# The European Research Journal

# The European Research Journal

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III

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University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital,
Department of Anesthesiology & Reanimation,
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Department of Cardiology,
Bursa, Turkey


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University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
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Bursa, Turkey


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Professor,
Medipol University School of Medicine,
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Istanbul, Turkey


## Ozen OZ GUL, MD

Associate Professor,
Uludag University School of Medicine,
Department of Endocrinology & Metabolism,
Bursa, Turkey

Ozkan KANAT, MD,

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Department of Medical Oncology,
Bursa, Turkey


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Professor,
Cadi Ayyad University School of Medicine,
Department of Neurosurgery,
Marrakech, Morocco


Sedat ALTIN, MD

Professor,
University of Health Sciences,Yedikule Training & Research Hospital,
Department of Chest Diseases,
Istanbul, Turkey


Semih HALEZEROGLU, MD, FETCS

Professor,
Acibadem University School of Medicine,
Department of Thoracic Surgery,
Istanbul, Turkey


Veysel TAHAN, MD, FACP, FACG, FESBGH

Assistant Professor,
University of Missouri,
Division of Gastroenterology and Hepatology,
Columbia, Missouri, USA


Yenal DUNDAR, MD

University of Liverpool School of Medicine,
Department of Psychiatry,
Liverpool, UK

# Table of Contents

## Original Articles

# Healing potentials of Marigold flower *(Tagetes erecta)* on full thickness dermal wound in caprine model

**Ajmary Sultana**[1], **Mahadi Hasan**[2], **Marzia Rahman**[2], **Md. Mahmudul Alam**[1]

[1]*Department of Surgery and Obstetrics, Bangladesh Agricultural University, Faculty of Veterinary Science, Mymensingh, Bangladesh*
[2]*Department of Microbiology and Hygiene, Bangladesh Agricultural University, Faculty of Veterinary Science, Mymensingh, Bangladesh*

## ABSTRACT

**Objectives:** In the modern days multiple drug resistance has been developed against many microbes due to the random use of existing antimicrobial drugs in the treatment of infectious diseases. This paves the way for reconsidering traditional medicine; hence we have carried out to evaluate the wound healing potentials of Marigold flower *(Tagetes erecta)* in the surgical wound model in black Bengal goats.
**Methods:** A total of sixteen surgical wounds were made in eight goats under proper restraint and analgesia. Wounds were topically treated with Marigold flower paste (Group A) and normal saline (Group B, control). Post-treatment information was recorded from day 1 to day 21. Planimetric features such as swelling of the wound area, elevation of suture line, and length of the wound were monitored. Histopathological and *in vivo* anti-microbial studies were also investigated.
**Results:** Results revealed that aqueous paste of *Tagetes erecta* flower modulated inflammation and promoted wound contraction leading to earlier healing than those with saline. Histological findings highlighted the normal cutaneous architecture of the marigold treated wound more than that appeared in the saline-treated wound. The antibacterial study revealed that the aqueous paste of marigold flower was highly effective against *Staphylococcus aureus* which is the ubiquitous bacterial pathogen both in humans and animals.
**Conclusions:** These results, thus, demonstrate that the aqueous paste of *Tagetes erecta* flower possesses wound healing activities and it could be a potential candidate for the treatment of dermal wounds by topical application.
**Keywords:** *Tagetes erecta*, wound healing, histopathology, antibacterial efficacy, goats

Wound healing or repairing is a process of replacing devitalized and missing cellular structures and tissue layers. This process can be categorized into four phases i.e. *hemostasis, inflammatory,* proliferative, and remodeling. The process involves growth factors, cytokines, extracellular matrix, and relevant enzymes along with the differentiated cells that modify molecular components of the matrix [1]. The initial events of the wound healing mechanism occurred smoothly under favorable condition but the healing rate may differ among species, tissues, nature of infection or infestation, even between sites of the same tissue as well as pathological features of tissues [2, 3].

The trend of using herbal medicines are not new, many herbal preparations are extensively utilized in rural areas. The uses of herbal products are not only because of the easier availability in local areas but also herbal products have no side effects on the body as

well as this is economic. The beneficial effect of herbal medicine typically results from the combination of secondary metabolites produced within the herbs like flavonoids, tannins, glycosides, alkaloids, gums, etc. The resistance of organisms against antibiotics is a matter of global concern and using herbal products may help to cope up with this problem. Plant-derived antimicrobials have received tons of attention recently [4]. The current threat of antimicrobial resistance and easy availability of Marigold flower (*Tagetes erecta* Linn) has drawn attention to use for therapeutic purposes.

Marigold has about 56 species but two basic types are *Tagetes erecta* Linn and *Tagetes patula* Linn. It is a good remedy for inflamed skin, open fresh wounds, and a range of microbial and parasitic infections [5]. Phytochemical constituents of Marigold indicate the presence of carotenes, flavonoids, resin, saponin, sterols, triterpenes, bitter glycosides, volatile oils, calcium, and alkaloids [5]. It shows different pharmacological activities like anti-bacterial, anti-microbial activity, hepatoprotective, insecticidal, mosquitocidal, larvicidal, nematocidal, wound healing, antioxidant, and analgesic [6]. In a combination of some substances with the high resin, content provide Marigold a powerful anti-inflammatory action.

Among different species of Marigold, *Tagetes erecta* is the most common and widely available in Bangladesh. Different study at a different place on Marigold was reported but with this species, there is still limited study. Based on having active constituents and therapeutic potentials mentioned above and easy availability of the plants in the country, we have conducted the study to investigate the healing potentiality of Marigold *(Tagetes erecta)* on full-thickness dermal wound in goat.

## METHODS

### Animals

This study was reviewed and approved by the Animal Experimental Ethics Committee (AEEC) of the Department of Surgery and Obstetrics, Bangladesh Agricultural University (Permission number: AEEC/DSO-BAU/01/2017) and strictly obeyed the rules of animal experiment ethics to reduced number as well as the suffering of animals. The research was

conducted on eight healthy goats with body weight ranged from 8-10 kg with irrespective of sexes. The animals were kept in the Veterinary Teaching Hospital (VTH), BAU under standard housing system and veterinary monitoring with no restrictions on water and food. They were acclimatized to the experimental conditions for 14 days. Deworming and vaccination against Peste des petits ruminants (PPR) virus were done. The overall health of the animals was monitored before and throughout the study.

### Preparation of Marigold Petal Paste

Fresh flower petals were collected from the premises of BAU. The petals were properly cleansed with distilled water and blended with sterile double distilled water to make homogeneous paste. The procedure was followed during each application on the wound.

### Surgical Wound Model

All the wounds were created aseptically considering animal welfare to provide minimal discomfort to the experimental goats. Local analgesia was done before wounding to minimize the pain sensation. A total of sixteen full-thickness cutaneous wounds of 2.0 cm length and 0.5 cm depth were made on either side of the vertebral column of eight goats with 2 wounds in each animal following the technique described by Tamanna *et al*. [7].

### Experimental Protocol

Following treatment, all the animals were maintained carefully to avoid contamination of wounds. Other than experimental herbal therapy, no other drugs were applied to the wound in order to avoid their influential effects on the healing process. Goats were divided into two groups:

*Group A* - Fresh aqueous Marigold flower paste was applied locally once daily to eight surgical wounds made in four animals.

*Group B* - This group received normal saline (NS) and served as control.

### Plenimetry

Follow-up information was obtained since day of surgical operation (day 1) up to the end of experiment (day 21). Gross morphology such as swelling of the wound area, elevation of suture line from the skin surface, length of wound area was recorded to evaluate

the healing potentials of Marigold flower. Elevation of suture line was recorded up to 7th days of wounding, length of suture line was measured on the day 1 (D1), day 3 (D3), day 7 (D7), day 14 (D14) and day 21 (D21) post-operation, for bacteriological study, wound swabs were collected from both the groups on D3 post-wounding. To examine histological changes of wounds following treatment, full-thickness lesion biopsies were collected at D3, D7, and D21 from both the groups.

## Monitoring and Data Collection

Swelling area (mm), elevation of suture line (mm), and length of suture line (mm) of the wound were measured using digital slide calipers (FstDgte, China). Healing progress of animals in both groups was monitored daily. Healing was scored as follows:

a) *Excellent* - No inflammation, no exudation, no infection, no dehiscence, gradual decrease of a width of the wound area.

b) *Good* - Minimum inflammation with minimum exudation, no dehiscence, gradual decrease of a width of the wound area.

c) *Poor* - Marked inflammation, presence of infection, and exudation.

## Antibacterial Evaluation

Antimicrobial activity was determined through the dilution method. All of the samples were collected aseptically from the wounds by using sterile cotton buds circling onto the wound and immediately transferred into screwed capped test tubes containing nutrient broth. The tubes were taken to the laboratory to culture and counting of viable bacteria. For this purpose, the spread method was used Swab collection, culture, and staining of collected bacteriological samples were done by following the method described by Jaman *et al*. [8].

## Histopathological Study

The full-thickness cutaneous biopsies (1.5 cm x 1.5 cm) were collected from the wound of each experimental animal on 3rd, 7th, and 21st days post-wound using the surgical procedure described by Tamanna *et al*. (2020). The samples were fixed in 10% formalin for 48 hours for proper fixation and slides were prepared and stained in the histopathology laboratory of the Department of Surgery and Obstetrics, BAU following the protocol described by Ashraf *et al*. [9]. The stained slides were assessed under a photographic microscope (Micros®, Austria) for epidermal thickness, inflammatory infiltration, fibroblasts generation, vascularization, and other essential histological changes and then images were captured accordingly.

## Statistical Analysis

All the data were expressed as Mean ± SE (Standard Error). To compare data among groups, one-way ANOVA (Analysis of variance) was performed using Statistical Package for the Social Sciences (SPSS) version 22.0.

## RESULTS

In this study, the efficacy of aqueous paste of Marigold flower was evaluated on the basis of morphological changes such as an area of swelling of wounds, elevation of suture line, length of wounds, mean time to get healed, histopathological changes and *in vivo* antimicrobial performance.

Morphological variables of wounds of group-A and B are shown in Table 1. Swelling of the wound edges was observed in both group of animals. Treatment with Marigold flower paste resulted in significant ($p < 0.05$) decreased swelling of the wound (4.02 ± 0.11 mm) than wound treated with NS (5.36 ± 0.12

**Table 1. Morphological characteristics of wounds after treatment with Marigold petal paste and NS**

| Groups | Area of swelling of wounds (mm) | Elevation of suture line (mm) | Length of wounds (mm) |
|--------|-------------------------------|-------------------------------|----------------------|
| Group A | 4.02 ± 0.11[a] | 2.73 ± 0.19[a] | 16.04 ± 0.06[a] |
| Group B | 5.36 ± 0.12[b] | 3.59 ± 0.12[b] | 17.11 ± 0.08[b] |

Data are shown as Mean± SE. Values with different superscript letter in the same column indicate significance ($p < 0.05$). NS = normal saline

mm), Elevation of suture line was remarkably lower in Marigold treated wound (2.73 ± 0.19 mm) than those of control (3.59 ± 0.12 mm). Decreased length of wounds after treatment with this herbal paste was recorded (16.04 ± 0.06 mm) whereas in NS treated wound, it was as high as 17.11 ± 0.08 mm.

The morphological changes of wounds of group A and group B at indicated days are shown in Fig. 1, Fig. 2 and Fig. 3.

In this study, we have found a significant difference ($p < 0.05$) in the average healing time between the groups. The mean days to complete healing for the treatment group was 14.17 ± 0.31 days where it was much higher in control wounds (18.67 ± 0.33 days) (Fig. 4) indicates that Marigold has potent accelerating wound healing effect.
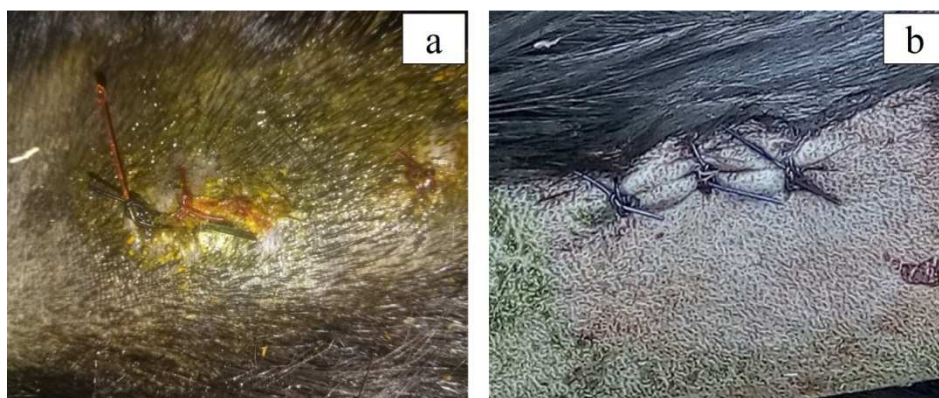


**Fig. 1. Gross observation of wounds on day 1 treated with (a) Marigold, (b) Normal saline (control).**



**Fig. 2. Gross morphology of wounds on day 7 treated with (a) Marigold, (b) Normal saline (control).**
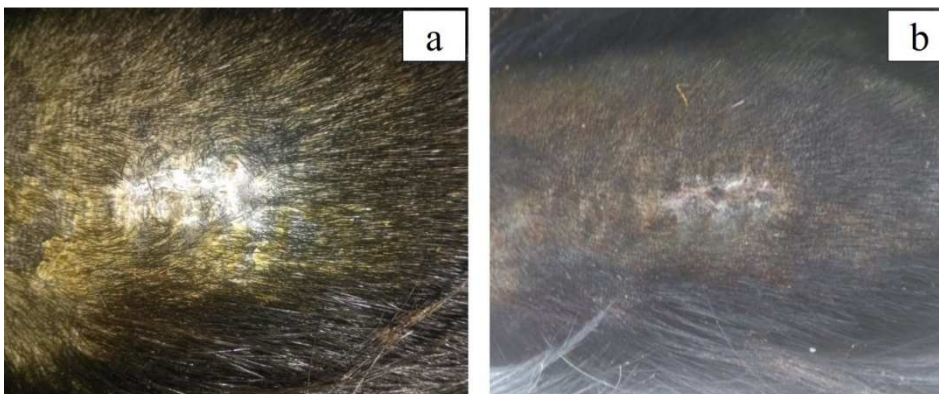


**Fig. 3. Gross observation of wounds on day 21 treated with (a) Marigold, (b) Normal saline (control).**
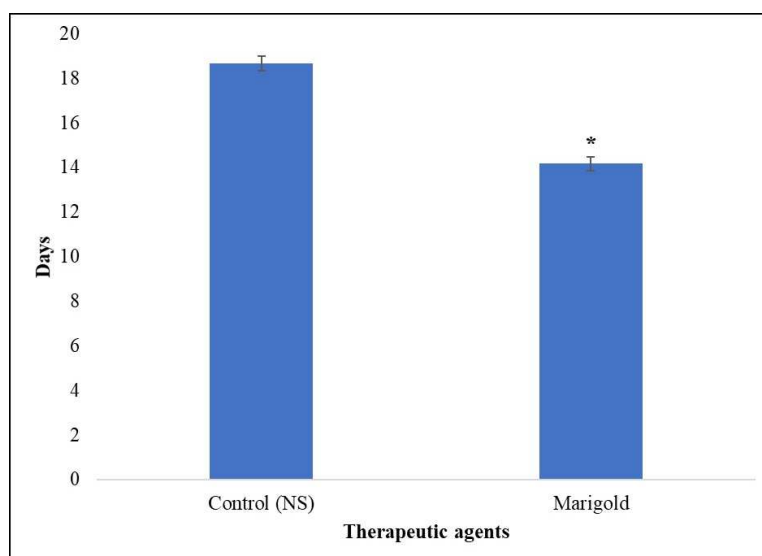
**Fig. 4.** Mean days to wound healing. * denotes healing time of Marigold is significantly lower than control group (*p* < 0.05).
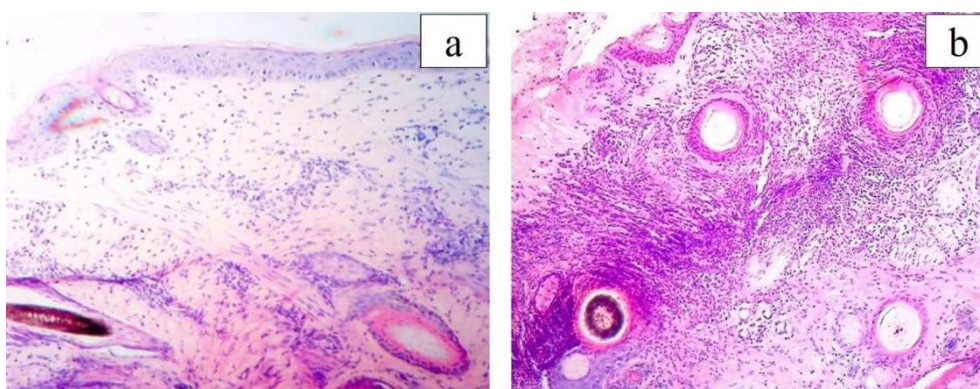


**Fig. 5.** Histological features on day 3. (a) Moderate infiltration of inflammatory cells with fibroplasia in group A, (b) Huge inflammation, and less fibroplasia in group B. (H & E stain, original magnification × 200).

The inflammatory lesions in the regenerating tissues were evaluated based on the infiltration of different reactive cells. Reactive cells decreased gradually in wounds treated with aqueous Marigold flower paste. Moderate infiltration of inflammatory cells with fibroplasia was seen on day 3 (Fig. 5a) and gradually decreased on day 7 (Fig. 6a) and on day 21, there was the least degree of inflammation present with marked thickening of the keratinized layer of the epidermis (Fig. 7a).

On the other hand, in the control group, huge inflammation and fibroplasia were encountered along with widespread hemorrhages and congestion on day 3 (Fig. 5b) which continued up to day 7 where fibroblastic proliferation with minimal infiltration inflammatory cells seen (Fig. 6b). On day 21, there was the

least degree of inflammation present with a thin keratinized layer of the epidermis (Fig. 7b). Based on the histological scoring as described in materials and methods, Marigold flower provided excellent score whereas NS has given poor score in this experiment. We have performed in vivo antibacterial efficacy study with the swab samples collected on day 3 post-operation. After culture and colony counting, we found that bacterial colonies were markedly reduced in Marigold treated wound (Fig. 8a) but there was a huge colony in saline-treated wound (Fig. 8b). To check the type of bacteria involved in the wound infection, we performed Gram's staining and it revealed Staphylococcus spp. characterized spherical shaped clustered Gram-positive bacteria (Fig. 9).
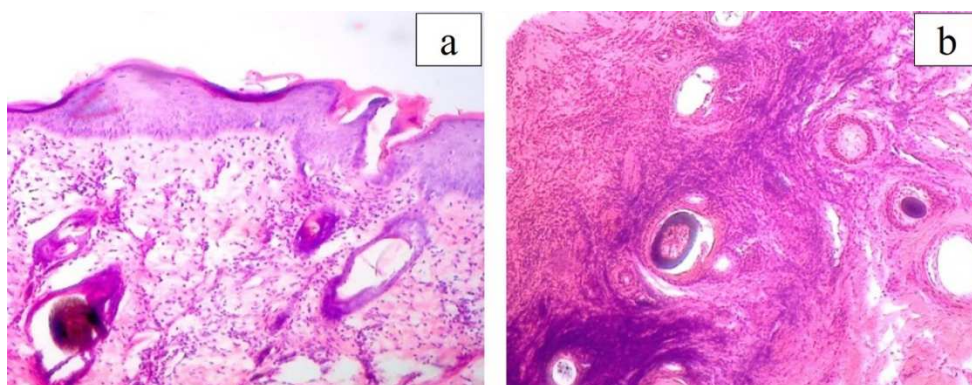
**Fig. 6.** Histological features on day 7. (a) Less inflammatory cells than those of fibroblastic cells in group A, (b) Fibroblastic proliferation with minimal infiltration inflammatory cells in group B. (H & E stain, original magnification × 200).
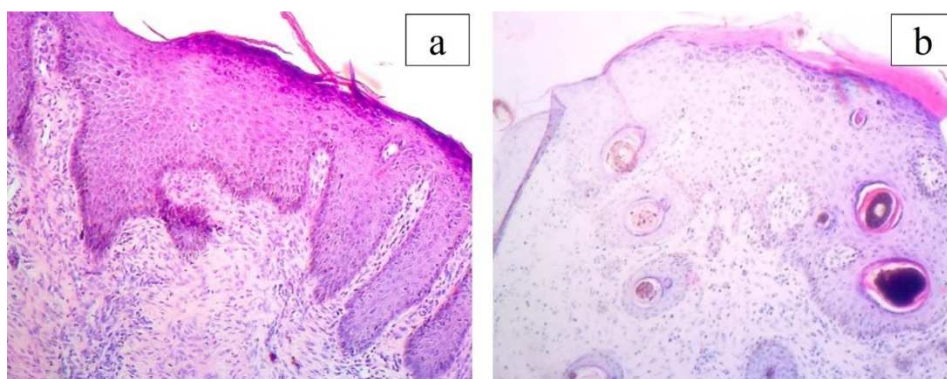


**Fig. 7.** Histological features on day 21. (a) Marked thickening of the keratinized layer of the epidermis in group A, (b) Thin keratinized layer of epidermis present in group B. (H & E stain, original magnification × 200).
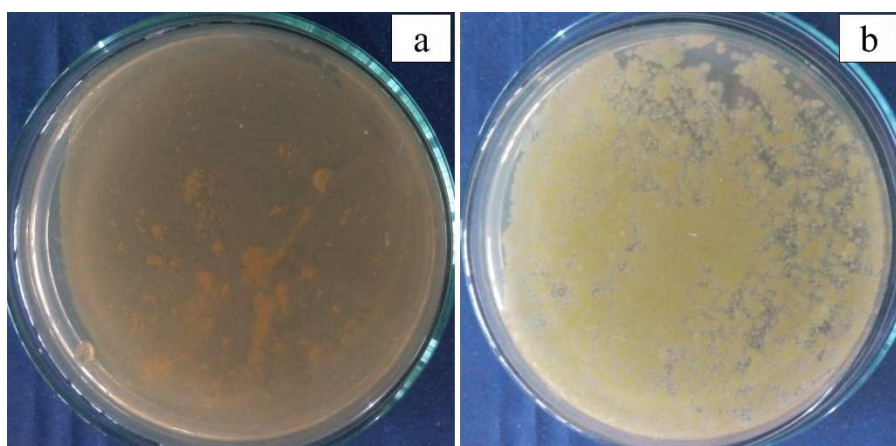


**Fig. 8.** Presence of bacterial colony in primary culture of Mannitol salt agar observed in samples collected from wound treated with (a) Marigold petal paste and (b) Normal saline (Control).

## DISCUSSION

Medicinal plants, their derivatives, and metabolites are widely used for medicinal purposes, either phamacopoeial, non harmacopoeial or synthetic drugs. They are becoming popular all over the world as a nat-

ural alternative to synthetically produced medicine. In developing countries like Bangladesh, most of the people lived in rural areas do not get access to sophisticated and expensive medical support. A greater portion of them rely on traditional or herbal therapy for both humans and animals [7, 10, 11]. The plant
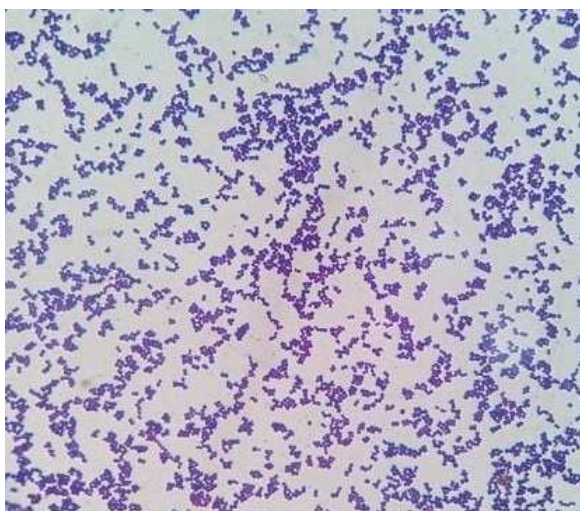
**Fig. 9.** The bacteria were arranged in a grape-like structure with spherical shaped indicating *Staphylococcus* spp.

*Tagetes erecta* has been shown to contain quercetagetin, phenolic, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercitin, thienyl, and ethyl gallate [12]. It has been reported that the *Tagetes erecta* flower acts as anti-hemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, and is useful in aromatherapy for its powerful skin healings properties [13]. In the current study, area of swelling, elevation of suture line, length of wounds was observed as gross indicators of wound healing potentials of *Tagetes erecta* petal paste. Results from our study showed that the mean value of swelling area, elevation of suture line and length of wound were remarkably lower in Marigold treated wound than those of control. The mean healing time significantly shorter in Group-A where wounds were treated with Marigold flower paste indicating that Marigold has outstanding healing accelerating properties.

Activation of fibroblasts, endothelial cells, and macrophages are keys of wound healing in which body cells respond to injury. The restoration of structure and function in the wound site is determined by fibroblast proliferation [14]. Therefore, therapeutic bioactive agents that are able to stimulate fibroblast growth and proliferation may be able to improve or promote wound healing as in the case of the present study, petal paste of *T. erecta* flower prepared was demonstrated to enhance the proliferation of fibroblast and collagen deposition in Black Bengal goats.

The mechanisms through which the *T. erecta* flower paste accelerates wound healing is unclear but further research could be done by examining whether this extract increased fibroblast migration and collagen deposition in wound tissue. It can be speculated that quick and maximum deposition collagen fibers in wounds treated with *T. erecta* flower paste may be the hidden mechanism of quick reformation of the wounded area by connective tissues.

The plant used as a wound healer may inhibit bacterial growth by different mechanisms than the presently used antibiotics. It has been shown that the essential oil of the *Tagetes erecta* flower showed noticeable antimicrobial activity against four gram-positive and fifteen gram-negative pathogenic bacteria *(Staphylococcus aureus, Bacillus mycoides, Bacillus pumilus, Bacillus subtilis, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella paratyphi C, Salmonella typhi H, Salmonella enteritides, Salmonella flexneri Salmonella typhimurium, Shigella sonnei, Shigella schimizii, Shigella shigae, Vibrio cholerae Inawa, Vibrio cholerae Ogawa, Vibrio cholera Eltor and Xanthomonas campestris)* strains with minimum inhibitory concentration (MIC) for the extract ranging between 12.5-100 µg/mL [15]. This would explain the wounds given *Tagetes erecta* flower paste in our study were protected from microbial or fungal infection that may compromise wound healing.

### CONCLUSION

In this study, the topical application of the *Tagetes erecta* flower paste on the surgical wound in goats caused a significantly higher rate of wound healing and reduced the epithelialization period. The study reveals that flower petals possess good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like alkaloids, and terpenoids present in the extract. Further investigations are necessary to determine the mode of action of constituents present in the paste to prove its potential in clinical studies.

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# Prenatal stress impairs recognition memory and leads to neurodevelopmental deficits in the hippocampus of adolescent rats with early acute pentylenetetrazole-kindling

Kübra Çelik[1], Petek Bilim[1, 2], Gurur Garip[3], Burak Durmaz[4], Eser Yıldırım Sözmen[4], Meral Baka[3]

[1]Department of Neuroscience, Ege University Institute of Health Sciences, İzmir, Turkey
[2]Department of Psychology, Toros University Faculty of Economics, Business Administration and Social Sciences, Mersin, Turkey
[3]Department of Histology and Embryology, Ege University School of Medicine, İzmir, Turkey
[4]Department of Medical Biochemistry, Ege University School of Medicine, İzmir, Turkey

## ABSTRACT

**Objectives:** This study aimed to investigate the effects of prenatal stress (PS) on hippocampus of early acute pentylenetetrazole (PTZ)-kindled offspring in adolescence. Recognition memory, morphological changes and synaptophysin levels in hippocampus were evaluated.
**Methods:** Restraint stress was induced to a group of pregnant dams and non-stressed (NA) group remained undisturbed. Next, male and female offspring were divided as 1. PS-PTZ, 2. PS -control, 3. NA-PTZ and 4. NA-control (n = 12 in each group). The object recognition test was performed following PTZ injection (45 mg/kg) on postnatal day 10 (P10). Brains were collected on postnatal day 35 (P35) to determine neuronal density and synaptophysin expression by immuno-/-histological studies. Further, oxidative stress products in hippocampus were analyzed with different biochemical assays.
**Results:** PS impaired recognition memory in PTZ group significantly ($p = 0.03$); however, the impairment of PS was reversible in control group compared to PTZ ($p = 0.04$). Furthermore, PS caused neuronal loss in CA1 ($p = 0.01$) and decreased synaptophysin expression in the CA3 area of hippocampus in PTZ group ($p = 0.03$). PS also increased the oxidative stress markers in PTZ group significantly ($p < 0.05$).
**Conclusions:** These results suggest that PS causes neurodevelopmental deficits in adolescent hippocampus and recognition memory after early-life seizures prominently. However, the damage of only PS in adolescence can be reversible. Therefore, the effects of PS in the adult hippocampus and other regions of brain need to be further studied
**Keywords:** prenatal stress, hippocampus, PTZ-kindling, seizure, recognition memory

Traumas in embryonic development usually cause major impacts in the neonatal period and later life of the infant [1]. Prenatal stress (PS) especially increases the tendency and frequency of seizures causing neurodevelopmental deficits in the brain [2]. PS impairs the regulation of the hypothalamic-pituitary-adrenal axis and decreases the seizure threshold through glucocorticoid enhancement [3]. Furthermore, suppression of the inhibitor GABAergic neurons and constant depolarization of glutamatergic neurons [4,

5] impairs the neonatal hippocampus through volume loss, dendritic atrophy, suppression of synaptic transmission [6, 7, 8]. Since the hippocampus is crucial for learning and memory processing, the damage of PS may cause cognitive deficits such as recognition problems in later life. However, recognition memory requires multiple pathways such as visual, entorhinal and perirhinal cortex. The role of hippocampus in recognition process is not known well. In this case, several studies indicated that PS increases seizure activity or improves learning and memory retention associated with hippocampus [9, 10]. Nevertheless, that improvement or impairment depends on timing, duration, intensity of PS and gender [1]. High levels of glucocorticoids as a physiological response to PS stimulate postsynaptic excitatory receptors by over-releasing glutamate. Consequently, increased intracellular $Ca^{+2}$ concentration leads to seizure susceptibility and oxidative stress which may cause neuronal loss in the developing hippocampus [11, 12]. However, the long-term effects of PS and early acute seizure on the oxidative stress markers, synaptic transmission and neuronal denstiy of hippocampus need to be clarified. Therefore, we investigated the effects of PS on hippocampus of early acute pentylenetetrazole (PTZ) - kindled offspring in adolescence. We aimed to understand the cognitive, biochemical and histological outcomes when hippocampus development is disrupted by acute early life seizures following PS exposure.

