45.861 ± 4.912), fathers with a marriage period of 11 years and over

were found to be higher than the marriage compliance scores (42.346 ± 4.996).

4. DISCUSSION

Attachment is a concept that has an important place in the human development process (2,4). Theoreticians who developed the infant attachment concept state that a reliable infant-parent attachment is created as a result of infant's sending signals for his needs and the caregiver adult's giving appropriate response to these needs (4,5). Attachment can be improved as long as the parents continue their positive communication behaviours with the children. High attachment scores in this study indicate fathers' positive relationships with their children.

Marital adjustment is acknowledged as one of the most important factors that affect the attachment process (3,5,7). While any adjustment disorders weaken the relationship between parents, it could also affect the relationship between the father and the infant, whose need for care from a mother is a must particularly in the first years (15,16). Of the present study which can promote development of secure infant-parent attachment relationships in the next generation. This study found that although there was astatistically significant relationship between paternal-infant attachment and a well-adjusted marriage, this relationship was a weak one. However, correlation analysis which aimed to identify the relationship between paternal-infant attachment total score and marital adjustment revealed 25,0% positive, significant relationship between the scores. Hence, marital adjustment score increases as the paternal-infant attachment total score increases.

Marital adjustment starts with healthy foundations formed by the parents whodecidedto share life together; and it continues throughout their life with right communication in the family, decision for pregnancy, pregnancy process, and birth and afterbirth relationships (5,17). Marital adjustment is couples' solving problems and sharing their happiness at every stage of marriage by talking (5,16,18). Findings of this study indicate that maritaladjustment is at good level and "tolerance" subscale is significantly associated with paternal-infant attachment level, which is line with the related literature.

Mercer states that maternal attachment starts naturally during the pregnancy period and highlights the importance of birth and afterbirth for the maintenance of this case (18). It is known that paternal attachment could be strengthened by starting this relationship – which starts between mother and infantduring pregnancy – for paternal-infant attachment during pregnancy and maintaining it after birth and in the first years of the baby (18-20). Significant relationships were detected between "pleasure in interaction in marriage", "affection and pride", "patience and tolerance" and infant attachment scale, which indicates positive relationships between the parents.

A number of studies have revealed that prospective fathers who follow the pregnancy process with their wives start to accept paternity roles by allocating sufficient time to their wife and babies after birth, taking care of the baby, and loving her (17-20). Studies report that patience, tolerance, pleasure, affection and pride have effects on attachment behaviours and maintenance of a well-adjusted marriage (5,15). This study indicates that fathers' definition of fatherhood had significant relationships with marital adjustment, marital adjustment subscales and PIAQ. This finding shows that marital adjustment is an effective factor in infant attachment.

Mutual understanding, cooperation, love, and respect are some of the things expected from a healthy marriage. The literature indicates that these concepts which have effects on marriage also affect maintenance of positive marriageas well as the relationships between children and other family members (18,19). The current study found no significant relationships between paternal-infant attachment and the factors affecting parental attachments reported in the literature (e.g. planned pregnancy, gender expectation, and number of children). However, marital adjustment subscales such as "patience and tolerance", "pleasure in interaction", and "affection and pride"were found to have important relationships with paternal-infant attachment.

A number of studies report that education level has also effects on the development of attachment (4,15). The present study found that the relationship between marital adjustment subscales of"patience and tolerance" and the father's education level have positive effects on paternal-infant attachment. Paternal-infant attachment increases with the increase in education level. Presence of the relationship between the increase in parents' education level and attachment is in line with the literature. Marriage is a process which has ups and downs between the couples (20,21). Living together/doing things together and behaviour styles could also be defined as adjustment by couples. Adjustmentis affected by factors such as spousal relationships, marriage duration, education, understanding, and respect (7). This study found a significant relationship between marital adjustment subscales of "patience and tolerance" and fathers' education and paternal-infant attachment. Paternal-infant attachment levelwas found to increase with the increase in the education level. The literature also has parallel information about the presence of the relationship between the increase in education level and attachment (4,5). The present study did not found relationship between marital adjustment and age, salary, health insurance, famillt type and sex of child.

Length of marriage duration affects marital adjustment (3,18,22). This study found that fathers with longer marriage durations demonstrate higher paternal-infant attachment levels. Besides, relationships with the spouse were found to affect the infant attachment level significantly, and paternal attachment levels were found to increase with the increase in adjustment between the couples. The literature mentions the effect of mother and father relationships on infant

attachment level (23,24). Importance of spousal relationship in marital adjustment has been supported with this study as well.

Limitations

There is a limitation of the current study. Participants were included in a non-randomized design, and only 68.7% of the target sample size was reached. Our inability to reach the targeted number in the sample might have increased the likelihood of type I errors. Therefore, our results cannot be generalized to the entire population.

5. CONCLUSION

This study found a significant relationship between marital adjustment andpaternal-infant attachment. Besides, there was a significant relationship between paternal-infant attachment and marital adjustmentsubscales of patience, pleasure and affection. Significant relationships were also found between marriage duration, fathers'education level, and spousal relationships and paternal-infant attachment and marital adjustment.

REFERENCES

[1] Shieh C, Kravitz M, Wang HH. What do we know about maternal–fetal attachment? J Med Sci 2001; 17: 448-445.

[2] Güleç D, Kavlak O. The study of reliability and validity of paternal-infant attachment scale in Turkish society. JHS 2013; 10(2): 170-181.

[3] Morsünbül Ü, Çok F. Attachmentand related variables. Current Approaches in Psychiatry, 2011; 3(3): 553-570.

[4] Newland LA, Coyl DD, Chen HH. Fathering and attachment in the USA and Taiwan: contextual predictors and child outcomes. Early Child Dev Care 2010; 180(1): 173-191.

[5] Marchand JF. Husbands' an wives' maritalquality: The role of adult attachment orientations, depressive symptoms, and conflict resolution behaviors. Attach Hum Dev 2005; 6(1): 99-112.

[6] Condon TJ, Corkindale C. The correlates of antenatal attachment in pregnant women. Br J Clin Psychol 1997; 70(4): 359-372.

[7] Monin JK, Feeney BC, Schulz R. Attachment orientation and reactions to anxiety expression in close relationships. Pers Relatsh 2012; 19(3): 535 – 550.

[8] Yılmaz SD, Beji NK. Therelationship between anxiety and depression level and coping styles with stress of pregnant women. Genel Tıp Dergisi 2010; 20(3): 99-108.

[9] Kelly RJ, Marks BT, El-Sheikh M. Longitudinal relations between parent–child conflict and children's adjustment: The role of children'ssleep. J Abnorm Child Psychol 2014; 42(7): 1175-1185.

[10] Dizman H, Gürsoy F. Aggression tendencies of children with and without mother who attend the fourth and fifth grades of primary education. Kastamonu Eğitim Dergisi 2005; 13(2): 437 – 446.

[11] Başal AH. Turkey's prenatal, birth and postpartum traditions related to child development and education, customs and beliefs. Eğitim Fakültesi Dergisi 2006; XIX(1): 45-70.

[12] Cranley SM. Roots of Attachments: The relationship of parets with their unborn. Birth 1981; 17(6): 59-83.

[13] Kişlak-Tutarel Ş. Validity and reliability study on marital adjustment scale (MAS). 3P Dergisi 1999; 7(1): 54-56.

[14] Üstünoz A, Güvenç G, Akyüz A, Oflaz F. Comparison of maternal–andpaternal–fetal attachment in Turkish couples. Midwifery 2010; 26 (2): 1-9.

[15] Marchand JF. Husbands' an wives' maritalquality: The role of adult attachment orientations, depressive symptoms, and conflict resolution behaviors. Attach Hum Dev 2005; 6(1): 99-112.

[16] Tüzün O, Sayar K. Attachment the oryand psychopathology. Dusunen Adam The Journal of Psychiatry and Neurological Sciences 2006; 19: 24-39.

[17] Erbek E, Beştepe E, Akar H, Eradamlar N, Alpkan RL. Marital adjustment. Dusunen Adam The Journal of Psychiatry and Neurological Sciences 2005; 18 (1): 39-47.

[18] Mercer RT. Becoming a mother versus maternal role attainment. J Nurs Scholarsh 2004; 36(3): 226-232.

[19] Lindgren K. Relationships among maternal–fetal attachment, prenatal depression, and health practices in pregnancy. Res Nurs Health 2001; 24: 203-217.

[20] Soysal AŞ, Bodur Ş, İşeri E, Şenol S. Attachment process in infancy: A review. J Clin Psychiat 2005; 8: 88-99.

[21] Şener T, Terzioğlu G. Investigation between some socio-economic, demographic variables and effect of communication on marital adjustment. Family and Society 2008; 4(13): 7-20.

[22] Greeff AP, Malherbe HL. Intimacy and marital satisfaction in spouses. J Sex Marital Ther 2001; 27: 247-257.

[23] Okanlı A, Tortumluoğlu G, Kırpınar İ. Determining postpartum social support needs of mothers and the support they received. Anatolian Journal of Psychiatry 2003; 4(2): 98-105.

How to cite this article: Demirbag BC, Kuytuncu M, Kivik SY. Relationship Between Marital Adjustment and Infant Attachment in Turkish Fathers. Clin Exp Health Sci 2020; 10: 125-130. DOI: 10.33808/clinexphealthsci.516309

Clinical and Experimental Health Sciences

Nursing Students' Perceptions of Nursing Diagnoses and Clinical Decision-Making

Demet Inangil[1], Sengul Uzen Cura[2]

[1] University of Health Sciences, Hamidiye Faculty of Nursing, Istanbul, Turkey.
[2] Canakkale Onsekiz Mart University, School of Medicine, Canakkale, Turkey.

Correspondence Author: Demet Inangil
E-mail: demet.inangil@sbu.edu.tr
Received: 21.05.2019 **Accepted:** 25.04.2020

ABSTRACT

Objective: The objective of this study was to evaluate nursing students' perceptions regarding nursing diagnoses and clinical decision-making skills level and to examine how these perceptions differ according to which nursing model was followed.

Methods: A descriptive, cross-sectional, comparative study was conducted. The study was carried out in one nursing department in Istanbul province and one nursing high school in Çanakkale province, Turkey. A total sample of 257 students participated. A Structured Information Form, Perception of Nursing Diagnoses Survey and Clinical Decision Making in Nursing Scale were used to collect data.

Results: 77.1% of the participants were women, with an average age of 19.09, and their average grade from the Fundamentals of Nursing course was 74.23 ± 9.41. The total of the PND score for the ADLs group was 2.45 ± 0.55, in the FHP group it was 2.31 ± 0.27. This difference in the incidence of value was not significant. The total of the CDMNS score means of the ADLs group was 147.44 ± 12.95, and the mean of the FHP group was 154.3 ± 12.29. A statistically significant difference was detected between the groups for the CDMNS score (p = 0.00).

Conclusion: From the results, it can be concluded that the use of nursing diagnoses is positively perceived by nursing students and their clinical decision-making perceptions are in the process of development. Nursing models that are used in nursing education may affect clinical decision making.

Keywords: Nursing models, diagnoses, decision making.

1. INTRODUCTION

Nursing is a profession which requires the combination of theoretical knowledge and psychomotor skills (1,2). In the past, nursing education was focused on medical science, treatment of patients and routine nursing functions. Today, nursing practice is based on its own specially-developed nursing theory, and its own research (3,4). The translation of scientific knowledge into nursing practice can take place only through the nursing process itself (5). The nursing process raises the quality of nursing health care and improves the effectiveness of individual patient care. Additionally, it improves the critical thinking and clinical decision-making skills of nurses (6).

It is expected from students graduating from nursing programs at Turkish universities that they meet the health care needs of patients using an individual and holistic approach, based on established nursing process and theory (7,8). Importantly too, the publication of the 2010 Nursing Regulations by the Turkish Ministry of Health in 2010, with the inclusion of the phrase 'the nursing service applies the nursing process', meant in fact that the application of the nursing process became a legal obligation (9).

Assessment is the first step of the nursing process. Assessment should include all aspects of the individual in order to determine his or her overall state of health, in accordance with a particular nursing model. Nursing models guide nurses in how to gather data in a systematic and organised manner. They also help ensure that nursing care is provided in a scientific, planned and systematic way, based on a sound theoretical foundation (10,11). The two models most commonly used today in nursing education are 'Activities of Daily Living: A Model for Nursing (ADLs)' developed by Roper, Logan, and Tierney (1996), and 'Functional Health Patterns (FHP)' developed by Gordon (1982). However, there is no data available to explain the reasons why one model has been chosen over the other for particular nursing

DOI: 10.33808/clinexphealthsci.568335

programs in our country. Also, there is very little data as to the distinctions between the two models and their relative effectiveness in the context of individual care planning. The ADLs model has been adopted both in education and in nursing practice. It provides an easy method of assessment within the nursing process, as well as being readily applicable to both ill and healthy individuals. On the other hand, nursing diagnoses under NANDA-I (North American Nursing Diagnosis Association – International) are categorised according to Gordon's FHP (7) and it is considered that this model best facilitates the determination of correct nursing diagnosis. Because of this, the purpose of this study was to evaluate nursing students' perceptions regarding nursing diagnoses and clinical decision making and to compare how these perceptions differ according to which nursing model was followed.

Research Questions

- What are the students' perceptions regarding nursing diagnosis?
- What are the clinical decision-making perception scores of students?
- What is the difference of two models on nursing students' perceptions regarding nursing diagnosis and clinical decision making?
- What is the correlation between students' perceptions regarding nursing diagnoses and clinical decision making?
- What is the relationship between the exam notes of the Fundamentals of Nursing course and the clinical decision-making perception/nursing diagnosis perception scores of students?

2. METHODS

2.1. Study Design

A descriptive, cross-sectional, comparative, study was conducted. The study was carried out in one nursing department in Istanbul and Çanakkale provinces, Turkey.

2.2. Sample

The population of the study consisted of all 264 second grade students who successfully passed the Fundamentals of Nursing course and studied in the academic year 2016-2017. Therefore, there was no need for sample selection. The study was in fact completed with a total sample of 257 students, as a result of factors such as failure to complete collection forms and absence due to sick leave, or other reasons.

The students in both schools received the Fundamentals of Nursing I and II theoretical classes and clinical skills laboratory teaching, and over the same period they met and observed the care needs of at least seven patients in accordance with the nursing process. However, in order to diagnose patients in the course of the nursing process, one school used ADLs, while the other used FHP. During the theoretical classes which accompanied practical lessons, NANDA-I diagnoses were applied to the sample cases examined by students as part of their nursing process learning. Although the assessment phase was based on two different models, NANDA-I was used for nursing diagnosis in both schools.

2.3. Instruments

Prior to collecting the data, a short description about the study objectives was offered to the students before distributing the questionnaires. Students were instructed about their anonymity and confidentiality. It was guaranteed that they might be able to withdraw from the study in case they would like. The data were collected via face-to-face interviews in the classroom after the Fundamentals of Nursing final exam was announced. The following methods were used for the collection of data: a 'Structured Information Form' prepared by researchers, the 'Perception of Nursing Diagnoses Survey (PND)' and the 'Clinical Decision Making in Nursing Scale (CDMNS)'.

2.4. Structured Information Form

The Structured Information Form was prepared by the researchers on intervening variables, including the students' age, gender, their average grade from the Fundamentals of Nursing course and the school they were attending.

Perception of Nursing Diagnoses Survey (PND)

The PND Survey was developed by Olsen, Frost, and Orth (1991) with the aim of determining how students perceive nursing diagnoses. The survey consists of 30 items and 4 subscales: 'Delineation and promotion of nursing profession', 'Clear representation of patient's situation', 'Ease of Use', and 'Conceptual orientation'. The validity and reliability of its Turkish version was tested in 2013 by Akın-Korhan et al. When PND adapted to Turkey, the number of items in PND decreased to 26 from 30. The Cronbach's alpha value of the PND was 0.84. (12). In this study, the Cronbach's alpha value of the PND was 0.83. 5-point Likert scale was used, ranging from the 5 points assigned for 'I strongly agree' to 1 point for 'I strongly disagree'. The results were determined by dividing the points by the number of items, with the total score varying from 1 to 5. A low total score on the scale indicates that students perceive the nursing diagnoses in a positive way (12). We are unaware of other research describing a significant difference in decision making between ADLs and FHP. We believe that this difference may explain when the research is carried out on this subject.

Clinical Decision Making in Nursing Scale (CDMNS)

The CDMNS measures the perceptions of nursing students in their own words with regard to clinical decision making. It was developed by Jenkins in 1983 and adapted for application in Turkey by Dicle and Durmaz in 2015. This original scale

consisted of a total of 40 items, divided equally into four categories as follows: 'search for alternatives or options', 'canvassing of objectives and values', 'evaluation and re-evaluation of consequences', and 'search for information and unbiased assimilation of new information'. The scale's Cronbach α value was found out to be 0.78 (13). In this study, the Cronbach's alpha value of the CDMNS was 0.71. Twenty-two of the items carried a positive meaning, and 18 a negative one. Each item was assigned a frequency value as follows: 5=always, 4=often, 3=sometimes, 2=rarely, and 1=never, with the scale's 18 negative value items graded in the opposite direction, from 5=never, down to 1= always. In theory, therefore, it is possible to obtain a score ranging anywhere from 40 to 200 points, with 10 to 50 points available from each sub-category. Also, there is no break point. A high score obtained from the scale indicates that the perception of decision making is high, while a low score indicates that perception is low (14,15).

2.5. Ethical Considerations

Approval was taken from Istanbul Medipol University's Ethics Committee (10840098-604.01.01-E.11891) before the study. Written permission was taken from the school where the study was conducted. The aims and intended benefits of the study were explained to the participating students by the researchers, who were in fact at the same time the instructors of the course. Verbal consent was obtained from students in accordance with the usual protocol for volunteers.

2.6. Data Analysis

The Licensed SPSS 16.0 (Statistical Package for Social science for Windows, Version 16.0) was used for the data analysis. Results were evaluated within the 95% confidence interval, at the significance level of $p < 0.05$. The nominal variables were rated for frequency and percentage; ordinal variables were rated for the minimum, maximum, median values, mean and standard deviation. Shapiro-Wilk test was used to examine the normal distribution. Because of non-normally distributed the Mann-Whitney U test was used for comparison between descriptive analyses (frequency, percentage, mean ± *SD*, median) and variables. Spearman's correlation technique was used to determine the relationship between Nursing Students' Clinical Decision Making in Nursing Scale and Subscales and Perception of Nursing Diagnoses Scale mean scores.

3. RESULTS

All the participants followed the 'Fundamentals of Nursing I and II' theoretical course and laboratory education programme, and over the same period provided for the care needs of at least 7 patients in line with the nursing process. 77.1% of the participants were women, with an average age of 19.09, and their average grade from the Fundamentals of Nursing course was 74.23 ± 9.41.

The total of the PND score for the ADLs group was 2.45 ± 0.55, while for the FHP group it was 2.31 ± 0.27. The difference in the incidence of value was not significant (p = 0.271). According to FHP and ADLs groups, there was not a statistically significant difference in the PND subscales (p > 0.05) (Table 1).

Table 1. *Nursing Students' Perception of Nursing Diagnoses Scale and Subscales: ADLs and FHP*

PND and Subscale		Groups			
		ADLs (n = 117)	FHP (n = 128)	The Total of Two Group (n=245)	[c] *p*
Definition of/ Introduction to the Nursing Profession	*Mean.± SD*	2.19 ± 0.76	2.43 ± 0.81	2.07 ± 0.6	*0.112*
Definitive Description of Patient's State	*Mean.± SD*	2.9 ± 0.53	2.82 ± 0.42	2.86 ± 0.48	*0.311*
Ease of Use	*Mean.± SD*	2.51 ± 0.52	2.48 ± 0.5	2.49 ± 0.51	*0.449*
Conceptual Orientation	*Mean.± SD*	2.64 ± 0.56	2.69 ± 0.42	2.67 ± 0.49	*0.13*
Value of PND	*Mean.± SD*	**2.45 ± 0.55**	**2.31 ± 0.27**	**2.38 ± 0.43**	*0.271*

[c]*Mann Whitney-U*

ADLs: A model of nursing care based on activities of daily living; FHP: Functional Health Patterns ; PND: Perception of Nursing Diagnoses Survey

The total of the CDMNS score mean of the ADLs group was 147.44 ± 12.95, and the mean of the FHP group was 154.3 ± 12.29. A statistically significant difference was detected between the groups (p = 0.00). Also the mean of the subscales score FHP group was found to be higher than that for the ADLs group and the differences statistically significant: 'canvassing of objectives and values' (p = 0.006), 'evaluation and re-evaluation of consequences' (p = 0.001), and 'search for information and unbiased assimilation of new information'(p = 0.00) (Table 2).

There were found to be significant differences between the total of CDMNS and subscale, and the total of PND (p < 0.01) (Table 3).

Also, according to the results comparing the relationship between the exam notes of the Fundamentals of Nursing course and the total scores of CDMNS-Tr, there was a statistically significant correlation (p < 0.01). But the relationship between the exam notes of the Fundamentals of Nursing course and the total scores of PND did not show a statistically significant correlation (p < 0.05).

Table 2. *Clinical Decision Making in Nursing Scale and Subscales: ADLs and FHP*

CDMNS and Subscale		Groups			
		ADLs (n=117)	FHP (n=128)	Total (n=245)	[c] *p*
Search for alternatives or options	*Min-Max (Median)*	26-50 (39)	30-48 (40)	26-50 (39)	***0.037***
	Mean.± SD	38.66 ± 4.47	39.66 ± 3.83	39.18 ± 4.17	
Canvassing of objectives and values	*Min-Max (Median)*	28-44 (35)	26-47 (36)	26-47 (35)	***0.006****
	Mean.± SD	35 ± 3.43	36.35 ± 3.87	35.71 ± 3.72	
Evaluation and re-evaluation of consequences	*Min-Max (Median)*	30-50 (39)	30-48 (41)	30-50 (40)	***0.001****
	Mean.± SD	38.58 ± 4.26	40.37 ± 4.02	39.5 ± 4.22	
Search for information and unbiased assimilation of new information	*Min-Max (Median)*	26-45 (35)	31-84 (37)	26-84 (37)	***0.00****
	Mean.± SD	35.17 ± 3.56	37.9 ± 5.39	36.6 ± 4.8	
Total of CDMNS-Tr	*Min-Max (Median)*	112-180 (146)	124-186 (154)	112-186 (151)	***0.00****
	Mean.± SD	147.44 ± 12.95	154.3 ± 12.29	151.02 ± 13.04	

[c]*Mann Whitney-U; *p<0.01*

ADLs: A model of nursing care based on activities of daily living; FHP: Functional Health Patterns; CDMNS: Clinical Decision Making in Nursing Scale

Table 3. *The Relationship between Nursing Students' Clinical Decision Making in Nursing Scale and Subscales and Perception of Nursing Diagnoses Scale*

CDMNS and Subscale	Total of PND (n=245)	
	r	*p*
Search for alternatives or options	-0.254	0.00*
Canvassing of objectives and values	-0.194	0.002*
Evaluation and re-evaluation of consequences	-0.232	0.00*
Search for information and unbiased assimilation of new information	-0.242	0.00*
The Total of CDMNS-Tr	-0.281	0.00*

*r. Spearman Correlation *p<0.01*

CDMNS: Clinical Decision Making in Nursing Scale; PND: Perception of Nursing Diagnoses Survey

4. DISCUSSION

Perceptions regarding nursing diagnosis of students and the difference of two models

The nursing diagnosis is the second step of the nursing process. Scientifically-based nursing diagnosis can be described as a clinical judgement about individual, family and community experiences or responses to actual or potential health problems or life processes (10,11). Nursing diagnoses determine the way that nursing knowledge is applied. At the same time they are important for their contribution to the autonomy of nursing as a distinct discipline and in providing a standard nomenclature (16).

The correct use of nursing diagnoses serves to increase patient safety and improve health care quality (17,18). Internalisation of the importance of correct nursing diagnoses is achieved through nursing education. A study conducted by El-Rahman et al. indicated that Jordanian nursing students perceived nursing diagnoses positively (19). In a study in Turkey by Hakverdioğlu et al., most of the students stated that nursing diagnoses were a priority in care practices (20). In the study which initially developed the PND scale, the value of the PND score was found to be 2.94. In the study into the validity and reliability of its Turkish version, the value was determined to be 2.48 (12). Another study, conducted by Halverson et al. on nurses from Minnesota, found the value of the PND score to be 3.09 (21). In the current study, the value of the PND score for nursing students from the two different schools was found to be 2.38 ± 0.43. These findings show similarities to the results of other studies. From the results, it can be concluded that the use of nursing diagnoses is positively perceived. It is considered that in both schools featured in this study, the results are compatible with the education offered. This accords with the statement commonly found in nursing program learning outcomes: 'He/she has acquired sufficient knowledge to enable them to meet the health needs of individuals, family and community by means of an individual and holistic approach, applied through the use of nursing process'. At the same time, the subscales of PND was perceived samely by students receiving an FHP and ADLs-based education.

Clinical decision-making perception of students and the difference of two models.

Decision making in clinical settings is a necessary factor in the provision of safe, quality care for the community and in improving patient care outcomes. Decision making in clinical settings is the way nurses express their knowledge in practice (22-24). It is important to determine the perception of nursing students in clinical decision making, and to evaluate their decision-making perception. In the current study, students' total CDMNS score of 151.02 ± 13.04 shows that their decision-making perception in clinical settings are in process of development. One study found that the undergraduate nursing students total of CDMNS score was 160.82 ± 10.75 (14). The study conducted by Dicle and Durmaz, students' total of CDMNS score was specified as 156.90 ± 11.11 and indicates that their decision-making perception in clinical settings are in process of development (13). Clinical decision making in a nursing context can be regarded as the process of putting acquired nursing knowledge into practice (23). In this study, the detection of a significant relationship between the

Fundamentals of Nursing exam score and the total of CDMNS score confirms this information.

It was determined that, with regard to the total CDMNS score, students receiving FHP based education did better than those receiving ADLs based education. This difference was found to be statistically significant. These results can be explained by the fact that NANDA-I diagnoses are classified according to FHP and thus it can make the decision easily. Besides, it should be noted that clinical decision making can also be affected by a range of other factors, such as: theoretical knowledge, personal traits, complexity of the decision-making situation (22).

Relationship between students' perceptions regarding nursing diagnoses and clinical decision making

The current study results show that there is a positive relationship between the subscales of CDMNS and the value of PND. Nursing diagnosis is a clinical judgment about the real or potential health problems / life processes of the individual, the family and the society (25). Positive perception of nursing diagnoses influences the clinical decision-making process of the individual's health care needs. Clinical decision-making perception being improved facilitates making a correct nursing diagnosis in the health care process. For this reason, there being a positive relationship between students' perceptions regarding nursing diagnoses and clinical decision making in our study is an expected outcome.

Limitations

The research was limited to the nursing departments in which the study was conducted and the results cannot be generalised. Ideally, the study should be repeated for further sample groups.

5. CONCLUSION

The two models most often used in nursing education in Turkey are ADLs and FHP. However, there is no information available as to why one is chosen over another in nursing programs across the country. At the same time, it is not clear how students perceive differences between the two models in terms of effectiveness or ease of use. This study attempts to explain the relationship between, on the one hand, the perception of nursing diagnoses and clinical decision making, and, on the other, the two models which constitute the basis of the education provided by nursing schools. No difference was found in perception of nursing diagnoses between the groups who were taught patient assessment with the two different models. It was observed that students receiving the FHP-based education were better than their counterparts who received the ADLs-based education with regard to clinical decision-making perception. However, it should be remembered that clinical decision making may be affected by a number of different factors. For example, in the study it was observed that students who attained good exam scores from the Fundamentals of Nursing course showed high clinical decision-making ability. In this light it is recommended that, ideally, a further evaluation should be carried out which takes more of these factors into account. It is likely too that there would be definite benefits from repeating the study with a larger sample and in different schools.

Implication for Nursing Knowledge

This study helps to understand the impact of two nursing models that are widely used in nursing schools in Turkey, on the perception of nursing diagnosis and clinical decision making. As a result of the study, it is seen that the two nursing models have different advantages in comparison to each other. Academic staff can decide which to choose in accordance with their expectations from the students. Thus, study results will shed light on the reason why they choose one model but not the other one.

REFERENCES

[1] April M, Bassendowski S. The history of evidenced-based practice in nursing education and practice. J Prof Nurs 2017; 33:51-55.

[2] Boztepe H, Terzioğlu F. Skill assessment in nursing education. Journal of Anatolia Nursing and Health Sciences 2012; 16:57-64.

[3] Güner P, Terakye G. The level of determination nursing diagnosis of senior nursing school students. Journal of Cumhuriyet University School of Nursing 2000; 4:9-15. (Turkish)

[4] Akça NK, Taşçı S. Nursing education and critical thinking. Mersin University Journal of the Faculty of Education 2009; 5:187-195. (Turkish)

[5] Gorton LK, Hayes J. Challenges of assessing critical thinking and clinical judgement in nurse practitioner students. J Nurs Educ 2014; 53:26-29.

[6] Andsoy II, Güngör T, Dikmen Y, Nabel E. B. Difficulties that nurses have in using care plan. J Contemp Med 2013; 3(2):88-94. (Turkish)

[7] Vicdan AB, Karabacak B, Alpar SE. Classification of 2012-2014 NANDA-I nursing diagnostic susing the nursing model based on activities of living. International Journal of Human Sience 2015; 12:1625-1626.

[8] Hakverdioglu G, Korhan AE, Erdemir F, Muller-Staub M. Nursing diagnoses determined by first year students: a vignette study. Int J Nurs Knowl 2014; 25:39-42.

[9] Hemşirelik yönetmeliğinde değişiklik yapılmasına dair yönetmelik 2011 (cited 2019 April 1) Available from: https://www.resmigazete.gov.tr/eskiler/2011/04/20110419-5.htm

[10] Birol L. Hemşirelik Süreci. İzmir: Etki Yayınları; 2009 (Turkish).

[11] Carpenito-Moyet LJ. Nursing Diagnosis: Application to Clinical Practice. 13th ed. Philadelphia: Lippincott, Williams & Wilkins; 2010.

[12] Akın-Korhan E, Hakverdioğlu-Yönt G, Ak B, Erdemir F. Analysis of Turkish validity and reliability of perception of nursing diagnosis. HEMAR-G. 2013; 15:13-25. (Turkish)

[13] Dicle A, Durmaz EA. Examination of clinical decision-making perceptions of nursing students. New Educ Review 2013; 33:134-144.

[14] Edeer AD, Sarikaya A. Adaptation of clinical decision making in nursing scale to undergraduate students of nursing: The study of reliablity and validity. IJPES 2015; 2:1-9.

[15] Jenkins H. A research tool for measuring perceptions of clinical decision making. J Prof Nurs 1985; 1:221-229.

[16] Aşti TA, Karadağ A. Fundemental of Nursing. İstanbul: Akademi Basın ve Yayıncılık; 2012 (Turkish).

[17] Turk G, Tugrul E, Sahbaz M. Determination of nursing diagnoses used by students in the first clinical practice. Int J Nurs Knowl 2013; 24:129-133.

[18] Zaybak A, İsmailoğlu GE, Özdemir H. Examining the difficulties experienced by nurses in the nursing process applications. Journal of Anatolia Nursing and Health Sciences. 2016; 19:269-277. (Turkish)

[19] El-Rahman MA, Kalaldeh MT, Malak MZ. Perceptions and attitudes toward NANDA-I nursing diagnoses: A cross-sectional study of Jordanian nursing students. Int J Nurs Knowl 2017; 28:13-18.

[20] Hakverdioglu GY, Khorsid L, Eser I. Examination of nursing diagnoses used by nursing students and their opinions about nursing diagnoses. Int J Nurs Terminol Classif 2009; 20:162-168.

[21] Halverson ER, Beetcher EL, Scherb AC, Olsen G, Frost M, Orth K. Minnesota nurses' perceptions of nursing diagnoses. Int J Nurs Terminol Classif 2011; 22:123-131.

[22] Sucu G, Dicle A, Saka O. Decision making in clinical nursing: decision-making models and affecting factors. HEMAR-G. 2012; 9:52-60. (Turkish)

[23] Yıldırım A, Taşcı S. Clinical decision-making on the effect of critical thinking in nursing. Balikesir Health Sciences Journal. 2013; 2:186-191. (Turkish)

[24] Janet LM, Kathie L, Sydnee S, Nathan D. New graduate nurse experiences in clinical judgment: What academic and practice educators need to know. Nurs Educ Perspect 2018; 39:201-207.

[25] Kaya H. NANDA International. Nursing Diagnoses: Definitions & Classification. 10th ed. Istanbul: Nobel Tıp Kitabevleri; 2017 (Turkish).

How to cite this article: Inangil D, Uzen Cura S. Nursing Students' Perceptions of Nursing Diagnoses and Clinical Decision-Making. Clin Exp Health Sci 2020; 10: 131-136. DOI: 10.33808/clinexphealthsci.568335

Clinical and Experimental Health Sciences

Reliability and Validity of Nurses' Experiences of Infection Prevention and Control Questionnaire

Eda Aktas[1], Cagri Covener Ozcelik[2], Ayse Ferda Ocakci[3]

[1] University of Health Sciences, Hamidiye Faculty of Nursing, Department of Pediatric Nursing, Istanbul, Turkey.
[2] Marmara University, Faculty of Health Sciences, Department of Nursing, Pediatric Nursing Division, Istanbul, Turkey.
[3] Koc University, Faculty of Nursing, Istanbul, Turkey.

Correspondence Author: Eda Aktas
E-mail: eda.aktas@sbu.edu.tr
Received: 13.11.2019 **Accepted:** 02.04.2020

ABSTRACT

Objective: Nosocomial infections are the primary reason of morbidity and mortality especially at children's hospitals. Thus, educating pediatric health care providers is rather crucial in order to protect children from exposure to infection. This study was planned to examine reliability and validity of *Nurses' Infection Prevention and Control Observation Questionnaire* in Turkish Language.

Methods: This methodological study was carried out at a randomly selected Children's Hospital from each of the 7 regions of Turkey. In total, 443 pediatric nurses from those hospitals participated in the study. Content validity, construct validity, inter-consistency reliability and item total correlation of the questionnaire were analysed in order to adjust reliability and validity.

Results: The questionnaire has good content, face and construct validity. The acceptable level for scales' items was above 0.30 and had one factor according to results of explanatory factor analysis. The questionnaire model fitted the data according to confirmatory factor analysis. A level of Cronbach's alpha at 0.74 was considered to be an acceptable level of reliability and item-total score correlation of the study –except for four items – was identified above 0.30.

Conclusion: The questionnaire was determined as a reliable and validate tool in evaluation of observation for nurses' infection prevention and control.

Keywords: Infection control, pediatric nurse, validity, reliability

1. INTRODUCTION

Nosocomial infections are among the most frequently reported problems arising in the provision of healthcare services the world over (1,2). Nosocomial infections represent the most significant health challenges to modern medicine in the broader sense that 'healthcare service-related infections' are one of the most important healthcare problems in modern medicine that are predictable and manageable (3-5). It has been estimated that nosocomial infections develop in 1.4 million people every day (6).

Nosocomial infections are not only a source of morbidity and mortality for patients, they also represent a serious financial loss for the countries involved (6-7). The key risk factors leading to nosocomial infections are:

- Interventional implementations at the hospital,
- Poor cleaning,
- Physical shortages,
- Insufficient number of personnel and
- Negative factors affecting the patient's immune system (7).

Risk factors leading to the development of nosocomial infection usually occur due to ignoring infection control principles (infrastructure insufficiency of hospitals, inadequate and uneducated healthcare providers, poor hand hygiene compliance by healthcare providers and their use of inappropriate types of medical gloves, unnecessary invasive interventions and not adhering to asepsis and antisepsis protocols) (5,7,8).

Nosocomial infection is the primary reason for morbidity and mortality, especially in children's hospitals (9). Thus, educating paediatric healthcare providers is crucial in order to protect children from exposure to infection. Since the number of brief and easily understood questionnaires is

DOI: 10.33808/clinexphealthsci.646357

limited in the Turkish language, this study aimed to prove the reliability and validity of the Nurses' Infection Prevention and Control Observation Questionnaire in the Turkish language.

2. METHODS

2.1 Type of Study

The study was methodological design.

2.2 Participants

The study was conducted at children's hospitals which were randomly selected from each of the 7 regions of Turkey. A total of 443 pediatric nurses working at these hospitals participated in the study. The inclusion criteria were a) having at list 1 month of clinical experience and holding a permanent position in the pediatric department, b) working full time, c) voluntarily signing the informed consent form

2.3 Measurements

Nurse Descriptive Information Form: This form consists of a total of 8 items, 5 close-ended questions and 3 open-ended questions and includes the sociodemographic characteristics of nurses working at the paediatric clinics. It was designed to be compatible with similar forms found in the literature.

Nurses' Infection Prevention and Control Observation Questionnaire: The questionnaire was developed to determine the observations of intern nurses on infection control and prevention. It consists of a total of 19 items, designed as a 5-point Likert-like questionnaire and scored as follows: 1 = never, 2 = not often, 3 = do not know/cannot remember, 4 = often and 5 = witnessed poor practice very often. The lowest score shows positive infection prevention behaviour. Gould et al. (2013) designed the items of the questionnaire, but validity and reliability analyses were not performed.

2.4 Data Collection

The required data were gathered from March 2014 to June 2015. The questionnaire was posted to relevant hospitals, and then the hospitals re-posted the questionnaires to the researchers. The study did not incur any loss of data.

2.5 Ethical Considerations

Required permissions were obtained from the Ethic Committee of Koc University (21.10.2013, 2013.209. IRB3.142). Participants were informed that the study was conducted solely for scientific means and that the data obtained from the study would never be shared with third parties. The participants confirmed their participation with their written approvals.

2.6 Statistical Analysis

Number Cruncher Statistical System (NCSS) 2007 Software (Utah, USA) was utilized to analyse the data. For validity analysis, the language validity, content validity and construct validity were tested. Explanatory factor analysis (EFA) and confirmatory factor analysis (CFA) were used to test the construct validity. Cronbach's alpha coefficient and item-total score correlation were calculated for reliability analysis. Further, sociodemographic data were calculated using descriptive statistical methods (averaging, standard deviation (SD) and percentage).

3. RESULTS

Mean ages of the participants were 30.76 ± 6.56 (18–52) years, and the duration of their professional career at clinics was 7.49 ± 7.06 (1 month to 34.41 years) years. A total of 87.4% (n = 387) of the participant nurses were females, and 12.6% (n = 56) were males.

3.1 Validity

3.1.1 Linguistic validity

The questionnaire was first translated from English to Turkish separately by two bilingual linguistic experts experienced in medical and nursing texts. The scales were then translated back from Turkish to English by two other bilingual language experts. The back-translated and original scales were compared, and they were found to be highly similar in terms of meaning. This completed the language validation.

3.1.2 Face validity

A pilot study was conducted with 30 paediatric nurses to ensure *face validity*. No changes in the questionnaire were deemed necessary.

3.1.3 Content validity

The Turkish scales were presented to 10 experts in communicable disease nursing and health sciences in order to test intelligibility and compatibility with the culture; *content validity* was ensured by making minor changes, such as word or sentence corrections, based on their suggestions. The content validity index (CVI) was calculated. The experts were asked to rate each item on a 4-point scale (1 = *not relevant*, 2 = *somewhat relevant*, 3 = *quite relevant* and 4 = *highly relevant*) according to the Davis technique (10). Then, for each item, item-CVI (I-CVI) was computed as the number of experts stating a rating of either 3 or 4, divided by the total number of experts. If 80% of the experts rated the item 3 or 4, the I-CVI score was 0.80. Then, the mean I-CVI was calculated across items for the questionnaire-CVI (Q-CVI) (11). The Q-CVI of the form was 1.0. Also, Kendall's W concordance analysis was utilized to assess content validity and was found to be concordant among the experts (Kendall's W = 0.221, df = 18, c^2 = 19.92, p = 0.337).

3.1.4 Construct validity

EFA was applied to ensure construct validity. The Kaiser-Meyer-Olkin (KMO) measure was 0.80, indicating the applicability of EFA. Bartlett's test of sphericity was statistically significant (χ^2 = 1220.27; p = 0.000). According to the results of factor analysis, the questionnaire had one factor and explained 21.63% of total variance. The acceptable level for the scales' items was above 0.30 according to the results of the EFA (Table 1).

Table 1. *Characteristics of Nurses' Infection Prevention and Control Observation Questionnaire*

Items	Factor load	Item to total correlations
1) Not cleansing hands between patient contacts	0.39	0.33
2) Wearing rings (excluding wedding bands)	0.44	0.30
3) Failure to apply isolation precautions (eg, not wearing PPE)	0.59	0.50
4) Poor cleaning (eg, lockers, trolleys, baths, wash bowls)	0.54	0.44
5) Not changing personal protective clothing between patients	0.47	0.39
6) Poor practice "sharps" management (eg, resheathing)	0.55	0.43
7) Using mobile telephones during patient contact	0.41	0.30
8) Reusing items without cleaning between patients	0.46	0.35
9) Items stained with blood or body fluids	0.45	0.32
10) Not being "bare below the elbow"	0.34	0.27
11) Dealing with body fluids without wearing gloves	0.58	0.45
12) Poor practice in relation to urinary catheters (eg, disconnecting catheter from drainage system)	0.57	0.41
13) Reuse of scissors during dressing procedures without cleaning	0.33	0.22
14) Poor management of intravenous therapy (eg, disconnecting lines from access device)	0.53	0.39
15) Inappropriate storage of sterile items (eg, torn or dusty outer wrapping)	0.48	0.32
16) Reuse of single-use item	0.40	0.27
17) Insertion of a urinary catheter without gloves	0.46	0.28

However, CFA was performed to test for construct validity. The factor model fitted the data (p = 0.000). Goodness of fit statistics follow: the root mean square error of approximation (RMSEA) was 0.066; comparative fit index (CFI) was 0.90; incremental fit index (IFI) was 0.90; root mean square residual (RMR) was 0.037, goodness of fit index (GFI) was 0.91 and χ^2/df index was 3.11, respectively (Fig. 1).

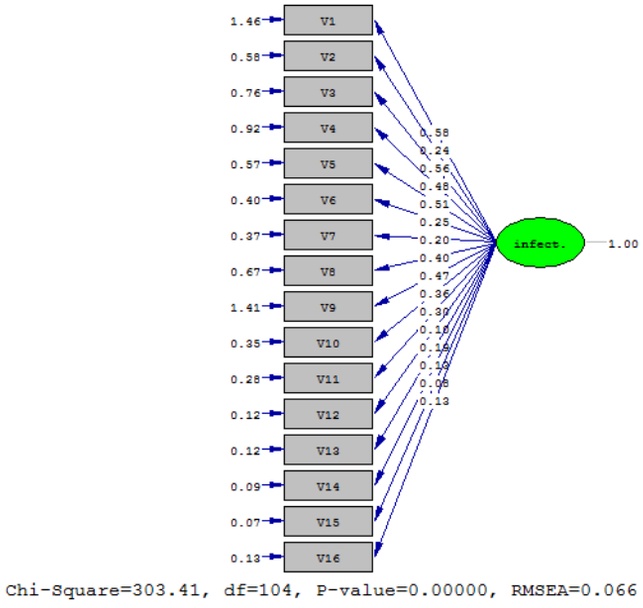


Figure 1. *Path Diagram of Confirmatory Factor Analysis for Nurses' Infection Prevention and Control Observation Questionnaire*

3.2 Reliability

The results of item-total score correlation are presented in Table 1. Cronbach's alpha coefficient of the questionnaire was determined as 0.74.

4. DISCUSSION

In the original text of the *Nurses' Infection Prevention and Control Observation Questionnaire,* validity and reliability analysis had not been performed (1). Consequently, in the Turkish adaptation of the questionnaire, validity and reliability analyses were performed using various methods.

Content validity refers to how accurately an assessment or measurement tool taps into the various aspects of the specific construct in question (11). In this case, the CVI was calculated. A CVI score of 0.80 or above means that all experts are in agreement about relevance. The Q-CVI of the form was 1.0, which means that the form has an acceptable content validity. However, according to Kendall's W concordance analysis, no meaningful difference was detected among the views of the experts. These results show that the questionnaire is appropriate for the Turkish culture.

Construct validity assesses how much the tool reached its goal to measure an abstract concept, difficult-to-measure behaviour or dimension. In the present study, explanatory and confirmatory factor analyses were utilized to ensure construct validity. EFA is a statistical method used to obtain information on the nature of the tool and factors being measured instead of testing a particular hypothesis (11).

According to KMO measure for sampling adequacy test values, 0.60–0.69 represents 'average', 0.70–0.79 is 'good', 0.80–0.89 is 'very good' and 0.90–1.00 is 'perfect'. Bartlett test evaluates the appropriateness of items for factor analysis (12). The value of 0.80 'very good' for the KMO measure for sampling adequacy test and p = 0.000 for the Bartlett test indicate that the results are statistically significant. Based on the findings, it may be concluded that the sample of the present study is appropriate for factor analysis. Factor loads ranged from 0.33 to 0.59. Factor loads were at acceptable levels as they were found above 0.30 (13). Two items were removed from the questionnaire because their factor loads were under 0.30. Although the original questionnaire has 19 items, the adapted questionnaire has 17 items.

CFA is a multivariate statistical procedure used to test whether the measured variables represent the number of constructs (11). The RMSEA fit index is used to assess goodness of fit statistics in CFA, with RMSEA ≤ 0.05 showing a perfect fit, while RMSEA ≤ 0.08 shows a good fit (14). The RMSEA value of the questionnaire was 0.066, which indicated a good fit. CFI ≥ 0.95 shows a perfect fit, and CFI ≥ 0.90 shows a good fit. The CFI fit index of the questionnaire, 0.90, represents a good fit. Another fit index is the IFI where ≥ 0.95 shows perfect fit, and IFI ≥ 0.90 shows a good fit (15). The IFI fit index of the questionnaire, 0.90, means a good fit. On the other hand, RMR fit index is a perfect fit if it is ≤ 0.05, and ≤ 0.08 is a good fit. In this study, the RMR index was 0.037, which shows a perfect fit. Another fit index, GFI, means a good fit if the value is ≥ 0.90, so the GFI fit index of the questionnaire, 0.91, represents a good fit. Similarly, a χ^2/df fit index of ≤ 3 shows a perfect fit, and ≤ 5 shows an acceptable model fit (14). The χ^2/df value for the questionnaire was 2.92, indicating a perfect fit. The questionnaire model fitted the data according to CFA.

Reliability refers to ensuring determinedness, adequacy, equality, consistency and stability (11). They are known as test-retest, parallel or alternate form, item-total correlation, split-half, Kuder-Richardson (KR-20), Cronbach's alpha and interrater reliability (16). In the present study, Cronbach's alpha reliability coefficient and item-total score correlation were used to analyse reliability. Cronbach's alpha coefficient of the study was determined as acceptable. A Cronbach's alpha coefficient of 0.70 or higher is considered to be acceptable for reliability (12,16).

The item-total score correlation of the study, except for four items, was identified as above 0.30. It was suggested in some sources that the correlation coefficient of the items should be higher than 0.30 and those items with lower values should be removed (11,12). The item-total score correlation value of four items ranged from 0.22 to 0.28. However, those items' factor loads were from 0.33 to 0.46, so researchers decided not to remove them in order not to ensure the integrity of the questionnaire.

Strengths and Limitations of The Study

The sample of the study included 443 paediatric nurses. It has been stated that reliability and validity analysis should include 5 to 10 times more participants than the total number of the items (11). Even though 190 participants would have been enough for the present study, it was conducted with a total of 443 participants. Findings of the study can be generalized to the country since the participants were selected from seven different regions in Turkey. All the reliability and validity analyses resulted in acceptable levels. However, the most significant limitation of the study was that it was carried out only with paediatric nurses working at children's hospitals.

5. CONCLUSION

These results indicated that this scale is a valid and reliable questionnaire. It is an useful questionnaire for evaluation of observation for nurses' infection prevention and control. Pediatric nurses can easily use this questionnaire. In addition, since, there is limited number of infection scales, this scale could be used in future studies and education programmes for nurses aiming at changing their behaviours and attributes.

REFERENCES

[1] Gould D, Drey N. Student nurses' experiences of infection prevention and control during clinical placement. Am J Infect Control 2013;41(9):760-763.

[2] Kaier K, Wilson C, Hulscher M, Wollersheim H, Huis A, Borg M, Scicluna E, Lambert ML, Palomar M, Tacconelli E, De Angelis G, Schumacher M, Wolkewitz M, Kleissle EM, Frank U. Implementing bundles for infection prevention and management. J Infect 2012;40(2):225-228.

[3] Jackson C, Lowton K, Griffiths P. Infection prevention as "a show": a qualitative study of nurses' infection prevention behaviours. Int J Nurs Stud 2013;51(3):400-408.

[4] Öztürk R. Hastane Enfeksiyonları: Sorunlar, Yeni Hedefler ve Hukuki Sorumluluk. In: Öztürk R, Saltoğlu N, Aygün G, editors. Hastane Enfeksiyonları Korunma ve Kontrol. İstanbul: Ohan Matbaacılık; 2008; pp.23-29 (Turkish).

[5] Pittet, D. The Lowbury lecture: behaviour in infection control. J Hosp Infect 2004;58(1):1-13.

[6] Alp E. Hastane enfeksiyonları tanımı, epidemiyolojisi ve risk faktörleri. In: Enfeksiyon Kontrol Programı. Kayseri: Erciyes Üniversitesi Hastaneleri Yayın No:55; 2012:5-7. (Turkish).

[7] Gürkan Karakuş Z, Ulupınar S. İnfeksiyon kontrol hemşirelerinin yaşadıkları güçlükler. Flora 2009;14(3):128-138.

[8] Morrow E, Griffiths P, Rao GG, Flaxman D. "Somebody else's problem": staff attributions of cause and risk of meticillin-resistant staphylococcus aureus. Am J Infect Control 2011;39(4):284-291.

[9] Pourakbari B, Rezaizadeh G, Mahmoudi S, Mamishi S. Epidemiology of nosocomial infections in pediatric patients in an Iranian referral hospital. J Prev Med Hyg 2012;53(4):204-206.

[10] Davis LL. Instrument review: getting the most from a panel of experts. Applied Nursing Research 1992; 5:194-197.

[11] Esin NM. Veri Toplama Yöntem ve Araçları & Veri Toplama Araçlarının Güvenirlik ve Geçerliği. In: Erdoğan S, Nahcivan N, Esin MN, editors. Hemşirelikte Araştırma. 1. Baskı. İstanbul: Nobel Tıp Kitabevi; 2014. pp.193-233 (Turkish).

[12] Tavşancıl, E. Tutumların Ölçülmesi ve SPSS ile Veri Analizi. 3. Baskı. İstanbul: Nobel Yayın Dağıtım; 2006 (Turkish).

[13] Şencan H. Cronbach Alfa Güvenirlilik Analizleri. In: Güvenirlik ve Geçerlilik. 1. Baskı, Ankara: Seçkin Yayıncılık; 2005. pp.230-247 (Turkish).

[14] Çokluk Ö, Şekercioğlu G, Büyüköztürk Ş. Sosyal Bilimler için Çok Değişkenli İstatistik SPSS ve LISREL Uygulamaları. 14. Basım. Ankara: Pegem Akademi; 2014 (Turkish).

[15] Seçer İ. Psikolojik Test Geliştirme ve Uyarlama Süreci SPSS ve LISREL Uygulamaları. 1. Baskı. Ankara: Anı Yayıncılık; 2012. pp. 98 (Turkish).

[16] Haber J, LoBiondo-Wood G. Reliability and validity. In: LoBiondo Wood G, Haber J, editors. Nursing research methods and critical appraisal for evidence-based practice. 6th ed. Missouri: Mosby Elsevier; 2006. pp.335-356.

How to cite this article: Aktas E, Covener Ozcelik C, Ocakci AF. Reliability and Validity of Nurses' Experiences of Infection Prevention and Control Questionnaire. Clin Exp Health Sci 2020; 10: 137-141. DOI: 10.33808/clinexphealthsci.646357

Clinical and Experimental Health Sciences

The Level of Serum C-Reactive Protein and Neutrophil Lymphocyte Ratio According to Thyroid Function Status

Fatih Kar, Zeynep Kusku Kiraz, Evin Kocaturk, Sema Uslu

Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Biochemistry, Eskisehir, Turkey.

Correspondence Author: Fatih Kar
E-mail: fkar@ogu.edu.tr

Received: 01.08.2019 **Accepted:** 23.02.2020

ABSTRACT

Objective: We aimed to investigate neutrophil lymphocyte ratio (NLR), leukocyte count (WBC), mean platelet volume (MPV) and C-reactive protein levels (CRP) as inflammatory markers according to thyroid function status in hypo-hyperthyroidism patients.

Methods: Data of patients (n=454, age>18) who applied to the Eskisehir Osmangazi University Hospital between March 2018 and December 2018 were evaluated retrospectively. There were 79 patients in hyperthyroidism group (TSH<0.27 µIU/ml, group I), 297 patients in euthyroid group (TSH=0.27-4.2 µIU/ml, group II) and 78 patients in hypothyroidism group (TSH>4.2 µIU/ml, group III).

Results: Serum TSH, fT4, fT3, anti-TG and anti-TPO levels were found statistically different between groups (p<0.001) but there were no significant difference in WBC, NLR and MPV between groups. There was a positive correlation between the NLR and CRP (r=0,295, p<0.01). In addition, NLR was positively correlated with WBC (r=0,412, p<0.001). Serum CRP levels were statistically higher in group I (3.5 mg/L [1.50-11]) than group II (2.1 mg/L [0.86-5.42]), (p<0.001). Although CRP levels were higher in group III (2.5 mg/L [1.18-5.73]) than group II, there was no significant difference. CRP showed weak positive correlation with fT4 (r=0,118, p<0.05) and negative correlation with TSH (r=-0,108, p<0.05).

Conclusion: High CRP levels may play an important role in the evaluation of hyperthyroidism in terms of thyroid dysfunction observed in the present study.

Keywords: Hyperthyroidism, hypothyroidsm, inflammation, TSH, CRP.

1. INTRODUCTION

The thyroid gland is an important endocrine organ that regulates basal metabolism and metabolic activity, secreting two major hormones triiodothyronine (T3) and thyroxin (T4). The secretion of these hormones are regulated by thyroid stimulating hormone (TSH) in the pituitary gland controlled by thyrotropin-releasing hormone (TRH) in the hypothalamus (1). Hyperthyroidism, hypothyroidism, and thyroid nodules are the most common disorders of the thyroid gland (2). The lack or absence of thyroid hormones (THs) causes hypothyroidism, and the excess of these hormones causes hyperthyroidism (3).

THs play a crucial physiological role and regulate human hematopoiesis. A study was performed in which all hematological parameters were normal in euthyroid state (4). In the evaluation of white blood cells and platelets, total leukocyte count and neutrophil levels decreased slightly in hypothyroid patients. Furthermore, total leukocyte count and neutrophil levels were found to be high, normal or mild in hyperthyroid patients (5).

The effects of THs were observed on the immune system in experimental research (6). TSH has been shown to be both produced and used by leukocytes as well as controlling thyroid hormone production and metabolic function (7). Moreover, lymphoid and myeloid cells also have a TSH receptor. There are studies showing the ability of TSH to influence lymphocyte function and it has been shown to have an enhancing effect on the proliferation of lymphocytes (8). Apart from these effects, T3 and T4 hormones may indirectly affect the immune system. Immune dysfunction has been demonstrated in cells with low TSh levels (9). The presence of a thyroid hormone receptor was also showed on haematopoietic progenitor cells (10).

Neutrophil/lymphocyte ratio (NLR) has been recently researched and is a marker of inflammation that can be easily

DOI: 10.33808/clinexphealthsci.600151

identified in the follow-up of the inflammatory process in patients with hyperthyroidism (11). One of the most commonly used acute phase reactants (APRs) is C-reactive protein (CRP) in the case of inflammation; a rapid increase is observed in the rate of damage and quickly returns to normal in recovery (12). Although thyroid disorders appear to cause changes in a large number of hematological parameters and the relationship between NLR and inflammation, there is inconsistent data on hypo-hyperthyroidism. In addition, many thyroid diseases involve inflammation processes, but so far, CRP is not used as a significant biomarker in thyroid diseases.

In the present study, the levels of hematological parameters (leukocyte, neutrophil, lymphocyte counts and mean platelet volume), serum inflammation markers (CRP) and their association with thyroid function tests (Serum free T4, free T3, TSH, anti-thyroglobulin and anti-thyroid peroxidase antibody) were evaluated in hypo-hyperthyroidism.

2. METHODS

Before obtaining the data, the research protocol was approved by Eskişehir Osmangazi University Non-invasive Clinical Research Ethics Committee (decision number:30, 30.04.2019) and handled according to the declaration of Helsinki.

Data of patients (n=454, age>18) who applied to the Eskişehir Osmangazi University Hospital between March 2018 and December 2018 were evaluated retrospectively. Patients with known chronic inflammatory disease, such as diabetes mellitus, with malignancy, hematological disease and acute infection were excluded from the study. The causes of hypothyroidism and hyperthyroidism such as chronic autoimmune response (Hashimoto thyroiditis), iodine status, genetic, drug-induced, transient thyroiditis, thyroid infiltration and escalated stimulation, Graves disease like secondary to TSH receptor antibodies were not specifically evaluated in our study.

TSH levels were used to detect the thyroid disorder group of all participants. There were 79 patients in hyperthyroidism group (TSH<0.27 µIU/ml, group I), 297 patients in euthyroid group (TSH=0.27-4.2 µIU/ml, group II) and 78 patients in hypothyroidism group (TSH>4.2 µIU/ml, group III)

Complete blood count (CBC), inflammation and thyroid function test parameters of the patients who visited our laboratory for routine control and were not included in the exclusion criteria were used in the study. fT4, fT3, TSH, anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase antibody (anti-TPO) levels for thyroid function tests; leukocyte (WBC), neutrophil (Neu), lymphocyte (Lym) counts, mean platelet volume (MPV) and CRP were recorded for all patients. NLR was calculated by dividing neutrophil counts by lymphocyte counts.

Sysmex XN-9000 (Sysmex Co., Kobe, Japan) automatic analyzer was used for hematological analyses. Thyroid hormone levels, CRP, anti-TG and anti-TPO levels were analyzed by Roche Cobas 8000 (Roche, Mannhaim, Germany). The original kits provided by the manufacturer were used for all analyzes.

2.1. Statistical analysis

All data were evaluated statistically with SPSS software (SPSS 21.0; SPSS Inc., Chicago, IL, USA). The results were evaluated using Kolmogorov-Simirnov and Shapiro Wilk normality tests. The data were performed according Kruskal-Wallis One Way Analysis of Variance on Ranks, Median (%25-%75) and p<0.05 values were considered significant.

3. RESULTS

There were 454 patients between the ages of 32-66 who were classified according to TSH levels. Hyperthyroidism patients (group I, n=79) mean age was 47.0 years (37.0, 66.0), euthyroid patients (group II, n=298) mean age was 44.5 (33.0, 57.0) and hypothyroidism patients (group III, n=78) mean age was 46.5 years (32.75, 58.25).

As shown in Table 1, TSH, fT3 and fT4 were significantly varied between groups (p<0.001). TSH levels were significantly higher in group III than group I and group II (p<0.001). The median serum anti-TPO values in group III was also significantly higher when compared with the group I and group II (p<0.001). The mean serum anti-TG levels were similar in group I and group II. However, serum anti-TG levels in group III were significantly higher in comparison with group II.