## METHODS

### Animals

Adult female Wistar albino rats (200-400 g) were obtained from Ege University, Laboratory of Animal Research and Application Center. They were housed with ad libitum food and water under 20-25Co, 12L/12D laboratory conditions. The estrous cycle of female rats was determined by vaginal smear and they were housed with male rats overnight. Next day, vaginal plugs were observed and presumed as the first day of pregnancy. Pregnant rats were divided into PS and non-stress (NA) groups. After the birth, offspring (n = 12) in each group stayed in their home cages until weaning and separated by their genders. Experimental groups were conducted as 1. PS-PTZ, 2. PS-control, 3. NA-PTZ and 4. NA-control. This study was carried out after the approval of the local ethical committee (2017-082). The experimental design is shown in Fig. 1.

### Prenatal Stress Procedure

Restraint stress was induced to a group of female rats (n = 6) between day 12.5-17 of their gestations individually due to hippocampus begins to form on embryonic day 12 in rats [14]. They were restrained in a Plexiglass transparent cylinder (19 cm × 6 cm) three times/day for 45 min under two bulbs (100W) as described previously [15]. NA group of pregnant rats was not disturbed.

### Pentylentetrazole Injection

Equal amount of male and female offspring from different litters received diluted PTZ (45mg/kg) in saline intraperitoneally on postnatal day 10 (Sigma Aldrich-P6500) [16]. After the injection, they were transferred to a transparent cage to observe behavioral outcomes. 4 Tonic-clonic generalized seizures characterized by myoclonic jerks, chewing scraping, contractions in all limbs, gradually decreasing tonic movements and freezing were observed in all animals for at least 1 hour following PTZ injection.

### Object Recognition Test

Object recognition test was developed by Ennaceur and Delacour states that the animal learns the object in natural conditions and retains information in memory depending on discovery behavior without any penalty or reward [17]. Object recognition test was performed to 10 animals in each group. First day, the animals were placed into a 60 × 60 × 40 box for 5 min
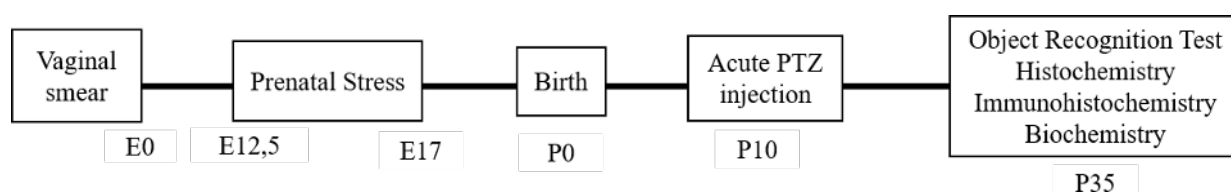


**Fig. 1.** The experimental design. E = embryonic day, P = postnatal day.

and expected to explore the box. Second day, two familiar objects were placed on different quadrants of the box symmetrically and rats discovered the objects for 10 minutes. Third day, the familiar object was replaced with a different shaped, sized and colored object. The animals placed into the box to explore familiar and novel objects for 10 minutes. All of the trials were recorded and recognition time was measured by two different stopwatches and mean was considered. The box and objects were cleaned with 70% alcohol between trials to avoid odor factor. Discrimination time was defined as when the animal approached the object less than 2 cm, sniffed, pawed and self-cleaned alongside the object. The discrimination index was calculated as follows: (Discrimination index = (new object/total exploration) × 100.)

### Immuno/-histochemistry and Image Analysis

The animals (n = 6) in each group were anesthetized and intra-cardiac perfusion was administered with 4% formaldehyde in 0.1 M phosphate-buffered saline (PBS) on P35. Brains were removed and washed in 0.1 M cacodylate buffer after storing in 4% formaldehyde in 0.1 M PBS for 3 days, respectively. Next day, brain slices were embedded into paraffin and sectioned (5 µm) for stainings. Firstly, Haematoxylin & Eosin staining (Merck, Germany) was 5 used to examine morphological differences of hippocampal regions. Secondly, another group of sections was incubated with anti-synaptophysin (1:100, Sigma Aldrich) primary antibody to detect synaptophysin, a presynaptic protein localized in synaptic vesicles [13]. Next day, the sections were washed and incubated with anti-mouse IgG (1:200, Sigma Aldrich) for 40 min. Finally, after DAB staining synaptophysin expressions were examined by light microscopy and microphotographs were captured (Olympus BX-51, light microscope, Olympus C-5050 digital camera). Synaptophysin immunoreactivity and neuronal density were examined in CA1 and CA3 areas by Image J (https://imagej.nih.gov/ij/). For this assessment, hippocampus of each animal was randomly chosen for unbiased counting and the average of 10 images from different sections was obtained by two researchers to ensure objectivity [18].

### Biochemistry

Another group of brains (n = 5) was removed without intra-cardiac perfusion and hippocampus was dissected from each brain (n = 5). Further, tissues were homogenized in phosphate buffer (0.5 M, pH:7.0), (1/10:w/v) and homogenates were centrifuged for 5 min at 700 × g at 4 ºC. Supernatants were collected immediately to determine myeloperoxidase (MPO), catalase (CAT) and thiobarbituric acid reactive substances (TBARS) levels. Tissue homogenates were incubated with TBARS solution (0.12 M TBA in 15% TCA and 1% HCl) for 30 min at 95 Co. TBARS levels were calculated using 1,1,3,3, tetramethoxypropane standard curve [19]. Hydrogen peroxide degradation was recorded at 240 nm by spectrophotometry and catalase activity was calculated [20]. MPO activities were measured following these stages: Homogenates were centrifuged at 10.000 rpm for 15 min. Pellets were re-homogenized in 0.5% HETAB (hexadecyltrimethylammonium bromide) in PBS (50 mM, pH: 6.0) and centrifuged 10.000 rpm for 10 min. Supernatants were added to a reactive solution containing 0.5 M o-dianisidine. Hydrogen peroxide solution (20 mM) was added and 6 absorbance was recorded at 492 nm with a microplate reader for 3 min with 15-sec intervals. Finally, MPO activity was calculated using the standard curve [21].

### Statistical Analysis

IBM SPSS Statistics 22.0 was used for statistical analysis. One-way ANOVA and post hoc Bonferonni tests were performed in normal distributed data according to Shapiro-Wilk test. Mann-Whitney test was used to compare PTZ and hypoxia subgroups in PS and NA groups for non-parametric data. The significance was considered as $p < 0.05$ and data is shown as mean ± standard error of the mean (S.E.M.)

## RESULTS

### *Object Recognition Test*

The discrimination index revealed that NA-PTZ group discriminated the new object better than PS-PTZ group ($p = 0.03$). However, the control offspring of the PS group (76.88 ± 9.95) discriminated the new object better than PS-PTZ group ($p = 0.04$, 42.91 ± 8.87) significantly. The object discrimination was worse in PTZ group compared to control for both PS and NA groups (Fig. 2). There was no statistical sig-
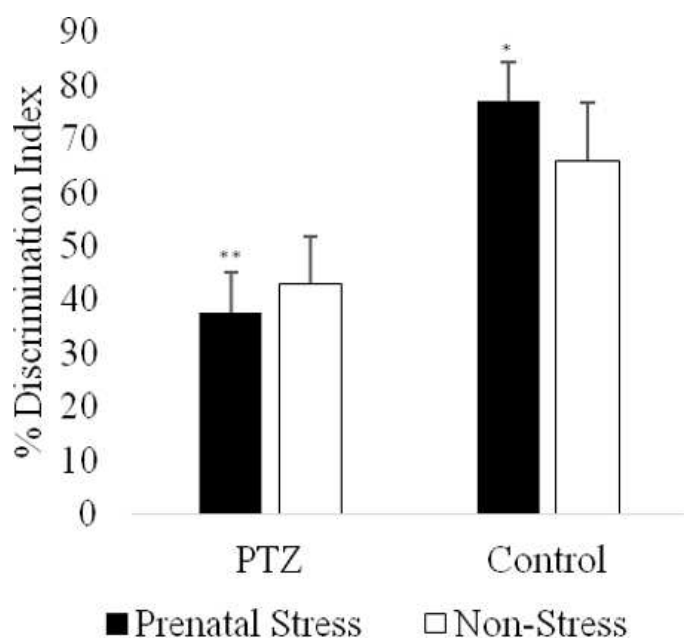
**Fig. 2.** Discrimination index of object recognition test. PS decreased the object discrimination in PTZ group and increased in control significantly (n=10 in each group). *p = 0.04 vs PS-PTZ, **p = 0.03 vs NA-control

nificance between PS and NA in the control group.

## Haematoxylin & Eosin (H&E)

CA1 and CA3 areas of hippocampus were examined to compare morphological differences (Fig. 3a and Fig. 3b). Gliosis and characteristic oligodendroglial nuclei were quite evident in PS group. Neuron loss was especially prominent in the CA1 area of PS-PTZ group significantly ($p = 0.015$) (Table 1). Further, large cavities between glias were observed and 7 gliosis in alveus proved the developmental deficits, especially in CA1. There was not a statistical difference between groups in CA3.

## Synaptophysin Expressions

Synaptophysin immunostaining was performed on CA1 and CA3 in each group and indicated as mean percentage. Post hoc test revealed that PS reduced synaptophysin immunoreactivity in CA3 of PTZ group ($p = 0.03$, $38.60 \pm 0.86$), contrary it increased synaptophysin in control group significantly ($p = 0.02$, $38.11 \pm 1.14$). There was no significant difference between groups in CA1 even though the mean percentage of PS-control was higher than NA-control (Fig. 4a and Fig. 4b).

## TBARS, MPO and CAT Levels

The current data demonstrated that PS increased TBARS ($p = 0.04$) and MPO levels ($p = 0.01$) in PTZ group significantly. Although the means of NA groups were higher than PS, there was not a statistical difference between CAT levels (Table 2). Furthermore, there was no statistical difference between PTZ and control groups in NA offspring. The results of biochemical assays proved that lipid peroxidation and oxidative stress were higher in PS-PTZ group significantly.
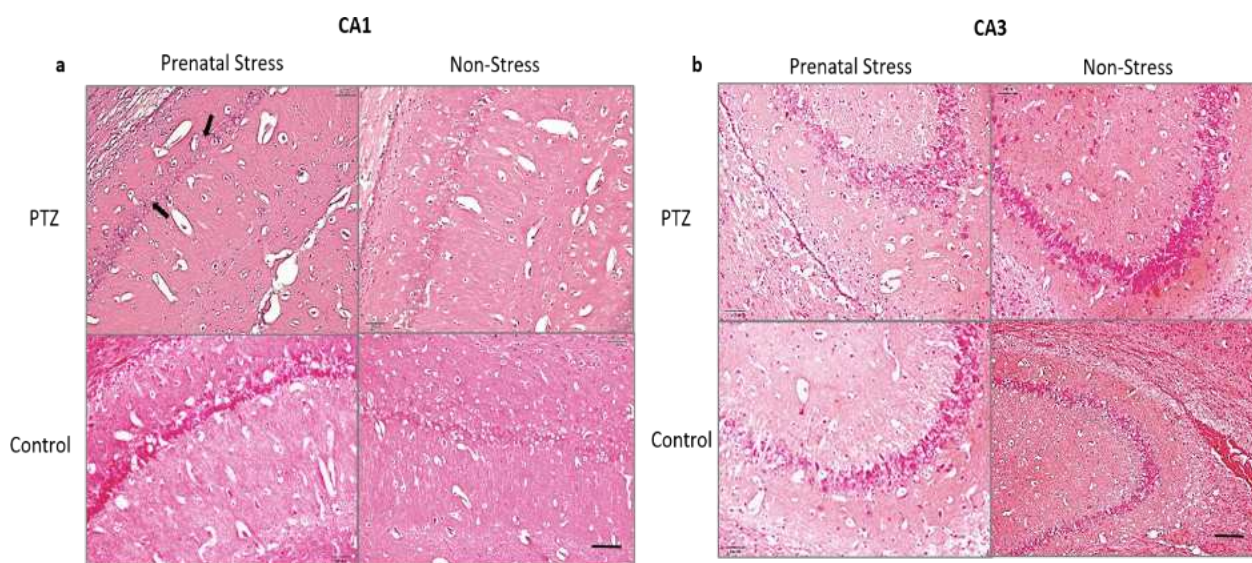


**Fig. 3.** Pyramidal layers of CA1 and CA3 areas H&E staining shows the morphological differences in the pyramidal layers in CA1 (a) and CA3 (b) regions. Neuron loss in PTZ group of PS was seen by arrows in CA1 (scale bar=50 μm, the magnification is 20×)

### Table 1. Quantitive measurement of the neuronal density by H&E staining

| Groups (n = 5) | CA1 | CA3 |
| --- | --- | --- |
| **Prenatal Stress (PS)** | | |
| PTZ | 129.83 ± 21.818* | 188.17 ± 26.077 |
| Control | 281.17 ± 25.375 | 223.17 ± 21.917 |
| **Non-Stressed (NA)** | | |
| PTZ | 235.33 ± 45.238 | 329.50 ± 33.985 |
| Control | 285.83 ± 25.088 | 683.50 ± 60.668 |

Data is presented as mean ± standard error of the mean (S.E.M.). CA1 and CA3 areas detected in hippocampus of each animal under light microscopy (20× magnification) and these areas were randomly chosen. The average of 10 images from different sections was obtained and counted by two researchers using Image J (https://imagej.nih.gov/ij/). PTZ = pentylenetetrazole, *$p$ = 0.015 vs NA-control
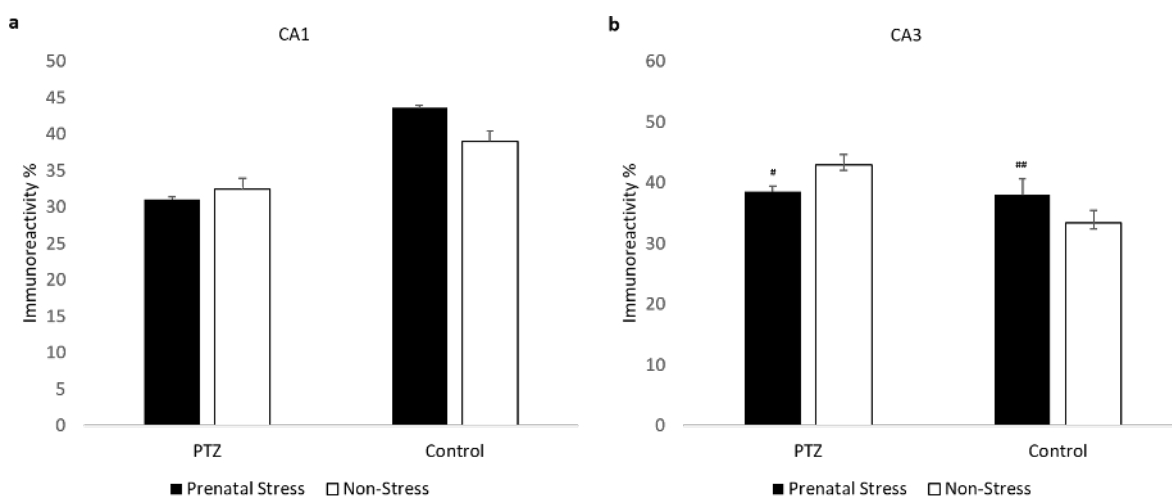


**Fig. 4. Synaptophysin immunoreactivity.** Synaptophysin positive areas were measured on 20× microphotographs by two researchers using Image J and the result is showed as a mean percentage. PS decreased the immunoreactivity in the CA3 region of the PTZ group and increased in control offspring significantly. There was no statistical significance between groups in the CA1 region. *$p$ = 0.03 vs NA-PTZ, **$p$ = 0.02 vs PS-PTZ

### Table 2. TBARS, MPO and CAT levels.

| Groups (n = 6) | TBARS (nmol/mgprt) | CAT (U/mgprt) | MPO (U/mgprt) |
| --- | --- | --- | --- |
| **Prenatal Stress (PS)** | | | |
| PTZ | 3.99 ± 1.08* | 0.44 ± 0.15 | 0.50 ± 0.009** |
| Control | 2.69 ± 0.62 | 0.38 ± 0.15 | 0.06 ± 0.01 |
| **Non-Stressed (NA)** | | | |
| PTZ | 2.13 ± 0.25 | 0.45 ± 0.06 | 0.13 ± 0.04 |
| Control | 2.79 ± 0.21 | 0.24 ± 0.10 | 0.05 ± 0.02 |

Data is shown as mean ± standard error of the mean (S.E.M.). In PS groups, values of TBARS, CAT, and MPO in PTZ group were higher than in control group. TBARS = thiobarbituric acid reactive substances, CAT = catalase, MPO = myeloperoxidase, PTZ = pentylenetetrazole. *$p$ = 0.04 vs NA-PTZ, **$p$ = 0.01 vs NA-PTZ

## DISCUSSION

PS is a risk factor for the occurrence and progression of seizures [22]. Therefore, it may lead to many neurodevelopmental and cognitive deficits in the developing brain. In this study, it was questioned whether PS causes neurodevelopmental deficits and recognition impairment in hippocampus of adolescent offspring with acute PTZ-kindling in early life. In this context, CA1 and CA3 regions of the hippocampus were examined regarding the detection of synaptic transmission and morphological changes. TBARS, MPO and CAT assays were conducted to demonstrate lipid peroxidation and antioxidant levels of the whole hippocampus. Furthermore, the object recognition test was performed to investigate recognition abilities and whether the hippocampus involves in the recognition process.

PS has different impacts on the hippocampus which is crucial for memory processing. However, the effects of PS on recognition memory of adolescent rats with early-life seizures are not known well. Ahmadi *et al*. [23] demonstrated that PTZ-kindled rats have exploratory deficits on object recognition. Current results similarly showed that recognition memory of PTZ-kindled rats was impaired associated with the neuron loss and reduced synaptophysin levels in CA1. Indeed, H&E staining showed that CA1 was more vulnerable than CA3 to the effects of PS. In contrary, PS increased the object discrimination in control group compared to PTZ-kindled group regarding synaptophysin levels in CA3. PS caused neuronal and synaptic damage in PTZ group, however that damage was reversible in the control group. These results referred that the long-term effects of PS enhance or reduce cognitive capabilities depending on seizure activity in the adolescent brain [9, 10]. Another important finding was that hippocampus is essential for the object recognition process. However, it is not the primary area for memory storage and retention since the entorhinal, perirhinal, and visual cortex are involved in possible mechanisms [24-27]. Therefore, despite the deficits in hippocampus PS increased the discrimination index in control group. Further, these regions of the cortex must be investigated to understand effects of PS on recognition memory.

TBARS, CAT, and MPO levels were determined to investigate lipid peroxidation and oxidative stress.

Several studies revealed that stress in the prenatal period increases TBARS, which is an end-product of lipid peroxidation [28-30]. Besides that, seizure activity also increases lipid peroxidation [31, 32]. Similarly, this study demonstrated that PS increases TBARS and MPO levels in PTZ group significantly. However there was no significance between NA groups. These results referred to epileptic seizures are associated with increased oxidative damage [33] due to reperfusion injury as well as the polymorphonuclear leukocyte recruitment in the brain [34, 35]. Consequently, increased oxidative damage and insignificant levels of catalase indicated that PS causes developmental disruptions by changing the oxidant & antioxidant balance of brain [36].

## CONCLUSION

Consequently, PS disrupted recognition memory, neuronal density and synaptic transmission in hippocampus of PTZ group in adolescence. Further studies are needed to understand the effects of PS in adult brain and other possible mechanisms as well as gender differences. This study provided a multidisciplinary evidence for the effects of PS on the recognition memory and hippocampus of adolescent rats with early acute PTZ-kindling.

TURKEY (18-SBE-004/ 2018).

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# Comparison of the effect of ethanol and potassium iodide in antibacterial photodynamic therapy on gram negative pathogens

**Nermin Topaloğlu Avşar**[1], **Melike Çağan**[1,2], **Emel Bakay**[1], **Aziz Kolkıran**[3]

[1]Department of Biomedical Engineering, Izmir Katip Celebi University, Faculty of Engineering and Architecture, Izmir, Turkey
[2]Department of Bioengineering, Izmir Institute of Technology, Izmir, Turkey
[3]Department of Engineering Sciences, Izmir Katip Celebi University, Faculty of Engineering and Architecture, Izmir, Turkey

## ABSTRACT

**Objectives:** Antibiotics is the most common treatment for bacterial infections. However, bacteria can change their genetic material, develop antibiotic resistance and cannot be treated. This brings the need for new treatment methods. Antibacterial Photodynamic Therapy is becoming a promising approach to treat bacterial infections. It is based on the use of photosensitizer to be activated by light with an appropriate wavelength and it will result in reactive oxygen species which are responsible for the destruction of pathogens. In this study, Antibacterial Photodynamic Therapy was examined for the treatment of *Pseudomonas aeruginosa* with the help of ethanol and potassium iodide.
**Methods:** The effect of Chlorin e6-based photoinactivation was studied on Multidrug resistant *P. aeruginosa* upon irradiation with 655-nm diode laser. Then ethanol and potassium iodide was added to the mechanism separately to increase the efficacy of photoinactivation. After each application, serial dilution method was used for the determination of viable cells.
**Results:** Outcomes showed that only Antibacterial Photodynamic Therapy causes a mortality rate of 75%. Addition of ethanol causes a mortality rate of 93% and addition of potassium iodide causes a mortality rate of 99.9% with less amount of Chlorin e6 and light dose.
**Conclusions:** Chlorin-e6 based photoinactivation did not provide high mortality rate on *P. aeruginosa*. The use of ethanol and potassium iodide increased the effect of photoinactivation. The highest mortality rate was obtained with potassium iodide. It was understood that potassium iodide was a better concomitant agent to increase the bactericidal effect of Antibacterial Photodynamic Therapy on *P. aeruginosa*.
**Keywords:** Antibacterial photodynamic therapy, chlorin e6, ethanol, potassium iodide, *pseudomonas aeruginosa*

**B**acteria are single cell organisms that can infect and contaminate any part of the body. Bacteria are generally classified as gram positive and gram negative. While gram positive bacteria have a thick cell wall, gram negative bacteria have a thin cell wall.

However, gram-negative bacteria are usually more harmful because their cell wall is tighter and their outer membrane is hidden by a capsule. If any treatment is not applied, these bacterial strains can even cause death in progressive stages [1]. The most com-

mon treatment of infections is the use of antibiotics. But, excessive use and misuse of antibiotics result in multidrug resistance which makes antibiotics become nonfunctional against multidrug resistant bacteria via horizontal gene transfer, mutations, etc. [2].

The resistance of pathogenic microorganisms to antibiotics has led to the search for alternative treatment methods for local infections [3]. A potential alternative is antibacterial photodynamic therapy (aPDT) that is defined as photoinactivation of microorganisms via reactive oxygen species that are produced by photosensitizers (PS) activated with appropriate wavelength of light. The best known advantage of aPDT is that both antibiotic-susceptible and resistant bacterial strains do not resist to photoinactivation and there is no possibility to induce resistance to aPDT after repetitive applications [4].

Strong light absorption capacity and high quantum yield of reactive oxygen species are the desired characteristics of an ideal PSs used in aPDT. Other desired properties are to show low toxicity and target selectivity. The wavelength of the light to be used must be selected according to the absorption spectrum of the PS. If the wavelength of the light reaches its peak value in the absorption spectrum of PS, the effect of the reaction will be high. In order to obtain the ideal therapeutic effect, the energy dose of light and the concentration of PS should be properly optimized during the applications [5]. aPDT leads to progressive lipid peroxidation by free radical attack on bacteria. This leads to cell death by the formation of lipid radicals, the elimination of cellular lipids resulting in oxidative damage to the cells. [4-7].

Chlorin e6 (Ce6) is a second-generation PS which is low toxic, easily synthesized, selectively accumulated in target tissue, and has high photosensitizing efficacy [8-10]. Ce6 absorbs 630-680 nm of wavelengths strongly [10]. For this reason, red part of the visible spectrum is an ideal choice to activate Ce6 [11]. Besides, the wavelengths around 600-nm is in therapeutic window in which the wavelengths can be safely used for the treatment of various diseases without giving serious harm to the biological tissues. Because of the limited absorption capacity of the biological tissue towards these wavelengths, they can travel deeper inside the biological tissues until its target without losing its energy. These features of the wavelengths in the therapeutic window makes them

favorable in the therapeutic light applications [12, 13]. Ce6 is more effective at destroying gram-positive bacteria, sometimes it needs some additional strategies to overcome problems related to gram-negative species [3]. The use ethanol (EtOH) as an adjuvant can be a good strategy to enhance the effect of PS. It is known that EtOH only at low concentrations (0.075% - 0.01%) slows the growth rate of bacteria in stationary phase [14]. In other studies, unlike water, the use of solvents with less polarity such as EtOH enhances the effect of aPDT on biofilms [15]. In the direction of this information, the use of EtOH as an adjuvant was thought to provide an advantage to increase the effect of PS.

Another possibility to potentiate the effect of aPDT is the addition of inorganic salt during applications. One of these inorganic salts is potassium iodide (KI). It has been shown that the mechanism of action of KI involves electron transfer reactions to produce iodine and molecular radicals [16]. KI could also potentiate aPDT to produce more reactive species [17]. This reaction seems to have singlet oxygens to produce peroxy iodide, which is subsequently converted into molecular iodine and hydrogen peroxide. A mixture of extracellular free iodine, and reactive iodine radicals causes bacterial killing depending on the degree of PS binding to the microbial cells [18]. In brief, KI can potentiate the effect of photoinactivation of microorganisms. If the PS can bind to the microbial cells and be activated by light absorption in the presence of KI, this mechanism results in short lived reactive iodine species and then molecular iodine produced as a result of singlet oxygen-mediated oxidation of iodide causes bacterial killing [19].

The aim of this study was to investigate and compare the effect of EtOH and KI on the photoinactivation of *Pseudomonas aeruginosa* in the presence of Ce6 irradiated by 655-nm diode laser.

## METHODS

In this study, photoinactivation efficacy of Ce6 and 655-nm diode laser was examined on multidrug resistant *P. aeruginosa* strain. Then EtOH and KI was used to increase the bactericidal effect of aPDT. Here is a list of groups which were formed and analyzed during these experiments.

1. Control group: The group which not received Ce6, KI, EtOH and laser treatment

2. Ce6 group: The group incubated only with Ce6

3. Laser group: The group treated only with laser light

4. KI group: The group incubated only with KI

5. Ce6 + KI group: The group incubated with KI and Ce6

6. EtOH group: The group incubated only with EtOH

7. Ce6 + EtOH group: The group incubated with Ce6 solution containing EtOH

8. aPDT group: The group incubated with Ce6 and irradiated by laser light

9. aPDT + KI group: The group incubated with Ce6 and KI and irradiated by laser light

10. aPDT + EtOH: The group incubated with Ce6 containing EtOH and then irradiated by laser light.

## Bacteria

Multidrug resistant clinical isolate of *P. aeruginosa* was used as gram negative pathogen. Stock bacteria was incubated in 5 ml Tryptic Soy Broth allowing them to grow overnight at 37°C. Then they were centrifuged, the supernatant was discarded. Bacterial pellet was dissolved in phosphate buffered saline (PBS) to obtain a concentration of 108 CFU/ml and this bacterial solution was used in experiments.

## Chemicals

Ce6 (Santa Cruz Biotechnology, Dallas, TX, USA) was used as PS. Fresh solutions in PBS were prepared at specific concentrations before each experiment and kept at dark to prevent photobleaching. 1, 2.5, 250 and 500 μM concentrations were examined throughout this study. These concentrations of Ce6 were determined according to our preliminary experiments. EtOH was used as an adjuvant to increase the effect of Ce6. The Ce6 solution was prepared together with absolute EtOH to obtain a solution containing 20% EtOH. The concentration of EtOH was defined as 20%, because it was proved previously that this concentration was effective to enhance the photodynamic action on pathogens [11]. KI pellets were dissolved in sterile dH2O to obtain 100 mM KI solution and it was used as a potentiator in aPDT. Similarly, 100 mM concentration of KI was also proved as a potentiator for the effective photoinactivation of different

bacterial species [20].