Table 1. *Thyroid function test and thyroid autoantibody levels between groups*

Thyroid Function Tests	Groups	Median (%25-%75)	P	Pairwise Comparisons
TSH (µIU/mL)	Group I	0,01 (0,01-0,07)	<0.001	1-3, 1-2, 2-3
	Group II	1,81 (1,04-2,62)		
	Group III	6,50 (5,28-9,51)		
fT3 (pg/mL)	Group I	3,94 (3,08-5,72)	<0.001	1-3, 1-2, 2-3
	Group II	3,10 (2,79-3,35)		
	Group III	2,77 (2,40-3,17)		
fT4 (ng/dL)	Group I	1,82 (1,48-2,31)	<0.001	1-3, 1-2, 2-3
	Group II	1,24 (1,13-1,37)		
	Group III	1,09 (0,92-1,24)		
Anti-TPO (IU/mL)	Group I	16,8(11,6-88,2)	<0.001	1-3, 2-3
	Group II	16,1 (11,5-35,9)		
	Group III	140 (13,3-235)		
Anti-TG (IU/mL)	Group I	15,2 (11,3-227)	<0.001	2-3
	Group II	13,4 (10,4-43,6)		
	Group III	64,78 (11,99-365,03)		

p<0.05 is significant, Kruskall Wallis Test

TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, Anti-TG: Anti-thyroglobulin

Table 2 summarizes levels of inflammation-related blood count parameters and CRP in hypothyroid and hyperthyroid patients. There were no significant differences for Neu, Lym, NLR, MPV and WBC between groups. In group I, NLR were slightly higher than other groups. However, no statistical difference was observed (p=0.485). Serum CRP levels were statistically higher in group I than group II (p<0.001). Although CRP values were higher in group III than group II, no significant difference was noted (p=0.342) (Table 2).

Table 2. *Levels of inflammation-related blood count parameters and CRP between groups*

Blood Count Patameters and CRP Levels	Groups	Median (%25-%75)	P	Pairwise Comparisons
Neu (10^3/uL)	Group I	3,95 (3,00-5,40)	>0.05	NS
	Group II	4,10 (3,20-5,00)		
	Group III	3,93 (3,10-5,20)		
Lym (10^3/uL)	Group I	1,90 (1,50-2,30)	>0.05	NS
	Group II	2,01 (1,61-2,50)		
	Group III	2,05 (1,59-2,40)		
NLR	Group I	2,00 (1,54-2,85)	>0.05	NS
	Group II	1,94 (1,46-2,56)		
	Group III	1,91 (1,56-2,83)		
MPV (fL)	Group I	9,20 (8,40-10,60)	>0.05	NS
	Group II	9,30 (8,50-10,20)		
	Group III	9,50 (8,55-10,20)		
WBC (10^3/uL)	Group I	6,60 (5,60-8,20)	>0.05	NS
	Group II	6,93 (5,80-8,26)		
	Group III	6,70 (5,30-8,45)		
CRP (mg/L)	Group I	3,50 (1,50-11,00)	<0.001	1-2
	Group II	2,10 (0,86-5,43)		
	Group III	2,50 (1,18-5,73)		

p<0.05 is significant, Kruskall Wallis Test; NS: Non significant. CRP: C-reactive protein levels, NLR: Neutrophil / lymphocyte ratio, MPV: Mean platelet volume, *WBC: Leukocyte count, CRP: C-reactive protein levels.*

We determined that there was a positive correlation between the NLR and CRP levels (r=0,295) as shown in Table 3. CRP levels were positively correlated with fT4 (r=0,118, p<0.05) and had a weak negative correlation with TSH (r=-0,108, p<0.05). No statistical difference was observed in the correlation of CRP with other parameters (p>0.05). In addition, NLR was positively correlated with WBC (r=0,412, p<0.001).

Table 3. *Correlation analyses between the variables in all groups.*

Spearman's rho		Neu	Lym	NLR	MPV	WBC	CRP	AntiTG	Anti TPO	fT3	fT4	TSH
NLR	Correlation Coefficient	,712**	-,538**	1,000	-,058	,412**	,295**	-,048	-,054	-,008	,041	-,076
	Sig. (2tailed)	,000	,000	.	,217	,000	,000	,311	,253	,872	,385	,108
CRP mg/L	Correlation Coefficient	,406**	,016	,295**	-,024	,381	1,000	-,004	-,054	-,090	,118*	-,108*
	Sig. (2-tailed)	,000	,731	,000	,606	,000	.	,938	,248	,055	,012	,021

**p<0.05, **p<0.01*

NLR: Neutrophil / lymphocyte ratio; CRP: C-reactive protein levels; MPV: Mean platelet volume, WBC: Leukocyte count; TSH: Thyroid stimulating hormone; Anti-TPO: Anti-thyroid peroxidase antibody; Anti-TG: Anti-thyroglobulin

4. DISCUSSION

Thyroid dysfunctions, which are identifiable and treatable endocrine diseases, can cause serious metabolic problems when ignored (13). Despite advanced laboratory techniques and increased awareness of the disease, there are still cases of excessive dysfunction. (14, 15). Hypo – and hyperthyroidism are usually caused by pathological processes in the thyroid gland, but may be caused by hypothalamus or pituitary (central hypothyroidism) or functional thyroid disorders in certain situations (16). In our study, CRP levels were found to be increased in patients with hyperthyroidism when compared with hypothyroidism and euthyroid patients. As far as we know this is the first reported relationship between hypo-hyperthyroidsm and inflammation such as NLR and CRP parameters.

Recently, NLR has been evaluated as a simple systemic inflammation indicator in experimental and observational studies in different clinical situations (17). In a study examining inflammation in different thyroid diseases it was found that NLR correlates with the size of thyroid tumors and functions (18). When compared with the healthy control group, patients with Hashimoto's thyroiditis (HT) had significantly lower TSH levels (control: 1.9±1; HT: 1.2±1) associated with this increased NLR (control: 1.9 [0.6-3.3]; HT: 2.1 [1.3-5.8]) (19). Keskin et al. (11) concluded that NLR was statistically elevated in euthyroid Hashimoto patients, but also positively correlated with NLR and autoantibody levels. Dagdeviren et al. found that NLR did not increase in hyperthyroid patients and this rate decreased in Graves' disease patients. They found a negative correlation between fT3 and NLR in hyperthyroid patients (r=-0.28, p=0.001). Oguzhan et al. found that NLR levels were higher in HT patients than the control group (HT: 2.37 ± 1.46, control: 1.80 ± 0.67; p<0.003). In the same study, they found a negative but not statistically significant correlation between NLR and anti-TG and anti-TPO antibodies (20). According to Turan's study, NLR decreased in untreated Graves' patients (21). Although previous studies have shown a negative relationship between the fT3 level and NLR (22, 23), we found no statistically significant correlation in our study (r=-0,008, p= 0,872). It is observed in the studies that NLR can be used as an inflammatory marker in thyroid diseases and other diseases. However, in our study, there was no statistically significant difference between the groups in the presence of an increased NLR in hyperthyroidism, but no significant correlation was observed with thyroid function tests (p>0.05).

There are some studies reporting changes in lymphocyte levels in thyroid diseases (24, 25). In one of these studies, there was no significant difference in lymphocyte levels between the hypo-hyperthyroid groups (p>0.05). This ratio is parallel to the data obtained in our study. However, we observed that these studies have inadequate and inconsistent results. We have shown that NLR is not an appropriate parameter in hypo-hypertyroid patients. The change in parameters (WBC) is also associated with thyroid dysfunction. According to the results of Dorgalaleh and colleagues, they did not observe a statistically significant difference in WBC levels in hypothyroidism and hyperthyroidism groups in a similar way to our results (p>0.05) (2). In another study, WBC levels in the euthyroid and hyperthyroid groups were slightly higher than those in the hypothyroid group. Although different results were obtained at the WBC levels between the groups, the underlying mechanism is unknown. These inconsistencies are largely attributable to differences in exclusion criteria and origin of thyroid dysfunction.

CRP responds quickly to inflammatory processes and is one of the best known inflammatory markers. Several studies have shown that NLR is correlated with CRP (26, 27). Steven et al. found this correlation value as r=0.438, whereas in our study it was found to be r=0.295 (26). Lee and colleagues found that CRP levels in hypothyroidism (0.99±0.36) were significantly higher than control (0.93 ± 0.37) and hyperthyroidism (0.43±0.08) groups. However, they did not find a direct correlation between CRP and fT4 (r=0.062) and TSH levels (r=0.038) (28). Thus, they concluded that there was a weak relationship between thyroid status and CRP. On the other hand, we concluded that CRP increased in the case of hyperthyroidism (3.50 mg/L [1.50-11.0]) and showed a weak correlation with fT4 and TSH in our results. According to the Savas and his friends' findings, CRP levels were significantly higher in both hypothyroidism (5.62±4.75 mg/L) and hyperthyroidism (5.62±4.75 mg/L) compared to the control group (3.59±0.87 mg/L) (p<0.001), but there was no statistically significant difference in the case of autoimmune (HT and Graves's disease) and non-autotoimmune thyroid dysfunctions (p=0.087) (29). In the same study of these researchers, they found that MPV levels of hypothyroid, hyperthyroid and non-autoimmune patients were minor compared to the controls (30). In our findings, there was no change in MPV levels between the groups. In contrast to our results, MPV levels increased in both hypothyroidism and hyperthyroidism patients in different studies evaluating thyroid functions (30-32). In a comprehensive study by Pearche et al., no statistically significant difference was detected in the distribution of high CRP levels (>10 mg/L) in different thyroid diseases, before and after treatment (33). However, we were unable to find any significant differences in CRP levels according to thyroid function status. These results show that CRP levels are limited in the evaluation of thyroid function states.

5. CONCLUSION

According to the data we obtained, patients with hyperthyroidism and hypothyroidism should be able to follow CRP and NLR changes regularly. Failure to include all risk factors for hypothyroidism and hyperthyroidism has limited our study. Elevated levels of CRP for thyroid function status observed in current study confirm that inflammation has an important role in pathogenesis of hyperthyroidism. The effect of inflammation parameters on thyroid dysfunction requires further investigation

REFERENCES

[1] Iddah MA, Macharia BN, Ng'wena AG, Keter A, Ofulla AV. Thryroid hormones and hematological indices levels in thyroid disorders patients at moi teaching and referral hospital, Western kenya. ISRN Endocrinology 2013, 385-940.

[2] Dorgalaleh A, Mahmoodi M, Varmaghani B. Effect of thyroid dysfunctions on blood cell count and red blood cell indice. IJPHO 2013; 3 (2): 73-77.

[3] Kandir S, Keskin E. Effects of hypothyroidism and hyperthyroidism on hematological parameters in rats. Thyroid 2016; 15: 30.

[4] Perlman JA, Sternthal PM. Effect of 131I on the anemia of hyperthyroidism. J Chronic Dis 1983; 36 (5): 405-412.

[5] Lima CSP, Zantut Wittmann DE, Castro V, Tambascia MA, Lorand-Metze I, Saad ST, Costa FF. Pancytopenia in untreated patients with Graves' disease. Thyroid 2006; 16 (4): 403-409.

[6] Jafarzadeh A, Poorgholami M, Izadi N, Nemati M, Rezayati M. Immunological and hematological changes in patients with hyperthyroidism or hypothyroidism. Clin Inves Med 2010; 33 (5): 271-279.

[7] Wang HC, Klein JR. Immune function of thyroid stimulating hormone and receptor. Crit Rev Immunol 2001; 21: 323-337.

[8] Klein JR. Physiological relevance of thyroid stimulating hormone and thyroid stimulating hormone receptor in tissues other than the thyroid. Autoimmunity 2003; 36 (6-7): 417-421.

[9] Klein JR. The immune system as a regulator of thyroid hormone activity. Exp Biol Med 2006; 231 (3):229-236.

[10] Grymuła K, Paczkowska E, Dziedziejko V, Baskiewicz-Masiuk M, Kawa M, Baumert B, Celewicz Z, Gawrych E, Machalinski B. The influence of 3, 3', 5-triiodo-l-thyronine on human haematopoiesis. Cell Proliferation 2007; 40 (3): 302-315.

[11] Keskin H, Kaya Y, Cadırcı K, Kucur C, Ziypak E, Simsek E, Gozcu H, Arikan S, Carlioglu A. Elevated neutrophil/lymphocyte ratio in patients with euthyroid chronic autoimmune thyreotidis. Endocrine Regulation 2016; 50: 148-153.

[12] Ansar W, Ghosh S. C-reactive protein and the biology of disease. Immunol Res 2013; 56: 131-142.

[13] Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. Lancet 2017; 390: 1550-1562.

[14] Rice SP, Boregowda K, Williams MT, Morris GC, Okosieme OE. Welsh-sparing dysphasia. Lancet 2013; 382: 1608.

[15] Taylor PN, Tabasum A, Sanki G, Burberry D, Tennat BP, White J, Okosieme O, Aldridge A, Das G. Weekly intramuscular injection of levothyroxine following myxoedema: a practical solution to an old crisis. Case Reports in Endocrinology 2015, 2015:1-3.

[16] Taylor PN, Albrecht D, Scholz A, Gutierrez Buey G, Lazarus JH, Dayan CM, Okosieme OE. Global epidemiology of hyperthyroidism and hypothyroidism. Nature Reviews Endocrinology 2018; 14 (5): 301-316.

[17] Moore MM, Chua W, Charles KA, Clarke SJ. Inflammation and cancer: causes and consequences. Clin Pharmacol Ther 2010; 87(4): 504-508.

[18] Liu CL, Lee JJ, Liu TP, Chang YC, Hsu YC, Cheng SP. Blood neutrophil-tolymphocyte ratio correlates with tumor size in patients with differentiated thyroid cancer. J Surg Oncol 2013; 107 (5): 493-497.

[19] Aktas G, Sit M, Dikbas O, Erkol H, Altinordu R, Erkus E, Savli H. Elevated neutrophil-to-lymphocyte ratio in the diagnosis of Hashimoto's thyroiditis. Rev Assoc Méd Bras 2017; 63 (12): 1065-1068.

[20] Aksu O, Aydin B, Koroglu BK, Tamer MN. An evaluation of the neutrophil/lymphocyte ratio in patients with Hashimoto's thyroiditis. Acta Medica Mediterr 2014; 30: 457-460.

[21] Turan E. Evaluation of neutrophil-to-lymphocyte ratio and hematologic parameters in patients with Graves' disease. Bratisl Med J 2019; 120 (6): 476-480.

[22] Kyritsi EMA, Yiakoumis X, Pangalis GA, Pontikoglou C, Pyrovolaki K, Kalpadakis C, Mavroudi I, Koutala H, Mastrodemou S, Vassilakopoulos TP, Vaiopoulos G, Kandarakis ED, Papadaki HA, Angelopoulou MK. High Frequency of Thyroid Disorders in Patients Presenting With Neutropenia to an Outpatient Hematology Clinic STROBE Compliant Article. Medicine 2015; 94 (23): e886.

[23] Aggarwal N, Tee SA, Saqip W. Treatment of hyperthyroidism with anti-thyroid drugs corrects mild neutropenia in Graves' disease. Clin Endocrinol (Oxf) 2016; 85: 949-953.

[24] Pedersen BK, Feldt-Rasmussen U, Bech K, Perrild H, Klarlund K, Madsen MH. Characterization of the natural killer cell activity in Hashimoto's and Graves' diseases. Allergy 1989; 44: 477-481.

[25] Corrales JJ, Orfao A, Miralles JM, Miguel JS. The relationship between hyperthyroidism and the distribution of peripheral blood T, NK and B-lymphocytes in multinodular goiter. Horm Metab Res 1994; 26: 104-108.

[26] Kao SC, Pavlakis N, Harvie R, Vardy JL, Boyer MJ, Zandwijk N, Clarke SJ. High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. Clin Cancer Res 2010; 16 (23): 5805-5813.

[27] Ohno Y, Nakashima J, Ohori M, Hatano T, Tachibana M. Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma. J Urol 2010; 184 (3): 873-878.

[28] Lee WY, Suh JY, Rhee EJ, Park JS, Sung KC, Kim SW. Plasma CRP, apolipoprotein A-1, apolipoprotein B and Lp (a) levels according to thyroid function status. Arch Med Res 2004; 35 (6): 540-545.

[29] Savas E, Sahin AZ, Aksoy SN, Tascan A, Sayıner ZA, Ozkaya M. Serum levels of inflammatory markers in patients with thyroid dysfunction and their association with autoimmunity status. Int J Clin Exp Med 2016; 9 (2): 4485-4490.

[30] Kim JH, Park JH, Kim SY, Bae HY. The mean platelet volume is positively correlated with serum thyrotropin concentrations in a population of healthy subjects and subjects with unsuspected subclinical hypothyroidism. Thyroid 2013; 23: 31-37.

[31] Erikci AA, Karagoz B, Ozturk A, Caglayan S, Ozisık G, Kaygusuz I, Ozata M. The effect of subclinical hypothyroidism on platelet parameters. Hematology 2009; 14: 115-117.

[32] Panzer S, Hausbenstock A, Minar E. Platelets in hyperthyroidism: studies on platelet counts, mean platelet volume, 111-indium-labeled platelet kinetics, and platelet-associated immunoglobulins G and M. J Clin Endocrinol Metab1990; 70 (2): 491-496.

[33] Pearce EN, Bogazzi F, Martino E, Brogioni S, Pardini E, Pellegrini G, parkes AB, Lazarus JH, Pinchera A, Braverman LE. The prevalence of elevated serum C-reactive protein levels in inflammatory and noninflammatory thyroid disease. Thyroid 2003; 13 (7): 643-648.

How to cite this article: Kar F, Kusku Kiraz Z, Kocaturk E, Uslu S. The Level of Serum C-Reactive Protein and Neutrophil Lymphocyte Ratio According to Thyroid Function Status. Clin Exp Health Sci 2020; 10: 142-147. DOI: 10.33808/clinexphealthsci.600151

Clinical and Experimental Health Sciences

Analysis of Selection Criteria of Dental Patients for General Anaesthesia and Conscious Sedation

Kader Cesur Aydin[1], Oguzhan Demirel[2]

[1] Istanbul Medipol University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Istanbul, Turkey.
[2] Bahcesehir University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Istanbul, Turkey.

Correspondence Author: Kader Cesur Aydin
E-mail: kadercesur@yahoo.com
Received: 11.09.2019 **Accepted:** 07.03.2020

ABSTRACT

Objective: The term general anaesthesia refers to a state of unconsciousness under control using pharmacological or non pharmacological agents in which patient reflexes are compeletely or partially lost. Concious sedation is a drug-induced state in which the conscious patient is rendered free of fear, anxiety, and apprehension while remaining comfortably relaxed. Both of these methods are used for various reasons in dental clinics for many years and their use in dentistry practice is increasing. Aim of this study is to quantify the number and demographic data of patients that received a dental general anaesthesia (DGA) or conscious sedation (CS) following referral from a general dental practice. Study also aimed to determine the reasons of referral and dental treatment modalities performed during the sessions.
Methods: This study includes patient records who had undergone dental general anaesthesia and conscious sedation in Medipol University Dental Hospital General Anaesthesia Clinic. Data were collected from records of the University Dental Hospital. The collected information included gender, age, type of DGA/ CS, reason for DGA, treatment modalities as tooth extraction, restorative, endodontics, periodontics and pedodontics.
Results: 896 patients were referred for DGA/CS during the two-year period. The mean age was 15.5 years and 27.3% were underaged children. The most common reason for DGA was dental anxiety (46.5%). 79.4% of patientd received DGA, while only 20.6% received CS. The highest mean in treatments is for decidious pulp capping (4.57) followed by decidious pulp amputation (3.57).

Conclusion: Majority of the patients receiving DGA/CS are formed by dental anxiety patients. Also, the rate of underaged children were very high (27.3%).

Keywords: Dentistry, General Anaesthesia, Conscious Sedation, Pediatric Dentistry, Dental Anxiety

1. INTRODUCTION

When behaviour management techniques are inadequate for patients with special needs, general anaesthesia and conscious sedation may take a role for these patients' dental treatments. Patients with dental anxiety, intellectual disability, dementia, physical limitations, behavioural disorders and chronic systemic conditions can be included into the group called 'patients with special needs' (1). Although treatment of pediatric patients may be held out without using any pharmacological agents, incorporation with some patients may require sedation and general anaesthesia (2,3).

The term general anaesthesia refers to a state of unconsciousness under control using pharmacological agents. Patient reflexes are compeletely or partially lost including airway control, response to physical or verbal contact is lost (4). In a study conducted by Nunn et al, it is described that 340 patients with varying ages had undergone general anaesthesia for their dental treatments between 1983 to 1993. Also, review made in the same study pointed out patient quantities in previous researches between 80 and 4000 in variable time periods (5). In 1984, Bennet defined the conscious sedation term as 'drug-induced state in which the conscious patient is rendered free of fear, anxiety, and apprehension while remaining comfortably relaxed' and explained this method is not a method of pain control (6). Pharmacological agents provide the depression of consciousness level, but complete loss is prevented and patient's airway control ability maintains. Patient's response to physical stimulation and verbal contact continues (7,8). In a study conducted by Varpio and Wellfelt included 146 children with dental phobia; while 48 % of children were treated with conventional techniques after been trained by 'tell-show-do' method, 25 % were treated with conscious sedation and 27 % with general anaesthesia (9). Differentiation of conscious sedation applied in dental clinics must be seperated from those made by general anaesthetists made in surgical environments where every kind of patient

DOI: 10.33808/clinexphealthsci.618749

monitoring agents are available. During sedation in dental clinics, patient communication must be maintained (10).

Regarding the benefits and risks of general anesthesia and conscious sedation; complex criteria are included for case and method selection. These criteria included patient anamnesis and evaluation by a dentomaxillofacial radiologist, evaluation of the dental operator, availability of the treatment plan during the selected method and lastly evaluation of the patient by the general anesthesician and planning the protocol.

For obtaining maximum treatment plan goals, patients were all administered for specialist dental operators, tried to comfort and convince for local anesthesia treatments. It is demonstrated that comprehensive discussion of the anesthesia techniques and the specialist reasoning may alter patients' attitude towards anesthesia selection (11).

The purpose of this study is to evaluate a group of dental patients that has undergone dental general anaesthesia (DGA) or conscious sedation (CS) in aspect of clinical necessities such as younger age, mental retardation, surgical necessities, gagging reflex or patient choice due to dental anxiety.

2. METHODS

2.1. Study Design

This study is held out in Medipol University, School of Dentistry. Data for the study cohort was collected retrospectively via general anesthesia electronic records. Total of 896 patients were analysed (425 female, 471 male) who received either conscious sedation or general anesthesia for their dental treatments. Patients were evaluated by age groups as child (age under 12) or adult (above age 12). Gender, reason of anesthesia and type of anesthesia are evaluated in age groups. Number and ratio of the operated treatments were also determined.

Because this study was a retrospective research based on data collection and informed consents of all patients were taken (which declares that patient information can be used for scientific research); no ethical regulation was required.

2.2. Statistical analysis

During evaluation of the cohort data, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for the statistical analysis. Correlation of data for normal distribution was determined by ShapiroWilks test. Mann Whitney U test was used for mean, standard deviation and frequency anlyses. Chi – square test, Fisher Freeman Halton test and Continuity (Yates) Correction was used for determination of the comparison of qualitative data. Statistical significance was determined as $p<0.05$.

3. RESULTS

This study demographics included 896 patients (425 female, 471 male) treated under general anaesthesia or conscious sedation. Mean age and standard deviation of the study group was 15,54±16,63 and female / male percentage was 47,4 % to 52,6 %. 67% of the study group were child patients (under age 12). Table 1 demonstrates the reasons for application to anaesthesia clinic and the technics used for the operation. It can be concluded that the most common cause for DGA/ CS selection in this study group is dental anxiety (46,5%), followed by underaged children (27,3%).

Table 1. *Reasons for application to anaesthesia clinic and selected anesthesia types for the operations.*

		n	%
Reasons for anaesthesia	Underaged child	245	27,3
	Patient with special needs	127	14,1
	Mental retardation	57	6,4
	Cerebral palsy	10	1,1
	Autism	27	3
	Down syndrome	24	2,7
	Visual/ hearing impaired	9	1
	Gagging reflex	28	3,1
	Surgical necessity	79	8,8
	Anxiety	417	46,5
Anesthesia type	**General anaesthesia**	711	79,4
	Sedation	185	20,6

No statistical significance is found among genders for anaesthesia reason, anaesthesia type and distribution of children with special needs ($p>0.05$) (Table 2).

Table 2. *Distribution of anesthesia reason and type in children aged ≤12 among genders.*

≤12 years		**Gender**		**p**
		Female (n=264)	**Male (n=336)**	
		n (%)	**n (%)**	
Reasons for anaesthesia	**Underaged child**	118 (%44,7)	127 (%37,8)	0,307
	Patient with special needs	32 (%12,1)	52 (%15,5)	
	Surgical necessities	3 (%1,1)	6 (%1,8)	
	Anxiety	111 (%42)	151 (%44,9)	
Anaesthesia type	**General anaesthesia**	259 (%98,1)	330 (%98,2)	0,922
	Sedation	5 (%1,9)	6 (%1,8)	
Patient with special needs	**None**	232 (%87,9)	285 (%84,8)	0,553
	Mental retardation	13 (%4,9)	16 (%4,8)	
	Cerebral palsy	3 (%1,1)	5 (%1,5)	
	Autism	5 (%1,9)	14 (%4,2)	
	Down Sydrome	9 (%3,4)	10 (%3)	
	Visual/hearing impaired	2 (%0,8)	6 (%1,8)	

Chi-square test p<0.01

Statistically significant differences were found in patients over 12 years old. In terms of reasons for general anaesthesia; anxiety was more frequent in females (p<0.01), while gagging reflex and surgical operation neccessities were more frequent in male patients (p<0.01). General properties of patients above 12 years old can be seen in Table 3. Chi-square test results reveal that female patients have statistically significant anxiety rates than man (p<0.01). Applied anesthesia type does not have significance in relation to gender.

Table 3. *Distribution of anesthesia reason and type in children aged >12 among genders*

>12 age		Gender		p
		Female (n=161)	Male (n=135)	
		n (%)	n (%)	
Reasons for anesthesia	Underaged Child	2 (%1,2)	0 (%0)	0,001**
	Patient with special needs	22 (%13,7)	24 (%17,8)	
	Gagging reflex	6 (%3,7)	22 (%16,3)	
	Surgical necessities	32 (%19,9)	37 (%27,4)	
	Anxiety	99 (%61,5)	52 (%38,5)	
Anaesthesia type	General anaesthesia	65 (%40,4)	57 (%42,2)	0,747
	Sedation	96 (%59,6)	78 (%57,8)	
Patient with special needs	None	141 (%87,6)	113 (%83,7)	0,219
	Mental retardation	14 (%8,7)	11 (%8,1)	
	Cerebral palsy	2 (%1,2)	0 (%0)	
	Autism	2 (%1,2)	6 (%4,4)	
	Down Syndrome	1 (%0,6)	4 (%3)	
	Hearing/ Visual Impaired	1 (%0,6)	1 (%0,7)	

*Chi-square Test **p<0.01*

Table 4 and 5 demonstrates the gender distribution of dental operations. No statistically significant difference was observed among genders in patients below 12 years with regard to dental treatments (p>0.05); on the other hand females had higher rate for permanent teeth extractions (p<0.01), subgingival curretage and periodontal flap surgeries (p<0.05).

Table 4. *List of dental operations and their relationship between genders in patients aged below 12 years old*

≤12 years	Gender		1p
	Female (n=264)	Male (n=336)	
	Mean±SD	Mean±SD	
Endodontic treatment	4,99±3,00	5,28±3,23	0,364
Decidous tooth extraction	2,09±2,42	2,19±2,39	0,362
Permanent tooth extraction	0,11±0,50	0,15±0,56	0,254
Restorative treatment	5,56±3,61	5,89±3,65	0,299
	n (%)	n (%)	2p
Surgery	4 (%1,5)	12 (%3,6)	0,195
Detartrage	8 (%3)	14 (%4,2)	0,606
Curretage	1 (%0,4)	0 (%0)	0,440
Flap surgery	1 (%0,4)	1 (%0,3)	1,000

1: Mann Whitney U Test
2: Continuity (Yates)Correction and Fisher's Exact Test

Table 5. *List of dental operations and their relationship between genders in patients aged above 12 years old.*

>12 years	Gender		1p
	Female (n=161)	Male (n=135)	
	Mean±SD	Mean±SD	
Endodontic treatment	0,34±0,94	0,58±1,41	0,128
Decidous tooth extraction	0,03±0,26	0,11±0,76	0,526
Permanent tooth extraction	1,75±3,36	1,16±2,62	0,033*
Implant surgery	0,45±1,37	0,87±2,49	0,184
Restorative treatment	1,14±2,63	1,43±2,74	0,123
	n (%)	n (%)	2p
Surgery	36 (%22,4)	40 (%29,6)	0,196
Lefort surgery	7 (%4,3)	6 (%4,4)	1,000
Detartrage	45 (%28)	34 (%25,2)	0,686
Curretage	11 (%6,8)	0 (%0)	0,005**
Flap surgery	8 (%5)	1 (%0,7)	0,043*
Prosthetic treatment	11 (%6,8)	10 (%7,4)	1,000

1: Mann Whitney U Test
2: Continuity (Yates) Correction and Fisher's Exact Test
**p<0.05 **p<0.01*

Table 6 demonstrates the distribution of dental treatments and their relationships between age groups. Restorative treatment modalities showed significant differences between age groups. While pulp capping operations of decidous teeth and fissure restorations in patients below 12 years age were more frequent than patients above 12 years old (p<0.01); permanent tooth pulp cappings and amalgam restorations were more common in patients above 12 years old (p<0.01).

No statistically significant differences were found in terms of tooth extractions between age groups (p>0.01).