## Laser Device

In this study, a diode laser (PS4 III.LED, Changchun New Industries Optoelectronics Co. Ltd., Changchun, China) which emits 655-nm of wavelength was used. This wavelength is in the range of the highest absorption band of the Ce6. The output power of this laser device is adjustable and was set to 200 mW for photoinactivation. Depending on our preliminary experiments, 200 mW was found quite safe when it was applied alone. For this reason, it was chosen as an output power and the application times were adjusted manually to obtain different values of energy densities (10, 25, 50, 100, 150 and 200 J/cm$^2$) which was examined during the laser applications.

## Experimental Procedure

In the experiments, each group was studied in 96-well plates as 3 samples and repeated at least three times. 50 μL bacterial solutions were added to the specific wells where light was irradiated on them. Then;

- 50 μL bacterial solution was mixed with equal volume of PBS in Control and only Laser groups.
- 50 μL bacterial solution was mixed with 50 μL of Ce6 in only Ce6, Ce6+KI, aPDT and aPDT+KI groups.
- 50 μL bacterial solution was mixed with 50 μL KI in only KI, Ce6+KI and aPDT+KI groups.
- 50 μL bacterial solution was mixed with 50 μL Ce6 solution containing 20% EtOH in only EtOH, Ce6+EtOH and aPDT+EtOH groups.
- The bacteria were incubated with specific solution in each experimental group for 15 minutes.
- Bacterial solutions were treated with laser light in only Laser, aPDT, aPDT+KI and aPDT+EtOH groups.
- After each of these applications, viable bacterial cell count was determined by serial dilution method.

## Statistical Analysis

Data collected from each group after colony counting was normalized by the data of control groups. Normalized data were statistically analyzed by two-tailed Student's t-test. A *p* value which was smaller than 0.05 was determined as statistically significant.

## RESULTS

### The Effect of Different Energy Doses on P. aeruginosa

10, 25, 50, 100, 150 and 200 J/cm$^2$ energy doses were used in Laser and aPDT groups. First of all, the effect of these energy doses was examined on bacteria without addition of any chemicals. As shown in Figure 1, the maximum bactericidal effect of only laser irradiation was obtained with 150 J/cm$^2$ and resulted in approximately 30% decrease in cell viability. And still none of these results were statistically significant when they were compared with control group (Fig. 1).

### The Photoinactivation with Chlorin e6 on P. aeruginosa

Throughout this study different Ce6 concentration (1, 2.5, 250 and 500 µM) was examined in aPDT groups. The maximum mortality rate was obtained with 500 µM Ce6 concentration. It reduced the cell viability nearly 50% and only this concentration showed a significant bactericidal effect (Fig. 2). In aPDT groups, 250 and 500 µM Ce6 were studied together with 50, 100, 150 and 200 J/cm$^2$ of energy doses. 100 J/cm$^2$ of energy dose together with 500 µM Ce6 provided maximum photoinactivation of *P. aeruginosa*. As shown in Figure. 2, the maximum reduction in bacterial cell viability with 100 J/cm$^2$ of energy dose and 500 µM Ce6 was nearly 75%. This effect was quite high when compared with other parameters that was used and it resulted in a statistical significant reduction

when compared with the control group. Unexpectedly, higher energy doses with the same Ce6 concentration did not provide similar or higher bactericidal effect (Fig. 2).

### The effect of Ethanol in Photoinactivation with Ce6 on P. aeruginosa

Ce6 solution was mixed with absolute EtOH to obtain 250 µM Ce6 concentration in a 20% EtOH solution. The effect of this combination was examined together with 4 different laser energy doses (50, 100, 150 and 200 J/cm$^2$). 20% EtOH solution decreased bacterial cell viability nearly 55%. When it was mixed with 250 µM Ce6, its bactericidal effect was not high as in the group of 20% EtOH only and both of these groups resulted in significant effects. Then this solution was used in aPDT groups. Maximum reduction was obtained with an energy dose of 200 J/cm$^2$ that resulted in 93% cell death and it was a statistical significant antibacterial effect (Fig. 3).

### The effect of Potassium Iodide in Photoinactivation with Ce6 on P. aeruginosa

In the last part of this study, 100 mM KI was used as a source reactive oxygen/iodine species to increase the effect of aPDT with Ce6. In the aPDT+KI group, firstly 500 and 250 µM Ce6 concentrations were used together with 100 and 200 J/cm$^2$ light energy doses re-



**Fig. 1.** Effect of light doses on the viability of bacterial cells. Cell viability for *P. aeruginosa* strains was assessed by colony counting after only laser applications and the number of viable cells was normalized with control group (Light dose: 10, 25, 50, 100, 150, and 200 J/cm2). * shows the statistical significance with respect to control group ($p \leq 0.05$).
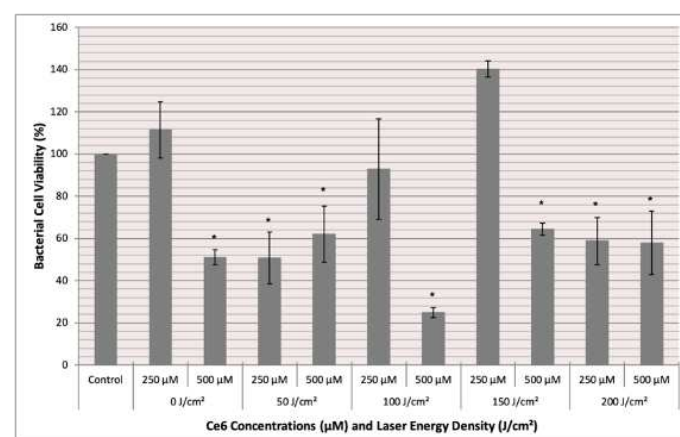


**Fig. 2.** Effect of Ce6 concentrations and PDT parameters on the viability of bacterial cells. Cell viability for *P. aeruginosa* strains was assessed by colony counting after Ce6 only and PDT applications and the number of viable cells in each experimental group was normalized with control group (Light dose: 50, 100, 150, and 200 J/cm2 and Ce6 concentrations: 250 and 500 µM. * shows the statistical significance with respect to control group ($p \leq 0.05$).
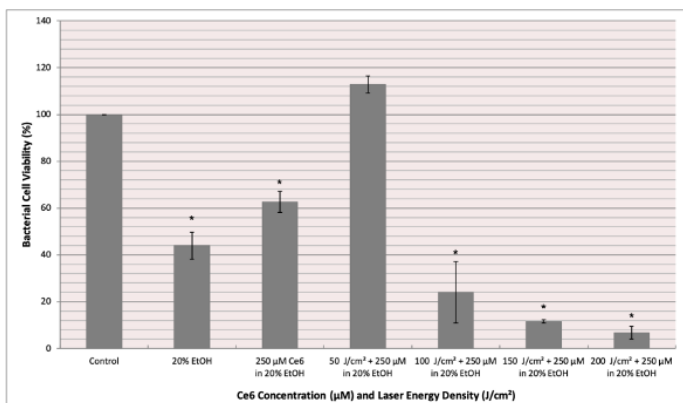
**Fig. 3.** Effect of 20% EtOH, 250 µM Ce6 in 20% EtOH and different PDT-EtOH parameters on the viability of bacterial cells. Cell viability for *P. aeruginosa* strains was assessed by colony counting after EtOH only, 250 µM Ce6 in 20% EtOH only and PDT-EtOH applications and the number of viable cells in each experimental group was normalized with control group (Light dose: 50, 100, 150, and 200 J/cm2 and Ce6 concentration: 250 µM). * shows the statistical significance with respect to control group (*p* ≤ 0.05)

**Fig. 4.** 4. Effect of 100 mM KI, 1 µM Ce6 and different PDT-KI parameters on the viability of bacterial cells. Cell viability for *P. aeruginosa* strains was assessed by colony counting after 100 mM KI only, 1 µM Ce6 in PBS only and PDT-KI applications and the number of viable cells in each experimental group was normalized with control group (Light dose: 10 and 25 J/cm2, Ce6 concentration: 1 µM and KI concentration: 100 mM). * shows the statistical significance with respect to control group (*p* ≤ 0.05)

spectively. These applications resulted in 100% mortality rate (data not shown). To find the optimum parameters that cause nearly 99.9% with nontoxic Ce6 concentration and lower energy doses, these parameters were decreased to 1 and 2.5 µM Ce6 and 10 and 25 J/cm2 energy doses and used together with 100 mM KI. As shown in Figure 4, 1 µM Ce6 was examined with 10 and 20 J/cm2 energy dose. These applications resulted in more than 98% decrease in cell viability (Fig. 4).

Then the concentration of Ce6 was increased to 2.5 µM and used with 10 and 20 J/cm² energy dose in the presence of 100 mM KI. The effect of 2.5 µM Ce6 with 10 J/cm² energy dose in the presence of 100 mM KI was again 98%. The highest mortality rate was observed with 99.97% when 25 J/cm² of energy dose used together with 2.5 µM Ce6 in the presence of 100 mM KI. The effect of 100 mM KI with and without these Ce6 concentrations showed no bactericidal effect (Fig. 5).

## DISCUSSION

aPDT has become a promising tool to combat bacterial infections recently and Ce6 has many advantageous characteristics against pathogens which has

been proved in many studies [21]. Therefore, aPDT with Ce6 was examined in this study for the treatment of gram-negative pathogens which is hard to be destroyed and need higher doses of PS and light intensities to be photoinactivated [22]. When the low concentrations of Ce6 were evaluated on *P. aeruginosa,* they did not exhibit any dark toxicity. But 500
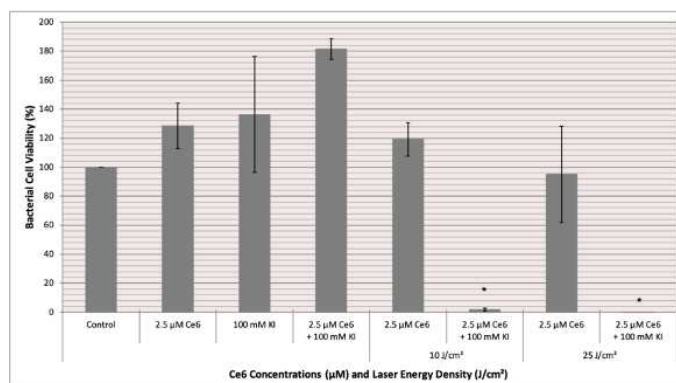


**Fig. 4.** Effect of 100 mM KI, 2.5 µM Ce6 and different PDT-KI parameters on the viability of bacterial cells. Cell viability for *P. aeruginosa* strains was assessed by colony counting after 100 mM KI only, 2.5 µM Ce6 in PBS only and PDT-KI applications and the number of viable cells in each experimental group was normalized with control group (Light dose: 10 and 25 J/cm2, Ce6 concentrations: 2.5 µM and KI concentration: 100 mM). * shows the statistical significance with respect to control group (*p* ≤ 0.05)

µM Ce6 caused nearly 50% reduction in cell viability which can be considered as a high dark toxicity and this cannot support idea of aPDT with less side effect in neighboring areas of biological tissues [23].

On the other hand, only laser light with various intensities ranging from 10 to 200 J/cm$^2$ did not result in meaningful reduction in the cell viability. The maximum mortality rate of 35% was obtained at the light energy dose of 150 J/cm$^2$. Thus, it can be explained that only laser application was not harmful on bacterial cell viability and it is a well-known characteristics of red light on cells [24].

Although the use of Ce6 was a promising drug in aPDT applications [3], our experiments showed that it was ineffective on the multidrug resistant *P. aeruginosa* strain. Even though light energy dose and drug concentration were increased in serious amounts, killing more than 99% was not obtained. The maximum reduction in cell viability nearly 75% was reached in the application of 100 J/cm$^2$ light dose and 500 µM Ce6 concentration (Fig. 2). According to many studies, it is shown that Gram negative bacteria opposed to aPDT due to their special wall structure [25-27]. Gram negative bacteria have a complicated many layered and hard-spun outer barrier structures [28]. This barrier excludes most of the PS, thus certain methods have to be used to confirm that PS can penetrate the bacteria and be effective on them [27]. *P. aeruginosa* strain which was used in this study cannot be destroyed efficiently solely using Ce6 in aPDT application. Therefore, this therapy needed some concomitant agents to amplify its activity.

Firstly, the effect of EtOH was examined as an adjuvant in order to increase the effect of the PS. The goal in this part of the study was to reduce the the drug concentration in a serious manner and achieve a mortality rate over 99%. 20% EtOH was chosen as a concentration to act as an adjuvant in aPDT application. Its antibacterial effect without Ce6 and light application was not significant, but still quite high. It reduced bacterial cell viability approximately 55%. Although an important decrease (around 93%) in cell viability with 20% EtOH in aPDT was obtained with the parameters of 200 J/cm2 energy dose and 250 µM Ce6, sufficient amount of antibacterial effect cannot be achieved (Fig. 3). The only advantage of using 20% EtOH as an adjuvant was to reduce the concentration of Ce6 to its half. The light dose has been increased to 200 J/cm2 when this was compared with only aPDT application. Using EtOH against P.aeruginosa raised antimicrobial effect of aPDT. This application exerts antibacterial activity including more production of singlet oxygen, destroying bacterial membrane, rapid denaturation of proteins, causing to cell lysis [29, 30]. But still the energy dose and PS concentration were quite high in this application.

Since the desired outcomes were not achieved in the applications of only aPDT and aPDT+EtOH, KI was examined as a potentiator at the last part of this study. Purpose of using KI was to increase effectiveness of PS for the production of more radical ions. For this reason, 100 mM KI concentration was chosen as an optimum parameter after literature survey [16-19]. When the effects of only KI and KI together with Ce6 were studied, the results showed that they had no lethal effects on the bacteria. Only KI treatment has increased the bacterial cell viability with a rate of 32% instead. Then, the effect of 100 and 200 J/cm2 light dose and 500 and 2500 µM ce6 concentrations respectively was evaluated together with 100 mM KI. This resulted in 100% mortality rate (data not shown). Then the concentration of Ce6 and light dose were reduced to obtain optimum energy dose and PS concentration for achieving a mortality rate of 99.9%. The highest mortality rate was 99.9%, when 25 J/cm2 light dose and 2.5 µM Ce6 were used together with 100 mM KI (Fig. 5). Results showed that nontoxic inorganic salt KI potentiates aPDT by increasing the effectiveness of Ce6 which was proved previously with different types of PSs on different strains of bacteria [17, 31, 32]. The presence of KI forms short-lived reactive iodine species and they induce the generation of more reactive species. Then these reactive iodine species damage bacterial cells [32, 33]. Use of 100 mM KI provides the opportunity to decrease the level of energy dose and PS concentration in serious levels. So, it eliminates the risk of dark toxicity and photodestruction depending upon the use of high level of PS and light dose.

## CONCLUSION

Although different light doses of 655-nm of wavelength and Ce6 concentrations were used in Ce6-based photoinactivation and their levels were increased quite

high values, it did not show efficient bactericidal effect on multidrug resistant *P. aeruginosa*. Therefore, EtOH was used as an adjuvant in aPDT applications to increase the mortality rate. Serious mortality rate was obtained, but it was still not sufficient and the parameters used were still high which may cause cytotoxicity on healthy cells. Then, KI was used as a potentiator in aPDT applications. Over 99.9% mortality rate was obtained with the help of KI. When results of each three different applications were examined, the use of KI resulted in a significant reduction in light dose (4 × reduction) and drug concentration (200 × reduction) by achieving high killing capability on P. aeruginosa which is multidrug resistant gram negative strain.

## Authors' Contribution

Study Conception: NTA, MÇ, EB, AK; Study Design: NTA, MÇ, EB, AK; Supervision: NTA, MÇ, EB, AK; Funding: NTA, AK; Materials: NTA, AK; Data Collection and/or Processing: NTA, MÇ, EB; Statistical Analysis and/or Data Interpretation: NTA, MÇ, EB; Literature Review: NTA, MÇ, EB; Manuscript Preparation: NTA, MÇ, EB, AK and Critical Review: NTA, MÇ, EB, AK.

## Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Mindfulness and related factors among addicted adolescents

**Mehmet Erdem Uzun[1]**, **Hüseyin Uslu[1]**, **Şenay Kılınçel[2]**, **Barış Uzunok[3]**, **Muhammet Furkan Korkmaz[4]**, **Hande Şirin[1]**

[1]Department of Child Psychiatry, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey
[2]Department of Child Psychiatry, Sakarya University Training and Research Hospital, Sakarya, Turkey
[3]Department of Physiology, Uşak University School of Medicine, Uşak, Turkey
[4]Department of Pediatrics, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey

## ABSTRACT

**Objectives:** Mindfulness has been defined as "the clear and single-minded awareness of what happens to us and in us, at the successive moments of perception". Mindfulness-based therapies have been used in the treatment of psychiatric diseases in recent years. There is no study on the mindfulness of addicted adolescents in our country. In this study, we aimed to determine the level of the mindfulness and the factors affecting the mindfulness among addicted adolescents.
**Methods:** Fourty drug-addicted adolescents and fourty five healthy adolescents were included in the study. Sociodemographic Data Form, Addiction Profile Index Adolescent Form (BAPI-E), Mindful Attention Awareness Scale (MAAS), Coping Orientation to Problems Experienced (COPE), Metacognition Questionnaire for Children and Adolescents (MCQ-C) and Child-Adolescent Social Support Scale (CASS) were applied to the participants. Clinical interviews (based on DSM-V) were performed to assess addiction.
**Results:** There were no significant differences in the mindfulness levels between the addicted and the non-addicted groups. The mindfulness levels of the patients who attempted suicide were lower than the patients who did not attempt suicide. Behavioral disengagement sub-scales of COPE were significantly higher in the substance use and the denial group.
**Conclusions:** Non-functional styles coping with stress were found to be significantly higher in the addicted group, however, we found no significant differences in the mindfulness levels.
**Keywords:** Mindfulness, adolescents, substance use, addiction

The word meaning of the Turkish equivalent of conscious mindfulness is expressed as a "state of deep awareness". Conscious mindfulness, which originates from Buddhism, is defined as "focusing only on what is now, without judgment "[1]. Mindfulness is a mind and the body activity that involves focusing attention on instant lives and observing inner experiences. The concept of Mindfulness originated from meditations practiced in the East. In this practice called mindfulness meditation, attention constantly pay attention to breathing, body feelings, emotions, or the mind's flow; that is, thoughts. All these inner experiences are observed through nonjudgmental acceptance. Mindfulness (mindfulness) is a non-pharmacological Mind-Body Therapy that first presented by Kabat-Zinn to the medical community in the Mindfulness-Based Stress Reduction Program. Kabat-Zinn explained the term mindfulness simply as "revealing

past experiences step by step without judgment and paying attention to the present purpose" [2].

Mindfulness has attracted considerable attention in behavioral medicine and psychiatric literature over the past 20 years. As a result, multiple mindfulness meditation has been designed to treat many diseases and conditions [3]. Although studies have shown that mindfulness reduces psychological problems in many patient populations, it is unclear how mindfulness causes that [1, 3, 4].

The number of research conducted on mindfulness in Turkey is very limited. When the literature is reviewed, we see that mindfulness studies conducted in the field of alcohol and substance addiction generally focus on using the mindfulness therapy for addiction [5].

In our country's literature, the mindfulness levels of addicts for the adult group were examined, and we did not find a study on the mindfulness in adolescents. This study aims to examine the level of mindfulness and related factors in adolescents with substance use disorder.

## METHODS

The study sample was composed of patients who were followed up in the Child and Adolescent Substance Addiction Treatment Center (ÇEMATEM According to DSM-V. The patients consisted of 40 adolescents with substance use disorders. The patient group's diagnoses were made by a child psychiatrist using a semi-structured interview that was created by us, taking into account the DSM-V Drug Use Disorders diagnostic criteria. The study's control group was composed of 45 adolescents from a high school in the city center who had similar characteristics in the terms of socioeconomic level, age and gender, with no substance use disorder and no psychopathology due to the clinical evaluation. The Clinical Research Evaluation Committee gave the ethics committee approval of the study. (Decision date / No: 14.01.2015 / 20478486-26).

### Personal Information Form

This form, which contains 12 questions, was created by researchers and is intended to learn the sociodemographic characteristics of participants.

### Addiction Profile Index Adolescent Form (BAPI-E)

BAPI-E was developed in 2012 by Ögel *et al*. [6] to evaluate the extent of addiction and measure the severity of addiction in the 15-18 age group adolescents. The scale consists of 25 items and includes five sub-dimensions: substance use characteristics, diagnostic criteria, effects on life, cravings and motivation. The response options are a quintet Likert-type scale, ranging from "ever, just 1-2 times, 1-3 times a month, 1-5 times a week and every day". In the scale's validity and reliability study, the Cronbach alpha coefficient was determined as 0.87 and the cut-off point as 2. In this study, the Cronbach alpha coefficient of the scale was calculated as 0.96.

### Mindful Attention Awareness Scale (MAAS)

The MAAS is designed to measure the attention and the mindfulness, focusing on the present. The MAAS is a 6-point Likert-type scale consisting of 15 items. Each item is scored between 1 (almost always) and 6 (almost never). Internal consistency was found to be $\alpha = 0.82$ (s = 327) in the student sample and $\alpha = 0.87$ (s = 239) in the general adult sample. MAAS's test-retest results are also quite high (r = 0.81). Internal consistency $\alpha = 0.78$ (s = 100), test-retest result 0.83 (s = 78), internal consistency $\alpha = 0.80$, (s = 78) and test-retest correlation result 0.86 were found in the adult sample studies [5].

### Coping Orientation to Problems Experienced (COPE)

The scale developed by Carver *et al*. is a self-report scale consisting of 60 questions and 15 subscales and each subscale consists of four questions. Each of these subscales gives information about a different coping attitude. As a result, the height of the subscales' scores provides the possibility to comment on which coping attitude is more used by the person [7].

### Metacognition Scale Child and Adolescent Form (MCQ-C)

The child and adolescent form of the upper cognition scale was developed by Bacow *et al*. [8] in 2009 and the original name of the scale is "Meta-Cognitions Questionnaire for Children (MCQ-C)". Each item in the MCQ-C is answered on a four-unit Likert-type Rating Scale with "(1) absolutely disagree" and "(4)

absolutely agree" ends. The scores that can be taken from the scale range from 24 to 96 and the rise of the score indicates an increase in negative upper cognitive activity. Sub factors and items covered; positive parent concerns (1, 7, 9, 16, 20, 23), negative parent concerns (2, 4, 8, 10, 13, 18), superstitions, punishment and responsibility beliefs (6, 12, 17, 19, 21, 22) and cognitive monitoring (3, 5, 11, 14, 15, 24) [9].

## Social Support Assessment Scale for Children (CASS)

The scale developed by Dubow and Ullman in 1989 aimed to assess students ' perceptions of the social support they receive from their families, friends, and teachers. On a quintet Likert-type scale, each item gets 1, 2, 3, 4, 5 points. Scale items are grouped into three sub-groups: friend Support (19 items), Family Support (12 items), teacher support (10 items). The score that can be taken from the scale is 41-205. The validity and reliability of the scale in our country was carried out by Gökler [10] in 2007.

## Statistical Analysis

The data was examined by the Shapiro Wilk test whether or not it presents normal distribution. Descriptive statistics are given as mean, standard deviation, frequency, and percentage. Independent samples t-test was used to compare two independent groups for normally distributed data. Pearson Chi-square test, Fisher's Exact Chi-square test, and Fisher-Freeman-

Halton test were used to analyze categorical data. The statistical significance level was determined as $\alpha = 0.05$. Statistical analyses were performed with IBM SPSS ver.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

## RESULTS

The sample's sociodemographic characteristics taken in the study are shown in the Table (Table 1). In the study, 40 adolescents were in the substance use disorder group and 45 adolescents were in the healthy group. No statistically significant difference was found between the patient and healthy groups in terms of age, gender, education level of the parents, the family's income level, place of residence, and presence of chronic physical disease ($p > 0.05$).

On the BAPI-E scale in the group with substance use disorder; substance use characteristics $1.71 \pm 0.63$, diagnosis $7.4 \pm 1.74$, effects on life $7.45 \pm 2.40$, cravings $1.28 \pm 0.78$, motivation $1.7 \pm 0.56$ and it was found to be $6.69 \pm 1.49$ in total.

There was no significant difference between the two groups in terms of MAAS mean scores, MCQ-C total and subscale total scores, COPE problem-oriented coping subscale scores, and COPE emotionally-oriented coping subscale scores. A significant difference was found between the scores obtained by

**Table 1.** Comparison of sociodemographic characteristics between groups

| Variables | Group without substance use disorder (n = 45) n (%) | Group with substance use disorder (n = 40) n (%) | *p* value |
|---|---|---|---|
| Age (years) (mean $\pm$ SD) | 16.51 ± 0.80 | 16.50 ± 0.95 | 0.972[a] |
| Gender (man) | 36 (80%) | 34 (85%) | 0.546[b] |
| Psychiatric ilness in the family (yes) | 2 (4.4%) | 12 (30%) | **0.002[b]** |
| Alcohol use in the family (yes) | 8 (17.8%) | 24 (60%) | **< 0.001[b]** |
| Previous psychiatric treatment (yes) | 5 (11.1%) | 26 (65%) | **< 0.001[b]** |
| Suicide attempt (yes) | 0 (0%) | 9 (22.5%) | **0.001[c]** |
| Smoking (yes) | 21 (46.7%) | 38 (95%) | **< 0.001[b]** |
| Alcohol use (yes) | 10 (22.2%) | 37 (92.5%) | **< 0.001[b]** |

SD = standard deviation

[a]t test, [b]Pearson's chi-square test, [c]Fisher's exact chi-square test

**Table 2. Comparison of total scale scores between groups**

|  | Group without substance use disorder (n = 45) (mean ± SD) | Group with substance use disorder (n = 40) (mean ± SD) | *p* value* |
|---|---|---|---|
| MAAS total | 53.69 ± 13.92 | 49.4 ± 11.52 | 0.146 |
| MCQ-C positive top concerns | 13.00 ± 3.98 | 13.65 ± 4.14 | 0.463 |
| MCQ-C negative top concerns | 16.27 ± 4.33 | 16.38 ± 4.31 | 0.909 |
| MCQ-C superstition punishment and responsibility | 16.36 ± 4.21 | 16.4 ± 4.95 | 0.964 |
| MCQ-C cognitive monitoring | 17.96 ± 3.57 | 15.65 ± 4.29 | **0.008** |
| MCQ-C total | 63.58 ± 9.12 | 62.08 ± 13.51 | 0.555 |
| COPE problem-focused coping | 13.61 ± 2.15 | 12.58 ± 2.89 | 0.083 |
| COPE emotionally focused coping | 13.38 ± 1.84 | 12.99 ± 2.65 | 0.466 |
| COPE dysfunctional coping | 10.15 ± 1.79 | 12.11 ± 2.63 | **< 0.001** |
| CASS friend Support | 2.97 ± 0.43 | 2.83 ± 0.45 | 0.181 |
| CASS family Support | 3.21 ± 0.24 | 3.1 ± 0.44 | 0.207 |
| CASS teacher Support | 2.6 ± 0.49 | 2.47 ± 0.55 | 0.291 |
| CASS total | 8.77 ± 0.90 | 8.40 ± 1.07 | 0.113 |

MAAS = Mindful Attention Awareness Scale, MCQ-C = Metacognition Questionnaire for Children and Adolescents, COPE = Coping Orientation to Problems Experienced, CASS =Child-Adolescent Social Support Scale (CASS), SD = standard deviation
*t test

patients and control groups from the "COPE dysfunctional coping subscales "($p < 0.05$) (Table2).

When the groups with and without substance use disorder were compared in terms of COPE substances, there were statistically significant differences in planning, positive reinterpretation and development, emotional social support use, behavioral disengagement and substance use and denial scores. It was found that the patient group scored lower than the healthy group in terms of planning, positive reinterpretation and development and the use of social-emotional support. The group's score with substance use disorder in terms of behavioral disengagement, substance use and denial was higher than the group without substance use disorder (Table 3).

A significant difference was found in the patients according to the gender in terms of suppression of other occupations, positive reinterpretation and development, use of emotional-social support and COPE problem-focused coping score scale scores ($p < 0.05$). Scale scores of girls were found to be lower than boys.

There is no difference between other scale scores in terms of gender ($p > 0.05$).

A statistically significant difference was found between the patients who attempted suicide and those who did not, only in terms of the MAAS score ($p < 0.05$). MAAS scores of those who attempted suicide were found to be lower. A moderately negative correlation was found between the MAAS and the MCQ-C positive higher concerns. A moderate positive correlation was found between the positive upper anxieties subscale of the MCQ-C and the backward, planning, acceptance, problem-focused coping, emotionally focused coping and dysfunctional coping subscales of COPE. A moderate positive correlation was found between the MCQ-C cognitive monitoring subscale and COPE's plan-making substance. There was a moderately positive relationship between the MCQ-C's total score and the plan-making clause of COPE.