With regard to endodontic treatments, statistical significance was evident between age groups. Decidous tooth pulp amputations and root canal therapies were more frequent in patients aged below 12 years (p<0.01) and permanent tooth root-canal therapies were more common in patients above 12 years old (p<0.01). No statistical significance was found between age groups according to permanent tooth pulp amputations (p>0.01).

Table 6. *Treatment modalities between age groups*

	Age group			P
	≤12 years	>12 years	Total	
	Mean±SD	Mean±SD	*Mean±SD*	
Decidous teeth pulp capping	5,55±3,54	0,13±0,80	*4,84±3,79*	**0,001****
Permanent tooth pulp capping	0,60±1,48	4,55±3,31	*1,12±2,26*	**0,001****
Decidous tooth extraction	0,10±0,71	0,00±0,00	*0,09±0,66*	**0,126**
Permanent tooth extraction	0,01±0,18	0,03±0,23	*0,01±0,18*	**0,126**
Fissure restorations	0,35±0,99	0,04±0,25	*0,31±0,93*	**0,004****
Amalgam restorations	0,04±0,29	0,18±0,58	*0,06±0,35*	**0,001****
Decidous tooth amputation	3,96±2,78	0,00±0,00	3,57±2,89	**0,001****
Permanent tooth amputation	0,03±0,29	0,00±0,00	0,03±0,27	**0,382**
Decidous tooth root-canal therapy	1,17±1,68	0,05±0,29	1,06±1,63	**0,001****
Permanent tooth root-canal therapy	0,45±1,16	1,21±1,51	0,52±1,21	**0,001****
Root extraction	0,05±0,32	0,88±0,97	0,13±0,5	**0,004****

Mann Whitney U Test
***p<0.01*

4. DISCUSSION

The study examined a considerable number of subjects (896) that a major percentage has undergone DGA (79,4%). The percentage ranking of the evaluated patient group is as follows; anxiety (46,5%), underaged child (27,3%), patient with cooperation problems (14,1%), surgical necessity (8,8%) and gagging reflex (3,1%).

Evaluating dental anxiety, it can both occur in children and adults. Varience in percentage of populations may occur due to parent attitudes, population norms, dentist attitude and cooperation for explanation and social status (12-15).

Studies regarding patient demographics reveal that dental anxiety is higher in young females than males (16) and dental anxiety decreases with age (17). In this study, our demographics also revealed higher dental anxiety in females above 12 years age, in comparison to males. Coric et al (18) and Wu and Gao (19) also stated that dental enxiety and fear in children is with coexistence of the parents, family style and increases with child age.

Studies about DGA and CS of children have also been carried out. Tyrer, in his study suggested that the increased need for dental treatments in many sexants led to more referral for DGA in children. He also concluded younger age as a referral need (11). Our study data also includes that 245/ 600 child patients were underaged. Looking at the total number of patients, 27,3% were underaged children in this study. Richards et al (20) determined similar percentage of underaged childred (30,7%) in his study that has evaluated 287 dental patients that has undergone GA in a 9 year period. Saxen et al (21) provided evidence that, due to widespread childhood caries, children having DGA had a peak below age 6.

Evaluating the type of anesthesia, our study group revealed DGA rates (79,4%) higher than CS (20,6%) in all age groups. In regard to childrens' group, these rates are higher for DGA than CS (98,1% to 1.9%). Whittle, in his study revealed that, the number of DGA's diminished 24%, and the number of CS or inhalation anesthetics have increased slightly from 1997/98 to 1999/2000 (22). Regarding the time limitation, in contrast to DGA, CS may be handled better in patients having limited number of treatments to avoid more treatment sessions (23,24).

5. CONCLUSION

The presented data here was collected from a single dental hospital that could manage daily hospitalization. Major maxillofacial surgeries in need of patient hospitalisation were not involved. This study indicates that a major percentage of all patients are formed by dental anxiety patients. As all patients were first evaluated for local anesthesia treatments, female patients older than 12 years tend to have higher and more persistent rate of dental anxiety.

REFERENCES

[1] Frassica JJ, Miller EC. Anesthesia management in pediatric and special needs patients undergoing dental and oral surgery. Int Anesthesiol Clin 1989;27(2):109-115.

[2] Lee PY, Chou MY, Chen YL, Chen LP, Wang CJ, Huang WH. Comprehensive dental treatment under general anesthesia in healthy and disabled children. Chang Gung Med J 2009;32(6):636-642.

[3] Nelson TM, Xu Z. Pediatric dental sedation: challenges and opportunities. Clin Cosmet Investig Dent 2015;7:97-106.

[4] Rosenberg J, Fuchs-Buder T. Why surgeons need to know about anaesthesia. Surg Endosc 2016 Sep;30(9):3661-3664.

[5] Nunn JH, Davidson G, Gordon PH, Storrs J. A retrospective review of a service to provide comprehensive dental care under general anesthesia. Spec Care Dentist 1995;15(3):97-101.

[6] Bennett CR. Conscious sedation: an alternative to general anesthesia. J Dent Res 1984;63(6):832-833.

[7] Rosenberg MB, Campbell RL. Guidelines for intraoperative monitoring of dental patients undergoing conscious sedation,

deep sedation, and general anesthesia. Oral Surg Oral Med Oral Pathol 1991;71(1):2-8.

[8] Rosenberg M; American Dental Association. New guidelines for the use and teaching of general anesthesia and sedation by dentists. J Mass Dent Soc 2010;58(4):22-27.

[9] Varpio M, Wellfelt B. Some characteristics of children with dental behaviour problems. Five-year follow-up of pedodontic treatment. Swed Dent J 1991;15(2):85-93.

[10] Craig DC, Wildsmith JA. Royal College of Anaesthetists; Royal College of Surgeons of England. Conscious sedation for dentistry: an update. Br Dent J 2007;203(11):629-631.

[11] Tyrer G L. Referrals for dental general anaesthetics – how many really need GA? Br Dent J 1999; 187: 440-443.

[12] Carter AE, Carter G, Boschen M, AlShwaimi E, George R. Pathways of fear and anxiety in dentistry: A review. World J Clin Cases. 2014;2(11):642-653.

[13] Storjord HP, Teodorsen MM, Bergdahl J, Wynn R, Kolset Johnsen JA. Dental anxiety: a comparison of students of dentistry, biology, and psychology. J Multidiscip Healthc 2014;7:413-418.

[14] Pop-Jordanova N, Sarakinova O, Markovska-Simoska S, Loleska S. Anxiety and personality characteristics in children undergoing dental interventions. Contributions. MASA (Sec Med Sci) 2013;34(3):93-103.

[15] Pop-Jordanova N, Sarakinova O, Pop-Stefanova-Trposka M, Zabokova-Bilbilova E, Kostadinovska E. Anxiety, Stress and Coping Patterns in Children in Dental Settings. Open Access Maced J Med Sci 2018;6(4):692-697.

[16] Pohjola V, Mattila A K, Joukamaa M, Lahti S. Anxiety and depressive disorders and dental fear among adults in Finland. Eur J Oral Sci 2011; 119: 55-60.

[17] Hill K B, Chadwick B, Freeman R, O'Sullivan I, Murray J J. Adult Dental Health Survey 2009: relationships between dental attendance patterns, oral health behaviour and the current barriers to dental care. Br Dent J 2013; 214: 25-32.

[18] Coric A, Banozic A, Klaric M, Vukojevic K, Puljak L. Dental fear and anxiety in older children: an association with parental dental anxiety and effective pain coping strategies. J Pain Res 2014;7:515-521.

[19] Wu L, Gao X. Children's dental fear and anxiety: exploring family related factors. BMC Oral Health 2018;18(1):100.

[20] Richards W, Razzaq K, Higgs G. An audit of dental general anaesthetic referral from a general dental practice in South Wales. Prim Dent Care 2009;16(4):143-147.

[21] Saxen MA, Urman RD, Yepes JF, Gabriel RA, Jones JE. Comparison of Anesthesia for Dental/ Oral Surgery by Office-based Dentist Anesthesiologists versus Operating Room-based Physician Anesthesiologists. Anesthesia Progress 2017;64(4):212-220.

[22] Whittle JG. The provision of primary care dental general anaesthesia and sedation in the north West region of England, 1996-1999. Br Dent J 2000; 189(9):500-502.

[23] Averley PA, Lane I, Sykes J, Girdler NM, Steen N, Bond S. An RCT pilot study to test the effects of intravenous midazolam as a conscious sedation technique for anxious children requiring dental treatment – an alternative to general anaesthesia. Br Dent J 2004;197(9):553-558.

[24] Lyratzopoulos G, Blain KM. Inhalation sedation with nitrous oxide as an alternative to dental general anaesthesia for children. J Public Health Med 2003;25(4):303-312.

How to cite this article: Cesur Aydin K, Demirel O. Analysis of Selection Criteria of Dental Patients for General Anaesthesia and Conscious Sedation. Clin Exp Health Sci 2020; 10: 148-152. DOI: 10.33808/clinexphealthsci.618749

Clinical and Experimental Health Sciences

Cytotoxicity and Collagen Expression Effects of Tideglusib Administration on Human Periodontal Cells: An In-Vitro Study

Buse Oncu[1], Ayse Mine Yilmaz[2], Betul Karademir Yilmaz[2], Elif Cigdem Altunok[3], Leyla Kuru[1], Omer Birkan Agrali[1]

[1] Marmara University, Faculty of Dentistry, Department of Periodontology, Istanbul, Turkey.
[2] Marmara University, Faculty of Medicine/Genetic and Metabolic Diseases Research and Investigation Center, Department of Biochemistry, Istanbul, Turkey.
[3] Yeditepe University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Istanbul, Turkey.

Correspondence Author: Omer Birkan Agrali
E-mail: omer.agrali@marmara.edu.tr
Received: 27.03.2020 **Accepted:** 20.04.2020

ABSTRACT

Objective: Tideglusib is a GSK-3 inhibitor activating Wnt/β-catenin signaling pathway which has significant importance in regenerative response. The aim of this study was to evaluate the cytotoxicity and protein expression impacts of Tideglusib on human periodontal cell lines.

Methods: Cytotoxicity effect of different concentrations (50nM, 100nM, 200nM) of Tideglusib application on human gingival fibroblast (hGF), periodontal ligament fibroblast (hPDLF), and osteoblast (hOB) cell lines was determined. Type-I and III collagen expressions were evaluated after 24-hour application of 50nM Tideglusib.

Results: The cytotoxicity of 200nM Tideglusib was higher in hGF and hOB ($p<0.05$), but no difference was found in hPDLF compared to the respective control group ($p>0.05$). The hGF and hOB treated with 50nM Tideglusib expressed an increased level of Type-I collagen ($p<0.05$), but no difference was detected in the hPDLF compared to the respective control ($p>0.05$). Type-III collagen expressions were similar between the test and control groups for each cell line ($p>0.05$).

Conclusion: Tideglusib is not cytotoxic at 50nM and 100nM concentrations and may have positive effect on bone regeneration rather than periodontal regeneration since it stimulated Type-I collagen production in hGF and hOB cells, but not in hPDLF.

Keywords: Cell biology, cell signaling biomolecules, Osteoblast(s), Tideglusib, Wnt/β-catenin signaling pathway.

1. INTRODUCTION

Periodontal diseases are inflammatory diseases leading to progressive loss of tooth supporting structures (1). Conventional periodontal therapies utilized for the treatment of advanced periodontal diseases usually result in the formation of long junctional epithelium with a quality of repair (2). However, nowadays a complete regeneration is aimed, and new alveolar bone, cementum and periodontal ligament lost by disease are attempted to be established to their original structure and function using regenerative periodontal procedures. Although several materials have been tested, so far, none of them succeeded to accomplish complete regeneration (3).

Reformative responses of an organism include various signaling pathway activation cascades both in embryonic and adulthood periods. The activation of Wnt signaling pathway (WSP), one of the marked trails in the organisms, represents an early response to tissue damage and is required for the stimulation of cellular-based repair in all tissues (4). Wnt signaling mechanism can be in either active or inactive state. Following binding of the Wnt protein to cellular receptors, glycogen synthase kinase-3 (GSK-3) molecule which has great importance in regulating cell division cycle, is inhibited after a series of phosphorylation. Then, the transcription of the target genes of WSP begins. This stimulation plays a crucial role in proliferation, cell cycle and differentiation activities of the cells. Transcription of the target genes of signaling pathway is suppressed when Wnt does not bind to the cellular receptors (5). Several studies have shown that reduction in Wnt signaling causes bone loss and reduces regenerative capacity (6, 7). If the Wnt signaling is inhibited in the skeleton after fracture, due to the decline in the proliferation and earlier differentiation of skeletal stem and progenitor cells, non-fused bone appears (8). WSP activation leads to the proliferation of osteoblasts and fibroblasts resulting in the improvement of the parameters related to bone growth (9). In mammals, the healing of skin injuries usually ends with

DOI: 10.33808/clinexphealthsci.709924

scar tissue, but when the WSP is activated, a fully functional epidermis can be achieved (10). Systemic use of Wnt agonist R-spondin has been shown to induce mucosal regeneration in oral mucositis seen after chemotherapy (11). Irregular anatomical formations were observed after the inhibition of WSP in planarians possessing a high regenerative capacity (12). Similarly, healthy fin regeneration was impaired due to the inhibition of Wnt signaling in zebrafish (13). Moreover, inhibition of WSPs in animals having continuous retinal regeneration capacity results in interruption of their capability (14).

Current knowledge about the role of WSP on the development and maintenance of the periodontium is limited (15-18). It was observed that root development discontinued in Wnt signaling inactivated mice (18). WSP-modulated mesenchymal mice odontoblast and cementoblast cells by persistent stabilization of ß-catenin have been shown to lead excessive dentin and cementum formation, and this finding was interpreted as Wnt signaling cascade might have therapeutic regenerative potential (15, 17, 19). However, Nemoto et al. (20) established that the proliferation of cementoblast cells was supported by WSP activation. Furthermore, activation of the WSP has been reported to play an active role in cementoblast differentiation and cementum regeneration in rat periodontal defect model, and in in-vitro studies of human periodontal ligament cells (9, 17, 21). In another study, it has been shown that lithium ions, known as activators of the WSP, increase the proliferation and differentiation of periodontal ligament cells after their release from bioactive scaffolds (9). Rooker et al. (17) found that the number of cells responding to the WSP was higher in the regions with increased proliferation of periodontal ligament cells. It is well-known that Wnt signaling plays a role in both osteoblast differentiation and proliferation, as well (22). A noteworthy increase in bone growth was observed in Wnt signaling activated mice (23). Popelut et al. (24) demonstrated that WSP activation might have a positive effect on implant osseointegration by enhancement of osteoblast activity, inhibition of osteoclast activity or differentiation of pluripotent stem cells.

Pharmacological inhibitors of GSK-3 are thought to be helpful in several areas such as Alzheimer's disease, some neurodegenerative diseases, Type-2 diabetes mellitus, cancer, psychiatric diseases and regenerative medicine (25). In an animal experimental periodontitis study, intraperitoneal administration of GSK-3 inhibitor was found to inhibit bone loss, suppress systemic cytokine response, local neutrophil infiltration and IL-17 expression (26). So far, many GSK-3 inhibitors including lithium, arylindolemaleimides, amino thiazoles, halomethylketone derivatives and thiadiazolidinones have been developed and shown to be applicable (26). Tideglusib belongs to the thiadiazolidinones group drug which acts as a non-ATP competitive GSK-3 inhibitor assessed in phase-II clinical trials on a small-scale of subjects with Alzheimer disease since 2015. In a recent study, interestingly, Tideglusib was found to support the process of natural and functional reparative dentin formation in mice teeth with deep decays (4).

Under the light of these evidences, it was hypothesized that periodontal regeneration might be obtained by the use of Tideglusib to activate the WSP. Therefore, the aim of this study was to evaluate the effects of Tideglusib on human gingival fibroblast (hGF), periodontal ligament fibroblast (hPDLF), and osteoblast (hOB) cell lines pointing to assess the potential efficacy of Tideglusib in periodontal regeneration by measuring the cytotoxicity and proliferation levels together with Type-I and III collagen expression in these cells.

2. METHODS

2.1. Study Plan

hGF (Catalog no: CRL-2014, ATCC®, USA), hPDLF (Catalog no: CC-7049, Lonza, Switzerland) and hOB (Catalog no: CRL-11372, ATCC®, USA.) cell lines were used to investigate the effects of Tideglusib administration. In the first stage of the study, MultiTox-Fluor Multiplex Cytotoxicity Test (Catalog no: G9200, Promega, USA) was applied to examine the effect of different Tideglusib concentrations on proliferation and cytotoxicity of the cells at the end of 24 hours. In the second stage, a common non-toxic dose of Tideglusib was determined and applied to all cell lines for 24 hours to investigate Type-I and Type-III collagen expressions evaluated by Western blot analysis. In accordance with this study plan, the cells treated with Tideglusib were designated as the test group, while the cells without Tideglusib served as the negative control group.

2.2. Cell Culture

Cells stored at – 152^{0}C were allowed to thaw rapidly and centrifuged in 10 ml medium at 1300 rpm for 5 minutes. After the supernatant was removed, the pellet was resuspended and plated into media containing flasks. hGF cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Catalog no: SLM-241-B, Sigma-Aldrich, Germany) supplemented with 10% fetal bovine serum (FBS) (Catalog no: 16000-036, GibcoTM, Thermo Fisher Scientific, USA), 2 mM sodium pyruvate, 1% L-glutamine (Catalog no: W368401, Sigma-Aldrich, Germany), high glucose, sodium bicarbonate and 0.1% amphotericin/gentamycin. Also, 0.1% fibroblast growth factor and 0.1% insulin-like growth factor were added to the culture medium for hPDLF cells. hOB cells were cultured in DMEM (Catalog no: P04-01549, Pan-Biotech, Germany) and DMEM/F12 (Catalog no: P04-41500, Pan BioTech, Germany) medium containing medium appended with 10% FBS, 1% L-glutamine (500µl) and 0.1% amphotericin/gentamicin.

Cells were incubated in medium in a humid environment with 5% CO2 at 37^{0}C, and cell proliferation was monitored for 24 hours. Cell passages with a confluence of 70% in the flasks were washed three times with phosphate-buffered saline (PBS) (Catalog no: P5493, Sigma-Aldrich, Germany)

and incubated with trypsin-EDTA (Catalog no: P10-0235SP, Pan-Biotech, Germany) at 37°C for 5 minutes. Following the end of the trypsinization, cells were centrifuged at 1300 rpm for 5 minutes and resuspended with the medium according to the pellet amount. In order to determine the cell count, 10µl cell suspension was mixed with 10µl trypan blue. Cells were counted at 10X magnification in the light microscope (ZEISS Primovert, Germany). The cells stained with trypan blue were regarded as dead, whereas, the non-staineds as alive. The number of cells per ml was calculated according to the following formula (Cell count/ml=Mean count value X dilution factor X 10^4 X 2).

2.3. Viability and Cytotoxicity Assays

MultiTox-Fluor Multiplex Cytotoxicity Test was used to investigate Tideglusib's biocompatibility and was repeated three times for each cell Type. Two protease activities were measured in order to determine the cell viability and cytotoxicity in this experiment. For the determination of cell viability, 100µl of cell suspension containing 5 000 cells was added to each well and incubated for 24 hours. After incubation, 50 nM, 100 nM and 200 nM Tideglusib (Catalog no: SML0339-50MG, Sigma-Aldrich, Germany) dissolved in dimethyl sulfoxide was applied to the test groups while the cells without Tideglusib served as negative control groups. At the end of 24 hours, 50µl of GF-AFC reagent was added to all wells for the viability test. Plates were orbitally mixed for 5 minutes to ensure homogeneity and incubated at 37^0C for 60 minutes. The plates were wrapped in aluminium foil and protected against light. The fluorescence was measured by the Multilabel Microplate Reader (EnSpire 2300 Multilabel Microplate Reader, Perkin Elmer, USA) with fluorescence of ~400 nmEx/~505 nm wavelength.

For the cytotoxicity test, 50µl AAF-Glo™ reagent was added to all wells and incubated in the dark at room temperature for 15 minutes. The absorbance values read in the Multilabel Microplate Reader were used for determining viability and cytotoxicity scores and specified as percentages of viability and cytotoxicity.

2.4. Western Blotting

Protein expressions of periodontal cells were examined by Western blotting method 24 hours after administration of 50 nM Tideglusib, which was selected as the non-cytotoxic and most viability supporting dose. After incubation, the medium was removed, the cells were washed with PBS followed by cell lysis buffer administration containing 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta glycerophosphate, 1 mM Na3VO4, 1 µg/ml leupeptin and 1 mM PMSF. Total protein extracts were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Blots were blocked with 5% milk powder-tris buffered saline with tween 20 for 1 hour and incubated with polyclonal antibodies against Type-I collagen (Collagen I alpha 1 Antibody, Catalog no: 84336S, Cell Signaling Technology, USA) and Type-III collagen (Collagen III alpha 1 Antibody, Catalog no: NBP2-3332, Novus Biologicals, USA). The membrane was incubated with chemiluminescence kit (Western blotting luminol reagent, Santa Cruz Biotechnology, USA) in dark medium for 1 minute. The resulting radiation (ChemiDoc™ MP, Bio-Rad, USA) was displayed, and the density of the protein bands was calculated. The experiment was performed with four replicates for all cell types.

2.5. Statistical Analysis

The data were analyzed by using the Statistical Package for Social Sciences package program (SPSS for Windows, Release 25.0, IBM Inc., USA). Descriptive statistics are shown as mean±standard deviation, median, minimum and maximum. Data distribution was evaluated with Kolmogorov-Smirnov test. Kruskal-Wallis test was used to compare the variables that did not show normal distribution. Mann-Whitney U test was used for paired comparisons. The results were interpreted with Bonferroni correction. Statistical significance was set as $p<0.05$ level.

3. RESULTS

3.1. The Effect of Tideglusib Administration on Cellular Viability and Cytotoxicity

Table 1 shows the viability results of different concentrations of Tideglusib administration in all cell groups for 24 hours. Viability result of Tideglusib on hGF cells revealed no significant difference among the control and test groups (Table 1). On the other hand, there was a significant difference among the control and different concentrations of Tideglusib administrations in terms of viability effect on hPdlf cells ($p=0.022$) (Table 1). 200 nM Tideglusib decreased the viability significantly compared to the 50 nM Tideglusib application to hPDLF cells ($p=0.037$) (Table 1). The viability of hOB cells showed no significant difference for all administered concentrations (Table 1).

Table 1. *Comparison of viability among different concentrations of Tideglusib administration in all cell groups.*

		Tideglusib Concentration				
w		**0 nM**	**50 nM**	**100 nM**	**200 nM**	*p**
hGF (%)	**N**	3	3	3	3	***0.639***
	Mean±SD	100.00±0.00	134.60±61.63	130.13±60.20	124.08±63.64	
	Median	100.00	100.86	100.24	91.33	
	Minimum	100.00	97.20	90.73	83.49	
	Maximum	100.00	205.74	199.44	197.44	
hPDLF (%)	**N**	3	3	3	3	*0.022*
	Mean±SD	100.00±0.00	107.51±6.23	105.02±2.36	97.63±1.68†	
	Median	100.00	110.93	105.11	97.63	
	Minimum	100.00	100.32	102.62	95.95	
	Maximum	100.00	111.30	107.35	99.32	
hOB (%)	**N**	3	3	3	3	***0.264***
	Mean±SD	100.00±0.00	115.77±3.20	110.28±25.34	101.98±8.90	
	Median	100.00	114.16	123.81	103.10	
	Minimum	100.00	113.69	81.04	92.57	
	Maximum	***100.00***	***119.46***	***126.00***	***110.27***	

*SD: Standard deviation, *Kruskal Wallis test, p<0.05, † Different from 50 nM Tideglusib administration, Mann Whitney-U Test with post-hoc Bonferroni correction, p=0.037*

Table 2 shows the comparison of cytotoxicity values of all cell lines after 24-hour administration of Tideglusib at 50 nM, 100 nM and 200 nM concentrations. Comparisons of the test and control groups in each cell line revealed significant cytotoxicity effect of Tideglusib in hGF (p=0.015), hPDLF (p=0.023) and hOB cells (p=0.015) (Table 2). However, paired comparisons demonstrated no significant differences between the test and control groups in hPDLF when post-hoc Bonferroni correction was applied (p>0.05). In the paired comparisons, the cytotoxic effects of 200 nM Tideglusib in the hGF and hOB cells were significantly higher compared to respective control groups (p=0.012, p=0.012, respectively) (Table 2).

3.2. The Effect of Tideglusib Administration on Type-I And Type-III Collagen Expression

Following administration of 50 nM Tideglusib onto the hGF cells, Type-I collagen expression in the test group was significantly higher than the control group (p=0.029) (Figure 1) (Table 3). There was no significant difference between the test and control groups in terms of Type-I collagen expression in the hPdlf cells (p>0.05) (Figure 1) (Table 3), Type-I collagen expression increased significantly in the test group compared to the control in the hOB cells (p=0.029). (Figure 1), (Table 3).

Table 2. *Comparison of cytotoxicity among different concentrations of Tideglusib administration in all cell groups.*

		Tideglusib Concentration (nM)				
		0 nM	**50 nM**	**100 nM**	**200 nM**	**p***
hGF (%)	**N**	3	3	3	3	**0.015**
	Mean±SD	0.00±0.00	1780.30±203.33	2342.23±82.75	3329.18±564.40†	
	Median	0.00	1816.17	2306.95	3290.34	
	Minimum	0.00	1561.43	2282.97	2785.20	
	Maximum	0.00	1963.32	2436.78	3912.0	
hPDLF (%)	**N**	3	3	3	3	**0.023**
	Mean±SD	0.00±0.00	1671.58±444.85	5029.05±3326.79	4782.91±2640.26	
	Median	0.00	1924.64	4350.70	4393.20	
	Minimum	0.00	1157.93	2093.71	2359.18	
	Maximum	0.00	1932.18	8642.74	7596.38	
hOB (%)	**N**	3	3	3	3	**0.015**
	Mean±SD	0.00±0.00	157.86±48.83	265.88±25.64	378.85±131.29†	
	Median	0.00	130.30	254.40	310.56	
	Minimum	0.00	129.05	247.99	295.79	
	Maximum	0.00	214.25	295.26	530.22	

*SD: Standart deviation, *Kruskal Wallis Test, p<0.05,†Different from the control group, Mann Whitney-U Test with post-hoc Bonferroni correction, p=0.012.*

Table 3. *Type-I collagen expression in all cell groups after 50 nM Tideglusib administration.*

Tideglusib (nM)		***hGF (Type-I Collagen/GAPDH)***	***hPDLF (Type-I Collagen/GAPDH)***	***hOB (Type-I Collagen/GAPDH)***
Control	**N**	4	4	4
	Mean±SD	1.57±0.09	0.11±0.03	0.06±0.02
	Median	1.56	0.12	0.06
	Minimum	1.47	0.07	0.03
	Maximum	1.70	0.14	0.09
50 nM	**N**	4	4	4
	Mean±SD	2.43±0.30	0.19±0.12	0.16±0.06
	Median	2.55	0.15	0.15
	Minimum	1.98	0.10	0.09
	Maximum	2.66	0.39	0.26
*p**		*0.029*	***0.343***	*0.029*

*SD: Standard deviation. *Mann Whitney-U Test with post-hoc Bonferroni correction. p<0.05.*

Type-III collagen expression analysis revealed no statistically significant difference between the test and control groups after 24 hours of 50 nM Tideglusib administration onto each cell line (p>0.05) (Figure 1) (Table 4).

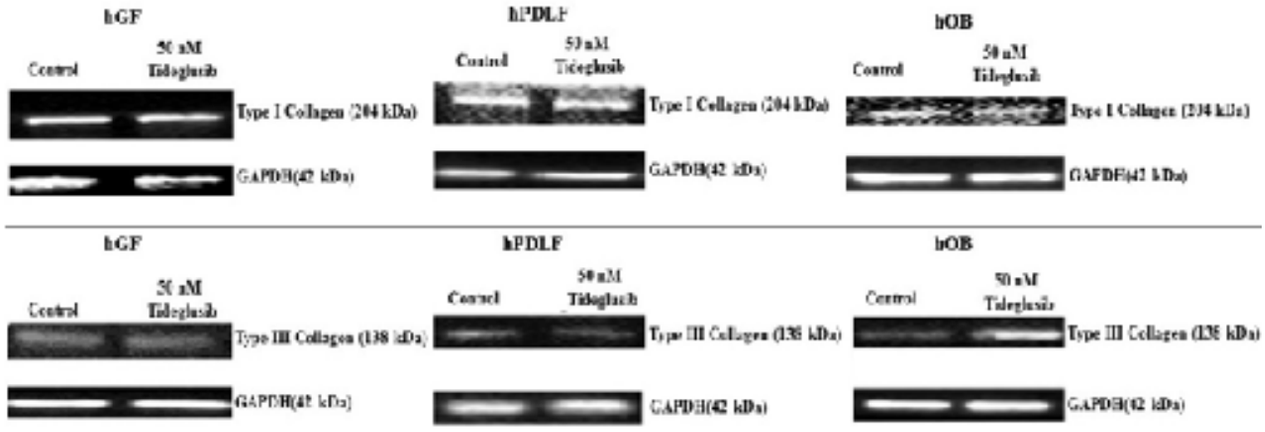


Figure 1. *Representative Western blot images of all cell types. Immunoblotting for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (bottom panel) showed equal loading of the proteins in each lane. Bands were normalized to glyceraldehyde-3-phosphate dehydrogenase by densitometry.*

Table 4. *Type-III collagen expression in all cell groups after 50 nM Tideglusib administration.*

Tideglusib (nM)		*hGF (Type-III Collagen/GAPDH)*	*hPDLF (Type-III Collagen/GAPDH)*	*hOB (Type-III Collagen/GAPDH)*
Control	N	4	4	4
	Mean±SD	0.03±0.01	0.24±0.07	0.81±0.32
	Median	0.03	0.25	0.73
	Minimum	0.02	0.15	0.54
	Maximum	0.05	0.33	1.24
50 nM	N	4	4	4
	Mean±SD	0.06±0.02	0.18±0.09	0.78±0.24
	Median	0.07	0.15	0.72
	Minimum	0.03	0.12	0.57
	Maximum	0.08	0.33	1.12
*p**		***0.20***	***0.34***	***1.00***

*SD: Standard deviation. *Mann Whitney-U Test with post-hoc Bonferroni correction. p<0.05.*

4. DISCUSSION

WSP activation is known as an early response to the natural repair mechanism against tissue damage in mammals (4). Therefore, the main target in repair/regeneration is WSP stimulation via several different conducts, one of which is GSK inhibition. Tideglusib is a small molecule GSK-3 inhibitor which is efficient in reparative dentin formation, osteoblast proliferation and tissue repair (4). In this study, the influence of Tideglusib on periodontal cells in terms of cytotoxicity, viability and Type-I and III collagen expressions were investigated in order to clarify any potential role of this molecule in periodontal regeneration. This is the first study evaluating the response of periodontal cells treated with Tideglusib.