COPE was found to have a moderately positive relationship between active coping, holding back, making plans, using useful social support, suppressing

**Table 3.** Comparison of people with and without substance use disorders in terms of coping attitude substances

| COPE sub items | Group without substance use disorder (n = 45) (mean ± SD) | Group with substance use disorder (n = 40) (mean ± SD) | *p* value* |
|---|---|---|---|
| Active coping | 2.90 ± 0.64 | 2.78 ± 0.73 | 0.446 |
| Restraint | 2.42 ± 0.58 | 2.51 ± 0.77 | 0.576 |
| Planing | 3.09 ± 0.60 | 2.46 ± 0.69 | **< 0.001** |
| Use of instrumental social support | 2.65 ± 0.83 | 2.41 ± 0.88 | 0.228 |
| Suppressing of competing activities | 2.55 ± 0.59 | 2.42 ± 0.57 | 0.334 |
| Positive reinterpretation and growth | 3.31 ± 0.49 | 2.74 ± 0.65 | **< 0.001** |
| Religious coping | 2.91 ± 0.82 | 3.20 ± 0.80 | 0.124 |
| Humor | 2.13 ± 0.86 | 2.29 ± 0.86 | 0.397 |
| Use of emotional social support | 2.74 ± 0.73 | 2.33 ± 0.69 | **0.01** |
| Acceptance | 2.29 ± 0.75 | 2.42 ± 0.66 | 0.434 |
| Behavioral disengagement | 1.56 ± 0.53 | 1.91 ± 0.71 | **0.018** |
| Substance use | 1.59 ± 0.97 | 2.91 ± 0.90 | **< 0.001** |
| Denial | 1.72 ± 0.58 | 2.26 ± 0.84 | **0.002** |
| Mental disengagement | 2.54 ± 0.59 | 2.51 ± 0.57 | 0.792 |
| Focusing on and venting of emotions | 2.74 ± 0.75 | 2.53 ± 0.77 | 0.228 |

COPE = Coping Orientation to Problems Experienced, SD = standard deviation
*t test

other preoccupations, acceptance, denial, problem-oriented coping, emotional-oriented coping, non-functional coping subscales and teacher support. A moderate positive relationship was found in COPE between the useful social support, religious coping, emotional-social support, acceptance and problem-focused coping subscales and the friend support subscale. A strong positive relationship was found with emotionally focused coping. A moderately positive correlation was found between the motivation of BAPI-E and the withdrawal of COPE. A positive correlation was found between the BAPI-E total score and the withdrawal subscale of COPE. A positive correlation was found between useful social support, positive reinterpretation, emotional-social support, sub-scales of COPE and all subscales of CASS. A moderate and positive correlation was found between CASS total scale score and COPE with positive reinterpretation, emotional-social support, problem-focused coping and emotionally focused coping.

## DISCUSSION

Our study investigated the mindfulness levels of adolescents with the substance use disorder and the factors affecting it. Simultaneously, there was no difference in mindfulness levels, perceived social support and metacognition in adolescents with and without substance use disorder. Differences were found in ways of coping with stress. It was found that patients mainly use dysfunctional coping methods more.

However, it was found that metacognitive monitoring decreased in addicted adolescents. Besides, it was also found that planning, emotional-social support use, positive reinterpretation and development characteristics among stress-coping attitudes decreased in adolescents with substance use disorder. The mindfulness levels of the patients who have attempted suicide are lower than those who have not attempted suicide.

When we look at the literature, there is no other study examining the level of the conscious mindfulness in adolescents with the substance use disorder in

our country. A 2014 study by Ögel *et al*. in adults showed that mindfulness levels in substance-using individuals were the same as in the control group and were similar to our study [1]. In the same study, it was stated that these patients might benefit from mindfulness-based therapies due to factors affecting mindfulness [5]. Shapiro *et al*. [11] in people with an increased level of conscious mindfulness, observed that substance use, depressive relapses, and stress levels decreased, immune system functions, empathy skills, motivation, and awareness of emotions increased. Bowen *et al*. [12] in a 2010 study, it was determined that the use of conscious mindfulness in the treatment of substance abuse is an effective method of bringing warmth and flexibility to the program as well as creating an area that supports discovery and development. In 2014, Robinson *et al*. [13] found that those with a high level of conscious mindfulness were associated with a low likelihood of lifetime alcohol or marijuana use. In the study conducted by Dabaghi *et al*. [14] in 2016; With the multidimensional prevention program designed to prevent substance use by adolescents, it has been proved that life skills (coping with stress, decision making, self-awareness), the individual's need to protect his body and positive attitudes towards substance use decrease, and as a result, it has been proved to be effective in preventing substance addiction. In two different studies conducted in 2006, it was found that impulsivity, problems with upper cognition and conscious suppression of thoughts were more significant in the addicted group. All these factors have been linked to low mindfulness levels in other studies [15, 16].

In the studies that examined the relationship between the mindfulness levels of drug addicts and suicide attempts, it was found that 25% of people with suicide attempts had alcohol abuse or substance abuse [17, 18]. Suicide rates have been shown to increase in direct proportion in countries with increased alcohol use [19]. In our study, parallel with the literature, mindfulness levels of the patients with substance use disorder and suicide attempts in their past were lower than those who did not have suicide attempts in their past.

In the studies in the literature, in adolescents with substance use disorder; emotionally focused coping styles are a risk factor; problem-focused coping styles;

It has been reported to be protective for alcohol addiction, depression and suicide attempts [20, 21]. Our study also supports the literature; A moderate and positive correlation was found between emotional-social support use, problem-focused coping and emotionally focused coping.

## Limitations

There are some limitations in our study. The small number of the sample, the parents' educational status, the economic status, the educational status of the adolescent make it difficult to generalize. Since the drugs used by the patients during the study were not evaluated, their relationship with clinical evaluation scales and cognitive functions could not be examined.

## CONCLUSION

As a result, substance use disorder is an increasing public health problem. We think that the application of therapies that will increase the level of conscious mindfulness, ways of coping with stress (especially functional coping methods), planning, use of emotional-social support, positive reinterpretation and improvement of development characteristics may be more effective in the treatment of substance use disorder. We believe that the number of psychological, biological and treatment effectiveness studies investigating the level of mindfulness of substance use among adolescents should increase.

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# Complications of upper urinary system laparoscopic surgery: a single center experience with 942 cases

**Mithat Ekşi[1]**, **Selçuk Şahin[2]**, **Nevzat Can Şener[3]**, **Kamil Gökhan Şeker[4]**, **İsmail Evren[2]**, **Serdar Karadağ[2]**, **Abdullah Hızır Yavuzsan[5]**, **Deniz Noyan Özlü[2]**, **Volkan Tuğcu[6]**

*[1]Department of Urology, Arnavutköy State Hospital, İstanbul, Turkey*
*[2]Department of Urology, Bakırköy Dr. Sadi Konuk Training and Research Hospital, İstanbul, Turkey*
*[3]Department of Urology, Adana City Training and Research Hospital, Adana, Turkey*
*[4]Department of Urology, Muş State Hospital, Muş, Turkey*
*[5]Department of Urology, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey*
*[6]Department of Urology, Memorial Bahçelievler Hospital, İstanbul, Turkey*

## ABSTRACT

**Objectives:** Since the introduction of laparoscopic nephrectomies, laparoscopic surgeries in the field of urology have become increasingly popular. Laparoscopic surgery has its advantages but carries the risk of complications like all interventions. In our study, we aimed to discuss our complication rates according to difficulty level by presenting our experiences with urological laparoscopic procedures for the upper urinary system.
**Methods:** This retrospective study includes 942 laparoscopic urological procedures performed by a single surgeon. The procedures divided into three groups according to the European Scoring System (ESS) Classification. The complication rate of each group was calculated separately.
**Results:** A total of 127 (13.4%) complications were observed. Partial nephrectomy, nephroureterectomy and ureterolithotomy had the highest complication rates. Renal cyst excision, simple nephrectomy and radical nephrectomy had the lowest complication rates. According to the Clavien Complication Classification, the distributions of Grade 1, 2 and 3 complications were 29.1% (n = 37), 57.4% (n = 73) and 13.3% (n = 17); respectively. The open conversion rate was 0.84%. When procedures were classified as "easy", "difficult" and "very difficult" according to the ESS classification; complication rates were found 7.3% in the easy group, 13.3% in the difficult group and 16.6% in the very difficult group. There was no significant difference between the three groups in terms of complication rates ($p = 0.329$).
**Conclusions:** Performing easier operations according to ESS in the first years of the learning curve is beneficial in preventing complications. With increasing experience, more complicated procedures can be performed with similar complication rates.
**Keywords:** Laparoscopy, urology, intraoperative complications, postoperative complications

Since the introduction of laparoscopic nephrectomies, laparoscopic surgeries in the field of urology have become increasingly popular and they are now routine procedures in many clinics [1]. Laparo- scopic surgery has its advantages, but carries the risk of complications like all interventions. Using common standardized systems for grading and recording surgical complications allows comparing operations per-

formed in different centers with different techniques to more meaningful criteria [2]. For this purpose, a grading system in 1992 by Clavien that allows classification of complications and this system was updated in 2004 by the same group [3]. The European Scoring System (ESS) is used to assess the degree of difficulty of laparoscopic surgeries [4].

In the literature, the articles reporting complications of laparoscopic urological procedures and investigating the factors associated with complications were published [5-13]. Many parameters have been associated with perioperative complications, such as the surgeon's experience [12-15]. Performing challenging laparoscopic procedures has increased the experience and therefore the subject should be reevaluated. In our study, we aimed to present our experience with consecutive 942 urological laparoscopic procedures for the upper urinary system.

## METHODS

After getting the approval of the Local Ethics Committee (2014/225); data from 942 laparoscopic surgical procedures for the upper urinary tract between December 2006 and January 2016 were evaluated. These surgeries were performed by a single surgeon (V.T). Outcomes were prospectively collected and retrospectively evaluated.

All patients received preoperative antibiotics and prophylaxis for deep vein thrombosis (compression stockings, subcutaneous low molecular weight heparin). A nasogastric tube and urethral catheter were placed at the beginning of the operation. A veress needle or Hasson technique was used to achieve pneumoperitoneum for the transperitoneal approach. Open access or balloon dilation was performed for the retroperitoneal approach.

The procedures divided into three groups according to the ESS Classification; renal cyst excision was regarded as "easy"; pyeloplasty, simple and radical nephrectomy, nephroureterectomy, ureterolithotomy and pyelolithotomy operations were regarded as "difficult", partial and donor nephrectomy operations were regarded as "very difficult" [4]. The complication rate of each group was calculated separately. Perioperative bleeding was defined as bleeding requiring blood transfusion. Open conversion was excluded from complications and analyzed separately. Written informed consent was taken from all patients.

## Statistical Analysis

Chi Square or Fisher exact test were used for categorical variables. Two-tailed $p$ values $< 0.05$ was considered as statistically significant. Data are shown as mean $\pm$ SD. Data were analyzed using Statistical Package for Social Sciences (SPSS) 20 software (SPSS Inc., Chicago, IL, United States).

## RESULTS

The mean age of the 942 patients included in the study was $43.7 \pm 14$ years. The study included 576 (61.1%) male and 366 (38.8%) female patients. The mean BMI was $25.1 \pm 2.3$ kg/m2, the mean operation time was $125.7 \pm 43.6$ minutes and the mean blood loss was $85.3 \pm 54.9$ cc. The duration of hospital stay was $3.5 \pm 1.4$ days. Data for all patients are given in Table 1. Procedures included pyeloplasty (n = 114, 12.1%), ureterolithotomy (n = 223, 23.6%), pyelolithotomy (n = 55, 5.8%), simple nephrectomy

**Table 1. Patients characteristics, peroperative and post operative datas**

| Parameters | mean ± SD or n (%) |
|---|---|
| Age (years) | 43.7 ± 4.7 |
| Male gender | 576 (61.1) |
| BMI (kg/m$^2$) | 25.1 ± 2.3 |
| Side (right/left) | 451 (47.88)/491 (52.12) |
| Retroperitoneal approach | 380 (40.3) |
| ASA | 1.5 ± 0.5 |
| CCI | 0.7 ± 0.9 |
| Operation time (min) | 125.7 ± 43.6 |
| EBL (cc) | 85.3 ± 54.9 |
| LOS (day) | 3.5 ± 1.4 |
| ESS | |
|     Easy | 41 (4,3) |
|     Diffucult | 787 (83.5) |
|     Very diffucult | 114 (12.1) |

SD = Standart Deviation, BMI = Body Mass Index, ASA = American Society of Anesthesiologists Score, CCI = Charlson Comorbidity Index, EBL = Estimated Blood Loss, LOS = Length of Stay, ESS = European Scoring System

**Table 2. Number of procedures and complications.**

| Procedure | ESS | CDCC | | | |
|---|---|---|---|---|---|
| | n (%) | Grade 1 | Grade 2 | Grade 3 | Total n (%) |
| Renal Cyst Resection | 5 | 41 (4.3) | 0 | 3 | 0 | 3 (7.3) |
| Ureterolitotomy | 7 | 223 (23.6) | 7 | 16 | 12 | 35 (15.6) |
| Pyelolithotomy | 7 | 55 (5.8) | 2 | 5 | 2 | 7 (12.7) |
| Simple Nephrectomy | 10 | 150 (15.9) | 0 | 13 | 1 | 14 (9.3) |
| Nephroureterectomy | 12 | 25 (2.6) | 1 | 4 | 0 | 5 (20) |
| Pyeloplasty | 13 | 114 (12.1) | 8 | 7 | 2 | 17 (14.9) |
| Radical Nephrectomy | 13 | 220 (23.3) | 0 | 22 | 5 | 27 (12.2) |
| Partial Nephrectomy | 15 | 59 (6.2) | 5 | 5 | 2 | 12 (20.3) |
| Donor Nephrectomy | 16 | 55 (5.8) | 0 | 7 | 0 | 7 (12.7) |

ESS = European Scoring System, CDCC = Clavien Dindo Complication Classification

(n= 150, 15.9%), radical nephrectomy (n= 220, 23.3%), donor nephrectomy (n= 55, 5.8%), nephroureterectomy (n= 25, 2.6%), partial nephrectomy (n= 59, 6.2%) and renal cyst excision (n= 41, 4.3%). Retroperitoneal approach was applied to 40.3% of the cases.

A total of 127 (13.4%) complications were observed. Partial nephrectomy, nephroureterectomy and ureterolithotomy had the highest complication rates, with rates of 20.3%, 20% and 15.6%, respectively. Renal cyst excision, simple nephrectomy and radical nephrectomy had the lowest complication rates, with rates of 7.3%, 9.3% and 12.2%, respectively. The distributions of the cases and the complication rates are shown in Table 2. According to the Clavien Complication Classification, the distributions of Grade 1, 2 and 3 complications were 29.1% (n = 37), 57.4% (n = 73) and 13.3% (n = 17) respectively. Grade 4 and 5 complications were not observed in any of the cases [3]. Data about complications are listed in Table 3.

The open conversion rate was 0.84% (n = 8); during pyeloplasty (n = 2), partial nephrectomy (n = 2), pyelolithotomy (n = 2), radical nephrectomy (n = 1) and ureterolithotomy (n = 1). There was no significant difference in the open conversion rates between the transperitoneal and retroperitoneal approaches. Open conversion was performed in two cases during partial nephrectomy due to massive bleeding; in five cases in pyeloplasty, pyelolithotomy and ureterolithotomy, due to technical difficulties, and in one case in radical nephrectomy due to massive perirenal adhesions.

Pyelolithotomy and partial nephrectomy had the highest open conversion rates with 3.6% and 3.3%, respectively.

The patients were divided into two groups according to the presence of complications. Operation time, estimated blood loss, open conversion rate and length of hospital stay were significantly higher in patients who had complications. In univariate and multivariate analyzes, none of the parameters could predict the presence of complications.

When procedures were classified as "easy", "dif-

**Table 3. Complications**

| Complications | n (%) |
|---|---|
| Fever | 37 (3.9) |
| Ileus | 34 (3.6) |
| Ureteral stent requirement | 12 (1.2) |
| Port side infection | 9 (0.9) |
| Prolonged drainage | 8 (0.8) |
| Peritoneal tear | 7 (0.7) |
| Secondary UPJ stricture | 4 (0.4) |
| Gonadal vein injury | 4 (0.4) |
| Liver injury | 4 (0.4) |
| Subcutaneous emphsema | 3 (0.3) |
| Colonic injury | 3 (0.3) |
| Vertebrale artery injury | 1 (0.1) |
| Renal vein Injury | 1 (0.1) |

UPJ = Uretero-pelvic junction

ficult" and "very difficult" according to the ESS classification; complication rates were found 7.3% in the easy group, 13.3% in the difficult group and 16.6% in the very difficult group. There was no significant difference between the three groups in terms of complication rates ($p = 0.329$).

## DISCUSSION

Many complications have been published in the urology field since the definition of laparoscopic surgery. Complications of laparoscopic urological procedures rates are variable and were given in the literature from 4.4% to 23.1% [6-16]. In their early laparoscopic experience, Parra et al. [5] reported their rate of complications 15%. Soulie et al. [8] found an overall complication rate of 6.9% in a large study with 1.085 cases. In parallel with the increasing number of performed more complex urological laparoscopic surgery, published complication rates have increased. Vallancien et al. [9] reported a complication rate as 22% and an open conversion rate as 1.2%. Sanli et al. [10] reported a 23.1% rate of overall complications. In our study, we have found a 13.4% rate of total complications with 10.9% and 2.5% of minor and major complication rates, respectively. Our open conversion rate was 0.84%.

Guillonneau et al. [4] have defined a novel scoring system that classifies all laparoscopic procedures according to their difficulty and complexity. The ESS is the most effective scoring system to provide the integrity of a scientific common language and to standardize the results of the studies about laparoscopic urologic procedures. Inoue et al. [11] divided each procedure into three groups according to the ESS. For the easy, difficult, and very difficult groups; the complication rates were 0%, 13.2% and 12.8%, respectively. They demonstrated that the complication rate was significantly low in the easy group and that the nephroureterectomy had the highest rate [11]. In our study, the procedures with the highest complication rates were partial nephrectomy, nephroureterectomy and ureterolithotomy. When the groups were separated as "easy", "difficult" and "very difficult", no significant difference was observed in complication rates.

It has been found that simpler procedures such as renal cyst excision or simple nephrectomy were gen-erally performed in the first years of the learning curve, and more complicated operations like donor nephrectomy or partial nephrectomy were performed with the increasing experience over the years. With this result, we thought that, despite more difficult procedures like partial nephrectomy or nephroureterectomy having higher complication rates, the complication rates were similar between three divided groups due to performing more complicated operations with more increased experience over the years. In the light of this analysis; we think that the complication rates can be kept low by preferring easier operations in terms of ESS score in the first year of learning curves.

Studies have shown that the presence of a high American Society of Anesthesiologists (ASA) score is associated with high complication rates [10-13, 17]. There was no relationship between any parameters with complications or open conversions in our study. We have found that the complication and open conversion rates were similar in transperitoneal and retroperitoneal procedures [18].

The main strengths of our study are the presentation of a large series, including all patients treated by a single surgeon from the beginning of the learning curve. The most important limitation is the retrospective nature.

## CONCLUSION

Performing easier operations according to ESS in the first years of the learning curve is beneficial in preventing complications. With increasing experience, more complex procedures can be performed with similar complication rates, but complication rates are still high in "very difficult" operations such as partial nephrectomy and nephroureterectomy.

*Conflict of interest*

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# Sleep disorders and relationship with comorbid anxiety and depression in carpal tunnel syndrome

**Şükran Çevik Yurtoğulları[1]**, **Meral Seferoğlu[2]**

[1]*Department of Neurology, University of Health Sciences, Ankara Gaziler Physical Therapy and Rehabilitation Training and Research Hospital, Ankara, Turkey*
[2]*Department of Neurology, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey*

## ABSTRACT

**Objectives:** In this study, it was aimed to assess relationship of disease severity with sleep quality, depression and anxiety in patients with carpal tunnel syndrome (CTS) and to compare findings with healthy controls.
**Methods:** The study included 35 patients diagnosed as CTS and 20 healthy individuals. In CTS patients, symptom severity and functional disorders were assessed using Boston Carpal Tunnel Questionnaire (BTCQ). The sleep quality was assessed using Pittsburg Sleep Quality Index (PSQI) in CTS patients and healthy controls. The Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) were used to evaluate depression and anxiety accompanying to CTS and sleep disorder.
**Results:** In the assessment of general sleep quality, it was found that PSQI scores were significantly higher in CTS group when compared to healthy controls ($p < 0.0001$). Again, the BDI and BAI scores were also significantly higher in CTS group than control group ($p < 0.0001$ and $p < 0.003$, respectively). There was a strong, positive correlation between BTCQ symptom severity scale (SSS) and PSQI (r = 0.774; $p < 0.0001$). A significant, positive correlation was observed between Boston SSS and BDI scores (r = 0.666; $p < 0.001$). When assessed correlation between anxiety scores and Boston SSS and functional state scale (FSS), it was found that anxiety was increased by increasing severity of CTS, indicating a positive, moderate correlation (r = 0.469, $p < 0.0001$ and r = 0.464, $p < 0.0001$).
**Conclusions:** The pain in patients with CTS can impair sleep quality, which, in turn, may increase risk for depression and anxiety. Thus, comorbid psychiatric symptoms and signs should be considered in the treatment of patients with CTS.
**Keywords:** Carpal tunnel syndrome, depression, anxiety, sleep disorder

**T**he carpal tunnel syndrome (CTS) is the most commonly seen entrapment neuropathy which results from compression of median nerve at level of wrist as it travels through carpal tunnel [1, 2]. Paresthesia at dermatome of median nerve is typical in CTS. It is often seen in middle-aged women and its prevalence confirmed by clinical examination and electroneuromyography (ENMG) is 2.7% in general population. It is seen that pain and paresthesia (numbness, tingling) are classically enhanced at night time in 80% of patients and that these complaints are relieved by repositioning or shaking hands [3-5].
Although the diagnosis is supported by Tinel's test and Phalen's test, the definitive diagnosis is made using

electrophysiological methods [6-8]. Since CTS symptoms are aggravated during night, it often causes sleep problems. Patients generally awake due to pain and paresthesia and attempt to relieve pain by shaking hands.

Sleep disorders can cause complaints such as concentration difficulty, mood disorder and increased pain, evolving to an important problem that negatively affects daily life of individual. The relationship between diseases and sleep has been investigated in many studies and it was shown that disorders characterized by pain affect sleep quality [9, 10].

In this study, it was aimed to compare depression, anxiety and sleep quality in patients diagnosed as CTS using ENMG with healthy controls and to identify severity of nerve injury on ENMG and depression, anxiety and sleep quality.

## METHODS

### Study Population

The study included 35 patients (aged 18-50 years) who presented to neurology outpatient clinic of Bursa Yuksek Ihtisas Training and Research Hospital and diagnosed as CTS with clinical findings and ENMG results according to American Association of Electrodiagnostic Medicine (AANEM) criteria between 2018 and December, 2019. In addition, 20 healthy controls were included to the study.

American Academy of Neurology criteria were used [11]. These criteria include: 1) Pain and paresthesia in the symptomatic hand at nights; 2) Sensational complaints during flexion and extension movements of wrist; 3) Pain and paresthesia in the morning; 4) Phalen's test positivity, 5) Sensation loss in median nerve innervation area; 6) Atrophy and weakness in median nerve innervated muscles. The patient was accepted to have CTS in the presence of at least one criterion.

The patients with systemic disease, B12 deficiency, cervical radiculopathy or rheumatoid disease, those with history of hand-wrist trauma or surgery, and those underwent corticosteroid injection to carpal tunnel area within prior 6 months were excluded. The study was approved by Ethics Committee of Bursa Yuksek Ihtisas Training and Research Hospital (approval#: 13.02.3030-E1-20-155). All patients and controls gave written informed consent. In all patients included, detailed history was taken and physical and neurological examinations were performed.

### Electroneurophysiological Assessment

All electroneurophysiological evaluations were performed by a single researcher and ENMG results were recorded. CTS staging was performed using Nihon Kohden Neuropack-S1 electromyography system (Tokyo, Japan) according to AANEM criteria [12].

Both hands were evaluated in all patients. Based on electroneurophysiological assessment, the patients were classified as follows:

Mild CTS: Prolonged distal sensory nerve conduction velocity and/or sensory nerve action potential amplitude below normal in median nerve.
Moderate CTS: Prolonged distal motor nerve action potential latency in median nerve in addition to above.
Severe CTS: Lack of sensory nerve action potential and decreased amplitude or delayed latency of motor response.

### Boston Carpal Tunnel Questionnaire

The Boston Carpal Tunnel Questionnaire (BTCQ) was used to assess symptom severity and functional disorders in the patients. The BTCQ was used to assess symptoms inn CTS patients, which includes 11 items and maximum score of 55 points. The BTCQ Turkish validity and reliability were proven [13]. There are two sub-domains in the questionnaire. The symptom severity scale includes 11 items rated by 5-points scale. Mean score was calculated by dividing total score into number of questions (mean score: 1-5 points). The functional status scale included 8 items rated by 5-points scale. Again, mean score was calculated by dividing total score into number of questions (n = 8). In both scale, maximum score was 5. Higher scores indicate higher severity of symptoms and disability.

### Pittsburgh Sleep Quality Index

The sleep quality was assessed using Pittsburgh Sleep Quality Index (PSQI) in CTS patients and controls. The PSQI provides a quantitative measure of sleep quality which differentiates good and poor sleep. The Turkish validity and reliability were studied by Agargun et al. [15] in 1996. It includes 24 items ad 7

sub-domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. Each item was rated by 0-3 points scale. Total score of 7 sub-domains provides PSQI score (range: 0-21). Higher total PSQI score indicates poorer sleep quality. PSQI score ≥ 5 indicates poor sleep quality [16].

### Beck Depression Inventory

The Beck Depression Inventory (BDI) was developed to assess depression by Beck *et al*. [17]. The BDI aims to measure depressive measures and higher total scores indicate severity of depression level. It included 21 self-rated items and maximum total point is 63. The Turkish validity and reliability were proven, and cut-off point was identified as 17. In our study, 0-9 points indicates minimal depression, 10-16 points mild depression, 17-29 points moderate depression and 30-63 points severe depression [18].

### Beck Anxiety Inventory

The Beck Anxiety Inventory (BAI) was developed to anxiety symptoms by Beck *et al*. [19] and Turkish validity and reliability study was performed by Ulusoy *et al*. [20]. It is a self-rated questionnaire. It is used to determine frequency of anxiety symptoms experienced. There are 4 choices in 21 items. Each item is rated by 0-3 points. Higher scores indicate severity of anxiety.

### Statistical Analysis

The statistical analyses were performed using SPSS (Statistical Package for Social Sciences) for Windows version 20. The normal distribution was assessed using Shapiro-Wilk test. For continuous variables, descriptive statistics are presented as mean, standard deviation, minimum and maximum. For data with skewed distribution, Mann Whitney U test was used to compare continuous variables while Chi-square test was used to compare discrete variables. Coefficient of correlation was used to assess relationships between data with skewed variables or ordinal variables and statistical significant was calculated using Spearman's test. A *p* value < 0.05 was considered as statistically significant.

## RESULTS

In our study, mean age was found as 46.1 ± 11.4 years in the CTS group and 40.6 ± 10.5 years in the control group. There was no significant difference in age between groups (*p* = 0.073). There were 27 women (77.1%) and 8 men (22.9%) in the CTS group while 17 women (85.0%) and 3 men (15.0%) in the control group (Table 1).

Mean CTS severity was calculated as 29.71 ± 6.84

**Table 1.** Demographic and clinical characteristics

|  | Total (n = 55) | CTS group (n = 35) | Control Group (n = 20) |
|---|---|---|---|
| **Age (years)** | 48.0 ± 14.5 | 46.1 ± 11.4 | 40.6 ± 10.5 |
| **Gender, n (%)** |  |  |  |
| **Female** | 44 (80.0) | 27 (77.1) | 17 (85.0) |
| **Male** | 11 (20.0) | 8 (22.9) | 3(15.0) |
| **Education, n (%)** |  |  |  |
| **Primary School** | 14 (25.5) | 10 (28.6) | 4 (20.0) |
| **High School** | 17 (30.9) | 10 (28.6) | 7 (35.0) |
| **University** | 24 (43.6) | 15 (42.9) | 9 (45.9) |
| **Dominant side, n (%)** |  |  |  |
| **Right** | 49 (89.1) | 31 (88.6) | 18 (90.0) |
| **Left** | 6 (10.9) | 4 (11.4) | 2 (10.0) |

Continuous data are presented as mean ± standard deviation whereas discrete data are presented as frequency (%). CTS = Carpal tunnel syndrome

for Boston SSS whereas $17.60 \pm 7.62$ for Boston FSS. According to electrodiagnostic evaluation, 8 patients diagnosed as severe CTS while 16 patient as moderate CTS and 11 patients as mild CTS. In the CTS group, mean symptom duration was $13.09 \pm 10.8$ months. There was unilateral involvement in 16 patients (45.7%) whereas bilateral in 19 patients (54.3%). In patients with CTS, most common symptom was numbness detected in 14 patients (40.0%) while pricking-tingling in one patient (2.9%) (Table 2).

In the assessment of sleep quality, PSQI scores were found to be significantly poorer in patients with CTS compared to controls ($p < 0.0001$). Again, BDI and BAI scores were also significantly higher in CTS patients compared to controls ($p < 0.001$ and $p < 0.003$, respectively) (Table 3).

There was a significant strong, positive correlation between Boston SSS and PSQI (r = 0.774; $p < 0.0001$). In addition, there was a significant strong, positive correlation between Boston FSS and PSQI (r = 0.666; $p < 0.0001$). It was found that there was significant, moderate, positive correlation between

Boston SSS and depression scores (r = 0.514; $p < 0.0001$). When assessing correlation between anxiety scores and Boston SSS and FSS scores, it was found that anxiety was increased by enhancing CTS severity, indicating a significant, moderate, positive correlation (r = 0.469, $p < 0.0001$; r=0.464, $p < 0.0001$). The numbness was the most common symptom in CTS patients. Mean PSQI score was $13.7 \pm 4.02$ in CTS patients with numbness whereas $16.6 \pm 2.6$ in CTS patients without numbness. A significant difference was fund in PSQI scores between CTS patients with or without numbness ($p < 0.045$) (Table 4).

## DISCUSSION

Our results demonstrate that sleep quality is significantly impaired in patients with CTS. In our study, we found that CTS symptom severity significantly affected sleep quality and that there was a significant correlation between symptom severity and scores of depression and anxiety as well.

In the CTS, most important feature is pain and paresthesia which awake patient from sleep. To relieve these symptoms, the patients are awakening at night and shake or flail their hands which are distinctive for CTS [21]. In previous studies, it was shown that sleep was disturbed more commonly in CTS patients compared to healthy individuals and that paresthesia is the major factor for disturbed sleep [22, 23].

In CTS, the increased pain at night triggers sleep disturbance in the patient, affecting sleep quality. Impaired sleep quality has direct effects on daily living activities and emotional status [24, 25]. Thus, quality of life is affected by both pain itself and resultant impairments in sleep quality and emotional status in the patients [26]. In addition, it was found that sleep quality is further impaired by increasing CTS severity in our study, as similar to the study by Geoghegan *et al*. [27].

In a similar study using Brief Pain Inventory to evaluate pain, Nicholson and Verma reported that severity of pain was correlated to sleep disturbance [28]. Thus, it is important to question pain upon presentation in these patients. It is also important to determine severity of pain and treat pain in CTS patients.

In previous studies, it was found that depression and anxiety prevalence were higher in CTS patients

**Table 2.** Comorbid symptom characteristics and disease severity in CTS patients

|  | CTS Group (n = 35) |
| --- | --- |
| **Hand involved, n (%)** |  |
| **Right** | 11 (31.4) |
| **Left** | 5 (14.3) |
| **Bilateral** | 19 (54.3) |
| **Comorbid symptom, n (%)** |  |
| **Pain** | 3 (8.6) |
| **Numbness** | 14 (40.0) |
| **Burning sensation** | 2 (5.7) |
| **Pricking, tingling** | 1 (2.9) |
| **Cramp** | 9 (25.7) |
| **Pain + Numbness + Cramp** | 4 (11.4) |
| **EMG, n (%)** |  |
| **Mild CTS** | 11 (31.4) |
| **Moderate CTS** | 16 (45.7) |
| **Severe CTS** | 8 (22.9) |

Data are shown as frequency (%). CTS = Carpal tunnel syndrome, EMG =Electomyography

**Table 3.** Comparison of groups regarding Pittsburgh sleep quality index total score, depression and anxiety

|                       | CTS (n = 35)   | Control (n = 20) | *p* value   |
|-----------------------|----------------|------------------|-------------|
| Total Pittsburgh score | 16.0 ± 3.20   | 10.4 ± 2.06      | **< 0.0001** |
| BDI score             | 13.45 ± 9.13   | 4.45 ± 2.75      | **< 0.0001** |
| BAI score             | 13.02 ± 11.97  | 4.90 ± 4.12      | **< 0.003** |

Data are shown as mean ± standard deviation. BAI = Beck anxiety inventory, BDI = Beck depression inventory, CTS = Carpal tunnel syndrome

**Table 4.** Correlation of Boston SSS and FSS scores with PSQI, depression and anxiety scores

|              | PSQI          | Beck Depression | Beck Anxiety   |
|--------------|---------------|-----------------|----------------|
| **Boston SSS** | r = 0.774   | r = 0.666       | r = 0.469      |
|              | *p* < 0.0001  | *p* < 0.0001    | *p* < 0.0001   |
| **Boston FSS** | r = 0.672   | r = 0.514       | r = 0.464      |
|              | *p* < 0.0001  | *p* < 0.0001    | *p* < 0.0001   |

PSQI = Pittsburgh Sleep Quality Index, SSS = symptom severity scale, FSS = functional state scale

compared to general population [29, 30]. In agreement with literature, anxiety and depression scores were found to be higher in patients with high CTS symptom severity score in our study. In another study, it was shown that there was a correlation between pain and anxiety levels regardless of pain severity [31]. It has been thought that impaired sleep quality and accompanying findings such as numbness, tingling, pain, muscle cramps and burning sensation predispose to anxiety. The finding that there was a positive correlation between impaired sleep quality by increasing sleep quality and scores of anxiety and depression in ENMG results also supports this concept. The previous studies showed that depression is most common psychiatric disorder accompanying to neuropathic pain. In addition, pain and depression may trigger each other. The pain predisposes to depression while resultant depression lowers pain threshold and pain tolerance [32]. It has been suggested that the likelihood for exaggerated symptoms and seeking behavior for medical attention and   altered pain perception in the presence of comorbid psychiatric disorders such as depression and anxiety is higher and that these patient have less flexibility to cope these diseases [33-35].

In diseases such as CTS where pain and paresthesia is prominent, the management generally focuses on treatment of pain. In most instances, comorbid sleep disorders, depression and anxiety aren't taken into consideration. The treatment of comorbid psychiatric disorders using short and simple scales such as BDI or BAI will improve success of pain management and quality of life in the patient. Adopting a wider perception before onset of vicious cycle including pain, sleep and psychiatric complaints and chronic disease will improve success of clinician and allow selecting appropriate treatment in the patient.

### Limitations

This study has some limitations including lack of structured psychiatric interview to determine whether patient has any psychiatric diagnosis, failure to use objective sleep assessment tools and relatively smaller sample size.

### Strengths

The strengths of study include assessment of psychological and physical factors together and presence of healthy control group.

## CONCLUSION

In conclusion, the risk for impaired sleep quality, depression and anxiety accompanying to pain is found

to be higher in CTS patients compared to healthy individuals. There is ease to used scales to detected comorbid condition in the management of CTS patients. The use of such scales in routine practice will improve treatment success and quality of life in the patients. There is a need for further studies using objective sleep assessment techniques and more detailed psychiatric interviews.

*Authors' Contribution*

Study Conception: ŞÇY; Study Design: ŞÇY; Supervision: ŞÇY, MS; Funding: ŞÇY, MS; Materials: ŞÇY; Data Collection and/or Processing: ŞÇY; Statistical Analysis and/or Data Interpretation: ŞÇY, MS; Literature Review: ŞÇY, MS; Manuscript Preparation: ŞÇY and Critical Review: ŞÇY, MS.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Landmark guided internal jugular vein catheterization in infants undergoing congenital heart surgery

Serkan Seçici®

*Department of Cardiovascular Surgery, Division of Pediatric Heart Surgery, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey*

## ABSTRACT

**Objectives:** Central venous catheterization is an important procedure for infants undergoing heart surgery. Ultrasound-guided methods have been shown to decrease failure and complications. The goal of the present study was investigate the effect of anatomical landmark technique during internal jugular vein access in low-weight infants undergoing congenital heart surgery.

**Methods:** A total 98 infants (median age was 16.5 days, median weight was 3275 g) who underwent internal jugular vein catheterization between January 2017 and October 2019 were retrospectively analyzed. All catherizations were performed by a pediatric heart surgeon. Data including demographic characteristics of the infants, success rate, and catheter-related adverse events were recorded.

**Results:** Success rate was 94.9% (n = 93). There were three arterial puncture (3.1%) and hematoma occurred in five patients (5.1%). Central venous catheterization of 78 (79.6%) patients were successful at the first attempt.

**Conclusions:** Our study suggest that landmark technique still seems useful by experienced operators and is safe and effective in infants weighing less than 5 kg undergoing congenital heart surgery.

**Keywords:** Central venous catheterization, internal juguler vein, infants

Central venous catheterization has vital importance during congenital heart surgery as well as post-operative care. Two techniques were defined for placement of central venous catheters (CVC): the traditional "landmark" approach or an ultrasound (US)-guided approach. The ultrasound guidance is recommended for locating the vascular structures and guiding the venous puncture, both in adults and in children. The use of US has been shown to increase the rate of success and reduce complications in many studies in which CVCs were placed by non-surgeons [1-5].

However, many surgeons continue to use the anatomical landmark (LM) technique rather than US-guidance, because three-dimensional, anatomy-based procedures constitute a significant part of the training process for surgeons, enabling them to be more facile with anatomy-based techniques for insertion of percutaneous CVCs compared with practitioners trained in other specialties [6].

Central venous catheter placement in neonates and small infants is considered a more challenging procedure. In such cases, vein size is small, advancement of the guidewire is often troublesome and puncture area is limited.

In the present study, we aimed to evaluate the clin-

ical effectiveness and safety of internal jugular vein (IJV) catheterization performed by a pediatric cardiovascular surgeon using LM technique in newborns and infants weighing less than 5 kg.

## METHODS

The study protocol was approved by the Local Ethical Review Committee. All neonates and infants with weight below 5 kg who had IJV catheterization due to congenital heart disease between January 2017 and October 2019 were retrospectively analyzed. Data including demographic characteristics of the infants such as age, gender, and body weight, catheter-related adverse events, surgical procedural details were recorded.

All cannulations were performed by a single pediatric cardiovascular surgeon under general anesthesia, after intubation in the operating room. Patients in this study were consecutive cases of central venous cannulation by the author.

Standard monitoring (electrocardiogram, blood pressure, and pulse oximeter) were applied to all patients. After orotracheal intubation, the patient was positioned with rolled towel under the shoulder with head slightly toward the left side (no more than 40°) (Fig. 1). The neck is fixed in more extension than normal position which helps to expose Sedillot's triangle, approximate the IJV to skin and, enables puncture of proximal IJV. For IJV cannulation, there are three approaches consisting of anterior, central, and posterior. In this study, central approach was preferred. The surface anatomy is comprised of the borders of Sedillot's triangle formed by sternal and clavicular heads of the sternocleidomastoid muscle , and the superior border of the medial third of the clavicle inferiorly [7]. The internal jugular vein lies posterior to the Sedillot's triangle. Cutaneous puncture was done at the midpoint of this triangle, directed toward the ipsilateral nipple at an angle 40-45° with the skin. Once a flash of blood encountered, standard Seldinger technique was followed.

A pass of the needle through the skin was defined as a single puncture attempt. Failure was described as a procedure where the operator could not place the catheter into the IJV, or venous flash could not be provided after 3 attempts. More than 3 attempts were not performed as complication rates are highest with more than 3 cannulation attempts [8].

The number of attempts, success rate, incidence of complications (i.e., arterial puncture, hematoma, pneumothorax, hemothorax, malposition of the catheter) were recorded.

### Statistical Analysis

Statistical analysis was performed using the SPSS for Windows version 22.0 software (SPSS

Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test the normal distribution of data. Descriptive data were expressed in mean ± standard deviation (SD), medians and number and frequency (%). Correlations between variables were tested by the chi-square analysis and Fisher's exact test. The influence of relevant variables on binary data was investigated by the logistic regression model. A $p$ value of < 0.05 was considered statistically significant.



**Fig. 1.** **Position of patient and Sedillot's triangle**

## RESULTS

The patients' weight ranged from 980 to 4999 gr with a median of 3275 gr (25%-Quartile: 2700 gr; 75%-Quartile: 4000 gr). The weight was not normally distributed (Kolmogorov–Smirnov test: $p < 0.01$). 21 patients (21.4%) were under 2501 gr. Median age was 16.5 days (2-315). 61 patients (62.2%) were in neonatal period, and 37 patients (37.8%) were older than 30 days (Table 1).

**Table 1. Demographic characteristics of patients (n = 98)**

| Characteristics | n | % |
|---|---|---|
| Age (days) | | |
| ≤ 30 | 61 | 62.2 |
| > 30 | 37 | 37.8 |
| Gender | | |
| Male | 55 | 56.1 |
| Female | 43 | 42.8 |
| Weight (g) | | |
| ≤ 2500 | 21 | 21.4 |
| 2501-4999 | 77 | 78.6 |

Successful cannulation of the IJV was 94.9% (93 of 98). In five patients IJV catheter placement was failed (Table 2). In these patients, four catheters were placed into left subclavian vein, and one was placed into femoral vein. In total, 78 CVC (79.6%) were successful at the first attempt and eleven (13.3%) at the second attempt, while seven patients (7.1%) required three punctures. Hematoma occurred in five patients (5.1%). Malposition of the catheter was observed in two patients during operation.

**Table 2. Outcomes**

| | ≤ 2500 g (n = 21) | 2501-4999 g (n = 77) | Total |
|---|---|---|---|
| Failure | 2 | 3 | 5 |
| Arterial Puncture | 2 | 1 | 3 |
| Hematoma | 2 | 3 | 5 |
| Malposition | 1 | 1 | 2 |
| Pneumothorax | 0 | 0 | 0 |

The patients were divided into two groups according to weight: ≤ 2500 g and > 2500 g. The median weight of these two groups were 2000 gr (980-2500) and 3500 gr (2600-4999), respectively. The success rates of low weight and normal weight groups were 90.5% vs 96.1, respectively ($p = 0.582$) (Table 2). However, complication rate was significantly higher in the low weight group (13.3% vs %7.1 $p < 0.05$). There was no significant difference between puncture attempts.

## DISCUSSION

Ultrasound guidance for central venous catheter placement has become increasingly common in the past decade [9]. Today, classical LM and US-guided techniques are being used during CVC placement. However, many surgeons continue to use LM technique rather than US guidance. Studies which cite higher rates of complications with LM technique are largely derived from studies performed by non-surgeons accessing the IJV [10-12].

Exposure during vascular access, vascular bypass, and vascular trauma cases provides surgeons with a valuable view of the vascular anatomy that they access during percutaneous CVC insertions. This perspective is unique to surgeons, as other specialists are accustomed to only seeing these vessels as two-dimensional images on US and CT scans [6].

A survey of pediatric surgeons regarding CVC placement practice patterns revealed that pediatric surgeons who continue to use LM technique did not believe that US was necessary or provided any benefit in regard to lowering complications rates [13]. The educational experience and anatomical knowledge may provide surgeons with a level of comfort.

Gurien *et al*. [14] pointed that pediatric surgeons were more likely to use LM technique and had decreased complication rates. A meta-analysis by Sigaut *et al*. [15]presented the use of US during CVC insertions in pediatric patients did not show any reduction in complications or failure rate when used by skilled operators. Also, Grebenik *et al*. [16] reported US guidance did not help experienced pediatric cardiac anesthetists to achieve better success rates in IJV cannulation in children.

In this cohort of 98 patients, internal jugular veins

were targeted by experienced pediatric cardiovascular surgeon using LTs. Successful cannulation occurred in 95.1% with a perioperative complication rate of 8.2%. No pneumothorax or hemothorax was observed.

Our failure rate to insert CVC into IJV using LT was 5.1% which compares favorably with 10.8% reported by Arujo *et al*. [3] using LT and 7.2% reported by Altun *et al*. [17] using US. Malbezin *et al*. [18] reported 1.3% of complication rate while complication was defined: failure to cannulate any vein, hemothorax, pneumothorax, right atrial perforation, extravenous wire positioning and mean weight was 19 kg. Weight under 3 kg was defined as predictive failure by Malbezin *et al*. [18]. However, we found no correlation with the weight and the failure rate. While group weighed under 2.5 kg had higher complication rate (OR 4.5 [1.2–14.5], p < 0.05). Altun *et al*. [17] also found higher complication rate in patients under 2.5 kg.

Bruzoni *et al*. [8] presented a study comparing LM to US-guided central venous access when performed by pediatric surgeons. In their study success at first attempt was achieved in 65% of patients in the ultrasound group vs 45% in the landmark group (p = 0.021). Success rates were 95% of ultrasound group vs 74% of landmark group (p = 0.0001). In this study 79.6% of CVC were successful at the first attempt, and overall success rate was 94.5%. Bruzoni *et al*. detected differences in success rate but not complication rates (4.5% in US vs 4.7% in LM), and arterial puncture was not defined as complication while it is 4.5% in US vs 8.3% in LM. And the patients mean age and weight were 5 years and 27 kg in US while 8 years and 36 kg in LM. However, in this study mean weight was 3.3kg and, mean age was 51 days which makes CVC insertion technically challenging even in experienced hands.

In a meta-analysis to compare outcomes for surgeon-performed with US CVC insertion versus LM presented that US improves success rate for surgeon performed CVC insertion, but no effect on complication rates. Also, studies in this meta-analysis were not designed on infants and underweight patients. According to Lamperti *et al*. [19] most of papers did not declare how the experience of operators were achieved, and history of using with landmark technique makes more difficult the learning curve. Furthermore, for achieving the minimal skills, at least 25

successful supervised ultrasound-guided vein accesses required.

The weight of the patients is also critical factors which affect the success and the complication rate. In a study represented by Froehlich *et al*. [20], there was a negative correlation between the weight of the patient and the success rate. However, we found no correlation with the weight and the failure rate, while there was a negative correlation between the weight and the complication.

Although the author appreciate the ability to image the internal jugular vein with US, there are some restriction of US guided cannulation. First, lack of space may prevent US use in small infants. Second, US device may not be available in some units. Third, intense training is required. In addition view of arterial pulsation may be difficult in small infants.

One of the most common problem in small infants is inability to pass guide wire. However, in our study, guide wires were easily advanced, as position of the neck enables to puncture of proximal IJV which is wider and closer to right innominate vein and vena cava superior. So J shaped guide-wire can be easily advanced.

## Limitations

The limitations of this study include lack of a control group to compare the usual technique of LT technique and retrospective design with small sample size.

## CONCLUSION

In our study, we found that LT technique still seems useful by experienced operators or when limited to the prelocation of the vein especially in small infants. Success rate of this study is high and, comparable with the US guided studies. In conclusion, our results support the theory that specifically trained skilled operators will have low failure rates when using LT technique. Larger studies needed which document experience level of surgeons as operators and the implementation of US technique.

Data Interpretation: SS; Literature Review: SS; Manuscript Preparation: SS and Critical Review: SS.

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# Intraoperative awareness during laparoscopic sleeve gastrectomy

**Ozan Şen**[1,2] , **Fatih Can Karaca**[3] , **Seniyye Ülgen Zengin**[4] , **Ahmet Gökhan Türkçapar**[2]

[1]*Department of Health Sciences, Nişantaşı University, İstanbul, Turkey*
[2]*Türkçapar Bariatrics, Center for Obesity Surgery, İstanbul, Turkey*
[3]*Department of Health Sciences, Bilgi University, İstanbul, Turkey*
[4]*Department of Anesthesiology and Reanimation, Bezmialem Vakif University, Faculty of Medicine, İstanbul, Turkey*

## ABSTRACT

**Objectives:** The aim of this study is to determine the incidence of intraoperative awereness (IA) in our patients who underwent laparoscopic sleeve gastrectomy (LSG) and the factors affecting the formation of this complication.

**Methods:** Four hundred ten patients who underwent LSG between March 2018 and September 2020 were included in the study. By April 2019, we started using the Bispectral index (BIS) monitoring, which measures the depth of anesthesia in all of our LSG cases (n = 167). Patients with and without BIS monitorization were divided into two groups and compared.

**Results:** In our series, IA was seen in 3 patients (2 males) in two different hospitals (0.7%; n = 410). They were all in the non BIS group (n = 243). The median duration of anesthesia was 120 minutes (ranging 90-180) in the non-BIS, and 113 minutes (ranging, 90-140) in the BIS group (*p* < 0.001). Hypotension developed in 63 patients in non-BIS and 12 patients in BIS group at the beginning of the operation (< 90/60 mm Hg). The total remifentanil infusion dose administered during the anesthesia period in the BIS group was 1310 ± 351 mcg, and 1330 ± 270 mcg in the non BIS group (*p* = 0.002). The effect of BIS monitorization between groups on IA, did not show statistical significance (*p* = 0.27).

**Conclusions:** Anesthesia techniques that work well for patients with normal weight may not be safe and appropriate for obese patients. Especially in patients with intraoperative hypotension, it is necessary to be more careful about dose adjustment of anesthetic drugs.

**Keywords:** Bariatric surgery, sleeve gastrectomy, general anesthesia, complication, intraoperative awareness

**B**ariatric surgery is one of the most effective treatment for morbid obesity. As obesity increases worldwide, people turning to bariatric surgery for weight loss also increases [1]. With increase in bariatric surgery, anesthesiologists face a new challenge in terms of management of obese patients during surgery.

Since the dose adjustment of the intravenous anesthetic agents specified in the anesthesia guidelines is usually based on the total body weight of people with normal weight, anesthesia management of morbidly obese patients requires extra care. Although there are no definitive guidelines for the dose adjustment of intravenous anesthetic agents in morbidly obese patients

[2, 3], it is generally recommended to adjust the dose according to lean body mass [4, 5]. Many comorbidities such as diabetes, hypertension, left ventricular hypertrophy, sleep apnea, and pulmonary hypertension accompany morbid obese people undergoing bariatric surgery. These comorbidities together with the excess adipose tissue and the lipophilic properties of many drugs used in general anesthesia affect the pharmacokinetics and pharmacodynamics of anesthetics, causing the drugs to show their effects in a very narrow spectrum [6]. In this respect, anesthesia management of bariatric surgery patients gains the utmost importance.

The patient regaining consciousness during general anesthesia is defined as awareness. Awareness is a serious condition that prompts patients into a panic situation even if they do not feel any pain at that time [7]. These patients experience insomnia, recurrent nightmares and post-traumatic stress disorder in postliminary periods [8, 9]. Intraoperative awareness (IA) is a rare but serious complication rating between 0.1-0.2% [10]. The most common cause is superficial anesthesia and the most common disciplines IA observed are in obstetrics and cardiac anesthesia. The ratio of IA was reported to be 1% in the high-risk cases [11, 12]. Obesity is defined to be an independent risk factor for IA [5, 13].

The aim of this study was to determine the incidence of IA in our patients undergoing laparoscopic sleeve gastrectomy (LSG), and the factors contributing to the development of this complication. To our knowledge, this is the first study in the literature reporting that IA might be observed during LSG.

## METHODS

Four hundred ten patients (56% female) who underwent primary LSG between March 2018 and September 2020 were included in the study.

### Preoperative Work-up

Each patient underwent a thorough evaluation of laboratory tests, upper GI endoscopy, and abdominal ultrasonography before the surgery. Also, patients were evaluated by a cardiologist, a pulmonology, and an anesthesiologist as a part of routine work-up.

### Anesthesia Management and Surgical Technique

LSG was applied to all patients using the same surgical technique. The operations were performed in two different hospitals with two different anesthesiology teams, using the same anesthesia protocol. Table 1 summarizes the doses and durations of the anesthetic agents administered during the procedures. Following the tracheal intubation and inhalation anesthesia, a 12 mm optical trocar (Endopeth Xcel®) was inserted into the abdomen from the left subcostal area under direct vision and abdomen was insufflated. LSG was performed using five trocar technique after applying a *45 degrees reverse Trendelenburg (modified litotomy) position.* After LSG was completed, the staple line was oversewn using 3.0 V-Loc™ suture in all patients.

### Evaluation of Intraoperative Awareness

In order to evaluate IA after surgery, each patient was questioned in terms of whether they remembered anything, heard anything or felt any pain during the operation.

### Bispectral Index Monitoring

The use of Bispectral Index monitoring equipment (BIS™ Medtronic), which monitors the depth of anesthesia was initiated for all of our bariatric surgery cases (n = 167) starting in April 2019. BIS moniterization was not used in any of our LSG cases before April 2019.

Patients with and without BIS monitoring were divided into two groups (Non-BIS and BIS group). BIS values between 40 and 60 indicate adequate anesthesia depth for the surgery.

Patients who developed intraoperative hypotension (systolic blood pressure below 90 mmhg) in both groups were recorded. Two groups compared in terms of demographics, the rate of IA, the factors affecting the condition, and the effect of BIS monitorization.

### Exclusion Criteria

Cases that included additional surgical procedures such as cholecystectomy and other bariatric procedures were excluded from this study in order to avoid skewness in the operation and anesthesia time. Patients with a previous history of IA were excluded from the study.

The study was approved by the institutional ethics commitee (ATADEK- 2019-17/11). All patients were

informed about the study in details, and written informed consents were obtained. All data, including intraoperative vital parameters were recorded prospectively and analyzed retsospectively.

### Statistical Analysis

Statistical analysis was performed using SPSS (version 21, SPSS, Inc., Chicago, IL, USA). Standard deviation and mean values were used for the variables with normal distribution and median values were used for the variables that were not normally distributed. Chi-square or Fisher's exact tests were used for categorical variables; while for continuous variables, independent-samples T-test or Mann-Whitney U test were performed. *P* values < 0.05 were considered statistically significant.

### RESULTS

Between March 2018 and September 2020 four hundred ten patients (56% female) with a mean age of $37.4 \pm 11.6$ years, a median body mass index (BMI) of 41.3 kg/m$^2$ (33-67) and ASA score of 2-3 had undergone LSG. In our series, IA was observed in three patients (2 male, 1 female) in two different hospitals (0.7%). Three of them were in the non BIS group (n = 243). The median duration of anesthesia was 120 minutes (90-180) in the non BIS group and 113 minutes (90-140) in the BIS group ($p < 0.001$).

Hypotension developed in 63 patients in non-BIS,

and 12 patients in BIS group at the beginning of the operation (< 90/60 mm hg). The total remifentanil infusion dose administered during the anesthesia period in the BIS group was $1310 \pm 351$ mcg, and $1330 \pm 270$ mcg in the non BIS group ($p = 0.002$). The demographic characteristics of the groups are shown in Table 2.

The first patient who experienced IA was 41 years old female with a BMI of 39 kg/m$^2$. The patient stated that she woke up at the beginning of the surgery, was fully awake, heard everything spoken during sterilization and surgical draping, wanted to say that she was awake. She was very scared of the situation and she was not able to say anything or move. She added that this situation lasted for 3-4 minutes, and she could not remember what happened subsequently.

The other two patients were males aged 40 years with a BMI of 46 kg/m$^2$ and 44 kg/m$^2$). Their experiences were very similar. According to their statements, awakenings were somewhere during the beginning of the surgery. Both patients experienced similar feelings of great pressure in the abdomen, surgical manipulation such as trocar entrance, which they described specifically as "there was a drill going into my abdomen". They emphasized that they felt enormous pain and this "nightmare" lasted for 10-15 minutes.

Except for a temporary period of hypotension at the beginning of the surgery, there was no abnormality in vital signs suggestive of IA in all 3 patients. Two male patients received psychotherapy support for 6 months due to post-traumatic stress disorder.

### Table 1. Applied anesthesia regimen for LSG patients

| | |
|---|---|
| Local anesthetic (Lidocaine) | 1 mg/kg |
| Propofol | 1.5-2 mg/kg bolus |
| Fentanyl | 1-1.5 µg/kg bolus |
| Rocuronium bromid | 0.6-0.8 mg/kg bolus<br>*repeated dosage intraoperativley every 20-30 minutes |
| Trakeal entubation | |
| Sevoflurane | 2% |
| Remifentanil (50 mcg/ml) | 10-25 ml/hour infusion |
| First trocar insertion and CO2 insuflation | |
| Reverse trendelenburg position | |
| Begin to operation | |

LSG = Laparoscopic sleeve gastrectomy

**Table 2. Patients' characteristics**

| Sleeve gastrectomy | Without BİS (n = 243) | With BİS (n = 167) | *p* value |
|---|---|---|---|
| Male/female | 118/125 | 62/105 | 0.02 |
| Age (years) | 37.5 ± 11.5 | 37.2 ± 11.8 | 0.78 |
| Preoperative weight (kg) | 121 (88-215) | 113 (87-215) | 0.001 |
| Preoperative body mass index (kg/m$^2$) | 42.4(33-67) | 39.7(32-62) | 0.001 |
| ASA score 2 | 81 | 88 | 0.001 |
| ASA score 3 | 162 | 79 | 0.001 |
| Anesthesia time (minutes) | 12 0(90-180) | 113 (90-140) | 0.001 |
| Intraoperative hypotension (< 90 mm Hg) | 63 | 12 | |
| Total remifentanil dosage (mcg) | 1330 ± 270 | 1310 ± 351 | 0.002 |
| Awereness | 3(1.2%) | 0 | 0.27 |
| Comorbidities (+) | | | |
|     Type 2 diabetes | 32 | 21 | 0.9 |
|     İnsüline resistance | 194 | 127 | 0.76 |
|     Hypertension | 68 | 37 | 0.18 |
|     Dyslipidemia | 132 | 75 | 0.06 |
|     Sleep apnea | 59 | 44 | 0.63 |

BIS = Bispectral index

## DISCUSSION

IA is a rare but serious complication with an incidence of 1% for high-risk individuals [11, 13]. In our study, IA was developed in three (0.7%) patients and there were all at the beginning of the operation. One patient with ASA 2 (n = 169) and two patients with ASA 3 (n = 241) experienced IA. We did not find a significant effect of the ASA score on IA (*p* = 0.20).

There are no definitive guidelines for dose adjustment of intravenous anesthetic agents in obese patients. Propofol is a highly lipophilic substance commonly used in anesthesia induction. While dose adjustment is performed according to the total body weight in normal-weight people, this approach should be avoided as it can easily cause adverse effects in obese people.

Some studies indicate that the current dose of propofol administered during induction in obese individuals is insufficient [14-16]. Agents used in the induction of anesthesia redistributes quicker in the fat

tissue. The meaning of this is that, obese patients awake earlier than non-obese people with single bolus application.

Anesthetic drugs such as propofol, remifentanil have a great hypotensive potential in higher doses. The reverse Trendelenburg or modified litotomy position for LSG induces the hypotensive effect of anesthetic drugs. İntraoperative hypotension is mostly undesirable by the surgeons. In most cases, it is preferable that the patient is normotensive in order to observe any signs of bleeding from the staple line or other surgical sites. It is worth mentioning that the caution of the anesthesiology team regarding the hypotension that might occur as a result of the surgical position at the beginning of the operation is one of the most important points to be considered for the prevention of this complication. Anesthesiologists' adjustment process of dose regulation in an attempt to avoid subsequent hypotension might be a factor in the manifestation of IA. In our study we also experienced intraoperative hypotension in our patients who underwent LSG. Hy-

potension developed in 63 patients in non-BIS, and 12 patients in BIS group at the beginning of the operation (< 90/60 mm Hg).