MultiTox assay protocol was applied to investigate the cytotoxicity and proliferation outcomes of Tideglusib on human periodontal cell lines. These tests are performed by the separation of live and dead cells based on protease and esterase activity (27). Since protease substrates do not disrupt cell viability throughout the experimental period, this assay can be repeated several times, unlike other dye exclusion (27). It is also possible to measure the number of both live and dead cells at the same time. Although other luminescence-based cytotoxicity tests without causing cell destruction are also available in the fields of toxicology and pharmacology, the stability of the tests mentioned above is relatively low as well as the half-life of the luciferase signals is short (27).

The periods in which cellular responses are intended to be evaluated in cell culture studies may vary according to the study plan. Osteoblast activity may be assessed either short-term or long-term ranging from 15 minutes to 60 hours (28-30), whereas 24-hour and 72-hour incubation periods were appraised in studies concerned with fibroblast activity (31). In this study, a 24-hour incubation period was performed as suggested by Kim et al. (32) and Scotchford et al. (33)

A recent study of Neves et al. (4) suggesting Tideglusib stimulated the renewal of living stem cells in tooth pulp inspired the concentrations used in our study. Different concentrations of Tideglusib (50 nM, 100 nM and 200 nM) were used for cytotoxicity and proliferation assays in this study. Cytotoxicity increased significantly after 24 hours administration of 200 nM Tideglusib in the hGF cells ($p=0.012$). In the proliferation experiment, there was no significant increase in the test groups compared to the control group. Some studies in the literature demonstrated that WSP activation causes fibrosis by increasing fibroblast activation and proliferation (34, 35). It has been shown that Wnt activation can lead to increased proliferation of lung fibroblasts, increased differentiation of fibroblasts to myofibroblasts together with increased myofibroblast count (36). Hamburg et al. (37) reported that continuous activation of β-catenin, a cytosolic protein that has been associated with numerous biological tasks utilizing a transcriptional co-activator in the WSP, may cause fibrosis depending on its enhancing result in the number of dermal fibroblasts. In contrast to these studies, Wnt activation and GSK-3 inhibition have been shown to decrease gingival growth by decreasing TGF-β1 expression in gingival fibroblasts (38). In our study for the first time, Tideglusib did not exert any proliferative effect on hGF cells.

The cytotoxicity and proliferation effects of Tideglusib on hPDLF cells were also evaluated in this study. Rooker et al. (17) found in their immunohistochemical study that the Wnt signaling response was higher in the proliferation areas in the periodontal ligament, however, so far no study exists in the literature investigating the impact of Tideglusib on hPDLF cells. Han et al. (9) showed that the proliferation of hPDLF cells increased compared to the control group when lithium, as a WSP activator, was applied. In our study, no significant difference was found between the test groups and the control group ($p>0.05$). While our result does not correlate with the previous studies (9, 17) investigating the influence of WSP activation on hPDLF cells using Wnt modulators such as lithium or Dickkopf molecules, this is the first study to asses the effect of Tideglusib as a WSP activator, and therefore, it may be necessary to evaluate the impact of Tideglusib on hPDLF cells for more extended periods of time.

While Tideglusib application did not demonstrate any upshot on the proliferation of hOB cells, the cytotoxicity of hOB cells increased compared to the control group after the use of 200 nM Tideglusib ($p=0.012$). Morvan et al. (39) observed raised proliferative impression on the osteoblasts of WSP induced mice. Babij et al. (23) stated that when the WSP is activated, there will be an increase in bone mass since the number of osteoblasts enhanced. Caetano-Lopes et al. (40) reported that activation of the WSP is a factor in osteoblast differentiation and proliferation. Moreover, Westendorf et al. (22) and Lerner et al. (41) have shown that WSP plays a critical role in the trabecular and cortical bone mechanism, but so far there are no studies published on the effect of Tideglusib on osteoblast cells. In the present study, osteoblast proliferation was not affected by WSP activation initiated with Tideglusib, which is a new promising molecule with its intriguing theraupeutic efficiency. Further detailed and comprehensive studies are needed to clarify the consequence of WSP activation on osteoblast cells through different pathways.

Based on the findings of cytotoxicity and proliferation assays, the non-cytotoxic dose of Tideglusib that supports the viability was determined as 50 nM, which was further used for Type-I and Type-III collagen experiments. Type-I collagen is the main component of connective tissues such as gingiva, periodontal ligament, dentin, cementum and bone. Also, Type-I collagen is considered as a marker for osteoblast differentiation (42). Type-III collagen exists in the gingiva, periodontal ligament, cementum, skin, blood vessels, fetal tissues and plays an essential role in collagen production/destruction mechanism (43). Type-III collagen provides tissue resistance at the early stages of healing and leaves its place to Type-I collagen as the healing is complete (43). However, there is a limited number of studies evaluating protein expression associated with the WSP. Minear et al. (6) created a biochemical approach to increase the duration and strength of Wnt signaling at the sites of the skeletal wound and demonstrated that Type-I collagen appeared faster in the wound area of WSP activated mutant mice as a result of more vigorous proliferation and earlier differentiation of skeletal stem/progenitor cells. Xiang et al. (44) established a significant decrease in Type-I collagen expression from cardiac fibroblasts of mice having a genetic deficiency of β-catenin molecule. In the light of these studies, Type-I and Type-III collagens were chosen as common proteins for three cell lines to examine the potential regenerative capacity of Tideglusib in our research.

After administration of 50 nM Tideglusib to the hGF cell line, Type-I collagen level was detected to increase significantly compared to the control group ($p=0.029$). Roh et al. (45) hypothesized that the stabilization of β-catenin molecule which exhibits regulatory properties on target gene expression for regeneration/repair after its translocation to the cell nucleus and has a manner of central transducer property for the WSP in fibroblasts might have a positive influence on Type-I collagen synthesis. They reported higher Type-I collagen expression in keloid tissue fibroblasts, presenting elevated fibrotic activity compared to the normal dermal fibroblasts. Similarly, improved expression of Type-I collagen was observed in the liver fibroblasts of WSP activated mice (46, 47). Moreover, Bergmann et al. (34) demonstrated that after inhibition of GSK-3 aiming to activate WSP, a significant upsurge in Type-I collagen expression was displayed in cultured mice dermal fibroblasts. Svegliati et al. (48) confirmed an escalation in the amount of Type-I collagen

expression in conjunction with WSP activation by fibroblasts causing fibrosis in the punch biopsies of systemic sclerosis patients. Our result that a rise in Type-I collagen level was observed in hGF cells was found to be compatible with the studies performed with other Types of fibroblast cells in the literature (34, 46-48).

On the contrary, hPDLF cells treated with Tideglusib expressed a similar level of Type-I collagen as the control cells ($p>0.05$). This finding is not parallel to the previous studies showing that Wnt signaling activation stimulates Type-I collagen expression in dermal, cardiac and liver fibroblasts (34, 45, 46). Nevertheless, the collagen expression in hPDLF cells treated with Tideglusib has not been investigated so far according to our knowledge. There is, however, a prosperity of data concerning the role of Wnt signaling in mediating the behaviours of other types of stem cells (17).

Besides mineralized nodule formation, expressions of Type-I collagen, alkaline phosphatase, osteonectin and Runx2 were evaluated as bone formation markers in studies investigating the result of WSP activation on osteoblast cells (15, 24, 39). Type-I collagen exists abundantly both in the organic matrix of gingiva and bone tissues (42, 47). In our study, as a common marker for both gingiva and bone tissues, Type-I collagen level was measured after the administration of 50 nM Tideglusib to the hOB cells and found significantly higher compared to the control group ($p=0.029$). Glass et al. (49) observed a significant increase in the bone mass accompanied by higher Type-I collagen expression in osteoblast cells of Wnt signaling activated mice. Kim et al. (15) presented higher Type-I collagen release and accelerated osteoblast differentiation together with Wnt signaling activation. Popelut et al. (24) examined the consequence of Wnt signaling activation on implant osseointegration and observed a substantial rise in Type-I collagen level after signal path stimulation. Activation of WSPs provides an escalation in bone mass and increases osteoblast activity, and the data revealed from our study may support the findings that Tideglusib may play a role in the intensification of bone mass.

There is a limited number of studies in the literature exploring the significance of WSP activation on Type-III collagen expression (45, 50). In our study, Type-III collagen expression was not statistically different between the test and control groups for each cell line. Similarly, Roh et al. (45) did not observe any sense of β-catenin stabilization on Type-III collagen release in dermal fibrosis tissue fibroblasts. In contrast to the findings of our study and Roh et al. (45), Ge et al. (50) reported Type-III collagen increase in hepatic tissue fibrosis and decreased hepatic cell proliferation after the blockage of Wnt signaling. However, the differences in the methods applied and cell lines may explain this inconsistency.

In our study, 24-hour incubation with Tideglusib did not cause any change in Type-I collagen expression in the hPDLF cell. Similarly, Tideglusib did not stimulate Type-III collagen expression at 24 hours in any cell line. Short evaluation period may be considered as a limitation of our study. Protein expressions were evaluated in the cell culture studies at periods ranging from 24 hours to 72 hours (15, 24, 28, 31). Although, Popelut et al. (24) did not observe an upturn in the amount of collagen after 24-hour protein release test, they found a significant upsurge after 48 hours of evaluation. Therefore, it was concluded that more prolonged periods of time are needed to examine the influence of Tideglusib on periodontal cells.

5. CONCLUSION

It can be concluded that tideglusib molecule is not cytotoxic at 50 nM and 100 nM concentrations and may have a possible positive effect on bone regeneration rather than periodontal regeneration since it causes type-I collagen increase in hGF and hOB cells, but not in hPDLF. However, our study is the first to investigate the impact of tideglusib on periodontal cells. Further studies are warranted to examine a wide range of healing markers to provide a clearer view for screening the power of Tideglusib on periodontal regeneration.

Acknowledgments: We thank our colleagues from Marmara University Genetic and Metabolic Diseases Research and Investigation Center who provided insight and expertise that greatly assisted the research.

REFERENCES

[1] Kocher T, Konig J, Dzierzon U, Sawaf H, Plagmann HC. Disease progression in periodontally treated and untreated patients—a retrospective study. J Clin Periodontol 2000; 27:866-872.

[2] Melcher AH. On the repair potential of periodontal tissues. J Periodontol 1976; 47:256-260.

[3] Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? Periodontol 2000 2009; 51:208-219.

[4] Neves VC, Babb R, Chandrasekaran D, Sharpe PT. Promotion of natural tooth repair by small molecule GSK3 antagonists. Scientific reports 2017; 7:39654.

[5] Willert K, Nusse R. beta-catenin: a key mediator of Wnt signaling. Curr Opin Genet Dev 1998; 8:95-102.

[6] Minear S, Leucht P, Jiang J, Liu B, Zeng A, Fuerer C, Nusse FR, Helms JA. Wnt proteins promote bone regeneration. Sci Transl Med 2010; 2:29ra30.

[7] Whyte JL, Smith AA, Helms JA. Wnt signaling and injury repair. Cold Spring Harbor perspectives in biology 2012; 4:a008078.

[8] Leucht P, Minear S, Ten Berge D, Nusse R, Helms JA. Translating insights from development into regenerative medicine: the function of Wnts in bone biology. Semin Cell Dev Biol 2008; 19:434-443.

[9] Han PP, Wu CT, Chang J, Xiao Y. The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/beta-catenin signalling pathway by Li+ ions released from bioactive scaffolds. Biomaterials 2012; 33:6370-6379.

[10] Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature 2007; 447:316-320.

[11] Zhao J, Kim KA, De Vera J, Palencia S, Wagle M, Abo A. R-Spondin1 protects mice from chemotherapy or radiation-induced oral mucositis through the canonical Wnt/beta-catenin pathway. Proc Natl Acad Sci U S A 2009; 106:2331-2336.

[12] Petersen CP, Reddien PW. A wound-induced Wnt expression program controls planarian regeneration polarity. Proc Natl Acad Sci U S A 2009; 106:17061-17066.

[13] Kawakami Y, Rodriguez Esteban C, Raya M, Kawakami H, Marti M, Dubova I, Belmonte JCI. Wnt/beta-catenin signaling regulates vertebrate limb regeneration. Genes Dev 2006; 20:3232-3237.

[14] Kubo F, Takeichi M, Nakagawa S. Wnt2b controls retinal cell differentiation at the ciliary marginal zone. Development 2003; 130:587-598.

[15] Kim JB, Leucht P, Lam K, Luppen C, Ten Berge D, Nusse R, Helms JA. Bone regeneration is regulated by wnt signaling. J Bone Miner Res 2007; 22:1913-1923.

[16] Lim WH, Liu B, Cheng D, Williams BO, Mah SJ, Helms JA. Wnt signaling regulates homeostasis of the periodontal ligament. J Periodontal Res 2014; 49:751-759.

[17] Rooker SM, Liu B, Helms JA. Role of Wnt signaling in the biology of the periodontium. Dev Dyn 2010; 239:140-147.

[18] Zhang R, Yang G, Wu X, Xie J, Yang X, Li T. Disruption of Wnt/beta-catenin signaling in odontoblasts and cementoblasts arrests tooth root development in postnatal mouse teeth. Int J Biol Sci 2013; 9:228-236.

[19] Kim TH, Lee JY, Baek JA, Lee JC, Yang X, Taketo MM, Jiang R, Cho ES. Constitutive stabilization of ss-catenin in the dental mesenchyme leads to excessive dentin and cementum formation. Biochem Biophys Res Commun 2011; 412:549-555.

[20] Nemoto E, Koshikawa Y, Kanaya S, Tsuchiya M, Tamura M, Somerman MJ, Shimauchi H. Wnt signaling inhibits cementoblast differentiation and promotes proliferation. Bone 2009; 44:805-812.

[21] Han P, Ivanovski S, Crawford R, Xiao Y. Activation of the Canonical Wnt Signaling Pathway Induces Cementum Regeneration. J Bone Miner Res 2015; 30:1160-1174.

[22] Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. Gene 2004; 341:19-39.

[23] Babij P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Bouxsein ML, Reddy SP, Bodine PVN, Robinson JA, Bhat BM, Marzolf J, Moran RA, Bex FJ. High bone mass in mice expressing a mutant LRP5 gene. J Bone Miner Res 2003; 18:960-974.

[24] Popelut A, Rooker SM, Leucht P, Medio M, Brunski JB, Helms JA. The acceleration of implant osseointegration by liposomal Wnt3a. Biomaterials 2010; 31:9173-9181.

[25] Meijer L, Flajolet M, Greengard P. Pharmacological inhibitors of glycogen synthase kinase 3. Trends Pharmacol Sci 2004; 25:471-480.

[26] Adamowicz K, Wang H, Jotwani R, Zeller I, Potempa J, Scott DA. Inhibition of GSK3 abolishes bacterial-induced periodontal bone loss in mice. Mol Med 2012; 18:1190-1196.

[27] Niles AL, Moravec RA, Eric Hesselberth P, Scurria MA, Daily WJ, Riss TL. A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. Anal Biochem 2007; 366:197-206.

[28] Bowers KT, Keller JC, Randolph BA, Wick DG, Michaels CM. Optimization of surface micromorphology for enhanced osteoblast responses in vitro. Int J Oral Maxillofac Implants 1992; 7:302-310.

[29] Campoccia D, Arciola CR, Cervellati M, Maltarello MC, Montanaro L. In vitro behaviour of bone marrow-derived mesenchymal cells cultured on fluorohydroxyapatite-coated substrata with different roughness. Biomaterials 2003; 24:587-596.

[30] Yliheikkila PK, Masuda T, Ambrose WW, Suggs CA, Felton DA, Cooper LF. Preliminary comparison of mineralizing multilayer cultures formed by primary fetal bovine mandibular osteoblasts grown on titanium, hydroxyapatite, and glass substrates. Int J Oral Maxillofac Implants 1996; 11:456-465.

[31] Kook SH, Lee D, Cho ES, Heo JS, Poudel SB, Ahn YH. Hwang JW, Ji H, Kim JG, Lee JC. Activation of canonical Wnt/beta-catenin signaling inhibits H2O2-induced decreases in proliferation and differentiation of human periodontal ligament fibroblasts. Mol Cell Biochem 2016; 411:83-94.

[32] Kim HJ, Kim SH, Kim MS, Lee EJ, Oh HG, Oh WM, Park SW, Kim WJ, Lee GJ, Choi NG, Koh JT, Dinh DB, Hardin RR, Johnson K, Sylvia VL, Schmitz JP, Dean DD. Varying Ti-6Al-4V surface roughness induces different early morphologic and molecular responses in MG63 osteoblast-like cells. J Biomed Mater Res A 2005; 74:366-373.

[33] Scotchford CA, Ball M, Winkelmann M, Voros J, Csucs C, Brunette DM, Danuser G, Textor M. Chemically patterned, metal-oxide-based surfaces produced by photolithographic techniques for studying protein – and cell-interactions. II: Protein adsorption and early cell interactions. Biomaterials 2003; 24:1147-1158.

[34] Bergmann C, Akhmetshina A, Dees C, Palumbo K, Zerr P, Beyer C, Zweina J, Distler O, Schett G, Distler JHW. Inhibition of glycogen synthase kinase 3β induces dermal fibrosis by activation of the canonical Wnt pathway. Annals of the rheumatic diseases 2011; 70:2191-2198.

[35] Burgy O, Königshoff M. The WNT signaling pathways in wound healing and fibrosis. Matrix Biology 2018.

[36] Akhmetshina A, Palumbo K, Dees C, Bergmann C, Venalis P, Zerr P, Horn A, Kireva T, Beyer C, Zwerina J, Schneider H, Sadowski A, Riener MO, MacDougald OA, Distler O, Schett G, Distler JHW. Activation of canonical Wnt signalling is required for TGF-beta-mediated fibrosis. Nat Commun 2012; 3:735.

[37] Hamburg EJ, Atit RP. Sustained beta-catenin activity in dermal fibroblasts is sufficient for skin fibrosis. J Invest Dermatol 2012; 132:2469-2472.

[38] Bahammam M, Black SA, Jr., Sume SS, Assaggaf MA, Faibish M, Trackman PC. Requirement for active glycogen synthase kinase-3beta in TGF-beta1 upregulation of connective tissue growth factor (CCN2/CTGF) levels in human gingival fibroblasts. Am J Physiol Cell Physiol 2013; 305:C581-590.

[39] Morvan F, Boulukos K, Clement-Lacroix P, Roman Roman S, Suc-Royer I, Vayssiere B, Ammann P, Martin P, Pinho S. Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. J Bone Miner Res 2006; 21:934-945.

[40] Caetano-Lopes J, Canhao H, Fonseca JE. Osteoblasts and bone formation. Acta Reumatol Port 2007; 32:103-110.

[41] Lerner UH, Ohlsson C. The WNT system: background and its role in bone. J Intern Med 2015; 277:630-649.

[42] Dacic S, Kalajzic I, Visnjic D, Lichtler AC, Rowe DW. Col1a1-driven transgenic markers of osteoblast lineage progression. J Bone Miner Res 2001; 16:1228-1236.

[43] Mariotti A. The extracellular matrix of the periodontium: dynamic and interactive tissues. Periodontol 2000 1993; 3:39-63.

[44] Xiang FL, Fang M, Yutzey KE. Loss of beta-catenin in resident cardiac fibroblasts attenuates fibrosis induced by pressure overload in mice. Nat Commun 2017; 8:712.

[45] Roh MR, Kumar R, Rajadurai A, Njauw C, Ryoo UH, Chung KY, Tsao H. Beta-catenin causes fibrotic changes in the

extracellular matrix via upregulation of collagen I transcription. Br J Dermatol 2017; 177:312-315.

[46] Beljaars L, Daliri S, Dijkhuizen C, Poelstra K, Gosens R. WNT-5A regulates TGFβ-related activities in liver fibrosis. American Journal of Physiology-Heart and Circulatory Physiology 2017.

[47] Beyer C, Schramm A, Akhmetshina A, Dees C, Kireva T, Gelse K, Sonnylal S, Crombrugghe B, Taketo MM, Distler O, Schett G, Distler JHW. β-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. Annals of the rheumatic diseases 2012; 71:761-767.

[48] Svegliati S, Marrone G, Pezone A, Spadoni T, Grieco A, Moroncini G, Grieco D, Vinciguerra M, Agnese S, Jungel A, Distler O, Musti AM, Gabrielli A, Avvedimento EV. Oxidative DNA damage induces the ATM-mediated transcriptional suppression of the Wnt inhibitor WIF-1 in systemic sclerosis and fibrosis. Sci Signal 2014; 7:ra84.

[49] Glass DA, 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 2005; 8:751-764.

[50] Ge W-S, Wang Y-J, Wu J-X, Fan J-G, Chen Y-W, Zhu L. β-catenin is overexpressed in hepatic fibrosis and blockage of Wnt/β-catenin signaling inhibits hepatic stellate cell activation. Molecular medicine reports 2014; 9:2145-2151.

How to cite this article: Oncu B, Yilmaz AM, Karademir Yilmaz B, Altunok EC, Kuru L, Agrali OB. Cytotoxicity and Collagen Expression Effects of Tideglusib Administration on Human Periodontal Cells: An In-Vitro Study. Clin Exp Health Sci 2020; 10: 153-162. DOI: 10.33808/clinexphealthsci.709924

Clinical and Experimental Health Sciences

Localizing Epileptic Focus with Interictal Scalp EEG in Patients with Focal Cortical Dysplasia

Ozgur Bilgin Topcuoglu[1], Ipek Midi[2]

[1] Sureyyapasa Chest Diseases and Thorax Surgery Training and Research Hospital, Neurology Clinics, Istanbul, Turkey.
[2] Marmara University, Faculty of Medicine, Department of Neurology, Istanbul, Turkey.

Correspondence Author: Ozgur Bilgin Topcuoglu
E-mail: ozgurbilgin1@yahoo.com
Received: 01.08.2019 **Accepted:** 23.08.2019

ABSTRACT

Objective: To assess the localizing value of interictal scalp EEG in epileptic focal cortical dysplasia (FCD) patients.

Methods: A retrospective observational study of EEG records of epileptic patients seen at the Marmara University Hospital was performed. EEG and MRI findings were evaluated to detect a possible correlation.

Results: EEG findings were consistent with MRI findings in 6 patients (37.5%).

Conclusion: EEG findings were irrelevant in 62.5% (n:10) of patients. Additional imaging techniques such as ictal SPECT, MEG etc. will clearly augment the success of lesion localisation in FCD patients.

Keywords: EEG, interictal, focal cortical dysplasia.

1. INTRODUCTION

Focal cortical dysplasia (FCD) is a common malformation of cerebral cortical development. FCD describes a wide range of abnormalities resulting from disruption in the migration of the maturing neurons, disturbance of programmed cell death in fetal brain and environmental factors (1). Association of FCD with epilepsy was first described by Taylor in 1971 (2) Five to 10% of all epilepsy patients have FCD (3). Intractable epilepsy remains the most common clinical presentation for all types of FCD (4). Seizures usually tend to start in early childhood, but may start at any age (5). In pediatric patients with drug-resistant epilepsy, up to 50% of patients have magnetic resonance imaging (MRI) visible FCD (3) Scalp electroencephalography (EEG) is one of the important tools to localize the epileptogenic zone (6). However, there are conflicting results from studies which evaluated the role of EEG in FCD (7,8).

In this study we aimed to analyze the interictal scalp EEGs of a refined group of FCD patients to see if interictal scalp EEG correctly localized the epileptogenic zone.

2. METHODS

A retrospective observational study of EEG records of epileptic patients seen at the Marmara University Hospital, Turkey between January 2012 to December 2018 was performed. Patients who were diagnosed as FCD were included. Age, sex, EEG findings and MRI findings of the patients were retrieved. All EEGs are 30 minutes interictal scalp EEGs which were performed using the 10-20 system. Patients were grouped according to their demographic variables. EEG and MRI findings were evaluated to detect a possible correlation.

This is a retrospective descripitive study thus there was no control group. Since the patient group was relatively small, the results were not statistically analyzed and given as ratios and percentages.

3. RESULTS

Sixteen epileptic patients who were diagnosed as FCD were included in the study. Nine patients (56.25%) were female while 7 patients (43.75%) were male. Mean patient age was 35,375 years with ranging from 18 to 72 years old.

DOI: 10.33808/clinexphealthsci.598395

Six patients(37.5 %) had FCD on left hemisphere, 5 patients (31.25%) had on right hemisphere and 5 patients (31.25%) had bilateral lesions.

43.75 % (n=7) of patients' lesions were found in frontal, 25 % (n=4) in perisilvie, 18.75% (n=3) in temporal, 6.25% (n=1) in parietal lobe. One patient (6.25%) had bilateral dual pathology (mesial temporal sclerosis with occipital FCD).

EEG findings were normal in 7 patients (43.75%) and pathological in 8 patients (56.25%). EEG findings were consistent with MRI findings in 6 patients (37.5%) and irrelevant in 62.5% (n=10) as shown at Table 1.

Table 1. *MRI and EEG findings of the patients.*

Patient no	Sex	Age(years)	MRI findings	EEG findings
1	1	22	Temporal(L)	Temporooccipital E.A.(L)
2	1	27	Frontal (L)	Normal
3	1	56	MTS+occipital (B)	Temporal E.A.(B)
4	0	35	Frontal (R)	Normal
5	0	44	Perisilvien (B)	Normal
6	1	34	Temporal (R)	Anterior temporal E.A.(R)
7	0	35	Perisilvien (B)	FIRDA
8	1	19	Frontal (L)	Bifrontal intermittant slowing
9	0	18	Temporal (L)	Normal
10	0	26	Frontal (L)	Normal
11	1	46	Superior frontal (R)	Normal
12	1	72	Frontal (L)	Normal
13	0	25	Parietal (R)	Frontocentral E.A.(R)
14	0	35	Frontal(R)	Frontocentral E.A.(R)
15	1	43	Perisilvien (B)	Jeneralized E.A.
16	1	29	Perisilvien(R)	Frontal E.A. (R)

0=male, 1= female ,L=left, R=right, B=bilateral, E.A=epileptifrom activity, FIRDA=Frontal intermittant rythmic delta activity

4. DISCUSSION

In this study we aimed to evaluate the localizing value of interictal scalp EEG in FCD. We found out that in 37.5% (n=6) of our patients, EEG findings were consistent with MRI lesions.

Epilepsy, the most common manifestation of FCD can ocur at any age but most commonly develops in childhood (9). Seizures from FCD are commonly refractory to medical treatment (10). With the evolving modern imaging technologies such as MRI, 18-fluorodeoxyglucose-positron –emission tomography (FDG-PET), ictal-single photon emission computed tomography (SPECT) and magnetoencephalography (MEG), cortical dysplasia are more frequently diagnosed in epileptic patients (11). Certainly the MRI lesion is not enough for epilepsy surgery decision. It is necessary to meticulously determine where exactly the seizure arises. Despite the variation between different types of FCD, rythmic epileptiform discharges on interictal scalp EEG have been determined to correlate with the anatomic extent of the lesion (7,8)

Interictal and ictal scalp EEG findings have been formerly studied to demonstrate electrographic signs by Noachtar et al. (12) Lerner et al. has shown that interictal and ictal scalp EEG correlated with the region of cortical dysplasia in 49-68% of patients (13). Our findings are close but less supportive than the former studies. We showed that interictal scalp EEG only localized 37.5% of the FCD patients. There may be a few reasons for this discordance. One of them is clearly the relatively small number of patients. The second reason may be the lack of ictal scalp EEG implementation.

5. CONCLUSION

As a conclusion, our relatively different results from the previous studies may be interpretad as the necessity of dual practice of ictal and interictal scalp EEG in FCD patients. Additional imaging techniques such as ictal SPECT and MEG etc. should be performed to augment the success of lesion localisation clearly in FCD patients.

Acknowledgement: Special thanks to Mrs. Berrak Kızılap for her generous support in the EEG laboratory.

REFERENCES

[1] Sullivan LR, Kull LL, Sweeney DB, Davis CP. Cortical dysplasia: zones of epileptogenesis. Am J Electroneurodiagnostic Technol 2005; 45: 49-60.

[2] Taylor DC, Falconer MA, Bruton CJ, Corsellis JA. Focal dysplasia of the cerebral cortex in epilepsy. J Neurol Neurosurg Psychiatry 1971; 34: 369-387.

[3] Leach JL, Greiner HM, Miles L, Mangano FT. Imaging spectrum of cortical dysplasia in children. Semin Roentgenol 2014;49: 99-111.

[4] Tassi L, Colombo N, Garbelli R, Francione S, Lo Russo G, Mai R, Cardinale F, Cossu M, Ferrario A, Galli C, Bramerio M, Citterio A, Spreafico R. Focal cortical dysplasia: neurpathological subtypes, EEG, neuroimaging and surgical outcome. Brain 2002; 125: 1719-1732.

[5] Crino PB. Focal Cortical Dysplasia. Semin Neurol 2015: 35: 201-208.