Nowadays, electroencephalogram-like devices that monitor brain functions are frequently used during surgery. The most commonly used is the BIS monitorisation, which measures the patient's level of consciousness by processing an electroencephalographic signal obtained from a single frontal electrode. The BIS interval is determined between 100-0, defining the complete awakeness and the absence of brain activity. BIS values between 40 and 60 indicate adequate anesthesia depth for the surgery whereas values below 40 indicate the deep hypnotic phase. It is recommended to keep the target value in the interval of 40-60 to prevent IA [17]. In our study, patients in the BIS group, median BIS score was 36 (26-47) during the operation.

The data on the effect of BIS monitorisation on the prevention of IA is controversial. The B-aware study reported that BIS monitorization provides a 0.74% of risk reduction in IA in high-risk individuals [18]. In a large multicentric cohort study, it was reported that BIS monitorization did not provide a positive effect on the IA [5].

In our LSG series, we experienced a fairly high rate of IA in three (0.7%) of our patients. All of these cases were in the non BIS group (n = 243; 1.2%). No incident of IA was observed in the BIS group. While we evaluated our patients for the effect of BIS monitorization on IA, the ratio did not show statistical significance ($p = 0.27$).

## CONCLUSION

Many factors affect the pharmacokinetics and pharmacodynamics of the anesthetics in obese patients. Anesthesiology practices that work well for patients of normal weight may not be safe and adequate for obese patients. Especially in patients with intraoperative hypotension, it is necessary to be more careful about dose adjustment of anesthetic drugs. Comprehensive studies are needed to create definitive guidlines in this area.

*Authors' Contribution*

Study Conception: OŞ, AGT; Study Design: OŞ, FCK, AGT; Supervision:; Funding: OŞ, AGT; Materials: ED, SK; Data Collection and/or Processing: OŞ, AGT; Statistical Analysis and/or Data Interpretation: OŞ; Literature Review: OŞ, FCK, SÜZ, AGT; Manuscript Preparation: OŞ, AGT and Critical Review: OŞ, FCK, SÜZ, AGT.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Quality of YouTube video resources on total knee arthroplasty: a search in Turkish

**Hakan Kocaoğlu**, **Abdullah Merter**, **Mustafa Onur Karaca**, **Emre Anıl Özbek**

*Department of Orthopedics and Traumatology, Ankara University School of Medicine, Ankara, Turkey*

## ABSTRACT

**Objectives:** We aim was to evaluate the quality and reliability of the information on knee arthroplasty available on YouTube in Turkish.

**Methods:** A systematic search was conducted using the term "diz protezi" (knee prosthesis) on 1 June 2020.Videos were filtered based on their degree of relevance alone. Videos that were not in Turkish, not on knee prosthesis or without audio and/or image were excluded. Copied videos were evaluated as a single video. The first 50 videos that came up in the search were included in the study. Video quality was scored using Global Quality Score (GQS). To assess reliability, modified DISCERN scale was used.

**Results:** The mean view count per video was $31.533 \pm 100.921$ and the total view count was 1.576.633. The mean duration per video was $337 \pm 475$ seconds. The median number of likes per video was 13 (0-426). The median number of dislikes per video was 2.5 (0-83). The mean GQS of the videos was $3.25 \pm 0.9$. When the reliability of the sources was evaluated using DISCERN, the mean score of the videos was $2.18 \pm 1.2$. It is striking that all videos were prepared by an orthopedic surgeon. Of the medical doctors preparing the resources, 44% had academic titles.

**Conclusions:** It was found that Turkish resources on arthroplasty on YouTube are promising in terms of quality. Content creators must make effort to increase their reliability according to DISCERN.

**Keywords:** Knee, arthroplasty, patient education, social media

**K**nee osteoarthritis is the most common arthritis and it constitute 3% of the total disease burden in our Turkey [1, 2]. ]The gold standard treatment for end-stage knee osteoarthritis is total knee arthroplasty (TKA). One of the important factors that affect the outcome after TKA, which is an elective surgery, is the patient's expectations of the treatment [3, 4]. Traditionally, when elective operations such as TKA are indicated, physician - patient consensus is required when deciding upon surgery [5]. In the meantime, the physician provides information to the patient on the procedure, its benefits, its potential complications and what the patient must/must not do after the procedure.

Today, the amount of medical information accessible via the internet increases every day [6]. It is clear that what patients see and read online highly affects their perception of the disease and expectations of the treatment. YouTube and other social media contents can be created by numerous sources and people, and they are not subjected to any monitoring or review.Thus, the accuracy and quality of these information is unknown.

The quality and reliability of increasing amount of online medical information found in sources in Eng-

lish are frequently discussed, but there are no studies on this subject regarding the sources in Turkish [7, 8]. In this study, our aim was to evaluate the quality and reliability of the information on knee arthroplasty available on YouTube in Turkish.

## METHODS

The approval of an institutional review board was not required for the present study. To find the relevant videos on knee arthroplasty, YouTube's search functionality was used. A systematic search was conducted using the term "dizprotezi" (knee prosthesis) on 1 June 2020. The reason behind using "protez" instead of "artroplasti" (arthroplasty) as the search term is the rare use of the word arthroplasty, which is not Turkish, in colloquial language. This can also be understood from the fact that the query "dizprotezi" has a higher search volume compared to "diz artroplastisi" in Google Trends, where online search tendencies are indexed [9]. The search on YouTube was performed using a web browser without a recorded history or "cookies". Videos were filtered based on their degree of relevance alone. Videos that were not in Turkish, not on knee prosthesis or without audio and/or image were excluded. Copied videos were evaluated as a single video.

The first 50 videos that came up in the search were included in the study. Although there is no consensus on this subject, this method is frequently used in similar studies [10, 11]. The contents of videos in question were evaluated by two independent researchers (HK, OK) and their duration in seconds and the number of days betweenthe date of upload to the date of evaluation were recorded. The source (creator) of contents, if indicated, was grouped as academic health professional (physician, nurse, physiotherapist, etc.), non-academic health professional, patient, and others. Moreover, the view count, number of likes, dislikes and comments of the videos were recorded.

Video quality was scored using Global Quality Score (GQS). GQS is a 5-point scoring system developed by Bernard *et al.* [12] for internet-based sources.The lowest score is 1, the highest is 5. Videos scored 4 or 5 were considered high quality, 3 were considered acceptable quality and 1 or 2 were considered low quality videos.

To assess reliability, modified DISCERN scale was used [13]. In this scale, using five yes/no questions, the reliability of the video regarding prejudice and objectivity, clarity and understandability, and reliability regarding references and additional resources are evaluated. Yes, corresponds to 1 point and no corresponds to 0 point. By this way, a maximum of 5 points can be obtained for reliability.

When there was a conflict between the two researchers in terms of scoring, a third independent researcher (AM) evaluated and scored the video and the decision was made by majority vote.

### Statistical Analysis

Logistic regression was used to analyze the relationship between GQS score and DISCERN score and other measured variables. A p value less than 0.05 was considered significant.

## RESULTS

For videos on knee arthroplasty, a total of 50 videos were analyzed. Of the videos in the first 50, 3 videos with no audio and 2 videos which were copied were excluded from the study. These 5 videos were replaced with the next 5 videos in line. The mean view count per video was $31.533 \pm 100.921$ and the total view count was 1.576.633. The mean duration per video was $337 \pm 475$ seconds. The median number of likes per video was 13 (0-426). The median number of dislikes per video was 2.5 (0-83).

The mean GQS of the videos was $3.25 \pm 0.9$. Of the videos, 6% (3/50) were bad and had the inappropriate or wrong content for the patients, 12% (6/50) were of poor quality but had limited information, 36% (18/50) were insufficient but still had information for the patients although limited, 42% were of sufficient quality and had appropriate amount of information for the patients and 4% (2/50) were of perfect quality and contained all the information necessary for the patients (Table 1).

When the reliability of the sources was evaluated using DISCERN, the mean score of the videos was $2.18 \pm 1.2$. While none of the videos had references to the appropriate sources, only 12% (6/50) had comments without any bias. Thus, there are no videos that meet all of the criteria and got full score from DIS-

**Table 1.** **Te video charachteristics according to the GQS and DISCERN scores (video duration, video age and view count was given by mean values and like and dislike numbers are represented by medianvalues of the specific subgroup)**

|  |  | n | Video duration (seconds) | Video age (days) | View count | Likes | Dislikes |
|---|---|---|---|---|---|---|---|
| **GQS** | 1 | 3 | 320 | 2709 | 29650 | 19 | 4 |
|  | 2 | 6 | 303 | 2819 | 33328 | 14 | 7 |
|  | 3 | 18 | 319 | 2613 | 38109 | 18 | 5 |
|  | 4 | 21 | 315 | 2780 | 33142 | 13 | 2 |
|  | 5 | 2 | 299 | 2400 | 27948 | 10 | 2 |
| **DISCERN** | 1 | 13 | 306 | 2699 | 37691 | 15 | 4 |
|  | 2 | 17 | 317 | 3005 | 32650 | 13 | 2 |
|  | 3 | 14 | 312 | 2778 | 29879 | 13 | 2 |
|  | 4 | 6 | 311 | 2516 | 30382 | 9 | 3 |
|  | 5 | 0 | - | - | - | - | - |

GOS = Global Quality Score, DISCERN = an instrument for judging the quality of written consumer health information on treatment choices

CERN (Table 1).

It is striking that all videos were prepared by an orthopedic surgeon. Of the medical doctors preparing the resources, 44% had academic titles. Advertisement of theemploying institution (private hospital) was detected in 70% (35/50) of the videos. In one video, identity of the patient was clearly visible during surgery, and although the patient gave consent, it was found ethically controversial [14]. The most commonly discussed subjects include post-operative physical therapy (62%) and complications after knee arthroplasty (22%). Options other than surgery and general information regarding surgery were the least discussed subjects. There was no correlation between GQS and DISCERN evaluation score and other characteristic variables of the videos.

## DISCUSSION

Results of our study demonstrate that, in Turkish sources on YouTube on knee arthroplasty, the level of information is at an acceptable level, but scientific reliability is low. Patients' access to data is increasing progressively through internet sources such as YouTube. Especially with the increase in the rate of internet accessibility from 2.9% to 59.6% from the start of the millennia in Turkey, the internet has almost become the strongest source of information [15].

There are numerous studies on the reliability and quality of information in online sources on surgery and numerous internal diseases [12, 16]. However, there is a limited number of similar studies on orthopedic diseases, and in particular, there are only two studies on arthroplasty in the literature [7, 8]. There are no studies in Turkish and as far as we know, this is the first study on Turkish content. Unlike the sources in English, approximately half (23/50) of the contents were categorized as acceptable and perfect according to GQS. In the study by Wong *et al*. [7], 66% of the videos were considered to have poor quality. This was attributed to the fact that all content creators were physicians and as previously shown, orthopedists have a high rate of social media use [17, 18].

However, unfortunately, scientific reliabilityof the sources is insufficient according to DISCERN. This is similar to other examples in the literature. Koller *et al*. [8] stated in their paperregarding hip osteoarthritis 84% of the videos were poor. Similarly Akpolat *et al*. [19] stated DISCERN score of videos about Bankart lesion was 2.35 ± 0.91. It is especially striking that scientific sources were not referred to in the videos. This is probably because since the content creators produced the videos for the general public, they did not refer to scientific sources. Another problem in the videos that led to poor quality was that information

transfer was biased,and most of the time, good outcomes were reported. This can be because some of the videos were broadcasts supported by the private hospital.

It is striking that all of the available content was created by orthopedists. The high quality of broadcasts can be attributed to the fact that 44% of these physicians had academic titles. In particular, there was one content creator, who was observed in 26% of the videos and was very active. Previous studies have shown the willingness of Turkish orthopedists to use social media [17, 18]. Since the information presented in the videos created by content creators include information provided to patients in face-to-face interviews, it is nevertheless clear that YouTube enables information to be widely accessible. In our case, the content on total knee arthroplasty reached to almost 1.5 billion users.

Internet, which provides a flow of information to and from various sources, does not always provide reliable and quality information. One of the areas most affected from this situation is the physician-patient relationship. Especially, the cases where the patients have unnecessary anxietiesand patient expectations of the treatment have increased without any foundation following misinformation are also troublesome for the physician undertaking the treatment.In a study performed in the US, it was found that nearly 40% of the physicians believe that their diagnosis and treatment becomes ineffective when the patient has certain prejudices due to information obtained online before consulting a physician [20].

## Limitations

We know our study is not free of limitations: first, there is no validated tool to assess the quality of video-based medical information. Yet GQS and DISCERN are widely used scales good inter-observer and intra-observer reliability. Second, YouTube's search code/algorithm is not openly disclosed, and the various factors effecting which videos delivered to a certain search is an important confounding factor. Even the internet protocol (IP) where the site is reached could impact the results. However, this variability is not adjustable, and we believe since the algorithm affects any search equally, we believe this discrepancy is negligible.

## CONCLUSION

Again, it was found that Turkish resources on arthroplasty on YouTube are promising in terms of quality. Content creators must make effort to increase their reliability according to DISCERN. Scientific facts should be cited with a plain language, and controversial topics about any procedure should always be highlighted. Any effort to increase the "social media abilities" to the medical doctors' armament, like addition of a social media course to medical curriculum, can be valuable for the future [21].

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*Anatomy*

# Bibliometric and altmetric analysis of publications examining education methods in realm of anatomy

**Emine Petekkaya**[1]●**, Mehmet Karadağ**[2]●**, Mehmet Dokur**[3]●

*¹Department of Anatomy, Kastamonu University School of Medicine, Kastamonu, Turkey*
*²Department of Biostatistics, Mustafa Kemal University School of Medicine, Hatay, Turkey*
*³Department of Emergency Medicine, Biruni University School of Medicine, Istanbul, Turkey*

## ABSTRACT

**Objectives:** This study aimed to investigate the significance of publications examining the effectiveness of education methods in the field of anatomy with the method of bibliometric and altmetric analysis, as well as online attention levels.
**Methods:** To search all publications, "Anatomy education" was entered as a search term on the Web of Science database. The topics, journal impact factors, publication years and research centers of the first 100 articles with the highest numbers of citations were examined, and their analysis was conducted with the "Altmetric it on website: http: almetric.com".
**Results:** Four thousand, three hundred fifty-six articles published in the period of 1975-2019 containing the key phrase "Anatomy education" were found on Web of Science. The study with the highest number of citations was the study published by McLachlan *et al.* titled "Teaching anatomy without cadavers, 2004". It was observed that the study titled "The production of anatomical teaching resources using three-dimensional (3D) printing technology, 2014" was the article with the highest rate of sharing on Twitter with the highest altmetric attention score (AAS) value. The AAS rates varied between 130 and 0.
**Conclusions:** Bibliometric and altmetric analysis provides significant but different points of view regarding the effects of an article in the world of science. The altmetrics score may provide contributions in determining the direction of studies regarding the high-level interests and perceptions of the public on dynamic science and the field of medicine.
**Keywords:** Bibliometric, altmetrics, analysis, anatomy, educational methods

**I**t is important to assess the effectiveness of medical education in today's conditions with evidence-based analyses, because these assessments have the potential of directing practices of developing curricula. While anatomy education is the building block of medicine, it is seen by students as a difficult to learn subject at the beginning of medical education [1]. The rapid development of technology has allowed development of various methods that present the practical ways of learning anatomy today. The practices of anatomy education have been enriched by cadaver dissection coming from traditional medical education, followed by plastic modellings of body parts and highly diverse online electronic sources that provide information gathering methods today, interactive three-dimensional (3D) visualization technologies and

"multimodal" approaches such as radiological imaging [2]. Especially 3D mobile applications that include virtual anatomy animations supported by videos, YouTube videos and animations obtained from other social sharing sites, colorful 3D images and problem-focused learning have taken their place as the current methods used in anatomy education [3, 4]. With the initiatives of anatomy educators to preserve traditional basic training methods such as dissection, there has been a face off between some clinical field educators that have transitioned to virtual simulative applications and conventional educators [2].

In this context, a process that gives rise to a necessity of updating the curriculum with various education and instruction methods has emerged. In addition to these methods, sharing of anatomy information via social networks today has started to be used by students as a learning method. For this reason, investigating the usage of existing anatomy learning methods and social platforms today for medical instruction purposes and shedding light on anatomy education have become a current need. For this purpose, we aimed to investigate publications that examine existing anatomy learning methods that are prevalently used by using metric methods. Especially bibliometric analysis (index criteria) is used in several fields to define the most significant studies [5]. This analysis method uses citation ranking to define the output with the largest intellectual effect in ranking articles.

The number of citations made to published articles is a reference in measuring the influence of the journal they are published in and assessing the capability of authors, and it is based on bibliometrics, which investigates such associations. Other important indicators that show the effect and quality of a journal are the journal impact factor (IF) which is based on citations and the h-index value provided by Web of Science (WoS), Scopus, Google Academics and Scimago Journal & Country Rank (SJCR) [6, 7]. IF is a significant bibliometric indicator that needs to be used carefully as it is known to be a value in which several criteria play a role on the final value. JCR calculates the citations and publications of the last two years and returns the value of IF every September [12]. It is clearly seen that although it allows the evaluation of large data sets, keeping track of changes in citation databases over time and evaluating journal IF that vary from year to year creates measurement and technical problems for bibliometric analysis.

Altmetrics, which is a new web-based metric analysis method, has started to be used as a current method in assessing the impact analyses of publications on social media platforms [14]. This method that conducts an analysis as the Altmetric Attention Score (AAS) and Altmetric feedback was designed to make it easier to define how much and what type of interest a certain research output receives [15]. AAS is calculated by an automated algorithm created by the company Altmetric based on the weighted quantity of the online interest received by a research output. This algorithm that is used under the name of Altmetric Explorer (Altmetric, London, the United Kingdom) Score is a web-based application that can use some research output resources to present the online activities of publications and the most relevant discussion forms in a current sense [15]. While making calculations, three main factors are used to determine the weights: volume, sources and authors. The role of social media platforms in the publicity, dissemination and presentation of the medical literature was increased substantially in the last few years [17]. Altmetrics are advantageous in that they can reflect significant non-academic effects and are visible before even academic citations occur, but they also have some disadvantages. At the beginning of these disadvantages, we can say that it is still not clear which general conclusion to draw from the altmetric analysis. In addition, data sparseness is an important disadvantage that makes altmetric analysis insufficient alone.

In this context, this study aimed to determine the relationship between bibliometric analysis on the number of citations of articles and journal IF values and Altmetric analysis (highest AAS and IF) which determine the social media usage score of studies in determining the quantitative impacts of methods in the field of anatomy in the world of medicine.

## METHODS

In this study, WoS Core Collection database was used for bibliometric citation analysis and PubMed was used for other article information. PubMed data was used to see the total number of authors of the article and the type of article (eg Review etc.) and to evaluate the altmetric score on the "altmetric it / alt-

metric.com" website. The WoS database was accessed (date of access: 15 January 2020) to determine the publications between 1975 and 2019 that contained the key phrase "Anatomy Education". As the WoS article database includes articles that have been published since 1975 on February 15, 2020, our access date, earlier articles could not be reached. As a result, 4356 articles related to the subject were obtained, and among these results, the 100 articles that received the most citations (T100) were subjected to bibliometric and altmetric score analysis. After writing "Anatomy education" in the "topic" criterion in WoS, there were articles outside the scope of anatomy education among the articles exhibited. For this reason, during the creation of the T100 article list, the Pubmed MESH terms were first examined to evaluate whether the studies were covered by the term "Anatomy education". In addition, the abstracts of the articles were read independently and carefully by the article researchers and the compatible articles with the subject of anatomy education were determined. The full texts of the articles, whose article summary was not clear enough, were also examined and carefully evaluated in such a way that bias was not allowed. At the end of these evaluations, articles that fall within the scope of "Anatomy education" are included in the study list, while other articles were excluded from the study as they are outside the scope of "Anatomy education". Subsequent articles were included within the scope to complement 100 in place of the articles that were omitted. For the studies published in the relevant field, name of the journal of publication, journal IF (2019 Journal Citation Reports (Clarivate Analytics)), year of publication, topic of article, type of article and sub-types were determined.

The AASs were obtained from the Altmetric.com website (https://www.altmetric.com/products/free-tools/bookmarklet/) by using the "Altmetric it" function. Each color in altmetric feedback represents a different source from the social media sharing network [18]. As Altmetric Explorer is a licensed application, Altmetric it was used instead. In the color spectrum of feedback, blue represents Twitter, dark blue represents Facebook, yellow represent blogs, red represents news stories, orange represents patents, pink represents Google, and brown represents Wikipedia (Fig. 1). The study included original research articles, review articles, conference manuscripts and letters to the editor. Additionally, PubMed was utilized to obtain additional data for the study. This study did not need to be approved by an ethics committee, because it only conducted bibliometric and altmetric analyses on classical studies that have been published.

## Statistical Analysis

To determine the relationships between the number of citations of the selected T100 anatomy education articles and AAS and between IF and AAS, descriptive statistics was revealed and evaluated, and Spearman's correlation analyses were carried out in SPSS package software.
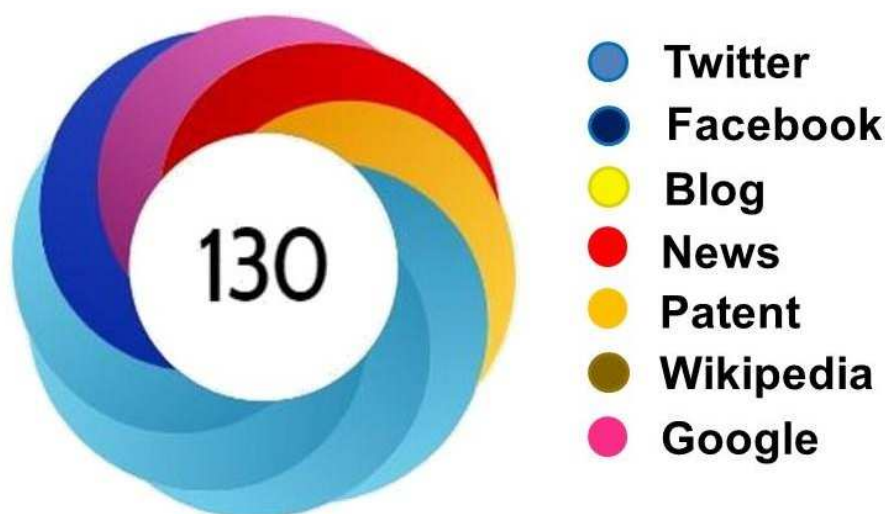


**Fig. 1. Example of altmetric feedback scoring sources.**

## RESULTS

In the search for publications in the period of 1975-2019 on WoS containing the key phrase "Anatomy education", 4356 articles were found. The T100 list on anatomy education shown in the literature was examined (Supplementary Table 1). In the list, the publications' numbers of citations, journals of publication, the highest numbers of publications based on years and AAS values were shown.

Sixty-eight of the T100 articles in this study were published in journals with an IF value of at least 2. According to Clarivate Analytics (2017), the average IF of 10 journals (Table 1) out of 26 journals in which at least two T100 articles were published was found, was 2.987, and the average h-index value was 80. The number of citations of the studies varied between 29 and 268. The study with the highest number of citations was published by McLachlan *et al*. [Suppl. Table 1 Rank 1] with the title "Teaching anatomy without cadavers, 2004". The one with the lowest number of citations was published by Baskaran *et al*. [Suppl. Table 1 Rank 100] with the title "Current applications and future perspectives of the use of 3D printing in anatomical training and neurosurgery".

Considering the publication years of the articles, it was determined that the time that passed since the article in the first place showed a change in favor of citations. Concerning the numbers of citations based on years, it was determined that the T100 publications almost did not receive any citations in 1998 and 1999,

their numbers of citations continued to increase in the following years, and the highest number of citations was in 2019 (Fig. 2). Considering the distribution of the publications on the topic of anatomy education based on years, while there was no publication in the years 1997 and 1998 among the articles, the lowest number of publications were in 1996 and 2003 by one article each, while the highest numbers of publications were in 2007 and 2016 (Fig. 3).

The journal with the highest number of publications in this field was "Anatomical Sciences Education" with 39 publications, which was followed by "Clinical Anatomy" in the second place with 19 and "Medical Education" in the third place with 13 publications. On the field of anatomy education, the Journals Anatomical Record, ANZ Journal of Surgery, Computers Education and Surgical and Radiologic Anatomy had 2 publications each (Table 1).

Among the T100 articles with the highest numbers of citations, 86 were research articles, 6 were reviews, 5 were verbal presentations, and 3 were letters to the editor. The highest number of original articles published in the relevant field was 34 for the period of 2006-2010, the highest number of reviews was 4 for 2011-2015, and the highest number of verbal presentations was 2 for 2011-2015 (Table 2).

Considering the countries of publication of these articles, the United States of America (USA) had the first place with 37 publications, while the United Kingdom was in the second place with 18 publications, and Australia was in the third place with 15,

**Table 1.** Journals where the T100 articles with most citations were published

| Rank | Journals | Amount | IF | H Index | Q Category |
|------|----------|--------|-----|---------|------------|
| 1 | Anatomical Sciences Education | 39 | 4.027 | 38 | Q1 |
| 2 | Clinical Anatomy | 19 | 1.813 | 62 | Q2 |
| 3 | Medical Education | 13 | 4.619 | 120 | Q1 |
| 4 | Annals of Anatomy | 3 | 2.241 | 45 | Q2 |
| 5 | Medical Teacher | 3 | 2.706 | 91 | Q1 |
| 6 | Academic Radiology | 2 | 2.110 | 87 | Q1 |
| 7 | Anatomical Record | 2 | 1.329 | 84 | Q2 |
| 8 | Anz Journal of Surgery | 2 | 1.605 | 69 | Q3 |
| 9 | Computers Education | 2 | 5.627 | 149 | Q1 |
| 10 | Surgical and Radiologic Anatomy | 2 | 1.039 | 52 | Q2 |

*Journals with 2 or more publications are listed.

Sum of Times Cited per Year
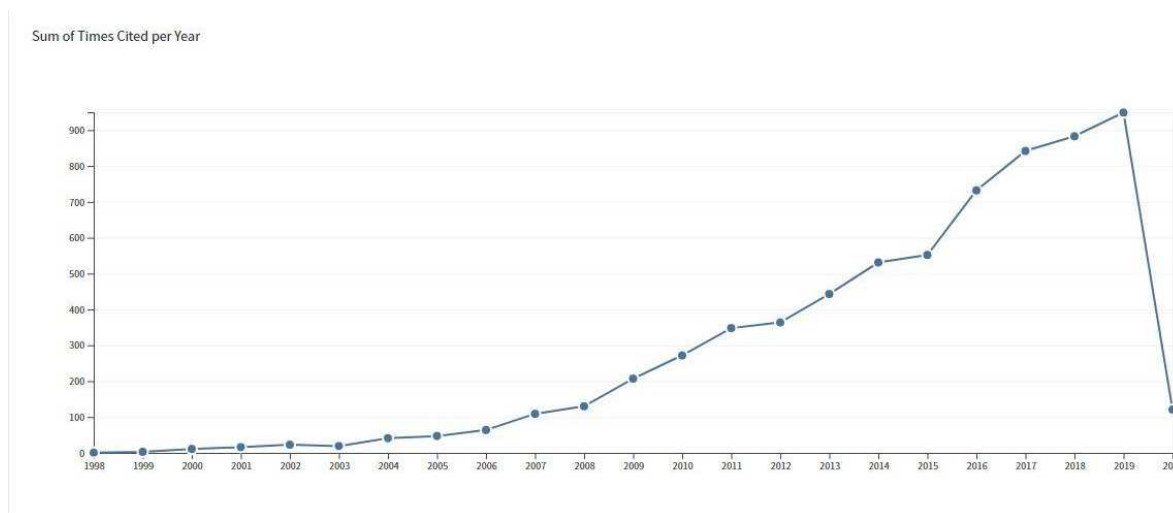


**Fig. 2. Number of citations of publications in the field of anatomy education by years.**

whereas 70% of the T100 articles were found to have been published by these three countries. The years with most publications for the USA and UK were 2006-2010, while Australia published more in the period of 2011-2015 in comparison to the other years (Table 2). From Turkey, only the article by Küçük. *et al*. [Suppl. Table 1 Rank 84] titled "Learning anatomy via mobile augmented reality: effects on achievement and cognitive load, 2016" was in this list.

When the types of research centers where the publications were made were examined, it was seen that these centers mostly operate in the fields of Education and educational research, Anatomy morphology and Health sciences. The highest number (68) and ratio (48.83%) of the articles were in the field of education research, which naturally included studies on educa-

tion. As seen in Table 3, it was observed that the number higher than 100 in the T100 list was caused by separate assessment of multidisciplinary studies and other fields.

The AAS values varied between 0 and 130. The article "The production of anatomical teaching resources using three-dimensional (3D) printing technology, 2014" (score 130) had the highest AAS score and most shares on Twitter. It was determined that it gained a fast scoring with social media shares although it was published on a close date. The T100 anatomy education articles' AAS, total number of citations, citations based on years, IF and h-index values are shown in Table 4. Accordingly, for the T100 articles ranked based on their total number of citations, the mean ASS was 5.29 ± 40.44, and the mean total num-

Total Publications

**100** Analyze
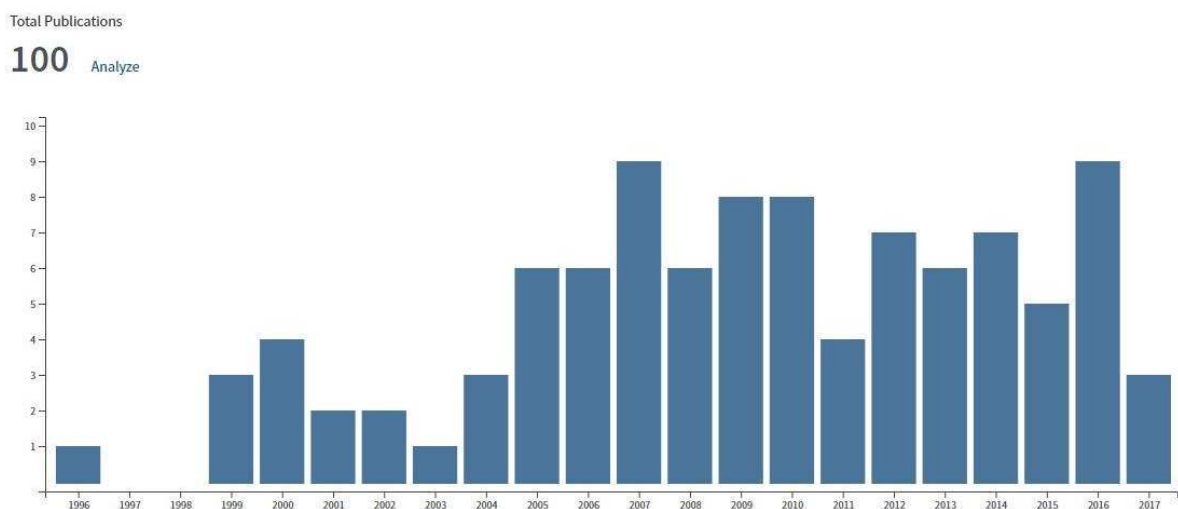


**Fig. 3. Distribution of publications in the field of anatomy education by years.**

**Table 2. Distribution of types of study and countries of the T100 articles with most citations based on years**

| | | < 2000 | | 2001-2005 | | 2006-2010 | | 2011-2015 | | 2016-2020 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **Sum** |
| **Study Type** | Original article | 8 | 88.90 | 12 | 92.30 | 34 | 91.90 | 21 | 75.00 | 11 | 84.60 | 86 |
| | Review | 0 | 0.00 | 0 | 0.00 | 1 | 2.70 | 4 | 14.30 | 1 | 7.70 | 6 |
| | Oral presentation | 1 | 11.10 | 0 | 0.00 | 1 | 2.70 | 2 | 7.10 | 1 | 7.70 | 5 |
| | Letter to the editor | 0 | 0.00 | 1 | 7.70 | 1 | 2.70 | 1 | 3.60 | 0 | 0.00 | 3 |
| **Countries** | Germany | 1 | 11.10 | 0 | 0.00 | 4 | 10.80 | 0 | 0.00 | 0 | 0.00 | 5 |
| | USA | 7 | 77.80 | 5 | 38.50 | 13 | 35.10 | 7 | 25.00 | 5 | 38.50 | 37 |
| | Australia | 1 | 11.10 | 1 | 7.70 | 4 | 10.80 | 5 | 17.90 | 4 | 30.80 | 15 |
| | United Arab Emirates | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 3 | 10.70 | 0 | 0.00 | 3 |
| | France | 0 | 0.00 | 0 | 0.00 | 1 | 2.70 | 1 | 3.60 | 0 | 0.00 | 2 |
| | England | 0 | 0.00 | 5 | 38.50 | 6 | 16.20 | 5 | 17.90 | 2 | 15.40 | 18 |
| | Ireland | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 2 | 15.40 | 2 |
| | Spain | 0 | 0.00 | 0 | 0.00 | 3 | 8.10 | 2 | 7.10 | 0 | 0.00 | 5 |
| | Sweden | 0 | 0.00 | 0 | 0.00 | 2 | 5.40 | 1 | 3.60 | 0 | 0.00 | 3 |
| | Italy | 0 | 0.00 | 1 | 7.70 | 0 | 0.00 | 1 | 3.60 | 0 | 0.00 | 2 |
| | Canada | 0 | 0.00 | 1 | 7.70 | 4 | 10.80 | 3 | 10.70 | 0 | 0.00 | 8 |
| | Sum | 9 | 100.0 | 13 | 100.0 | 37 | 100.0 | 28 | 100.0 | 13 | 100.0 | |

**Table 3. Research centers where the T100 articles with most citations were conducted.**

| Rank | Research type | n | % |
|---|---|---|---|
| 1 | Educational Research | 63 | 48.83 |
| 2 | Morphological Anatomy | 27 | 20.93 |
| 3 | Health Sciences | 18 | 13.95 |
| 4 | Surgery | 6 | 4.65 |
| 5 | Radiological Nuclear Medicine | 5 | 3.87 |
| 6 | Computer Science | 3 | 2.32 |
| 7 | Neuroscience, Neurology | 2 | 1.55 |
| 8 | Acoustic | 1 | 0.78 |
| 9 | General Internal Medicine | 1 | 0.78 |
| 10 | Science Technology Other Subjects | 1 | 0.78 |
| 11 | Social Sciences Other Subjects | 1 | 0.78 |
| 12 | Veterinary Sciences | 1 | 0.78 |
| | Sum | 129 | 100 |

**Table 4. Number of citations, AAS and IF of the journals published the T100 anatomy education articles**

|  | Mean ± SD | P25 | Median | P75 |
|---|---|---|---|---|
| Altmetric score | 5.29 ± 40.44 | 0.00 | 0.00 | 3.00 |
| Total citations | 66.84 ± 1.23 | 39.50 | 48.50 | 87.50 |
| Number of citations per year | 6.33 ± 35.73 | 3.33 | 5.47 | 7.86 |
| IF | 3.31 ± 15.70 | 1.81 | 4.03 | 4.03 |
| H Index | 65.75 ± 3.87 | 38.00 | 54.00 | 84.00 |

ber of citations was 66.84 ± 1.23. The mean number of citations per year was 6.33 ± 35.73, the mean IF was 3.31 ± 15.70, and finally, the mean h-index was 65.75 ± 3.87 (Table 4).

Correlation analyses were conducted to determine the relationship between AAS and the number of citations based on years and h-index. While there was a positive moderate relationship between AAS and number of citations based on years (r = 0.428), AAS and h-index had a negative weak relationship (r = -0.358). Likewise, there was a positive weak relationship between IF and h-index and number of citations based on years. There was no significant relationship between total number of citations and AAS (Table 5). The variation of the instruction methods used in anatomy education was determined (Table 6). A broad scale of anatomy learning methods was determined to include current methods in the form of virtual and augmented reality applications, mobile technology applications in the form of ultrasonography, virtual simulators, laparoscopy and other radiological imaging methods, clinical anatomy instruction methods, outputs obtained by 3D printer technology, plastination, web-based interactive 3D visualization, enriched multimedia e-book applications, close peer, problem-focused instruction, clay models, and YouTube and social media sharing. Comparisons of education with and without cadavers had a significant place. While there was also an education method in the form of integrated multimodal-multidisciplinary and blended instruction method examinations, this method involved comparison of several models.

## DISCUSSION

For the purpose of determining the effectiveness of methods that are used in the field of anatomy education, determining the online interest value and social media sharing scores of articles published on this topic and received the highest numbers of citations is highly

**Table 5. Correlation analysis of the relationship between AAS and number of citations by year and h-index**

|  |  | Total citations * | Number of citations per year | H Index | IF |
|---|---|---|---|---|---|
| Altmetric scoring | r | -0.051 | 0.428 | -0.358 | **0.002** |
|  | *p value* | 0.616 | **< 0.001** | **< 0.001** | 0.983 |
| Total citations * | r |  | 0.555 | 0.266 | 0.166 |
|  | *p value* |  | **< 0.001** | **0.009** | 0.106 |
| Number of citations per year | r |  |  | -0.192 | 0.277 |
|  | *p value* |  |  | 0.060 | **0.006** |
| H Index | r |  |  |  | 0.123 |
|  | *p value* |  |  |  | 0.231 |

r = Spearman's correlation coefficient

**Table 6.** **The most prevalent instruction modalities that are currently used in anatomy education**

| Rank | Anatomy education methods | Classification | Amount |
|---|---|---|---|
| 1 | Teaching with cadaver and dissection | I | 14 |
| 2 | Teaching with virtual reality, mobile augmented reality and mobile technology | II | 18 |
| 3 | 3D computer modeling and digital animated teaching | III | 10 |
| 4 | Anatomy teaching in clinical applications (Through Ultrasound imaging, Laparoscope imaging, Surgical simulators, Other radiological imaging techniques) | IV | 12 |
| 5 | Computer assisted teaching | V | 10 |
| 6 | Integrated multimodal-multidisciplinary and blended teaching | VI | 11 |
| 7 | Teaching with Youtube | VII | 7 |
| 8 | Teaching with 3D printer technology | VIII | 4 |
| 9 | Other: | IX | 14 |
| | Problem-based teaching (3) | | |
| | Near peer teaching (3) | | |
| | Teaching with clay models (1) | | |
| | Teaching with plastination (2) | | |
| | Anatomy teaching with social media (1) | | |
| | Teaching with enriched multimedia Ebook application (1) | | |
| | Teaching with web-based interactive 3D visualization (3) | | |

important in terms of determining trends towards the future. There are numerous studies and discussions carried out on suitable methods in obtaining academic information. Measuring the value and impact of studies and determining the trending topics are a significant criterion in determining the direction of studies. Considering that studies covering the comparative examinations of current practices used in anatomy education were conducted mostly in the period of 2007-2016, it was thought that this situation may be related to the date where especially virtual reality applications started to enter education. Virtual reality studies started to be carried out in 2006, and virtual reality became included in the education process as a technology-integrated instrument [19].

AAS assessment in our study revealed that the articles shared via social networks were rather on looking for answers to the question of "how to learn anatomy", and the effects of current practices such as "the contribution of 3D applications on anatomy edu-

cation" were investigated. As Marsland and Lazarus [20] in 2018 stated in their work, information sharing via social media is more popular with students due to the continuous existence of technology throughout the lives of young people, and that it is an international academic platform for sharing knowledge and education research experiences among academicians. This situation showed that youths focused on studies on learning methods involving current techniques rather than conventional ones. When studies on learning with YouTube videos, which is another method used in anatomy education, is examined by bibliometric analysis, it was stated that YouTube videos would display low educational value in the case that they are not checked by member of academia [21-23]. In their study on the learning levels of students with anatomy education videos uploaded on YouTube, Jaffar [24] in 2012 emphasized that videos could be useful, but their effect on examination performance is weak, and students prefer to learn surface anatomy by cadavers and

models. This idea was supported by the findings that YouTube videos and three-dimensional virtual reality videos have lower value in terms of education then dissections [25]. On the other hand, Winkellman [26] in 2007 reported that anatomy teaching with living beings and medical imaging methods is dominant over cadaver teaching. They added that these methods, which are especially advantageous for visual learners, are more compatible with clinical examination [26]. However, teaching about the 3D structure of the human body using 2D images is highly complicated. It is especially more difficult to perform volumetric examinations on the organs and structures that are desired to be imaged. For this reason, anatomic applications created in 3D are seen as an important method in making it easier to access structures by three-dimensional imaging.

Besides, teaching with cadavers should not be underestimated by relying solely on social media posts. Wilson *et al*. [27] in 2018 advocated the idea that the anatomical knowledge obtained by teaching with cadaver dissection remains in mind for a longer term in the temporal course in their studies where anatomy teaching methods were examined using meta-analysis method. However, they still recommended the examination and dissection of cadavers in the post-graduation surgical training of cadaver training.

When the social media sharing frequency of the article with the highest AAS (130) in our study, "The production of anatomical teaching resources using three dimensional (3D) printing technology," is examined, it was determined that it was shared via Twitter 30 times, Facebook 7 times, Google users 6 times, blogs 4 times and other networks 10 times. The highest number of shares made for the article with the second highest AAS, "Human cadaver vs. multimedia simulation: a study of student learning in anatomy," was again on Twitter. These findings confirm the comments of previous studies concluded that students and academicians have increased their interest in teaching styles with increased visual aspects. Regardless, as reported by Marsland and Lazarus [20], MD discussions around the anatomy teaching on twitter shows the need to develop potential educational resources to eliminate the difficulty in teaching and learning difficult areas in anatomy education.

It is highly important to comprehend basic anatomy in terms of being able to make the connections between anatomy knowledge and the clinic and integrate knowledge in surgical practices. For this reason, developing effective methods to teach anatomy is very important for medical practices. Since the Renaissance, cadaver dissection is still considered to be the most ideal and universal method for this purpose, and thus, it continues to be used as the building block of anatomy education [1, 28]. Moreover, considering the increasing prevalence of robotic surgeries, it may be stated that application of cadaver surgery and virtual simulations together could be more ideal. Students are no longer satisfied with looking at the pictures of a textbook or observing from the corner of a crown surrounding a cadaver [29]. Therefore, it was thought that in could be sufficient in meeting educational needs for educators to plan a modern anatomy education that will integrate "new and old" approaches for these searches of students and put these plans into practice.

Altmetric scoring assesses an interest shown in a publication from a different perspective. While a relationship is expected between bibliometric and altmetric assessment, such a relationship could not be found in the analyses that were conducted. An interesting finding in our study was that the articles with the highest IF values did not receive more interest than the ones with low IF values. For example, journals like Computers Education, Medical Education and Anatomical Sciences Education that are included in the high-IF journal list or contributed to a large number of articles in the field of anatomy education were observed to be not in the highest rating in terms of Altmetric scoring. While it was expected for the relationship between altmetric scoring and number of citations based on years to be stronger, it was determined that this relationship was moderate. Due to the contribution of the public in addition to the scientific world to the scores of articles shared on social networks, it was considered that the main factor determining the direction is the attractiveness of the topic. eBizMBA Inc. [30] in 2020 listed the most popular social websites worldwide as Facebook, YouTube, Instagram and Twitter and stated Facebook as the most popular among these. The popular social networks in the list followed as WhatsApp, Pinterest, Reddit, Ask.fm, Tumblr, Flickr, SnapChat, VK, LinkedIn, Tagged and

Meetup.

Although eBizMBA stated that Facebook is the most popular social network for sharing, it was seen that literature sharing was carried out more on Twitter. Marsland and Lazarus [20] in 2018 reported that an online community has formed on Twitter regarding literature sharing, but the impact of Twitter has not been completely demonstrated in anatomy instruction. They showed that Twitter could only be an instrument that strengthens the collaboration and communication of students with academicians, and social platforms may only have a useful impact under the appropriate guidance of academicians. Facebook is the most popular social media site visited daily by university students in integrating social media technologies into education. In their study that examined the usage activity of Facebook for the purpose of education in the field of anatomy, Jaffar [21] in 2014 reported that it has a natural potential in increasing learning in students, and it may be accepted as an instruction tool supportive of conventional education.

AASs are observed to provide new points of view in collecting scientific information. However, as the Altmetric system has started to collect data since the end of 2011, this method is only sensitive for later news. It may be considered that new articles may receive more AAS in time and lead to some incomplete interpretations. For this reason, by using bibliometric analysis, the weak aspect of the method was strengthened. Another important issue that should not be overlooked in AASs is social media policies of journals. Managers of some journals can develop planning digital scientific marketing practices for such social media metrics, which can influence the social media visibility of the journal and hence the journal's publications. This can be an important study topic that needs careful consideration. Even so it may be stated that AAS may be an indicator of the perceptions of the public on the dynamic field of science and medicine, and it will make it easier to determine the direction of studies in line with the high levels of interest by the public in time. It may also be considered as a catalyst that encourages reading scientific articles on a topic that is considered valuable by the public.

## CONCLUSION

In this study, articles that examined instruction resources and strategies that are used in the discipline of anatomy were evaluated with analysis methods that allow looking from a broad perspective. The necessity of planning an anatomy education that comprehensively integrates conventional and modern approaches and the necessity of presenting these in practice were determined by the analyses. It may be stated that there is no strong evidence yet to suggest that social networks are strong anatomy instruction instruments. While the interest received by a scientific study in the literature is usually measured by the number of citations it receives, the interest received by it in the public is measured with parameters such as the number of news stories published on it and the speed of being shared on social platforms. Consequently, for being able to more comprehensively assess scientific research outputs, we recommend assessment of AAS and conventional metrics in combination.

*Authors' Contribution*

Study Conception: EP; Study Design: MD; Supervision: EP, MD; Funding: EP; Materials: EP, MD; Data Collection and/or Processing: EP, MD; Statistical Analysis and/or Data Interpretation: MK; Literature Review: MK, EP, MD; Manuscript Preparation: EP, MD, MK and Critical Review: EP, MD, MK.

**Supplementary Table 1.** Details of the T100 list on anatomy education

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|------|---------------|---------|--------|------|--------------|---------|------------------------------|-----------------|
| 1 | Teaching anatomy without cadavers | Medical Education | 38 | 2004 | McLachlan, JC | 268 | 15.76 | 7 |

**Supplementary Table 1. Cotinued.**

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|---|---|---|---|---|---|---|---|---|
| 2 | The production of anatomical teaching resources using three dimensional (3D) printing technology | Anatomical Sciences Education | 7 | 2014 | McMenamin, PG | 174 | 24.86 | 130 |
| 3 | Anatomical dissection as a teaching method in medical school: a review of the evidence | Medical Education | 41 | 2007 | Winkelmann, A | 174 | 12.43 | 4 |
| 4 | Can virtual reality improve anatomy education? A randomised controlled study of a computer-generated three-dimensional anatomical ear model | Medical Education | 40 | 2006 | Nicholson, DT | 148 | 11.47 | 1 |
| 5 | Do we need dissection in an integrated problem-based learning medical course? Perceptions of first- and second-year students | Surgical and Radiologic Anatomy | 29 | 2007 | Azer, SA | 148 | 10.57 | 3 |
| 6 | A pilot study of comprehensive ultrasound education at the Wayne state university school of medicine - A pioneer year review | Journal of Ultrasound in Medicine | 27 | 2008 | Rao, S | 143 | 11.00 | 3 |
| 7 | "Let's Get Physical": Advantages of a physical model over 3D computer models and textbooks in learning imaging anatomy | Anatomical Sciences Education | 6 | 2013 | Preece, D | 129 | 16.13 | 5 |
| 8 | The dissection course - necessary and indispensable for teaching anatomy to medical students | Annals of Anatomy-Anatomischer Anzeiger | 190 | 2008 | Korf, HW | 129 | 9.92 | 0 |
| 9 | Near-peer teaching in anatomy: an ppproach for deeper learning | Anatomical Sciences Education | 2 | 2009 | Evans, DJR | 125 | 10.42 | 4 |
| 10 | Web-based interactive 3D visualization as a tool for improved anatomy learning | Anatomical Sciences Education | 2 | 2009 | Petersson, H | 121 | 10.08 | 0 |
| 11 | Mortui vivos decent? The evolving purpose of human dissection in medical education | Academic Medicine | 75 | 2000 | Dyer, GSM | 121 | 5.76 | 0 |
| 12 | Survey of clinicians' attitudes to the anatomical teaching and knowledge of medical students | Clinical Anatomy | 18 | 2005 | Waterston, SW | 118 | 7.38 | 0 |
| 13 | Perceptions of dissection by students in one medical school: beyond learning about anatomy. A qualitative study | Medical Education | 39 | 2005 | Lempp, HK | 114 | 7.13 | 0 |
| 14 | Does problem-based learning lead to deficiencies in basic science knowledge? An empirical case on anatomy | Medical Education | 37 | 2003 | Prince, KJAH | 114 | 6.33 | 0 |
| 15 | Reciprocal peer teaching: Students teaching students in the gross anatomy laboratory | Clinical Anatomy | 18 | 2005 | Krych, AJ | 109 | 6.81 | 0 |

## Supplementary Table 1. Continued.

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|------|---------------|---------|--------|------|--------------|---------|------------------------------|-----------------|
| 16 | Effectiveness of using blended learning strategies for teaching and learning human anatomy | Medical Education | 41 | 2007 | Pereira, JA | 108 | 7.71 | 0 |
| 17 | The relationships between learning outcomes and methods of teaching anatomy as perceived by professional anatomists | Clinical Anatomy | 21 | 2008 | Patel, K | 105 | 8.08 | 0 |
| 18 | Integrating professionalism in early medical education: the theory and application of reflective practice in the anatomy curriculum | Clinical Anatomy | 19 | 2006 | Lachman, N | 105 | 7.00 | 1 |
| 19 | The gross anatomy course: an analysis of its importance | Anatomical Sciences Education | 3 | 2010 | Boeckers, A | 99 | 9.00 | 0 |
| 20 | Modernization of an anatomy class: from conceptualization to implementation. A case for integrated multimodal-multidisciplinary teaching | Anatomical Sciences Education | 5 | 2012 | Johnson, EO | 97 | 10.78 | 2 |
| 21 | YouTube: an emerging tool in anatomy education | Anatomical Sciences Education | 5 | 2012 | Jaffar, AA | 96 | 10.67 | 15 |
| 22 | Medical students' learning of anatomy: memorisation, understanding and visualisation | Medical Education | 41 | 2007 | Pandey, P | 94 | 6.71 | |
| 23 | Evaluation of computer-aided instruction in a gross anatomy course: a six-year study | Anatomical Sciences Education | 2 | 2009 | McNulty, JA | 92 | 7.67 | |
| 24 | Use of 3D printed models in medical education: a randomized control trial comparing 3D prints versus cadaveric materials for learning external cardiac anatomy | Anatomical Sciences Education | 15 | 2016 | Lim, KHA | 89 | 17.80 | 12 |
| 25 | A novel three-dimensional tool for teaching | Human Neuroanatomy. | 3 | 2010 | Estevez, ME | 88 | 8.00 | 7 |
| 26 | Virtual reality and brain anatomy: a randomised trial of e-learning instructional designs | Medical Education | 41 | 2007 | Levinson, AJ | 87 | 6.21 | 0 |
| 27 | Teaching anatomy: cadavers vs. computers? | Annals of Anatomy-Anatomischer Anzeiger | 188 | 2006 | Biasutto, SN | 87 | 5.80 | 0 |
| 28 | Using multimedia and Web3D to enhance anatomy teaching. | Computers & Education | 49 | 2007 | Brenton, H | 84 | 6.00 | 0 |
| 29 | Best teaching practices in anatomy education: a critical review. | Annals of Anatomy-Anatomischer Anzeiger | 208 | 2016 | Estai, M | 83 | 16.60 | 0 |

## Supplementary Table 1. Continued.

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|---|---|---|---|---|---|---|---|---|
| 30 | Computer-aided learning: an overvalued educational resource? | Medical Education | 33 | 1999 | Devitt, P | 76 | 3.45 | 0 |
| 31 | Restructuring a basic science course for core competencies: an example from anatomy teaching | Medical Teacher | 31 | 2009 | Gregory, JK | 75 | 6.25 | 0 |
| 32 | Teaching and learning gross anatomy: dissection, prosection, or "both of the above?" | Clinical Anatomy | 12 | 1999 | Dinsmore, CE | 73 | 3.32 | 0 |
| 33 | Anatomical dissection: Why are we cutting it out? Dissection in undergraduate teaching. | ANZ Journal of Surgery | 31 | 2002 | Parker, LM | 71 | 3.74 | 2 |
| 34 | Ultrasound anatomy: a practical teaching system in human gross anatomy | Medical Education | 30 | 1996 | Teichgraber, UKM | 71 | 2.84 | 0 |
| 35 | The relative effectiveness of computer-based and traditional resources for education in anatomy | Anatomical Sciences Education | 6 | 2013 | Khot, Z | 69 | 8.63 | 3 |
| 36 | Integration of ultrasound in the education programme in anatomy | Medical Education | 11 | 2005 | Tshibwabwa, ET | 69 | 4.31 | 0 |
| 37 | Importance of dissection in learning anatomy: personal dissection versus peer teaching | Clinical Anatomy | 15 | 2002 | Johnson, JH | 67 | 3.53 | 0 |
| 38 | Dissection as a modulator of emotional attitudes and reactions of future health professionals | Medical Education | 42 | 2008 | Arraez, A | 66 | 5.08 | 0 |
| 39 | Advanced 3D visualization in student-centred medical education | Medical Teacher | 30 | 2008 | Silen, C | 62 | 4.77 | 0 |
| 40 | The role of three-dimensional information in health care and medical education: the implications for anatomy and dissection | Clinical Anatomy | 13 | 2000 | Marks, SC | 62 | 2.95 | 0 |
| 41 | Complementing anatomy education using three-dimensional anatomy mobile soware applications on tablet computers | Clinical Anatomy | 27 | 2014 | Lewis, TL | 60 | 8.57 | 7 |
| 42 | Medical students' approaches to learning anatomy: students' experiences and relations to the learning environment. | Clinical Anatomy | 23 | 2010 | Smith, CF | 55 | 5.00 | 1 |
| 43 | Anatomy education for the YouTube generation | Anatomical Sciences Education | 9 | 2016 | Barry, DS | 54 | 10.80 | 15 |
| 44 | Evaluation of a surgical simulator for learning clinical anatomy | Medical Education | 38 | 2004 | Hariri, S | 53 | 3.12 | 0 |

### Supplementary Table 1. Continued.

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|------|---------------|---------|--------|------|--------------|---------|------------------------------|-----------------|
| 45 | Ultrasound and cadaveric prosections as methods for teaching cardiac anatomy: a comparative study | Anatomical Sciences Education. | 5 | 2012 | Griksaitis, MJ | 52 | 5.78 | 3 |
| 46 | Mirracle: an augmented reality magic mirror system for anatomy education | İEE Virtual Reality Conference | … | 2012 | Blum, T | 52 | 5.78 | 0 |
| 47 | The value of teaching sectional anatomy to improve CT scan interpretation | Clinical Anatomy. | 14 | 2001 | De Barros, N | 52 | 2.60 | 0 |
| 48 | From Chalkboard, slides, and paper to e-learning: how computing technologies have transformed anatomical sciences education | Anatomical Sciences Education. | 9 | 2016 | Trelease, RB | 49 | 9.80 | 5 |
| 49 | Back to the future: teaching anatomy by whole-body dissection | Medical Journal of Australia. | 193 | 2010 | Ramsey, G | 49 | 4.45 | 0 |
| 50 | Learning of cross-sectional amatomy using clay Models | Anatomical Sciences Education | 2 | 2009 | Oh, CS | 49 | 4.08 | 1 |
| 51 | Can "YouTube" help students in learning surface anatomy? | Surgical and Radiologic Anatomy | 34 | 2012; | Azer, SA | 48 | 5.33 | 3 |
| 52 | Computer visualizations: factors that influence spatial anatomy comprehension | Anatomical Sciences Education | 5 | 2012 | Ngan, N | 48 | 5.33 | 0 |
| 53 | A meta-analysis of the educational effectiveness of three-dimensional visualization technologies in teaching anatomy | Anatomical Sciences Education | 8 | 2015 | Yammine, K | 46 | 7.67 | 0 |
| 54 | The application of 3D printing in anatomy education | Medical Education Online | 20 | 2015 | AbouHashem, Y | 46 | 7.67 | 6 |
| 55 | Virtual reality anatomy: is it comparable with traditional methods in the teaching of human forearm musculoskeletal anatomy? | Anatomical Sciences Education | 4 | 2011 | Codd, AM | 46 | 4.60 | 7 |
| 56 | Problem-based learning: is anatomy a casualty? | Plastic and Reconstrictive Surgery | 3 | 2005 | Hinduja, K | 46 | 2.88 | 0 |
| 57 | Progress and perspectives in computational neuroanatomy | Anatomical Record | 257 | 1999 | Ascoli, GA | 46 | 2.09 | 3 |
| 58 | Experimental evidence for improved neuroimaging interpretation using three-dimensional graphic models | Anatomical Sciences Education | 5 | 2012 | Ruisoto, P | 45 | 5.00 | 0 |
| 59 | How useful is plastination in learning anatomy? | Journal of Veterinary Medical Edcuation | 34 | 2007; | Latorre, RM | 45 | 3.21 | 3 |
| 60 | Using QuickTime virtual reality objects in computer-assisted instruction of gross anatomy: Yorick - the VR skull | Clinical Anatomy | 13 | 2000 | Nieder, GL | 45 | 2.14 | 0 |

## Supplementary Table 1. Continued.

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|------|---------------|---------|--------|------|--------------|---------|------------------------------|-----------------|
| 61 | Tendons, ligaments, and capsule of the rotator cuff. Gross and microscopic anatomy | Anatomical Sciences Education | 7 | 2014 | Clark JM | 44 | 6.29 | 0 |
| 62 | Building the dody: active learning laboratories that emphasize practical aspects of anatomy and integration with radiology | Anatomical Sciences Education | 3 | 2010 | Zumwalt, AC | 44 | 4.00 | 0 |
| 63 | The effectiveness of virtual and augmented reality in health sciences and medical anatomy | Anatomical Sciences Education. | 10 | 2017; | Moro, C | 43 | 10.75 | 34 |
| 64 | Teaching anatomy in the XXI century: new aspects and pitfalls | Scientific World Journal | --- | 2013 | Papa, V | 43 | 5.38 | 1 |
| 65 | Teaching anatomy with surgeons' tools: use of the laparoscope in clinical anatomy | Clinical Anatomy | 14 | 2001 | Fitzpatrick, CM | 43 | 2.15 | 0 |
| 66 | Social media and anatomy education: using Twitter to enhance the student learning experience in anatomy | Anatomical Sciences Education | 9 | 2016 | Hennessy, CM | 42 | 8.40 | 47 |
| 67 | The impact of alternating dissection on student performance in a medical anatomy course: are dissection videos an elective substitute for actual dissection? | Clinical Anatomy | 20 | 2007 | Granger, NA | 42 | 3.00 | 1 |
| 68 | Virtual anatomy: an anatomist's playground | Clinical Anatomy | 19 | 2006 | Spitzer, VM | 42 | 2.80 | 0 |
| 69 | Building virtual models by postprocessing radiology images: a guide for anatomy faculty | Anatomical Sciences Education. | 3 | 2010 | Tam, M | 40 | 3.64 | 0 |
| 70 | Virtual temporal bone: an interactive 3-dimensional learning aid for cranial base surgery | Neurosurgery | 64 | 2009 | Kockro, RA | 40 | 3.33 | 3 |
| 71 | The poor, the black, and the marginalized as the source of cadavers in United States anatomical education | Clinical Anatomy | 20 | 2007 | Halperin, EC | 40 | 2.86 | 22 |
| 72 | Teaching methods in anatomy courses in North American medical schools: the role of radiology | Academis Radiology | 13 | 2006 | Ganske, I | 40 | 2.67 | 0 |
| 73 | Anatomy instruction in medical schools: connecting the past and the future | Advances in Health Sciences Education | 11 | 2006 | Leung, KK | 40 | 2.67 | 0 |
| 74 | Evaluation of computer-aided instruction in the medical gross anatomy curriculum | Clinical Anatomy | 17 | 2004 | McNulty, JA | 40 | 2.35 | 0 |