[6] Rosenow F, Luders H. Presurgical evaluation of epilepsy. Brain 2001; 124: 1683-1700.

[7] Krsek P, Pieper T, Karlmeier A, Hildebrandt M, Kolodziejczyk D, Winkler P, Pauli E, Blümcke I, Holthausen H. Different presurgical characteristics and seizure outcomes in children with focal cortical dysplasia type I or II. Epilepsia 2009; 50:125-137.

[8] Gambardella A, Palmini A, Andermann F, Dubeau F, Da Costa JC, Quesney LF, Andermann E, Olivier A. Usefulness of focal rythmic discharges on scalp EEG of patients with focal cortical dysplasia and intractable epilepsy. Electroencephalogr Clin Neurophysiol 1996; 98: 243-249.

[9] Gaitanis JN, Donahue J. Focal cortical dysplasia. Pediatric Neurology 2013; 49: 79-87.

[10] Wyllie E, Comair YG, Kotagal P, Bulacio J, Bingaman W, Ruggieri P. Seizure outcome after epilepsy surgery in children and adolescents. Ann Neurol 1998; 44: 740-748.

[11] Hauptman JS, Mathern GW. Surgical treatment of epilepsy associated with cortical dysplasia: 2012 update. Epilepsia 2012;53:98-104.

[12] Noachtar S, Bilgin O, Rémi J, Chang N, Midi I, Vollmar C, Feddersen B. Interictal regional polyspikes in noninvasive EEG suggest cortical dysplasia as etiology of focal epilepsies. Epilepsia 2009; 50: 550-555.

[13] Lerner JT, Salamon N, Hauptman JS, Velasco TR, Hemb M, Wu JY, Sankar R, Donald Shields W, Engel J Jr, Fried I, Cepeda C, Andre VM, Levine MS, Miyata H, Yong WH, Vinters HV, Mathern GW. Assessment and surgical outcomes for mild type I and severe type II cortical dysplasia: a critical review and the UCLA experience. Epilepsia 2009 ;50: 1310-1335.

How to cite this article: Topcuoglu OB, Midi I. Localizing Epileptic Focus with Interictal Scalp EEG in Patients with Focal Cortical Dysplasia. Clin Exp Health Sci 2020; 10: 163-165. DOI: 10.33808/clinexphealthsci.598395

Clinical and Experimental Health Sciences

Effects of Cold Band Application Treatment on Pain and Quality of Life in Migraineurs: A Self-Controlled Study

Sengul Uzen Cura[1], Rengin Acaroglu[2]

[1] Department of Nursing, Faculty of Health Science, Canakkale Onsekiz Mart University, Canakkale, Turkey.
[2] Istanbul University-Cerrahpasa, Department of Fundamentals of Nursing, Florence Nightingale Faculty of Nursing, Istanbul, Turkey.

Correspondence Author: Sengul Uzen Cura
E-mail: snglzn@gmail.com
Received: 23.07.2019 **Accepted:** 25.04.2020

ABSTRACT

Objective: The aim of this study was to examine the effect of applying cold band treatment to migraineurs on the duration and severity of migraine headaches, and on migraine-specific life quality.

Methods: Patients referred to neurology clinic and diagnosed with migraine by a neurologist were examined prospectively. A self-controlled research design was applied to minimize individual differences. The sample group participating in the study were monitored over the course of four migraine attacks: two before and two during application period.

Results: Comparison of the mean values of pain duration for pre-application and application periods revealed no statistically significant difference ($p>0.05$). However, there was found to be a significant difference between mean pain intensity total scores ($p<0.05$). At 30th and 60th – minute points, it was noted that the mean value of the total pain scores for the application period decreased significantly ($p<0.05$). Also, it was recognized that the 24-hour Migraine Quality of Life Scale showed statistically significant increase in total score and in subscale scores when compared to pre-application period ($p=0.0001$).

Conclusion: The application of a cold band to forehead was found to have a positive effect in reducing pain severity and also improving quality of life.

Keywords: Cold application, migraineurs, nursing, pain, quality of life.

1. INTRODUCTION

The International Headache Society (IHS) defines migraine as a headache which reoccurs, which is so intense that it can seriously affect quality of life and which might last for hours; it emphasizes that migraine can alter individuals' physiological and emotional states, as well as their social and economic conditions (1). It is evident that the unpredictable nature of migraine can often cause individuals to experience fear and anxiety (2). Also, it can negatively affect their daily life activities, work / school performance and their general social functioning (3-5). Furthermore, it is commonly felt that the pain and incapacity experienced during a migraine attack is not limited only to the period of the attack itself, but also continues to affect the individual between attacks (6).

The main goal in the care and treatment of migraineurs is to enhance quality of life by reducing the frequency of attacks, and the severity and duration of the pain (6).

It is necessary to teach these individuals methods and practices for coping with pain by raising the level of their physical, social and psychological well-being, thereby improving their quality of life (4, 6).

A number of studies have advocated the use of herbal products, vitamins and minerals and also the benefits of methods such as applied pressure and hot and cold applications to prevent or relieve migraine (3, 7-9). In particular, cold application to the head is a method that has been used for many years in migraine treatment and is generally a responsibility of nursing staff (10-13) (Turkish Nurses Association, 2011). Some previous studies into the effects of cold application on headaches employed different application methods, such as: a complex helmet system that combines cold, heat and pressure (14); a cold gel cap attached at the neck and surrounding the whole head (15); and a cold neoprene band wrapped around the neck (13).

DOI: 10.33808/clinexphealthsci.595444

However, to date, there do not appear to have been any studies carried out on this subject by nursing practitioners. The focus of this study was the use of a cold band applied to the forehead during migraine attacks. This method was chosen because the time of occurrence of migraine attacks is uncertain and this method is easy to use in any setting. Similarly, the forehead is the most commonly-reported locus for the experience of migraine and the band can be easily applied there.

In this context, the study was designed to evaluate the effect of local cold application to the forehead on headache intensity level and duration, and also on quality of life. It is anticipated that the results of this study might provide useful evidence for the improvement of patient care guidelines in future.

In this study, the following hypotheses were tested during any follow-up that was conducted.

Hypothesis (H) 1: Local cold application on the forehead of the migraineurs shortens the length of pain.

Hypothesis (H) 2: Local cold application on the forehead of the migraineurs decreases the severity of pain.

Hypothesis (H) 3: Local cold application on the forehead of the migraineurs increases the quality of life.

2. METHODS

2.1. Study Design and Sampling

The study was completed within one year (August 2015 – August 2016) using self-controlled pre-test and post-test research design at a single center. Ethical approval from Medipol University Clinical Research Ethics Committee was obtained (No: 108400987-379).

After the research sample was determined, an effect test was performed using the Visual Analogue Scale (VAS) scores from the study results previously obtained by Suprouse-Blum and colleagues (2013). Based on these calculations, it was considered necessary to include 22 migraineurs in the sample with 0.80 power value, 0.05 level of significance, ± 0.05 deviation. Finally, it was decided to proceed with the study with 30 patients who met the inclusion criteria, so that parametric tests could be applied, bearing in mind it was likely there would be some drop-out from the total number starting the study. These individuals were monitored throughout the duration of four attacks in total, two in the pre-application period and two in the application period, for pain severity, pain duration and quality of life.

Individuals who were diagnosed by a physician with migraine according to International Classification of Headache Disorders-II (CHD-II) criteria, and who met certain specified criteria, were included in the study. The participants had to be 18 years of age or above, who were not pregnant, who took only nonsteroidal anti-inflammatory (NSAID) analgesics, who had not used the cold application method before in order to relieve migraine, who had no record of cold allergies and whose attack frequencies were usually less than two months.

Patients with migraine who visited the neurology polyclinic and who met the research criteria, were informed on admission about the aims of the research, its scope, duration and what was expected from them. Written consent was obtained from the volunteers before including them in the sample. During the pre-application period, participants were not informed about the nature of the treatment in case they were tempted to try it before application. They were told only that it was known to be effective as a migraine treatment, it had no side effects, and it was a non-pharmacological intervention. They were assured that they would be able to quit at any time if they did not want to proceed. In fact, however, no participant withdrew from the study.

2.2. Instruments

The following items were employed in the data collection process: Patient Information Form, Patient Inspection Form, VAS and The 24-hour Migraine Quality of Life Questionnaire (24 h-MQoLQ). **Patient Information Form** consisted of three parts. The first included participants' personal details such as age, gender, marital status, occupation and educational status. Part 2 featured questions regarding the duration of the migraine attack, the location in which it took place, the frequency of attacks, aura presence, and any methods used to stop the pain.The third part contained questions about the effect of the cold application in relieving pain, whether or not there were any side effects (and, if so, the nature of these side effects), their intention to use the cold application again for migraine relief or not, and whether or not they were using other non-pharmacological applications. **Patient Inspection Forms** were prepared separately for the pre-application and application periods. They listed the contact details of the researcher and specified the type of data the patients were required to enter in the course of the attacks. **Pre-application Inspection Form** was designed to record the patients' own descriptive account of the starting-point and end-point of migraine attacks and their experience of pain levels. In the same way **application period inspection form,** collected the patients' descriptions of their experience at the start and end of the cold application period. **VAS** was used to translate the participants' subjective judgements into numerical values. The participants were required to indicate their perception of the pain level experienced on a 10 cm (100 mm) ruler. This registered the complete absence of pain at one end and severe pain at the other. The **24 h MQoLQ** is a migraine-specific questionnaire which aims to quantify short-term changes in quality of life within the 24-hour period after the onset of pain. There are fifteen items in total covering five pre-defined subscales of quality of life: Migraine Symptoms, Emotions-Anxiety, Work Functionality, Social Functioning and Energy/Vitality. It is possible to get a minimum of 1 point and a maximum of 7 points from each item in the seven-point Likert – type scale, and a minimum of

3 and maximum of 21 points from each item on the quality of life subscale. The minimum possible score is 15, and the maximum 105. The higher the score on the scale, the better the quality of the individual's life in relation to his or her migraine. The lower the score on the scale, the worse the quality of life. The Cronbach Alpha Value of the scale was found to be 0.84. In this study a value of 0.79 was obtained for the pre-application period, and 0.89 for the application period (16, 17).

2.3. Data Collection

Individuals participating in the survey were observed during four migraine attacks: two before the cold application and two after. During the pre-application period, the first and second parts of the Patient Information Form were introduced to the participants, followed by the Pre-application Patient Observation Form, VAS and the 24h MQoLQ. The forms were explained in detail and any points requiring clarification were answered. During the application period, the information on the Patient Information Form was reviewed and any changes were recorded. The Application Period Patient Observation Form, VAS and the 24h MQoLQ were then issued. Instruction was provided on how to complete the forms. The cold application process was demonstrated and explanatory information provided about it and the material to be used. Feedback was received from the patients. The 'Cold Migraine Bands', with a form explaining the application method, were distributed to the participants so that they could apply them to their foreheads as directed as soon as the pain started.

The participants were asked to call the researcher after every attack in order the ensure all the relevant data was collected in full. Patients were called every two weeks by the researcher and questioned as to whether they had experienced a migraine attack. Those who said they had were invited to the polyclinic and their forms collected. They were issued with new forms to complete in the event of a further attack. The participants were invited to the polyclinic for interview after the application period had ended. Their forms were collected and the third part of the Patient Observation Form, containing the questions for evaluation after application, was issued to them.

2.4. Statistical Analysis

The data were analyzed using SPSS for Windows (Version 16.0). Descriptive statistics, such as mean, SD, number, and frequency, were used to characterize the research participants. The Wilcoxon Marked-Rank Test was employed to test the difference between groups in non-parametric data. Since the samples were collected independently from each other, the t test and ANOVA were used to determine whether the results were rational. The results were evaluated at 95% confidence interval, and at $p<0.05$ significance level.

3. RESULTS

The study was carried out with 30 patients. The average age of the patients included in the research was 35.47±8.95. 23 of the patients were women, 16 had a university degree, 15 were government officers, and 16 were married.

When the sickness characteristics of the participants were analyzed: 43.3% were found to have been diagnosed with migraine for 11 years or more; the same figure of 43.3% was found for those who experienced pain in the pre-frontal area; 40% experienced a migraine attack once a month; 6.6% experienced a migraine attack every day; and 70% experienced aura symptoms before pain. The most common aura symptom was visual disorder, reported by 71.4%; 80% of participants used medicine to relieve pain; 60% of them used non-pharmacological methods; and the most common non-pharmacological method was simply to rest in a dark and quiet environment, as stated by 33.3% (Table 1).

Table 1. *Comparison of pain duration averages during pre-application and application periods (n=30)*

	Pre-application	Application Period		
	Avg±SD	Avg±SD	Z*	P
Total Pain Duration	18.29±15.85	20.21±13.27	-1.48	0.138

**Wilcoxon Signed Rank Test*

When the pain duration experienced in the pre-application and application periods was compared, no significant difference was detected ($p>0.05$) (Table 2).

Table 2. *Comparison of pain level averages in the pre-application and application periods (n=30)*

	Pre-application Period	Application Period			Percentage Difference Between Pre-Application and Application Periods
Total Pain Duration					
	Avg±SD	*Avg±SD*	*t**	*p*	
Start	5.2±1.6	5.6±1.4	1.634	0.113	%8
30.Minute	6.4±1.4	5.8±1.0	2.169	0.038	-%9
60.Minute	7.3±1.5	5.7±1.0	5.227	0.0001	-%22

**t-test *Anova*

When the pain level averages in the pre-application and application periods were compared, no statistically significant difference was observed between them at the beginning (0 min), ($p=0.113$). The application period pain level at the 30th and 60th minutes points dropped by a significant level compared to the pre-application period ($p<0.05$). This drop in pain level percentages was observed clearly between the two application periods. The pain level total points averages obtained were transformed from 0 min <30 min <60 min in

the pre-application period into 0 min <30 min> 60 min in the application period (Table 3).

Table 3. *Comparison of point averages in the 24 h-MQoLQ during the pre-application and application periods (n=30)*

	Average of pre-application period	Average of application period		
Subscales of the 24-hour QOLS	Attack 1 and Attack 2	Attack 1 and Attack 2		
	Avg±SD	*Avg±SD*	*Z**	*p*
Migraine Symptoms	6.92±1.67	8.70±2.22	-3.430	,001
Emotion/Anxiety	6.95±5.53	8.38±2.22	-3.114	,002
Work Functionality	6.18±2.40	8.62±2.30	-4.324	,0001
Social Functioning	6.20±2.04	8.83±2.50	-4.001	,0001
Energy-Vitality	6.58±1.88	8.22±2.35	-3.230	,001
Total	32.83±6.84	42.75±8.98	-4.314	,0001

**Wilcoxon Signed Rank Test*

It was noted that there was a statistically meaningful increase in the total points averages of the 24 h-MQoLQ in the application period compared to the pre-application period (z=4.314, p=0.0001).

4. DISCUSSION

After the data on pain duration from the migraineurs were collated, the total points average of pain duration for the pre-application period was determined to be 18.29±15.85 hours, and for the application period the corresponding figure was 20.21±13.27 hours (Table 1). It is generally agreed that migraines are rarely shorter than 3 hours and usually last between 8 and 24 hours. However, they can last a few days and sometimes even up to a week (6, 9). In this study, our findings with regard to pain duration are consistent with the literature though there are only a limited number of studies on this aspect. However, the evaluation carried out after implementation of the cold application treatment revealed no significant difference between the total points averages over the pre-application and application periods (p>0.05). Although there are a few studies in the literature examining the effect of cold applications on migraine pain, none have focused on their effect on the duration of pain application period (11-13, 15). With regard to this, H1 hypothesis was rejected.

In migraineurs the severity of the pain experienced varies from individual to individual. A high degree of severity might prevent them from carrying out their normal everyday activities (18). The data collected from participants during attacks showed that there was a statistically significant increase in the median severity of pain in the case of both attacks in the pre-application period (p<0.001; Table 2). For data collected during attacks within the application period, the median values of pain severity showed similar distributions (p<0.05, Table 2). When the total pain level points averages in the pre-application and application periods were compared, no statistically significant difference was observed between the averages at the start point (0 min), (p=0.113). However, at the 30th and 60th minutes points a significant difference was detected (p=0.038; p=0.0001) (Table 2). Thus, H2 hypothesis was confirmed. Similar results can be found in previous studies of the effect of cold application treatment on headaches (13, 15, 18, 19). In addition, the effect of cold application was investigated with regard to the experience of pain across a wide range of conditions, for example: following total hip surgery; after knee surgery; in cases of acute low back pain; postpartum episiotomy; for heel pain; in the post-operative period following an open abdominal operation; for pain due to chest tube removal; and for patients with primary osteoarthritis. The results of these studies indicate that cold application treatment has a positive effect (19-26).

For migraineurs, recurring episodes of headache, and persistent fear and anxiety about the uncertainty of when the next one will occur, can all have a disturbing effect on themselves and their family, as well as on their work and social life. A study by Powers et al (2003) found that children with migraine had similar quality of life scale scores to those with arthritis and cancer (27). Researchers in Canada revealed that half of migraineurs were unable to continue their daily activities during attacks and one third needed bed rest (28). Özkan and Ongun (2017), found that 53.1% of migraineurs were severely restricted in carrying out their normal daily activities, while the figure obtained by Gul and Mollaoğlu (2012) was 68.3% (29, 30).

When the data on quality of life with migraine were analyzed, it was found that, for the first and second attacks in the pre-application period, the total average score for the 24 h-MQoLQ was 32.83±6.84, rising to 42.75±8.98 in the application period. Scale point averages were compared at the end of the pre-application and application period attacks. As a result, a significant increase in total quality of life scores in the application period was observed, compared to the pre-application period (p<0.05, Table 3). With regard to this finding, H3 hypothesis was confirmed. Although to our knowledge no studies have been conducted into the direct effect of cold application on quality of life, it is recognized as a frequently used treatment method (31, 32). It is asserted that the higher the individual's score on the 24 h-MQoLQ, the higher their migraine-related quality of life will be (16, 17). The increase in migraine-related quality of life scale scores during the application period when compared to the pre-application period showed that the cold application had a positive effect in improving the quality of life of individuals suffering from migraine. However, given that the highest possible score on the 24 h-MQoLQ is 105, it was noticeable that quality of life had not reached the expected level even at the end of the application period. This was taken as a reflection of the overall quality of life of the patients.

5. CONCLUSION

In conclusion, the application of a cold band to the forehead of migraineurs was found to be an effective intervention in reducing the level of pain and improving migraine – related quality of life, but it had no effect on the duration of pain. It is recommended that a further study should be carried out with migraine patients, using different non-pharmacological methods to relieve the pain and then the results compared.

Limitations of the Study

The limitations of the study are as follows; the data's being dependent on self-report, the time between the migraine attacks being too long, the research's being limited to the sample group and not being able to perform any generalizations.

Source of Funding: This study was supported by Istanbul University Scientific Research Projects Unit. Project no: TDK-2016-3781

REFERENCES

[1] Adams J, Barbery G, Lui CW, Pain F. Complementary and alternative medicine use for headache and migraine: a critical review of the literature. Headache 2013; 53(3): 459-473.

[2] Abu Bakar N, Tanprawate S, Lambru G, Torkamani M, Jahanshahi M, Matharu M. Quality of life in primary headache disorders: a review. Cephalalgia 2016; 36(1): 67-91.

[3] Evans RW, Taylor FR. "Natural" or alternative medications for migraine prevention. Headache 2006; 46(6): 1012-1018.

[4] Goolsby MJ. Migraine Headaches. J Am Acad Nurse Pract 2003; 15(12): 536-538.

[5] Mollaoğlu M. Trigger factors in migraine patients. J Health Psychol 2013; 18(7): 984-994.

[6] Wright WL. Assessing functional impairment during and between migraine attacks. Mayo Clin Proc 2008; 4(3): 201-207.

[7] Landy SH, Griffin B. Pressure, heat, and cold help relieve headache pain. Archives of Family Medicine 2000; 9(9): 792.

[8] Wells RE, Bertisch SM, Buettner C, Phillips RS, McCarthy EP. Complementary and alternative medicine use among adults with migraines/severe headaches. Headache 2011; 51(7): 1087-1097.

[9] Silberstein S, Holland S, Freitag F, Dodick DW, Argoff C, Ashman E. Evidence-based guideline update: pharmacologic treatment for episodic migraine prevention in adults: report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. Neurology 2012; 78(17): 1337-1345.

[10] Diamond S, Freitag FG. Cold as an adjunctive therapy for headache. Postgraduate Medicine 1986; 79(1): 305-309.

[11] Friedman M, Peterson S, Behar C, Zaidi Z. Intraoral chilling versus oral sumatriptan for acute migraine. Heart Disease 2001; 3(6): 357-361.

[12] Vanderpol J, Bishop B, Matharu M, Glencorse M. Therapeutic effect of intranasal evaporative cooling in patients with migraine: a pilot study. J Headache Pain 2015; 16(1): 5.

[13] Sprouse-Blum AS, Gabriel AK, Brown JP, Yee MH. Randomized controlled trial: targeted neck cooling in the treatment of the migraine patient. Hawaii J Med Public Health 2013; 72(7): 237-241.

[14] Lance JW. The Controlled application of cold and heat by a new device (Migra-Lief Apparatus) in the treatment of headache. Headache 1988; 28(7): 458-461.

[15] Ucler S, Coskun O, Inan LE, Kanatli Y. Cold therapy in migraine patients: open-label, non-controlled, pilot study. eCAM 2006; 3(4): 489-493.

[16] Hartmaier SL, Santanello NC, Epstein RS, Silberstein SD. Development of a brief 24-hour migraine-specific quality of life questionnaire. Headache 1995; 35(6): 320-329.

[17] İltuş F, Karadakovan A. The reliability and validity of Turkish form of "the 24-hour migraine quality of life questionnaire". Turkiye Klinikleri J Neur 2008; 3(2): 59-63.

[18] Freitag FG. The cycle of migraine: patients' quality of life during and between migraine attacks. Clinical Therapeutics 2007; 29(5): 939-949.

[19] Arankalle D, Wardle J, Nair PM. Alternate hot and cold application in the management of heel pain: a pilot study. The Foot 2016; 29: 25-28.

[20] Paiva CdSB, de Oliveira SMJV, Francisco AA, da Silva RL, Mendes EdPB, Steen M. Length of perineal pain relief after ice pack application: A quasi-experimental study. Women and Birth 2016;29(2): 117-122.

[21] Saito N, Horiuchi H, Kobayashi S, Nawata M, Takaoka K. Continuous local cooling for pain relief following total hip arthroplasty. J Arthroplasty 2004;19(3): 334-337.

[22] Kullenberg B, Ylipaa S, Soderlund K, Resch S. Postoperative cryotherapy after total knee arthroplasty – A prospective study of 86 patients. J Arthroplasty 2006; 21(8): 1175-1179.

[23] Watkins AA, Johnson TV, Shrewsberry AB, Nourparvar P, Madni T, Watkins CJ, Feingold PL, Kooby DA, Maithel SK, Staley CA, Master VA. Ice packs reduce postoperative midline incision pain and narcotic use: a randomized controlled trial. J Am Coll Surg 2014; 219(3): 511-517.

[24] Demir Y, Khorshıd L. The effect of cold application in combination with standard analgesic administration on pain and anxiety during chest tube removal: a single-blinded, randomized, double-controlled study. Pain Manag Nurs 2010; 11(3): 186-96.

[25] Yilmaz N, Kiyak E. The effects of local cold application on fibromyalgia pain. Int J Rheum Dis 2017;20(8): 929-934.

[26] İnan Ç, Kıyak E. The effect of hot and cold application on pain, stiffness and physical function in patients with knee osteoarthritis. HEMAR-G 2014; 16(2): 1-10

[27] Powers SW, Patton SR, Hommel KA, Hershey AD. Quality of life in childhood migraines: clinical impact and comparison to other chronic illnesses. Pediatrics 2003; 112(1): e1-e5.

[28] Leroux E. Impact of a nurse for migraine management: the IMPACT Project (IMPACT). 2013.

[29] Özkan İ, Olgun N. The relationship between self efficacy, general self disease management strategies in coping with pain and quality of life in migraine patients: Testing a theoretical method Journal of Human Sciences 2017; 14(4): 3389-3404.

[30] Gül S, Mollaoğlu M, editors. Characteristics of migraine pain and effects on the daily activity of pain in individuals with migraine. Yeni Symposium; 2011.

[31] Kazan E. Cold applications and nursing care. Hacettepe University Faculty of Health Sciences Nursing Journal 2011; 18(1): 73-82.

[32] Bag B, Karabulut N. Pain-relieving factors in migraine and tension-type headache. J Clin Pract 2005; 59(7): 760-763.

How to cite this article: Uzen Cura S, Acaroglu R. Effects of Cold Band Application Treatment on Pain and Quality of Life in Migraineurs: A Self-Controlled Study. Clin Exp Health Sci 2020; 10: 166-171. DOI: 10.33808/clinexphealthsci.595444

Clinical and Experimental Health Sciences

Efficacy of Hyaluronic Acid Gel as an Adjunct to Non-Surgical Periodontal Treatment in Smokers with Periodontitis: A Retrospective Case Control Study

Hafize Ozturk Ozener, Omer Birkan Agrali, Hatice Selin Yildirim

Marmara University, Faculty of Dentistry, Department of Periodontology, Istanbul, Turkey.

Correspondence Author: Hafize Ozturk Ozener
E-mail: hafize.ozturk@marmara.edu.tr

Received: 26.03.2020 **Accepted:** 20.05.2020

ABSTRACT

Objective: To investigate the clinical effectiveness of adjunctive hyaluronic acid (HA) application to non-surgical periodontal treatment (NSPT) on periodontal parameters and gingival crevicular fluid (GCF) volume in smoking patients with stage III and grade C periodontitis.

Methods: Records of patients who underwent previously NSPT with or without HA gel application were reviewed. The clinical examinations and radiographs of patients, which were collected, are used as data. At baseline, 1 and 3 months, periodontal parameters such as plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL), and GCF volume were assessed.

Results: Total 20 patients (male/female: 15/5) were included in the study who met the eligibility criteria. For 10 of these patients only NSPT was performed (Control group), the other 10 patients received NSPT with HA gel application (Test group). Intra-group differences in both groups were significant in periodontal parameters: PI (both, $p<0.001$), GI (both, $p<0.001$), BOP (both, $p<0.001$), PD (both, $p<0.05$) and CAL (both, $p<0.05$); and in GCF volume (both, $p<0.05$). No statistically significant differences were determined between the treatment groups at any assessment time periods in any outcomes (all, $p>0.05$) nor in the changes (Δ) with time (all, $p>0.05$).

Conclusion: Compared to NSPT alone, the application of HA as adjunct to NSPT did not have any additional clinical effectiveness in smokers having periodontitis.

Keywords: Gingival crevicular fluid, hyaluronic acid, periodontitis, root planing

1. INTRODUCTION

Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan with a high molecular weight, which is existing in various body fluids, including saliva, gingival crevicular fluid (GCF), serum and synovial fluid. HA plays complex roles in maintaining homeostasis in the body and regulating various biological processes (1). It is also a major element of the extracellular matrix of non-mineralized and mineralized tissues, such as the skin, the joints, the eyes, and the periodontium (2-4). HA shows bacteriostatic (5), anti-inflammatory (6), fungastatic (7), osteoinductive (8, 9), anti-oedematous (10) and pro-angiogenetic (11). To date, HA-based biomaterials and exogenous HA have shown success in an extraordinarily broad range of biomedical usage for the treatment of wound healing and inflammation in ophthalmology, dermatology and orthopedics (10).

Hyaluronan synthase enzymes in cells in periodontal tissues (fibroblasts and keratinocytes in the gingiva and periodontal ligament, cementoblasts in the cementum and osteoblasts in the alveolar bone) synthesize hyaluronan in periodontal tissues (12). It exists a higher amount in the periodontal ligament and gingiva compared to the cementum and alveolar bone (10, 13-18). HA is a critical component in maintaining of healthy periodontal tissue, the most abundant glycosaminoglycan in the periodontal ligament matrix (19). Antioedematous, anti-inflammatory and antibacterial effects of HA are also demonstrated in the treatment of periodontal diseases (20). HA is effective on the reduction of plaque index and sulcus bleeding index in gingivitis patients (20, 21). However, there are no differences in bacterial profile and clinical parameters when HA was applied subgingivally as adjunctive to scaling and root planning (SRP) in chronic periodontitis patients (22). On the other hand several clinical studies and reports have revealed additional clinical improvements in probing depth (PD) reduction and clinical attachment level (CAL) gain after the application of HA adjunctively to periodontal operation (23-25). A systematic review (26) concluded that the use of

DOI: 10.33808/clinexphealthsci.709327

HA as an adjunct to both SRP in chronic periodontitis patients and surgical periodontal therapy provided positive effects on the bleeding on probing (BOP), PD, CAL and bone fill. In a recent systematic review and meta-analysis, it was stated that adjunctive use of HA may improve the clinical outcomes when used in conjunction with non-surgical and surgical periodontal therapy (27).

Smoking is one of the main risk factors for the initiation and progression of periodontal diseases. In addition to increasing periodontal destruction, previous data revealed that smoking also adversely affects the response to periodontal treatment (28-30).

To the best of the authors' knowledge, despite all aforementioned unique properties, there is no information about the effectiveness of HA as an adjunct to non-surgical periodontal treatment (NSPT) in smokers with periodontitis. Thus, the present retrospective study aimed to evaluate the clinical effectiveness of HA as adjunctive to NSPT in the management of periodontitis. The null hypothesis was that additional HA gel application during NSPT would not influence the outcomes in smokers.

2. METHODS

This retrospective clinical study was approved by Ethical Committee of Clinical Studies in the Marmara University with the reference number 2019-345.

2.1. Study Population

The data used in this study were extracted from the records of subjects who received NSPT from July 1, 2018 to January 1, 2019 at the Periodontology Clinics, Marmara University.

The inclusion criteria of the subjects were as follows: (a) systemically healthy, (b) periodontitis, (c) smoker (≥10 cigarettes/day; >5 years), (d) aged between 18 and 65 years, (e) not existed any kind of periodontal surgery. Subjects with incomplete documentation and/or without at least 3-week follow-up observation were excluded.

A total of 4625 patients' data were screened and evaluated based on the inclusion criteria. Among them, 4605 patients' records were excluded from the study: 2778 patients had systemic disease and/or were non-smoker and/or presented presence of less than 16 teeth and/or gingivitis and/or history of antibiotic, anti-inflammatory; records of 256 patients were incomplete; 977 patients were not within the defined age range. Thus, our study proceeded with records of 20 patients who fulfilled the inclusion criteria. A written informed consent form was obtained from all subjects before NSPT.

Radiographic (Fig 1) and clinical data of 20 enrolled subjects were retrieved and the procedures and follow-up sessions were reviewed in their records, retrospectively. The patients included in study comply with the periodontitis stage III with regard to the extent and severity and grade C depending on the smoke (31). The same treatment protocol was applied to the clinical records of patients, who underwent either only NSPT or HA gel as adjunct to NSPT, as detailed below.

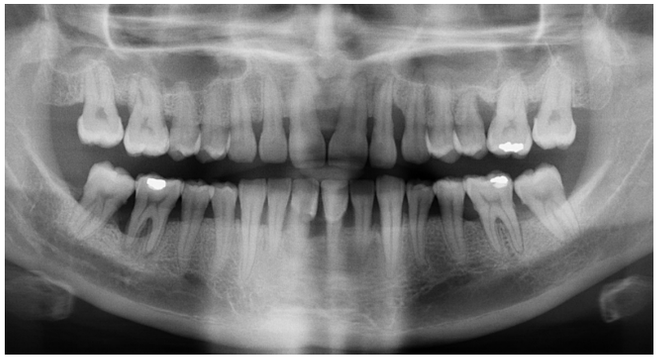


Figure 1. *Radiographic view of one of the patient*

2.2. Treatment protocol

Participants received oral hygiene instructions 1 week before the experimental period. At baseline, 1 and 3 months clinical parameters were recorded and GCF samples were collected. The GCF samples were collected from four distinct deeper pockets with standardized paper strips (PerioPaper, Proflow, Amityville, NY) which inserted at 1 mm depth at the entrance of the periodontal pocket, regardless of the PD, for 30 seconds following isolation of the sampling area. Strips visibly contaminated with microbial dental plaque, saliva or blood were excluded. A calibrated Periotron 8000 (OraFlow, Inc., Smithtown, NY, USA) was used to measure the GCF volume. SRP was performed with hand instruments (Gracey, SG 5/6, 7/8, 11/12, 13/14, Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA) and an ultrasonic scaler (Cavitron®, BOBCAT® Pro, Dentsply International, USA) in two separate sessions with one week interval to both group. In the test group HA gel (Periosyal, Teoxane SA, Geneva, Switzerland) was applied to the gingival crevicular as an adjunct to NSPT in sessions. The gel inside the syringe was appled with a needle that allows it to reach the depth of the pocket until the pocket was filled with gel. Following this procedure, patients were asked not to eat or drink for one hour.

2.3.Data extraction

Clinical parameters in terms of plaque index (PI) (32), gingival index (GI) (33), BOP, PD, and CAL using the manual periodontal probe (UNC15; Hu-Friedy, Chicago, IL, USA) were considered at baseline, 1 and 3 months. The PI and GI were scored at 4 periodontal sites and the PD, CAL and BOP values were measured at 6 periodontal sites per tooth. The average scores for the whole-mouth PI, GI, BOP, PD and CAL; and GCF volume were calculated for each patient. The numerical readings of GCF volume were converted into actual volume (µl) with the reference to formulation obtained from the standard curve.

2.4. Statistical analysis

SPSS 20 (SPSS Corporation, Chicago, USA) was used for statistical analysis with a significance level of 5%. The distribution for normality was checked via the Kolmogorov–Smirnov test. The Mann-Whitney U, the Kruskal-Wallis and Chi-Square tests were used to analyze intergroup differences. The Wilcoxon signed-rank and Friedman's test were performed to check repeated measurements of clinical parameters.

3. RESULTS

In Table 1, the demographic data of subjects without significant differences in gender and age (p>0.05) is presented. No adverse effects were reported throughout the study period.

Tablo 1. *Baseline data of patients*

Characteristic	Total (N=20)	Control Group (N=10)	Test Group (N=10)	*p*
Gender (F/M)	5/15	2/8	3/7	0.606*
Age (Mean±SD)	31.706.23	31.10±6.02	32.30±6.70	0.739†
(Max-Min)	21-46	21-42	23-46	

**Chi Square, †Mann-Whitney U test, p<0.05*

The periodontal clinical parameters in the control and test groups are shown in Table 2. At baseline, clinical parameters for all patients were similar for both groups (p>0.05). The 1 and 3 months' evaluations yielded significant reductions compared with the baseline values in all periodontal parameters in both treatment groups (p<0.05).

Table 2: *Overview of all examined parameters*

		Mean±SD			Delta±SD		
	Time Point	**Control Group (N=10)**	**Test Group (N=10)**	***P****	**Control Group (N=10)**	**Test Group (N=10)**	***P****
Clinical Parameters							
PI	**Baseline**	1.49±0.34	1.48±0.64	0.315			
	1st month	0.43±0.21[a]	0.26±0.35[a]	0.075	0.77±0.37	1.00±0.30	0.143
	3rd month	0.32±0.14[a]	0.19±0.17[ab]	0.105	0.18±0.09	0.15±0.14	0.075
Intragroup *P* value		**0.000‡**	**0.000‡**				
GI	**Baseline**	1.26±0.29	1.52±0.46	0.874			
	1st month	0.49±0.28[a]	0.51±0.34[a]	0.143	0.77±0.37	1.00±0.30	0.218
	3rd month	0.32±0.22[ac]	0.36±0.28[ab]	0.971	0.18±0.09	0.15±0.14	0.247
Intragroup *P* value		**0.000‡**	**0.000‡**				
BOP (%)	**Baseline**	31.94±10.14	39.61±10.02	0.075			
	1st month	4.27±1.26[a]	4.37±2.92[a]	0.481	27.66±9.93	35.24±10.71	0.190
	3rd month	3.41±0.70[a]	3.23±2.45[a]	0.529	0.86±1.53	1.14±1.85	0.529
Intragroup *P* value		**0.000‡**	**0.000‡**				
PD (mm)	**Baseline**	2.92±0.60	3.20±0.58	0.315			
	1st month	2.42±0.29[a]	2.35±0.56[a]	1.000	0.49±0.48	0.85±0.24	0.089
	3rd month	2.36±0.33[d]	2.30±0.72[a]	0.853	0.06±0.14	0.05±0.42	0.529
Intragroup *P* value		**0.000‡**	**0.001‡**				
CAL (mm)	**Baseline**	2.98±0.58	3.35±0.54	0.165			
	1st month	2.48±0.28[a]	2.79±0.89[a]	0.436	0.55±0.49	0.56±0.55	0.912
	3rd month	2.43±0.28[d]	2.75±0.78[a]	0.436	0.06±0.18	0.04±0.15	0.796
Intragroup *P* value		**0.002‡**	**0.001‡**				
GCF volume							
GCF (×10^5 CFU/ml)	**Baseline**	0.42±0.12	0.59±0.24	0.105			
	1st month	0.24±0.06[a]	0.34±0.15[e]	0.105	0.18±0.09	0.18±0.09	0.143
	3rd month	0.14±0.07[ac]	0.24±0.14[fg]	0.143	0.25±0.26	0.25±0.26	0.853
Intragroup *P* value		**0.000‡**	**0.002‡**				

**Mann-Whitney U* test, † *Student-t* test, ‡*Friedman's* two way test

PI: Plaque Index, GI: Gingival Index, BOP: Bleeding on Probing, PD: Probing Depth, CAL: CAL: Clinical Attachment Level, GCF: Gingival Crevicular Fluid [a]Wilcoxon Signed Rank, p=0.005, compared to the baseline, [b]Wilcoxon Signed Rank, p=0.008, compared to the 28th day, [c]Wilcoxon Signed Rank, p=0.005, compared to the 28th day, [d]Wilcoxon Signed Rank, p=0.007, compared to the baseline, [e]Wilcoxon Signed Rank, p=0.028, compared to the baseline, [f]Wilcoxon Signed Rank, p=0.017, compared to the baseline, [g]Wilcoxon Signed Rank, p=0.007, compared to the 28th day

No statistically significant inter-group differences were detected in any of the clinical parameters at any time points (all, $p>0.05$) and no significant differences in the changes (Δ) of clinical outcomes between baseline and 1 month (all, $p>0.05$) or changes between 1 and 3 months (all, $p>0.05$).

The mean values of GCF volume were similar in both groups at baseline and the treatments led a significant reduction in the GCF volumes (µl) in both groups at 1 and 3 months ($p<0.05$) (Table 1). Comparisons between baseline and follow-up measurements, and changes (Δ) revealed no significant differences in GCF volume between control and test groups at all evaluation time periods (Table 1).

4. DISCUSSION

The present study aimed to evaluate whether the use of HA gel may improve the outcomes obtained by NSPT in smoking patients periodontitis. NSPT is the most usual periodontal therapy that is effective in reducing PD, BOP and improving CAL (34-36). Smoking patients with periodontitis are a therapeutically challenging subpopulation who may not respond to periodontal treatment as favorably as non-smoking patients (37-40). Generally, the outcomes have indicated that smoking promotes an unfavorable clinical response such as less PD reductions and lower clinical attachment gains after non-surgical and surgical periodontal treatments. As the smoking decreases the clinical effectiveness of SRP, some previous researches have suggested the use of therapeutic approaches such as local or systemic anti-inflammatory and antimicrobials agents as an adjunct to augment mechanical treatment to enhance the effects of initial periodontal treatment in smokers (41-43). Considering the potential side effects of antibiotics together with limited benefits of short-term duration, in this study, the effect of HA application in addition to NSPT was evaluated. In a systematic review (26), it is concluded that most of clinical studies (44, 45) described a beneficial effect of HA with statistically significant reduction in BOP (2.28–19.5%), PD (0.2–0.9 mm) and CAL gain comparing to controls; it was also reported that there were no side effects. Similarly, in a recently published meta-analysis (27) revealed that non-surgical treatment with adjunctive HA resulted in additional PD and BOP reduction (mean −0.36 mm and −15%, respectively) and CAL gain (mean 0.73 mm) compared with conventional SRP after 3 months. In the present study, NSPT with and without HA gel application resulted in an improvement in all clinical parameters. A similar positive change of all periodontal parameters in both groups shows that HA has no adjunctive clinical effect in smokers with periodontitis. The result of study is consistent with Xu et al. (22) who studied with chronic periodontitis population and did not find any difference in PD, BOP and CAL between test and control groups at 6 and 12 weeks follow-up after NSPT with or without HA gel. Engstrom et al. (46) also reported no difference in PD between the groups at 6 and 12 months after SRP. Furthermore, there is no study evaluating the use of HA in additional to NSPT in smokers, for that reason we cannot directly compare our results.

GCF, is a biological fluid used to assess the clinical status of periodontal tissues, is important for providing site-specific data. GCF also helps to evaluate periodontal tissue formation and destruction, disease activity and treatment effectiveness (47). In this study, the assessment of GCF samples throughout the study period aimed to support the clinical evaluation. The volume of GCF has been shown to be associated with the status of periodontal disease and is an indicator of gingival inflammation (48). A positive relationship between gingival inflammation and GCF volume has been reported (47, 48), as well as result of periodontal treatment, with reduced periodontal inflammation, a decrease in GCF volume is expected (49). Kanmaz et al. (50) reported similar volumes of GCF sample at baseline in the smoker and non-smoker patients with periodontitis stage III or IV, and grade C and significant reduction in the volumes of GCF following NSPT in both groups at 1-, 3-, and 6-months. Similarly, in this study it was found that both groups exhibited a significant and similar reduction in GCF volume throughout the 3-month study period.

Small sample size and the absence of a positive control group including placebo gel application may be the limitations of the present study. On the other hand, the outcomes obtained from this study will make important contributions to the limited information about the subject currently available in the literature.

5. CONCLUSION

Within the limits of the present study, it may be concluded that the additional HA gel application to NSPT of smoker patients with periodontitis may not demonstrate any further contribution to the rehabilitation.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Leyla Kuru, Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey, for the contributions.

REFERENCES

[1] Necas J, Bartosikova L, Brauner P, Kolar J. Hyaluronic acid (Hyaluronan): A review. Veterinarni Medicina 2008; 8: 397–411.

[2] Embery G, Oliver WM, Stanbury JB, Purvis JA. The electrophoretic detection of acidic glycosaminoglycans in human gingival sulcus fluid. Arch Oral Biol 1982; 27(2): 177-179.

[3] Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. J Intern Med 1997; 242(1): 27-33.

[4] Pogrel MA, Lowe MA, Stern R. Hyaluronan (hyaluronic acid) in human saliva. Arc Oral Biol 1996; 41(7): 667-671.

[5] Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. Bacteriostatic Effects of Hyaluronic Acid. J Periodontol 1999; 70: 370-374.

[6] Laurent TC, Laurent UB, Fraser JR. Functions of hyaluronan. Ann Rheum Dis 1995; 54(5): 429-432.

[7] Kang JH, Kim YY, Chang JY, Kho HS. Influences of hyaluronic acid on the anticandidal activities of lysozyme and the peroxidase system. Oral Dis 2011; 17(6): 577-583.

[8] Sasaki T, Watanabe C. Stimulation of osteoinduction in bone wound healing by high-molecular hyaluronic acid. Bone 1995; 16(1): 9-15.

[9] Kawano M, Ariyoshi W, Iwanaga K, Okinaga T, Habu M, Yoshioka I, Tominaga K, Nishihara T. Mechanism involved in enhancement of osteoblast differentiation by hyaluronic acid. Biochem Biophys Res Commun 2011; 405(4): 575-580.

[10] Dahiya P, Kamal R. Hyaluronic Acid: a boon in periodontal therapy. N Am J Med Sci 2013; 5(5): 309-315.

[11] Deed R, Rooney P, Kumar P, Norton JD, Smith J, Freemont AJ, Kumar S. Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan. Int J Cancer 1997; 71: 251-256.

[12] Ijuin C, Ohno S, Tanimoto K, Honda K, Tanne K. Regulation of hyaluronan synthase gene expression in human periodontal ligament cells by tumour necrosis factor-alpha, interleukin-1beta and interferon-gamma. Arch Oral Biol 2001; 46(8): 767-772.

[13] Bartold PM, Wiebkin OW, Thonard JC. Glycosaminoglycans of human gingival epithelium and connective tissue. Connect Tissue Res 1981; 9(2): 99-106.

[14] Bartold PM, Page RC. Hyaluronic acid synthesized by fibroblasts cultured from normal and chronically inflamed human gingivae. Coll Relat Res 1986; 6(4): 365-377.

[15] Bartold PM. Proteoglycans of the periodontium: structure, role and function. J Periodontal Res 1987; 22(6): 431-444.

[16] Waddington RJ, Embery G, Last KS. Glycosaminoglycans of human alveolar bone. Arch Oral Biol 1989; 34(7): 587-589.

[17] Giannobile WV, Riviere GR, Gorski JP, Tira DE, Cobb CM. Glycosaminoglycans and periodontal disease: Analysis of GCF by safranin O. J Periodontol 1993; 64(3): 186-190.

[18] Shimabukuro Y, Ichikawa T, Takayama S, Yamada S, Takedachi M, Terakura M, Hashikawa T, Murakami S. Fibroblast growth factor-2 regulates the synthesis of hyaluronan by human periodontal ligament cells. J Cell Physiol 2005; 203(3): 557-563.

[19] Sukumar S, Drizhal I. Hyaluronic acid and periodontitis. Acta Medica (Hradec Kralove) 2007; 50(4): 225-228.

[20] Jentsch H, Pomowski R, Kundt G, Göcke R. Treatment of gingivitis with hyaluronan. J Clin Periodontol 2003; 30(2): 159-164.

[21] Pistorius A, Martin M, Willershausen B, Rockmann P. The clinical application of hyaluronic acid in gingivitis therapy. Quintessence Int 2005; 36(7-8): 531-538.

[22] Xu Y, Höfling K, Fimmers R, Frentzen M, Jervøe-Storm PM. Clinical and microbiological effects of topical subgingival application of hyaluronic acid gel adjunctive to scaling and root planing in the treatment of chronic periodontitis. J Periodontol 2004; 75(8): 1114-1118.

[23] Briguglio F, Briguglio E, Briguglio R, Cafiero C, Isola G. Treatment of infrabony periodontal defects using a resorbable biopolymer of hyaluronic acid: a randomized clinical trial. Quintessence Int 2013; 44(3): 231-240.

[24] Fawzy El-Sayed KM, Dahaba MA, Aboul-Ela S, Darhous MS. Local application of hyaluronan gel in conjunction with periodontal surgery: a randomized controlled trial. Clin Oral Investig 2012; 16(4): 1229-1236.

[25] Vanden Bogaerde L. Treatment of infrabony periodontal defects with esterified hyaluronic acid: clinical report of 19 consecutive lesions. Int J Periodontics Restorative Dent 2009; 29(3): 315-323.

[26] Bertl K, Bruckmann C, Isberg PE, Klinge B, Gotfredsen K, Stavropoulos A. Hyaluronan in non-surgical and surgical periodontal therapy: a systematic review. J Clin Periodontol 2015; 42(3): 236-246.

[27] Eliezer M, Imber JC, Sculean A, Pandis N, Teich S. Hyaluronic acid as adjunctive to non-surgical and surgical periodontal therapy: a systematic review and meta-analysis. Clin Oral Investig 2019; 23(9): 3423-3435.

[28] Heasman L, Stacey F, Preshaw PM, McCracken GI, Hepburn S, Heasman PA. The effect of smoking on periodontal treatment response: a review of clinical evidence. J Clin Periodontol 2006; 33(4): 241-253.

[29] Labriola A, Needleman I, Moles DR. Systematic review of the effect of smoking on nonsurgical periodontal therapy. Periodontol 2000 2005; 37: 124-137.

[30] Mullally BH, Breen B, Linden GJ. Smoking and patterns of bone loss in early-onset periodontitis. J Periodontol 1999; 70(4): 394-401.

[31] Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018; 89:173-182.

[32] Silness J, Loe H. Periodontal Disease in Pregnancy. II. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand 1964; 22: 121-135.

[33] Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. Acta Odontol Scand 1963; 21: 533-551.

[34] Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. J Clin Periodontol 1981; 8(1): 57-72.

[35] Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy. II. Severely advanced periodontitis. J Clin Periodontol 1984; 11(1): 63-76.

[36] Loos B, Nylund K, Claffey N, Egelberg J. Clinical effects of root debridement in molar and non-molar teeth. A 2-year follow-up. J Clin Periodontol 1989; 16(8): 498-504.

[37] Ah MK, Johnson GK, Kaldahl WB, Patil KD, Kalkwarf KL. The effect of smoking on the response to periodontal therapy. J Clin Periodontol 1994; 21(2): 91-97.

[38] Kaldahl WB, Johnson GK, Patil KD, Kalkwarf KL. Levels of cigarette consumption and response to periodontal therapy. J Periodontol 1996; 67(7): 675-681.

[39] Kinane DF, RadvarM. The effect of smoking on mechanical and antimicrobial periodontal therapy. J Periodontol 1997; 68(5): 467-472.

[40] Trombelli L, Cho KS, Kim CK, Scapoli C, Scabbia A. Impaired healing response of periodontal furcation defects following flap debridement surgery in smokers. A controlled clinical trial. J Clin Periodontol 2003; 30(1): 81-87.

[41] Machion L, Andia DC, Benatti BB, Carvalho MD, Nogueira-Filho GR, Casati MZ, Nociti FH Jr, Sallum EA. Locally delivered doxycycline as an adjunctive therapy to scaling and root planing in the treatment of smokers: a clinical study. J Periodontol 2004; 75(3): 464-469.

[42] Machion L, Andia DC, Lecio G, Nociti FH Jr, Casati MZ, Sallum AW, Sallum EA. Locally delivered doxycycline as an adjunctive therapy to scaling and root planing in the treatment of smokers: a 2-year follow-up. J Periodontol 2006; 77(4): 606-613.

[43] Machion L, Andia DC, Saito D, Klein MI, Gonçalves RB, Casati MZ, Nociti FH Jr, Sallum EA. Microbiological changes with the use of locally delivered doxycycline in the periodontal treatment of smokers. J Periodontol 2004; 75(12): 1600-1604.

[44] Eick S, Renatus A, Heinicke M, Pfister W, Stratul SI, Jentsch H. Hyaluronic Acid as an adjunct after scaling and root planing: a prospective randomized clinical trial. J Periodontol 2013; 84(7): 941-949.

[45] Johannsen A, Tellefsen M, Wikesjö U, Johannsen G. Local delivery of hyaluronan as an adjunct to scaling and root planing in the treatment of chronic periodontitis. J Periodontol 2009; 80(9): 1493-1497.

[46] Engstrom PE, Shi XQ, Tronje G, Larsson A, Welander U, Frithiof L, Engstrom GN. The effect of hyaluronan on bone and soft tissue and immune response in wound healing. J Periodontol 2001; 72(9): 1192-1200.

[47] Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontol 2000 2003; 31: 32-42.

[48] Goodson JM. Gingival crevice fluid flow. Periodontol 2000 2003; 31: 43-54.

[49] Gomes SC, Piccinin FB, Oppermann RV, Susin C, Marcantonio RA. The effect of smoking on gingival crevicular fluid volume during the treatment of gingivitis. Acta Odontol Latinoam 2009; 22(3): 201-206.

[50] Kanmaz B, Lappin DF, Nile CJ, Buduneli N. Effects of smoking on non-surgical periodontal therapy in patients with periodontitis stage III or IV, and grade C. J Periodontol 2019; 91(4):442-453.

How to cite this article: Ozturk Ozener H, Agrali OB, Yildirim HS. Efficacy of Hyaluronic Acid Gel as an Adjunct to Non-Surgical Periodontal Treatment in Smokers with Periodontitis: A Retrospective Case Control Study. Clin Exp Health Sci 2020; 10: 172-177. DOI: 10.33808/clinexphealthsci.709327

Clinical and Experimental Health Sciences

Neuroprotective effects of thymoquinone against ketamine -and MK-801-induced neurotoxicity in SH-SY5Y cells: From the perspective of glutamatergic dysfunction in schizophrenia

Gokhan Unal, Beyza Erdogan

Erciyes University, Faculty of Pharmacy, Department of Pharmacology, Kayseri, Turkey.

Correspondence Author: Gokhan Unal
E-mail: ecz_gokhanunal@hotmail.com

Received: 09.05.2020 **Accepted:** 28.05.2020

ABSTRACT

Objective: Schizophrenia is a chronic disorder with approximately 1% prevalence and related to disrupted neurodevelopment process. It has been known that N-methyl D-Aspartate (NMDA) receptor antagonists such as ketamine and MK-801 mimic schizophrenia-like behaviors in rodents and cellular changes in cell culture. There are certain preliminary reports showing the beneficial effects of Nigella sativa L. extracts or its main active ingredient, thymoquinone, on psychiatric disorders. In our study, we aimed to investigate the neuroprotective effects of thymoquinone against ketamine – and MK-801 – induced neurotoxicites, which may be relevant to schizophrenia.

Methods: The neurotoxic concentrations of ketamine and MK-801, and non-toxic concentrations of thymoquinone were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test at the 24th hour of administrations in SH-SY5Y cells. Seven different concentrations of thymoquinone (0.5 µM, 1 µM, 2.5 µM, 5 µM, 10 µM, 20 µM, 30 µM) were tested against two different concentrations of ketamine (250 µg/ml, 500 µg/ml) and one concentration of MK-801 (100 µM).

Results: Ketamine (250 µg/ml and 500 µg/ml) and MK-801 (100 µM) decreased ($P<0.05$) the cellular viabilities at the 24 hour of administrations. Thymoquinone pretreatment prevented ($P<0.05$) the decrease of cell viabilities against ketamine (250 µg/ml) and ketamine (500 µg/ml) at 1 uM, 2.5 µM, 5 µM, 10 µM, 20 µM, and 2.5 µM concentrations, respectively. Thymoquinone pretreatment also increased ($P<0.05$) cell viability compared to MK-801.

Conclusion: We suggested that thymoquinone had neuroprotective effects on the NMDA receptor antagonists induced neurotoxicity and encourage researchers for further in vivo studies for schizophrenia.

Keywords: Thymoquinone, ketamine, MK-801, SH-SY5Y cells, schizophrenia

1. INTRODUCTION

Schizophrenia is a severe psychiatric disorder with its complex symptoms. There are certain strong hypotheses about its neurobiology, even though the exact neuronal mechanisms are still unknown. According to the dopaminergic hypothesis, which is the oldest and well-accepted hypothesis, dopaminergic D2 receptor activity increased in the mesolimbic dopaminergic pathway while it decreased at the mesocortical pathway (1). One of the recognized complementary hypotheses, the glutamatergic N-methyl D-Aspartate (NMDA) receptor hypofunction hypothesis, emphasizes that the decrease in glutamatergic neurotransmission from cortical regions to brainstem cause a schizophrenia-related dopaminergic dysregulation. The fact that NMDA receptor antagonists such as ketamine and MK-801 lead to schizophrenia-like behaviors in humans and rodents is one of the reliable findings supporting this hypothesis (2). In addition, it has been shown that NMDA receptor antagonists mimic schizophrenia-like cellular and molecular alterations in cell culture (3). For these reasons, glutamatergic NMDA receptor antagonists are commonly used to set a schizophrenia model in rodents and cell culture.

There are also two essential hypotheses when the brain is considered structurally and functionally in schizophrenia. The first one is the neurodegenerative hypothesis, which was widely accepted by scientists until the 1990s. According to this hypothesis, individuals with normal brain functions and functions until young adulthood have degenerative damage that causes progressive impairment of neuronal functions in this period (4). In the neurodevelopmental hypothesis, which has become more accepted by the scientists in recent years, it is mentioned that a pathological condition that occurs during the development of the brain at early stages such as neuronal migration, neuronal survival, and plasticity causes disorders, and symptoms of schizophrenia occur in later periods (5). In both hypotheses, it has been seen that

DOI: 10.33808/clinexphealthsci.734422

cell survival or cell death constitutes one of the main factors for the beginning of the disease. Therefore, cell viability has been accepted as an essential parameter in both *in vivo* and *in vitro* schizophrenia researches.

It has been demonstrated that glutamatergic NMDA receptor antagonist decreased cellular viabilities in various studies. Xia et al. (6) have demonstrated that the NMDA receptor antagonist Phencyclidine, which behaviorally creates a well-validated schizophrenia model in rodents, significantly increases programmed cell death, apoptosis, in rat corticostriatal cell culture. Another study has also shown that MK-801 significantly reduces cell viability in rat prefrontal cortex neuron culture, and paliperidone, an antipsychotic drug, can reverse the effect of MK-801 (7). Lei et al. (8) have shown that administration of Phencyclidine triggers caspase-3, an apoptotic protein in rat embryonic forebrain cell culture, cause apoptosis in these cells. They also have shown that ketamine significantly decreased cell viability as a result of increased apoptosis triggered by the caspase-3 protein in cells (8). Our previous studies also indicated that MK-801 decreased the neuronal viability, and this effect was prevented by an antipsychotic drug in SH-SY5Y cells (3).

Nigella Sativa L. (Ranunculaceae) is a widely used medicinal plant for various diseases worldwide. It has been proved that thymoquinone is one of the main constituents of *N. sativa* and thought to be responsible for the beneficial effects of *N. sativa* in several studies. It has been demonstrated that thymoquinone has a therapeutical potential with its antiinflammatory, antioxidant, hepatoprotective, anticancer, neuroprotective properties. Besides, studies have indicated it has therapeutical potential for certain neurological and psychiatric disorders such as Alzheimer's disease, Parkinson's disease, stroke, epilepsy, depression, and anxiety (9, 10). However, no study examined the effects of thymoquinone on schizophrenia related neurotoxicity in previous studies.

As a result of the knowledges presented above, it has been shown that neuronal survival is an important parameter for schizophrenia researches. Therefore, neuroprotective agents in cellular schizophrenia models promise hope for further studies. It has been repeatedly indicated that thymoquinine had neuroprotective effects against various neurotoxic agents in previous studies. However, there is no study that investigates the effects of thymoquinone in schizophrenia-related cellular and molecular deficits in rodents or cell culture. In this study, we aimed to investigate the neuroprotective potential of thymoquinone in ketamine –and MK-801–induced neurotoxicity, which represents certain cellular aspects of schizophrenia, in SH-SY5Y human neuroblastoma cell line.

2. METHODS

2.1. Cell culture and drugs

SH-SY5Y human neuroblastoma cells (ATCC®CRL-2266, VA, USA) were cultured with Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100U/ml penicillin, and 100µg/ml streptomycin. Cells were cultivated at 37 °C under humidified conditions with 5% CO2 and were routinely tested for any contamination. Thymoquinone was dissolved in dimethylsulfoxide (DMSO, Sigma Aldrich, Germany) and added to the incubation media, with the final concentrations of 0.5 – 100 µM in DMSO at 0.5% (v/v). (+)-MK-801 hydrogen maleate, (SigmaAldrich, Germany) dissolved in a trace amount (<0.01 w/v) of DMSO and diluted with media. Ketamine (Ketalar®, Pfizer Pharmaceuticals, USA) was directly diluted with an appropriate volume of media.

2.2. Experimental design

The neuronal viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analyses. In order to investigate the neuroprotective effects of thymoquinone, it is aimed to determine the neurotoxic concentrations of ketamine and MK-801 and non-toxic concentrations of thymoquinone in SH-SY5Y cells. For these aims, ketamine and MK-801 were used at concentrations of 50, 100, 250, 500, 1000 µg/ml, and 25, 50, 100, 250, 500 µM, respectively. Thymoquinone was used at concentrations of 1, 3, 10, 30, 100 µM in concentration determination experiments. After the first experiments (concentration determination), non-toxic concentrations of thymoquinone were treated to media 1 hour before the administrations of selected toxic concentrations of ketamine (250 µg/ml and 500 µg/ml) and MK-801 (100 µM). Twenty four hours after the ketamine or MK-801 administrations, cellular viabilities of SH-SY5Y cells were evaluated by MTT test.

2.3. MTT test

SH-SY5Y cells were seeded at a density of 10.000 cells / 100 µl per well in 96-well plates. MTT test was conducted for evaluating the cell viability of SH-SY5Y neuroblastoma cells according to the prior studies (11). In sum, cells were allowed to adhere to wells for 24 hours. After that, the cells were exposed to 25 µl of thymoquinone, ketamine, or MK-801 administrations for 24 hours. Then, the medium carefully aspired from all wells with a syringe and incubated with 100 µl MTT solution (5 mg/ml in medium) at 37 °C for 4 hours. The medium aspired from wells with a syringe, and 50 µl DMSO was added per wells for dissolving MTT salts. The plate was shaken for 5 minutes in a plate shaker. The absorbances of each well were measured by a microplate reader at 560 nm (Biotek, Synergy HT, VT, USA). The relative viabilities of thymoquinone, ketamine, and MK-801 administered cells were calculated by the following formula: "Absorbance of treatments /Absorbance of medium x 100."

2.4. Statistical analyses

GraphPad Prism 8 was used to perform the statistical analyses of this study. One-way analysis of variance (ANOVA) was used for statistical analysis. Multiple comparisons of groups were

made by Dunnett's post hoc test. The data were shown as mean ± standard error of the mean (SEM), and *P*<0.05 was accepted for the value of significance.

3. RESULTS

The cellular viabilities were measured by MTT test at 24th hours of administration to determine non-toxic concentrations of thymoquinone in a range of 1 uM – 100 µM. In parallel with this, cellular viabilities were investigated 24th hour after ketamine and MK-801 administrations to find toxic concentrations in a range of 50 µg/ml – 1000 µg/ml and 25 µM – 100 µM, respectively. After the determination of non-toxic concentrations of thymoquinone and toxic concentrations of ketamine and MK-801, the potential neuroprotective effects of thymoquinone were evaluated against ketamine and MK-801 induced neurotoxicity in SH-SY5Y cells.

Thymoquinone treatments (1 µM and 3 µM) increased (*P*<0.05) the cellular viability compared to medium at the 24th hour of administration in SH-SY5Y cells. Thymoquinone (100 uM) decreased (*P*<0.001) the cell viability, while 10 uM and 30 uM concentrations of its did not alter the viability compared to medium treated cells (Figure 1).

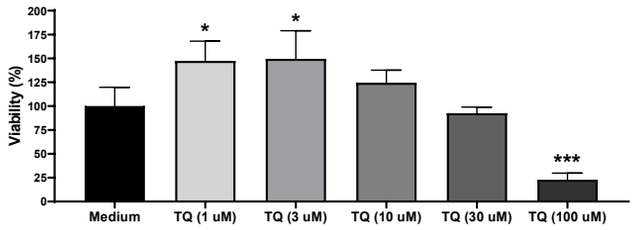


Figure 1. *The effects of thymoquinone on cellular viabilities in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. * P<0.05, *** P<0.001 compared to medium treated cells. (TQ: Thymoquinone)*

Ketamine at the concentrations of 50 µg/ml and 100 µg/ml did not alter the cell viabilities compared to medium at the 24th hour of administrations in SH-SY5Y cells. However, ketamine concentration-dependently decreased the cell viabilities at the 250 µg/ml, 500 µg/ml, and 1000 µg/ml concentrations compared to medium treated cells (Figure 2).

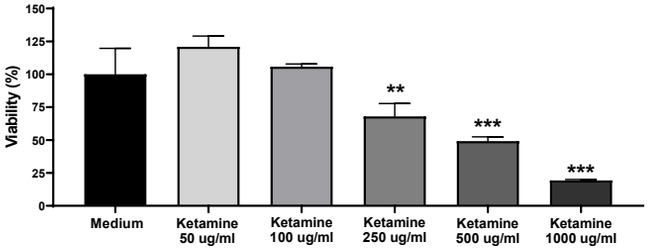


Figure 2. *The effects of ketamine on cellular viabilities in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. ** P<0.01, *** P<0.001 compared to medium treated cells.*

MK-801 administrations did not alter the cell viabilities at the concentrations of 25 µM and 50 µM compared to medium treated cells. MK-801 markedly decreased (*P*<0.001) the viabilities at the concentrations of 100 µM, 250 µM, and 500 µM (Figure 3).

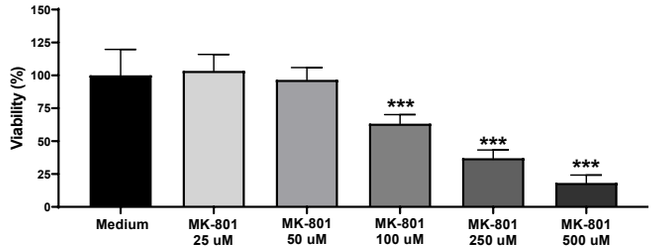


Figure 3. *The effects of MK-801 on cellular viabilities in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. *** P<0.001 compared to medium treated cells. (TQ: Thymoquinone)*

Ketamine (250 µg/ml) administration significantly decreased (*P*<0.001) cell viability compared to medium in SH-SY5Y cells. Thymoquinone pretreatments at the concentrations of 1 µM (*P*<0.01), 2.5 µM (*P*<0.001), 5 µM (*P*<0.001), 10 µM (*P*<0.001), 20 µM (*P*<0.01) markedly increased viability compared to ketamine (250 µg/ml) administrated cells. However, there is no significant difference between ketamine and thymoquinone pretreated cells (Figure 4).

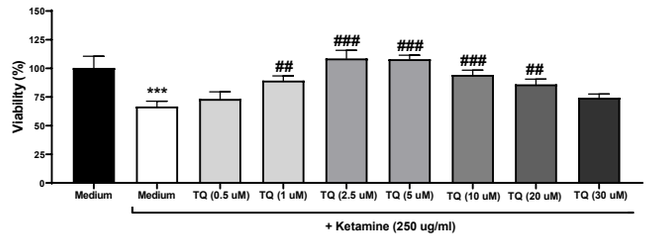


Figure 4. *The effects of thymoquinone pretreatments on ketamine-induced cellular death in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. *** P<0.001 compared to medium, ## P<0.01 and ### P<0.001 compared to ketamine (250 ug/ml) treated cells. (TQ: Thymoquinone)*

It has been found that ketamine (500 µg/ml) administration markedly decreased (*P*<0.001) the number of living cells compared to medium treated ones. Thymoquinone pretreatment at the 2.5 µM concentrations increased the cell viability compared to ketamine (500 µg/ml) administered cells while did not alter the cell viabilities at the other concentrations in SH-SH5Y cells (Figure 5).

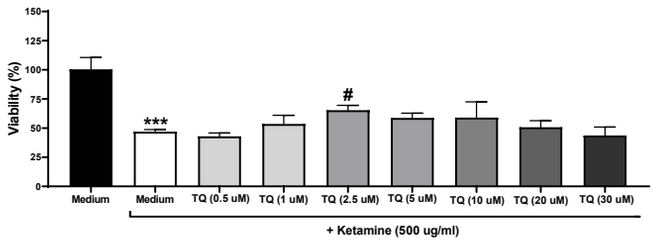


Figure 5. *The effects of thymoquinone pretreatments on ketamine (500 ug/ml) induced cellular death in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. *** P<0.001 compared to medium, ### P<0.001 compared to ketamine (500 µg/ml) treated cells. (TQ: Thymoquinone)*

It has been seen that MK-801 (100 µM) administration markedly (*P*<0.001) decreased the cellular viabilities in SH-SY5Y cells. Thymoquinone pretreatment significantly increased the survival at the concentrations of 1 (*P*<0.01), 2.5 (*P*<0.001), 5 (*P*<0.01), 10 (*P*<0.001) µM whereas it is ineffective at the concentrations of 0.5, 20 and 30 µM against MK-801 induced cell death in SH-SY5Y cells (Figure 6).

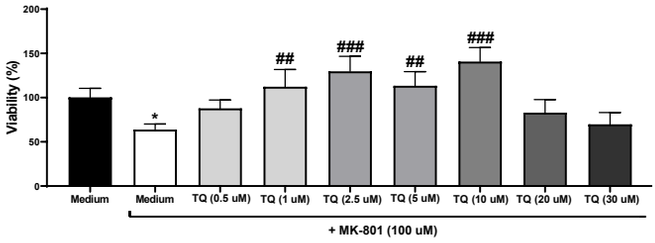


Figure 6. *The effects of thymoquinone pretreatments on MK-801 induced cellular death in SH-SY5Y cells. Data were presented as mean ± SEM, and statistical analyses were done with one way ANOVA followed by Dunnett's post hoc test. * P<0.05 compared to medium, ## P<0.01, ### P<0.001 compared to MK-801 treated cells. (TQ: Thymoquinone)*

4. DISCUSSION

In our study, the potential neuroprotective effects of thymoquinone on ketamine and MK-801, which are widely used to create an experimental models of schizophrenia and cell culture, induced neurotoxicity was investigated in SH-SY5Y cells. Prior to this, it has been examined whether single thymoquinone administration has neuroprotective or neurotoxic effects on SH-SY5Y cells and determined the toxic and non-toxic concentrations of it. After that, toxic concentrations of ketamine and MK-801 were also determined in SH-SY5Y cells. Our results showed that thymoquinone has a neuroprotective effect at the low concentrations (1 µM and 3 µM) and neurotoxic effect at the high concentration (100 µM) in SH-SY5Y cells. It has also been found that NMDA receptor antagonism caused toxicity at the higher concentrations than 250 µg/ml, and 100 µM for ketamine and MK-801, respectively. When the neuroprotective potential of thymoquinone was investigated, it has been seen that thymoquinone prevented the neurotoxicity induced by both NMDA receptor antagonists in SH-SY5Y cells.

The studies which aim to enlighten the neurobiology of schizophrenia have revealed that the hypofunction of glutamatergic NMDA receptors plays a critical role in the neurobiology of disease. The fact that NMDA receptor antagonists, such as ketamine, caused schizophrenia-like manifestions in healthy volunteers and mimick schizophrenia-like behaviors and neurobiological findings in preclinical studies supports this hypothesis (12, 13). In recent years, some parts of the psychiatry researches shifted to *in vitro* studies because of its certain advantages such as low cost, time-effective, and lack of ethical approval compared to *in vitro* or clinical studies. In these studies, neuronal survival and intracellular signaling pathways are commonly investigated in certain neuronal cell lines. Zhao et al. (14) showed that ketamine (5 mM) administration caused neurotoxicity in embryonic stem cell-derived neuron culture. In another study, it has been indicated that ketamine (300 µM and 1000 µM) increased cell death at the 24th hour of administrations in rat hippocampal cells (15). It has also been demonstrated that ketamine (100 µM) administration induced apoptosis and decreased neuronal survival in rat cortical neuron culture (16). When our results were investigated from this aspect, it has been shown that ketamine administrations at the 250 µg/ml (900 µM) and 500 ug/ml (1,8 mM) had neurotoxic effects on SH-SY5Y neuroblastoma in accordance with the previous researches. For MK-801, our previous study also showed the neurotoxic effects of 100 uM MK-801 at the 6th, 12th, and 24th hours of administrations in SH-SY5Y cells (3). Besides, it has been shown that MK-801 caused neuronal death in prefrontal cortical neurons, and this effect was reversed by atypical antipsychotic drugs (17). In accordance with these studies, MK-801 caused neurotoxicity at the 24th hour of administrations in SH-SY5Y cells in our study.

There are limited number of studies about the effects of thymoquinone on schizophrenia. It has been indicated that thymoquinone treatment decreased certain schizophrenia-related behaviors in mice (18). In addition to this, our previous studies showed that the hydroalcoholic extract of *N. sativa* reversed schizophrenia-like behaviors in acute ketamine models of schizophrenia in rats (19). Besides, certain studies may indirectly contribute to the investigation of the effects of thymoquinone on schizophrenia. It has been known that neurodegeneration and related inflammatory alterations play a role in schizophrenia pathophysiology (20). Certain studies showed that thymoquinone had an antiinflammatory effect in microglial cell culture besides of its neuroprotective effect against some neurotoxic agents (21). In these studies, it has been demonstrated that the usage of thymoquinone at the concentrations of 0.1 – 1000 µM protected neurons from neurotoxicity induced by 1-methyl-4-phenylpyridinium (MPP) and amioid beta, which present Parkinson and Alzheimer like neuronal conditions (21). At this point, we first reported that thymoquinone had neuroprotective effects on ketamine and MK-801 induced neuronal toxicities. Our results provide a confirmation for neuroprotective effects of thymoquinone

against different neurotoxic agents. Moreover, our results suggest a therapeutical potential of thymoquinone on schizophrenia, even though our study provides a preliminary perspective to schizophrenia pathophysiology and does not mimic the complete alterations of schizophrenia.

In conclusion, we showed that ketamine and MK-801 caused a neuronal injury in parallel with its schizophrenia-like effects in clinical and preclinical studies. We have firstly demonstrated that thymoquinone may have beneficial effects on ketamine and MK-801 induced cellular models of schizophrenia. We suggest that our study will provide a basis for the studies that will investigate the beneficial effects of thymoquinone in further studies and will give a promising prospect for effective treatment of schizophrenia. Also, investigating the potential beneficial effects of thymoquinone with all aspects such as behavior and *ex vivo* analyses will be valuable for further studies.

REFERENCES

[1] Van Os J, Kapur S. Schizophrenia. Lancet 2009;374(9690):635-645.

[2] Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, Snigdha S, Rajagopal L, Harte MK. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol Ther 2010;128(3):419-432.

[3] Unal G, Dokumaci AH, Ozkartal CS, Yerer MB, Aricioglu F. Famotidine has a neuroprotective effect on MK-801 induced toxicity via the Akt/GSK-3β/β-catenin signaling pathway in the SH-SY5Y cell line. Chem Biol Interact 2019;314:108823.

[4] Gürsu Hariri A, Uzuner Özer G, Ceylan ME, Ceylan N, Yazan B, Önal AO. Şizofreni etyolojisinde nörogelişimsel hipotez. Bull Clin Psychopharmacol 1999; 9(2):99-103.

[5] Lieberman JA. Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective. Biol Psychiatry 1999;46(6):729-39.

[6] Xia Y, Wang CZ, Liu J, Anastasio NC, Johnson KM. Brain-derived neurotrophic factor prevents phencyclidine-induced apoptosis in developing brain by parallel activation of both the ERK and PI-3K/Akt pathways. Neuropharmacology 2010;58(2):330-336.

[7] Peng L, Zhu D, Feng X, Dong H, Yue Q, Zhang J, Gao Q, Hao J, Zhang X, Liu Z, Sun J. Paliperidone protects prefrontal cortical neurons from damages caused by MK-801 via Akt1/GSK3β signalingpathway. Schizophr Res 2013;147(1):14-23.

[8] Lei G, Xia Y, Johnson KM. The role of Akt-GSK-3beta signaling and synaptic strength in phencyclidine-induced neurodegeneration. Neuropsychopharmacology 2008;33(6):1343-1353.

[9] Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. Phytomedicine 2004;11(1):56-64.

[10] Jakaria M, Cho DY, Ezazul Haque M, Karthivashan G, Kim IS, Ganesan P, Choi DK. Neuropharmacological potential and delivery prospects of thymoquinone for neurological disorders. Oxid Med Cell Longev 2018;2018:1209801.

[11] Zhang L, Yu H, Sun Y,Lin X, Chen B, Tan C, Cao G, Wang Z. Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. Eur J Pharmacol 2007;564(1-3):18-25.

[12] Cadinu D, Grayson B, Podda G, Harte MK, Doostdar N, Neill JC. NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacology 2018;142:41-62.

[13] Moore JW, Turner DC, Corlett PR, Arana FS, Morgan HL, Absalom AR, Adapa R, de Wit S, Everitt JC, Gardner JM, Pigott JS, Haggard P, Fletcher PC. Ketamine administration in healthy volunteers reproduces aberrant agency experiences associated with schizophrenia. Cogn Neuropsychiatry 2011;16:364-381.

[14] Zhao X, Shu F, Wang X, Wang F, Wu L, Li L, Lv H. Inhibition of microRNA-375 ameliorated ketamine-induced neurotoxicity in human embryonic stem cell derived neurons. Eur J Pharmacol 2019;844:56-64.

[15] Cao, C, Zhang Y, Zhang Z, Chen Q. Small interfering LncRNA-TUG1 (siTUG1) decreases ketamine-induced neurotoxicity in rat hippocampal neurons. Int J Neurosci 2019;129(10):937-944.

[16] Takadera T, Ishida A, Ohyashiki T. Ketamine-induced apoptosis in cultured rat cortical neurons. Toxicol Appl Pharmacol 2006;210(1-2):100-107.

[17] Peng L, Zhu D, Feng X, Dong H, Yue Q, Zhang J, Gao Q, Hao J, Zhang X, Liu Z, Sun J. paliperidone protects prefrontal cortical neurons from damages caused by MK-801 via Akt1/GSK3β signaling pathway. Schizophr Res 2013;147(1):14-23.

[18] Khan RA, Najmi AK, Khuroo AH, Goswami D, Akhtar M. Ameliorating effects of thymoquinone in rodent models of schizophrenia. Afr J Pharm Pharmacol 2014;8(15):413-421.

[19] Unal G, Keles R, Taskin T, Aricioglu F. *Nigella sativa* extract improved sensorimotor gating deficit on acute ketamine model of schizophrenia in rats. Eur Neuropsychopharmacol 2017;27:921-922.

[20] Aricioglu F, Ozkartal CS, Unal G, Dursun S, Cetin M, Müller N. Neuroinflammation in schizophrenia: a critical review and the future. Bull Clin Psychopharmacol 2016;26(4):429-437.

[21] Samarghandian S, Farkhondeh T, Samini F. A Review on Possible Therapeutic Effect of Nigella sativa and Thymoquinone in Neurodegenerative Diseases. CNS Neurol Disord Drug Targets 2018;17(6):412-420.

How to cite this article: Unal G, Erdogan B. Neuroprotective effects of thymoquinone against ketamine -and MK-801-induced neurotoxicity in SH-SY5Y cells: From the perspective of glutamatergic dysfunction in schizophrenia. Clin Exp Health Sci 2020; 10: 178-182. DOI: 10.33808/clinexphealthsci.734422

Clinical and Experimental Health Sciences

Rare Location and Clinical Presentation of Gout Disease: Distal Ulna Localized Gout Disease and Acute Gout Attack of the Wrist

Recep Ozturk, Huseyin Dogan, Emek Mert Duman, Bedii Safak Gungor

Dr. Abdurrahman Yurtslan Ankara Oncology Training and Research Hospital, Department of Orthopaedics and Traumatology, Ankara, Turkey

Correspondence Author: Recep Ozturk
E-mail: ozturk_recep@windowslive.com
Received: 03.03.2019 **Accepted:** 28. 02. 2020

ABSTRACT

Gout is one of the most common inflammatory arthritis in adults. This disorder is caused by the accumulation of monosodium urate crystals in soft tissues and joints because of the hyperuricemia. One of the lower extremity joint especially the first metatarsophalangeal joint is affected by more than 75% of the gout attacks. In this study, we reported a rare presentation of gout disease, the acute gout attack of the wrist, in a woman, 72 years aged, who has not been diagnosed before. Gout which occurs with the involvement of the wrist is very rare and no other cases with distal ulna localization have been reported according to our knowledge. We reported the first case of gout disease which started from distal ulna. The patient was operated because the symptoms did not recover with conservative treatments.

Keywords: Gout, wris, acute attack, ulna, tophi.

1. INTRODUCTION

Gout disease is the most common inflammatory arthritis in the elderly (1). One of the lower extremity joint especially the first metatarsophalangeal joint is affected by more than 75% of the gout attacks that is known as podogra (2). Acute gout arthritis of the wrist is a rare case and we could not find any other gout lesion which is localized in the distal ulna by our recent literature search. The incidence of the gout disease is increasing by aging and is more common in men (3). The prevalence of self-reported physician-diagnosed gout is more than 2% in men older than 30 years and in women older than 50 years. The hand and the wrist involvement is more common in women (4).

We aimed to present a rare arthritis case which is the first example of the wrist localized gout in the distal ulna.

2. CASE REPORT

Seventy-two years aged a woman, who complaining swelling and pain on the right wrist applied to our department. Her complaints were present for about last 15 days and their severity was increasing recently. The redness, tenderness and swelling on the right wrist is very remarkable and this situation restricted the range of motion of the wrist. C-reactive protein was high (41.0 mg/dl) and serum uric acid level was normal (4.7 mg/dl, normal range is between 2.4 and 7 mg/dl for men) found by laboratory results.

She had swellings on her all fingers and wrist, had extreme sensitivity to palpation and marked motion loss. When we look at her detailed history, she applied to another hospital complaining of pain in her left wrist about 3 years ago. She was told there was a mass lesion on her left wrist around distal ulna and she should be followed up for her lesion. Although the patient suffered from pain time to time she never applied to the hospital for following up again.

There was an expansile lytic view with irregular borders accompanied by peripheral periost reaction around the epiphyseal-metaphyseal region of distal ulna on direct graphy. Besides, a volume increase related to soft tissue edema or effusion was observed most prominent around the volar side (Figure 1). Also, there was a view that is heterogenously hyperintense on t2 weighted image and that is isointense with muscle on t1 weighted image around distal ulna on MRI. There has been minimal effusion on distal radioulnar and proximal carpal joints. Effusion related to tenosynovitis around carpal tunnel and extensor carpi ulnaris tendon. A degenerative cystic lesion was observed in lunate bone (Figure 2).

DOI: 10.33808/clinexphealthsci.560202

Figure 1. *There is an expansile lytic view with irregular borders accompanied by peripheral periost reaction around the epiphyseal-metaphyseal region of distal ulna on direct graphy.*

Figure 2. *There is a view that is heterogenously hyperintense on t2 weighted image and that is isointense with muscle on t1 weighted image around distal ulna on MRI. There has been minimal effusion on distal radioulnar and proximal carpal joints. Effusion related to tenosynovitis around carpal tunnel and extensor carpi ulnaris tendon. A degenerative cystic lesion was observed in lunate bone.*

The acute gout attack was thought by considering the diagnosis criteria of American College of Rheumatology and clinical, radiological and the history of the patient evaluation results (5).

Resolution of the attack was seen in five days after the start of 25 mg diclofenac sodium three times a day. The operation was planned because of persistent pain, swelling, loss of motion and suspicious diagnosis. The macroscopic appearance of the lesion gave rise to thought gout in intraoperative evaluation. Fresh frozen examination was reported as it is coherent with gout. The lesion was debrided and carpal tunnel release were performed. The area of involvement in the distal ulna was completely curetted and debrided. Static wrist splint was worn for 20 days. The patient was consulted to rheumatology department for medical treatment.

3. DISCUSSION

Gout is an inflammatory arthritis caused by the accumulation of monosodium urate(MSU) crystals in synovial fluid and it is associated with hyperuricemia. It was described for the first time by Egyptians in B.C. 2640 (3).

The incidence increases by aging and gout is more common among men. The definitive diagnosis of gout disease is best established by demonstration of monosodium urate crystals in the synovial fluid or biopsy (3, 6).

Tophaceous gout occurs years after recurrent attacks of acute inflammatory arthritis (7). Hand and wrist tophaceous gout, often seen as the first symptom of the disease process in the elderly (4). Synovitis and bone lesions on direct radiography and MRI are common lesions of the wrist even in the first episode of acute gout arthritis (8). Tophaceous involvement of the wrist may be limited to either the flexor or extensor compartment, or it may be permeative and include the wrist joint itself. Tendon infiltration, tendon rupture and rarely skin ulcers may develop in neglected aggressive cases (4). The involvement of radiocarpal, intercarpal and distal radioulnar joints is rare. Uncontrolled or untreated gout in these joints may lead to scapho-lunate dislocation and severe joint damage (2, 4, 8). The involvement of the flexor surface of the wrist may lead to carpal tunnel syndrome which is often seen in chronic cases (9, 10).

In addition, Bouaziz et al. reported a case of infected gout tophus located at the wrist and Skedros et al. reported compartment syndrome in the wrist in 2018 (7, 11). Kamimura et al. reported a case of acute gout attack on the wrist in 2004 (2). Jacob et al. reported a rare and unusual gout attack on the wrist, induced by a change in the dosage of anti-hypertensive drug in 2007 (1).

Radiographies and magnetic resonance imaging are usually helpful to understand the changes in the bone and soft tissue related to gout and tophus. Also they are helpful to differentiate gout and tophus from the other joint diseases and tumoral lesions (12).

The patients with typical podagra can be diagnosed as gout on clinical examination. Joint aspiration is recommended for the patients who has atypical joint involvement to demonstrate monosodium urate crystals and exclude other causes of acute arthritis (13).

Diseases such as rheumatoid arthritis, osteoarthritis, septic arthritis, calcium pyrophosphate dihydrate crystal deposition disease, psoriatic arthritis and tumoral lesions should be considered in the differential diagnosis of gout (1, 14).

Gout disease has no a specific treatment. Antihyperuricemic therapies may provide benefit, and low dose steroids may control the pain and inflammation. Surgical treatment is indicated in cases refractory to medical therapy and

suspicious diagnosis. Surgical treatment may be simple as squeezing the pasty tophaceous material and fluid aspiration but sometimes surgical decompression may be needed. Following surgical decompression, pharmacological treatment and lifestyle modifications are of paramount importance (15-18).

4. CONCLUSION

It should be kept in mind that inflammatory signs on the wrist of elderly patients may be related to the first presentation of gout and that radiographical and MRI changes may be present even in the first presentation. Surgical treatment may be used in cases that does not benefit from medical treatment or are difficult to diagnose. In addition, a multidisciplinary approach is principle in the treatment of gout.

REFERENCES

[1] Jacobs CL, Stern PJ. An unusual case of gout in the wrist: the importance of monitoring medication dosage and interaction. A case report. Chiropr Osteopat. 2007;15(1):1-5.

[2] Kamimura T, Hatakeyama M, Okazaki H, Minota S. Acute gout attack in the wrist joint. Intern Med. 2004;43(7):641-642.

[3] Gaviria JL, Ortega VG, Gaona J, Motta A, Medina Barragán OJ. Unusual dermatological manifestations of gout: review of literature and a case report. Plast Reconstr Surg Glob Open 2015;3(7):1-6.

[4] Fitzgerald BT, Setty A, Mudgal CS. Gout affecting the hand and wrist. J Am Acad Orthop Surg. 2007;15(10):625-635.

[5] Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977;20(3): 895–900.

[6] Purohit MB, Purohit TM, Tandon RK. FNAC of gouty tophi—a case report. Indian J Pathol Microbiol 2006;49(1):42–43.

[7] Bouaziz W, Rekik MA, Guidara AR, Keskes H. Infection of a tophaceous nodule of the wirst and hand. BMJ Case Rep 2018;28(1):81-84.

[8] Cimmino MA, Zampogna G, Parodi M, Andracco R, Barbieri F, Paparo F, Ferrero G, Garlaschi G. MRI synovitis and bone lesions are common in acute gouty arthritis of the wrist even during the first attack. Ann Rheum Dis. 2011;70(12):2238-2239.

[9] Kalia KK, Moossy JJ. Carpal tunnel release complicated by acute gout. Neurosurgery. 1993;33(6):1102-1103.

[10] Lu H, Chen Q, Shen H. A repeated carpal tunnel syndrome due to tophaceous gout in flexor tendon: A case report. Medicine (Baltimore). 2017;96(9):1-4.

[11] Skedros JG, Smith JS, Henrie MK, Finlinson ED, Trachtenberg JD. Upper extremity compartment syndrome in a patient with acute gout attack but without trauma or other typical causes. Case Rep Orthop. 2018;23(1):1-6.

[12] Bousson V, Hamze B, Wybıer M, Daguet E, Parlier C, Petrover D, Bossard P, Laredo JD. Soft tissue tumors and pseudotumors of the foot and ankle. j radiol 2008; 89(1): 21-34. [Article in French]

[13] Abhishek A, Roddy E, Doherty M. Gout – a guide for the general and acute physicians. Clin Med (Lond). 2017;17(1):54-59.

[14] Atalay İB, Yapar A, Öztürk R, Ulucaköy C, Toptaş E. Surgical outcomes of synovial osteochondromatosis: an evaluation of 15 cases. Acta Oncol Tur. 2019; 52(3): 379-382

[15] Ochoa CD, Valderrama V, Mejia J, Rondon F, Villaroya N, Restrepo JF, Espinoza LR, Iglesias-Gamarra A. Panniculitis: another clinical expression of gout. Rheumatol Int. 2011;31(6):831-835.

[16] Öztürk R, Atalay İB, Bulut EK, Beltir G, Yılmaz S, Güngör BŞ. Place of orthopedic surgery in gout. Eur J Rheumatol. 2019;6(4):212-215.

[17] Weberschock T, Gholam P, Hartschuh W, Hartmann M. Gouty panniculitis in a 68-year-old man: case report and review of the literature. Int J Dermatol 2010;49(4):410–413.

[18] Martin D, Joliat GR, Fournier P, Brunel C, Demartines N, Gié O. An unusual location of gouty panniculitis: a case report. Medicine (Baltimore). 2017;96(16):1-3.

How to cite this article: Ozturk R, Dogan H, Duman EM, Gungor BS. Rare Location and Clinical Presentation of Gout Disease: Distal Ulna Localized Gout Disease and Acute Gout Attack of the Wrist. Clin Exp Health Sci 2020; 10: 183-185. DOI: 10.33808/clinexphealthsci.560202