**Supplementary Table 1. Continued.**

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|---|---|---|---|---|---|---|---|---|
| 75 | Animated PowerPoint as a tool to teach anatomy | Conference: Annual Meeting of the AAA held at the EB | 261 | 2000 | Carmichael, SW | 40 | 1.90 | 0 |
| 76 | Effectiveness of three-dimensional digital animation in teaching human anatomy in an authentic classroom context | Anatomical Sciences Education | 3 | 2014 | Hoyek, N | 39 | 5.57 | 3 |
| 77 | Undergraduate perspectives on the teaching and learning of anatomy | Anz Journal of Surgery | 79 | 2009 | Mitchell, R | 39 | 3.25 | 0 |
| 78 | Direct manipulation is better than passive viewing for learning anatomy in a three-dimensional virtual reality environment | Computers & Education | 247 | 2017 | Jang, S | 38 | 9.50 | 10 |
| 79 | How useful is YouTube in learning heart anatomy? | Anatomical Sciences Education | 7 | 2014 | Raikos, A | 38 | 5.43 | 35 |
| 80 | Cadaveric dissection as an educational tool for anatomical sciences in the 21st century | Anatomical Sciences Education | 10 | 2017 | Ghosh, SK | 37 | 9.25 | 7 |
| 81 | A change in paradigm: giving back identity to donors in the anatomy laboratory | Clinical Anatomy | 26 | 2013 | Talarico, EF | 37 | 4.63 | 0 |
| 82 | A head in virtual reality: development of a dynamic head and neck model | Anatomical Sciences Education | 2 | 2009 | Nguyen, N | 37 | 3.08 | 0 |
| 83 | Transforming clinical imaging data for virtual reality learning objects. | Anatomical Sciences Education | 1 | 2008 | Trelease, RB | 37 | 2.85 | 0 |
| 84 | Learning anatomy via mobile augmented reality: effects on achievement and cognitive load | Anatomical Sciences Education | 9 | 2016 | Küçük, S | 36 | 7.20 | 1 |
| 85 | Perceptions of a mobile technology on learning strategies in the anatomy laboratory | Anatomical Sciences Education | 6 | 2013 | Mayfield, CH | 36 | 4.50 | 1 |
| 86 | Use of plastinated prosections for teaching anatomy-The view of medical students on the value of this learning resource | Clinical Anatomy | 24 | 2011 | Fruhstorfer, BH | 36 | 3.60 | 1 |
| 87 | Explorable three-dimensional digital model of the female pelvis, pelvic contents, and perineum for anatomical education | Anatomical Sciences Education | 3 | 2010 | Sergovich, A | 36 | 3.27 | 0 |
| 88 | Developing medical students as teachers: an anatomy-based student-as-teacher program with emphasis on core teaching competencies | Anatomical Sciences Education | 6 | 2013 | Erie, AJ | 35 | 4.38 | 34 |

**Supplementary Table 1. Continued.**

| Rank | Article Title | Journal | Volume | Year | First Author | TCited* | Average Citations per Year** | Altmetric Score |
|------|---------------|---------|--------|------|--------------|---------|------------------------------|-----------------|
| 89 | Human cadavers vs. multimedia simulation: a study of student learning in anatomy | Anatomical Sciences Education | 7 | 2014 | Saltarelli, AJ | 34 | 4.86 | 52 |
| 90 | An enriched multimedia eBook application to facilitate learning of anatomy | Anatomical Sciences Education | 7 | 2014 | Stirling, A | 34 | 4.86 | 14 |
| 91 | Enhancement of temporal bone anatomy learning with computer 3D rendered imaging sowares | Medical Teacher | 332 | 2010 | Venail, F | 34 | 3.09 | 0 |
| 92 | How spatial abilities and dynamic visualizations interplay when learning functional anatomy with 3D anatomical models | Anatomical Sciences Education | 8 | 2015 | Berney, S | 33 | 5.50 | 3 |
| 93 | ARBOOK: Development and assessment of a tool based on augmented reality for anatomy | Journal of Science Education and Technology | 24 | 2015 | Ferrer, TJ | 33 | 5.50 | 1 |
| 94 | Fabrication and assessment of 3D printed anatomical models of the lower limb for anatomical teaching and femoral vessel access training in medicine | Anatomical Sciences Education | 9 | 2016 | O'Reilly, MK | 32 | 6.40 | 2 |
| 95 | Virtual cerebral Ventricular system: an MR-based three-dimensional computer model | Anatomical Sciences Education | 4 | 2011 | Adams, CM | 32 | 3.20 | 0 |
| 96 | Using 3D modeling techniques to enhance teaching of difficult anatomical concepts | Academic Radiology | 23 | 2016 | Pujol, S | 31 | 6.20 | 2 |
| 97 | Utilising mobile-augmented reality for learning human anatomy | Conference: 7th World Conference on Educational Sciences Location: Athens, GREECE | | 2015 | Jamali, SS | 31 | 5.17 | 0 |
| 98 | A "Second Life" for gross anatomy: applications for multiuser virtual environments in teaching the anatomical sciences | Anatomical Sciences Education | 4 | 2011 | Richardson, A | 31 | 3.10 | 0 |
| 99 | Comparison of computer-based and paper-based imagery strategies in learning anatomy | Clinical Anatomy | 18 | 2005 | Khalil, MK | 30 | 1.88 | 0 |
| 100 | Current applications and future perspectives of the use of 3D printing in anatomical training and neurosurgery | Frontiers in Neuroanatomy | 24 | 2016 | Baskaran, V | 29 | 5.80 | 2 |

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# Magnetic resonance imaging changes in multifidus and psoas muscles in patients with lumbar spinal stenosis

**Gökhan Çavuş[1]**, **Yeliz Çavuş[2]**

[1]*Department of Neurosurgery, University of Health Sciences, Adana City Training and Research Hospital, Adana, Turkey*
[2]*Department of Radiology, University of Health Sciences, Adana City Training and Research Hospital, Adana, Turkey*

**ABSTRACT**

**Objectives:** The aim of this study is to evaluate the relationship between the spinal canal cross-sectional area (SCCSA) at the stenosis level, the cross-sectional areas of the multifidus (MF) muscle at the L3-S1 levels, and the cross-sectional areas of the psoas muscle (PSCSA) at the L3 level in patients with nerve compression due to lumbar spinal stenosis.

**Methods:** A total of 280 patients were included in the study, 140 in the control group and 140 with lumbar spinal stenosis that had been detected with magnetic resonance imaging (MRI). The patients' ages, gender, SCCSA at the level of stenosis, PSCSA at L3 level, MFCSA at L3-S1 levels, and degrees of atrophy caused by multifidus muscle fatty degeneration were evaluated and compared with the control group whether the comparison was statistically significant or not.

**Results:** PSCSA values at the L3 level and MFCSA at the L3-4 level (r = 0.555, $p < 0.01$), MFCSA at the L4-L5 level (r = 0.559, $p < 0.01$), and MFCSA at the L5-S1 level (r = 0.429, p < 0.01) were found to have a statistically significant positive correlation. In addition, MFCSA at the L3-4 level ($p < 0.001$), MFCSA at the L4-5 level ($p < 0.001$), MFCSA at the L5-S1 level ($p < 0.001$), the L3-L4 SCCSA ($p < 0.001$), and the L4-L5 SCCSA ($p < 0.001$) mean values were found to be statistically significantly lower than the mean of the cases in the control group.

**Conclusions:** Compared to the control group, a significant correlation was found with a decrease in the CSA of the multifidus muscle and the presence of fatty degeneration of the multifidus muscle in patients with spinal stenosis. However, in the CSA of the psoas muscle at the L3 level, it was found that there was no correlation between patients with spinal stenosis and the control group.

**Keywords:** fatty degeneration, stenosis, multifidus, spinal, psoas, cross-sectional area

Lumbar spinal stenosis is the narrowing of the entrance and exit of the spinal canal, nerve root canal, and intervertebral foramen due to bone or soft tissue compression. As a result of disc degeneration, bone structures, and ligament hypertrophy, the spinal canal becomes narrow. If direct compression of the nerve root is involved in the physiopathology, it is accepted that the lumbar muscles will also play a role in the clinical symptoms. Clinical symptoms of degenerative lumbar stenosis are usually seen in patients over 60 but may be seen at an earlier age in patients with developmental and acquired stenosis. The man/woman ratio in gender distribution is 2:3, but it usually manifests clinically with neurogenic claudica-

tion or radicular symptoms [1].

The psoas muscle and the paraspinal muscles are important in maintaining the function of the lumbar spine. The multifidus muscle is the largest and most medial of the lumbar paraspinal muscles and is innervated by the medial branch of the segmental nerve root. The psoas muscle is innervated at the L1-L4 levels of the lumbar plexus by the branches of the anterior rami [2] Nerve root compression with spinal stenosis can cause morphological changes in the psoas and multifidus muscles [3].

Patients with clinical symptoms should be monitored by radiological studies to confirm that they have lumbar stenosis. MRI provides the most comprehensive imaging of the paraspinal muscle groups, psoas muscle, intervertebral discs, ligamentum flavum, spinal canal, and neuroforamen [4]. Computed tomography (CT) scanning allows accurate evaluation of the bone structures including vertebral bodies and facet joints [5]. When evaluating the spine, an SCCSA below 145 mm2 at the level of stenosis is radiologically accepted as spinal stenosis [6].

Muscle morphology may be changed by muscle atrophy and fatty degeneration, both of which affect muscle performance. Many studies report using imaging methods such as MRI or CT scanning to evaluate this change [7, 8]. Fat infiltration of the multifidus muscle on MRI was graded as "grade 0" for 0–10% fat, "grade 1" for 10–50% fat, and "grade 2" for > 50% fat (Fig. 1) [9]. Apart from these studies, there are studies evaluating muscle cross-sectional areas [10, 11]. Farshad *et al*. [3] measured the distance of the

multifidus muscle to the lamina in addition to the cross-sectional area. Yagi *et al*. [12] reported that trunk muscles play an important role in the spinal structure and that paraspinal muscle degeneration is associated with spinal deformity based on the evaluation of the cross-sectional area. However, there has been no study in which the cross-sectional areas of the psoas and multifidus muscles were evaluated together in patients with lumbar spinal stenosis.

The aim of this study is to demonstrate the cross-sectional changes in the multifidus and psoas muscles in patients with lumbar spinal stenosis and those in the control group and to compare the degree of fatty degeneration in the multifidus muscles at the spinal stenosis level using MRI to evaluate whether the results are statistically correlated.

## METHODS

A total of 280 patients were included in the study, 140 patients in the control group and 140 patients with lumbar spinal stenosis as detected with MRI in the radiology clinic between 01/01/2018–12/25/2020. Patients with previous spinal surgery, spondylolisthesis, spinal mass, vertebral fracture, or scoliosis were not included in the study. The control group patients were selected to correlate with the age and gender of the patient group. In the study, there were 54 men in both the patient and control group (38.6%), and 86 women in the patient and control group (61.4%). The mean age of the patient group was 58.2 ± 8.34 years and that
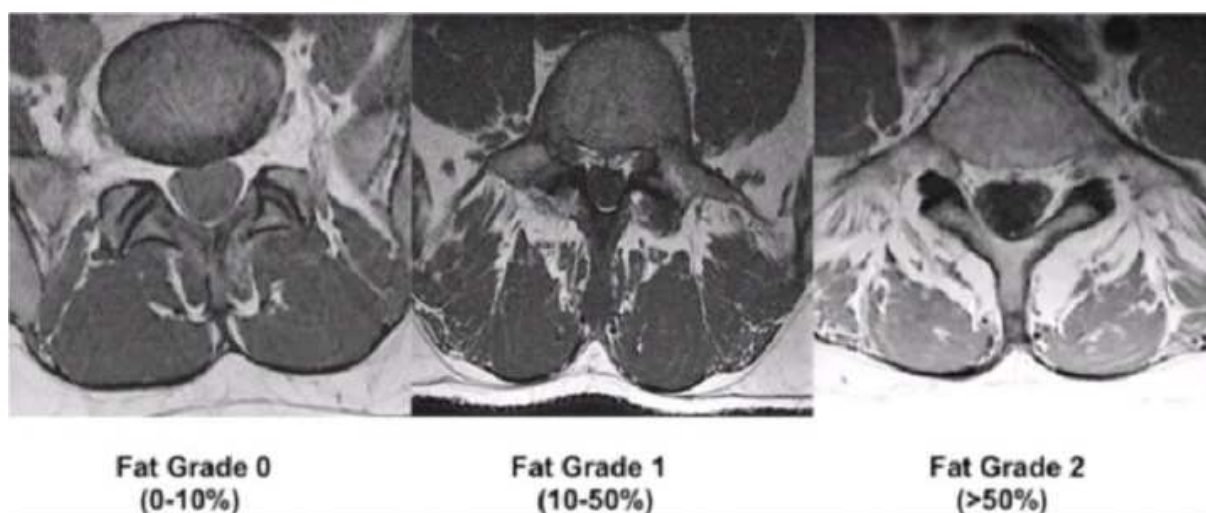


**Fig. 1.** Grading of MR images with different muscle-fat components of the lumbar multifidus muscle.

**Fig. 2.** SCCSA measurement at L4-5 level.

of the control group was 58.34 ± 7.7 years. The age range of the study group was 39-75 years and that of the control group was 39-76. SCCSA at the stenosis level (mm2), PSCSA (mm2) at the L3 level, MFCSA (mm2) at the L3-L4 and the L4-L5 levels, and the degrees of multifidus muscle atrophy were evaluated using MRI and compared with the control group. Adana City Training and Research Hospital Clinical Research Ethics Committee approved the study. Registration number: 1202, registration date: 30/12/2020.

**Imaging Parameters**

An MRI of the lumbar spine was performed using a 1.5-T unit (Philip, Ingenia; Philips Medical Systems, Best, the Netherlands). The imaging protocols included axial T2-weighted images (TR/TE 2770/100, matrix size 200 × 138, slice thickness 4 mm) and sagittal T2-weighted images (TR/ TE 3403/100, matrix size 160 × 232, slice thickness 4 mm).

**Image Analysis**

The lumbar MR images were evaluated by an experienced neuroradiologist, blinded to the symptoms, using a digital workstation. The measurements were done by one radiologist who repeated the measurements three times on different days to reduce the possibility of a bias. First, T2-weighted sagittal and axial images were used to determine the level and the cross-sectional area of the stenosis. The severity of multifidus fatty degeneration was graded according to the fat infiltration grading system.

T2-weighted axial MR images were used for the evaluation of the multifidus muscles and psoas muscles. MFCSA, PSCSA, and SCCSA at the stenosis level were examined to assess muscle atrophy. The SCCSA was measured on the axial T2-weighted MR images at the level of the stenosis, and the PSCSA at the L3 level. SCCSA was measured by measuring the area between the posterior edge of the posterior longitudinal ligament and the inner edge of the ligamentum flavum (Fig. 2). The MFCSA (Fig. 3) and PSCSA (Fig. 4) were traced using the manual cursor technique. The MFCSA and the PSCSA were measured on the right side of the spine.

**Statistical Analysis**

In the study, the demographic and clinical characteristics of the participants in the patient and control



**Fig. 3.** MFCSA measurement at L4-5 level.



**Fig. 4.** PSCSA measurement at L3 level.

groups were evaluated with descriptive statistical analysis regarding the number, percentage, mean, standard deviation, and median values. The degree of multifidus fatty degeneration among the participants in the patient group and the control group was evaluated using Fisher's exact test. In addition, gender ratios between the patient and control groups were evaluated according to the Pearson chi-squared analysis. The mean L3 PSCSA between the two groups was determined by the Mann-Whitney U test; MFCSA at the L3-4 level, MFCSA at the L4-5 level, MFCSA at the L5-S1 level, the L3-L4 spinal blood CSA, and the L4-L5 spinal blood CSA were compared with an Independent Groups t test. Among the men and women in both the patient group and the control group; PSCSA at the L3 level, MFCSA at the L3-4 level, MFCSA at the L4-5 level, MFCSA at the L5-S1 level, L3-L4 SCCSA and the L4-L5 SCCSA averages were compared using the Mann-Whitney U test. PSCSA at the L3 level, MFCSA at the L3-4 level, MFCSA at the L4-5 level, MFCSA at the L5-S1 level, the L3-L4 SCCSA and the L4-L5 SCCSA averages were compared using the Independent Groups t test. PSCSA at the L3 level, MFCSA at the L3-4 level, MFCSA at the L4-5 level, MFCSA at the L5-S1 level, the L3-L4 SCCSA, and the L4-L5 SCCSA relationships were analyzed using the Pearson's correlation analysis for normally distributed data, and the Spearman's correlation analysis for non-normally distributed data. The normal distribution compliance of the Independent Groups t test and the Pearson's correlation analysis were checked with kurtosis and skewness values ($\pm$ 1.5). The significance level for all analyses was set as $p < 0.05$. The IBM SPSS 22.0 program application was used for the analysis.

## RESULTS

The mean age of the patients evaluated was 58.20 $\pm$ 8.34 years (min. 39.00; max. 75.00), and the mean age of the participants in the control group was 58.34 $\pm$ 7.70 (min. 39.00; max. 76.00). According to the t test, the mean age of the two groups were statistically similar ($p = 0.916$). There were 54 (38.6%) men in the patient group and the control group and according to the Pearson chi-squared analysis, it was found that the gender ratio between the two groups was similar ($p = 0.999$).

In the patient group, 60 (42.9%) had multifidus muscle fatty atrophy grade one, 76 (54.3%) had fatty atrophy grade two, and four (2.9%) had fatty atrophy grade three. The control group was found to have 116 (82.9%) of the participants with multifidus muscle fatty atrophy grade one, and 24 (17.1%) had fatty atrophy grade two. According to the Fisher exact test results, it was found that the degree of multifidus muscle fatty atrophy between the patient and control groups was statistically significantly different.

According to the Mann-Whitney U test, it was found that the mean PSCSA at the L3 level in the patient group was not statistically significantly different from the control group ($p = 0.067$). According to the Independent Groups t test, the MFCSA at the L3-4 level ($p < 0.001$), the MFCSA at the L4-5 level ($p < 0.001$), the MFCSA at the L5-S1 level ($p < 0.001$), L3-

**Table 1.** Comparison of the averages of L3 psoas area, MFCSA L3-4, MFCSA L4-5, MFCSA L5-S1, L3-L4 SCCSA and L4-L5 SCCSA between patient (PG) and control groups (CG)

| | PG | | | CG | | | *p* value |
|---|---|---|---|---|---|---|---|
| | mean | SD./med | min-max. | mean | SD./med. | min-max. | |
| L3 psoas area | 816.02 | 798.93 | 400.98–1421.41 | 917.46 | 832.50 | 484.01–2006.54 | 0.067[a] |
| MF L3-4 | 431.10 | 80.11 | 260.79–609.05 | 535.83 | 102.78 | 361.92–879.31 | **< 0.001**[b] |
| MF L4-5 | 574.71 | 105.32 | 370.69–818.35 | 719.97 | 115.59 | 490.14–976.84 | **< 0.001**[b] |
| MF L5-S1 | 666.32 | 133.73 | 410.19–1011.64 | 820.06 | 132.52 | 514.20–1157.53 | **< 0.001**[b] |
| L3-L4 | 91.77 | 18.40 | 57.39–124.36 | 208.48 | 36.48 | 149.71–292.26 | **< 0.001**[b] |
| L4-L5 | 97.52 | 21.81 | 49.43–136.32 | 201.29 | 39.22 | 146.04–286.68 | **< 0.001**[b] |

MFCSA = cross-sectional areas of the multifidus, SCCSA = spinal canal cross-sectional area, med. = median, min. =minimum, max. = maximum, [a]Mann-Whitney U test, [b]Independent Groups t test

L4 SCCSA ($p < 0.001$) and the L4-L5 SCCSA ($p < 0.001$) were found to be statistically significantly lower than the mean of the cases in the control group (Table 1).

According to the Mann-Whitney U test, the means of the PSCSA at the L3 level ($p < 0.001$), MFCSA at the L3-4 level ($p < 0.001$), MFCSA at the L4-5 level ($p < 0.001$), and the MFCSA at the L5-S1 level ($p = 0.001$) was found to be statistically significantly higher in the men than the means of the women in the patient group. According to the Mann-Whitney U test, the means of the PSCSA at the L3 level ($p < 0.001$), the MFCSA at the L3-4 level ($p < 0.001$), the MFCSA at the L4-5 level ($p < 0.001$), and the MFCSA at the L5-S1 level ($p < 0.001$) for the men in the control group was found to be statistically significantly higher than the means of women. According to the Mann-Whitney U test, the means of the MFCSA at the L3-4 level ($p < 0.001$), MFCSA at the L4-5 level ($p < 0.001$), and MFCSA at the L5-S1 level ($p = 0.001$), the L3-L4 SCCSA ($p < 0.001$) and the L4-L5 SCCSA ($p < 0.001$) for the men in the patient group was found to be statistically significantly lower than the means for the men in the control group. According to the Independent Groups t test of the women in the patient group the means of the multifidus psoas muscle CSA at the L3 ($p = 0.043$), the MFCSA at the L3-4 level ($p < 0.001$), MFCSA at the L4-5 level ($p < 0.001$), MFCSA at the L5-S1 level ($p < 0.001$), and the L4-L5 SCCSA ($p < 0.001$) was found to be statistically significantly lower than the means of women in the control group. In addition, according to the Mann-Whitney U test, the L3-L4 level of the women in the patient group was found to be statistically significantly lower ($p < 0.001$) than the mean SCCSA of women in the control group (Table 2).

According to the Spearman correlation analysis, PSCSA values at the L3 level and the MFCSA at the L3-4 level (r = 0.555, $p < 0.01$), the MFCSA at the L4-5 level (r = 0.559, $p < 0.01$), and the MFCSA at the L5-S1 level (r = 0.429, $p < 0.01$), were found to have a statistically significant positive correlation. According to the Pearson correlation analysis, the L4-L5 level of the cases in the control group was found to have a statistically significant positive relationship between the SCCSA values and the MFCSA at the L3-4 level (r = 0.343, $p < 0.01$), the MFCSA at the L4-5 level (r = 0.404, $p < 0.01$), and the MFCSA at the L5-S1 level

**Table 2. Evaluation of study results with Mann-Whitney U test and Independent groups t test**

| | PG | | | | | CG | | | | | | |
| | Male (I) | | Female (II) | | | Male (III) | | Female (IV) | | | I-III | II-IV[b] |
| | mean | med. | mean | med | p value | mean | med. | mean | med | p value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L3 psoas area | 1040.52 | 1140.78 | 675.06 | 640.09 | <0.001[a] | 1183.92 | 1160.27 | 750.14 | 737.30 | <0.001[a] | 0.210[a] | 0.043[b] |
| MF L3-4 | 479.11 | 468.75 | 400.97 | 415.60 | <0.001[a] | 602.40 | 578.05 | 494.02 | 485.95 | <0.001[a] | <0.001[a] | <0.001[b] |
| MF L4-5 | 636.28 | 645.54 | 536.05 | 536.51 | <0.001[a] | 784.80 | 785.89 | 679.27 | 690.10 | <0.001[a] | <0.001[a] | <0.001[b] |
| MF L5-S1 | 730.57 | 748.56 | 625.98 | 633.76 | 0.001[a] | 890.75 | 898.62 | 775.68 | 791.11 | <0.001[a] | <0.001[a] | <0.001[b] |
| L3-L4 | 85.78 | 78.47 | 94.77 | 97.06 | 0.175[a] | 211.22 | 213.64 | 204.02 | 202.02 | 0.375[a] | <0.001[a] | <0.001[a] |
| L4-L5 | 98.82 | 99.48 | 96.64 | 99.66 | 0.801[a] | 209.75 | 213.28 | 195.7 | 194.51 | 0.132[a] | <0.001[a] | <0.001[b] |

MF = multifidus, PG = patient group, CG = control group, med. = median, min. = minimum, max. = maximum, [a]Mann-Whitney U test, [b]Independent Groups t test, MF = multifidus

**Table 3.** Correlations between L3 psoas area, MFCSA L3-4, MFCSA L4-5, MFCSA L5-S1 in patient and control groups

| Group | | L3-L4[a] | L4-L5[a] | L3 psoas area[b] | MF L3-4[a] | MF L4-5[a] | MF L5-S1[a] |
|---|---|---|---|---|---|---|---|
| PG | L3-L4[a] | 1.000 | | | | | |
| | L4-L5[a] | 0.357 | 1.000 | | | | |
| | L3 psoas area[b] | -0.080 | -0.026 | 1.000 | | | |
| | MF L3-L4[a] | 0.013 | 0.121 | 0.555** | 1.000 | | |
| | MF L4-L5[a] | 0.108 | 0.122 | 0.559** | 0.810** | 1.000 | |
| | MF L5-S1[a] | -0.237 | 0.114 | 0.429** | 0.715** | 0.840** | 1.000 |
| CG | L3-L4[a] | 1.000 | | | | | |
| | L4-L5[a] | 0.856** | 1.000 | | | | |
| | L3 psoas area[b] | 0.039 | 0.123 | 1.000 | | | |
| | MF L3-L4[a] | 0.197 | 0.343** | 0.622** | 1.000 | | |
| | MF L4-L5[a] | 0.264* | 0.404** | 0.470** | 0.795** | 1.000 | |
| | MF L5-S1[a] | 0.190 | 0.336** | 0.468** | 0.777** | 0.865** | 1.000 |

MF = multifidus, PG = patient group, CG = control group, [a]Pearson Correlation Analysis, [b]Spearman Correlation Analysis, ** < 0.001. * < 0.01

($r = 0.336$, $p < 0.01$). According to the Spearman correlation analysis, a statistically significant positive relationship was found between the PSCSA values at the L3 level and the MFCSA at the L3-4 level ($r = 0.622$, $p < 0.01$), the MFCSA at the L4-5 level ($r = 0.470$, $p < 0.01$), and the MFCSA at the L5-S1 level ($r = 0.468$, $p < 0.01$) (Table 3).

## DISCUSSION

Lumbar spinal stenosis is a common disease. The occurrence rate of this disease has increased significantly in recent years due to the increase in life expectancy. The introduction of new diagnostic methods in clinical practice has brought new recommendations for radiological criteria in spinal canal evaluation. MRI has become the preferred diagnostic tool for lumbar spinal stenosis because it can visualize muscle and other soft tissues. Evaluation of multifidus muscle atrophy in a diagnostic lumbar MRI will be advantageous in guiding the treatment process.

A limited number of studies have examined changes in lumbar paraspinal muscle cross-sectional areas. None of these studies have compared PSCSA, MFCSA, and SCCSA at the spinal stenosis level in patients with lumbar spinal stenosis. Takayama *et al.*

[13] studied the cross-sectional area of the paraspinal muscles using T2-weighted MRI in 160 patients aged between 10-88 years. They showed that the cross-sectional area of the paraspinal muscles tends to decrease with age. Sasaki *et al.* [14] achieved the same result in their studies. In our study, we found that the cross-sectional area of the paraspinal muscles decreases with increasing age in agreement with the literature. We anticipate that exercise may prevent narrow canal development caused by age-related multifidus muscle and psoas muscle atrophy.

In addition, men are known to have a larger paraspinal muscle cross section than women [13], and in our analysis, we found a statistically significant difference in that the cross-sectional area of the psoas muscle and multifidus muscle in men is higher than in women.

Zhao *et al.* [15] and Yoshihara *et al.* [16] reported histological studies showing significant atrophy of the multifidus muscles in patients with lumbar disc herniation. Hodges *et al.* [17] found rapid atrophy of the multifidus muscles after root compression in his experimental studies. In our study, we found statistically significant atrophy due to fatty degeneration in multifidus muscles due to root compression in patients with spinal stenosis with narrowed spinal canal incisional area similar to lumbar disc herniation. At the same

time, we found that there was no statistically significant difference in the PSCSA at the L3 level in patients with spinal stenosis compared to the control group. In our study, it was found that atrophy and fatty degeneration of the multifidus muscle due to compression of the spinal root at the spinal canal level were statistically significantly associated, but the PSCSA did not change at the L3 level in patients with spinal canal stenosis. The diagnostic evaluation of patients with lumbar MRI, together with an indication of the degree of multifidus muscle fatty degeneration atrophy should be considered in terms of stenosis prognosis and treatment. However, additional studies will be required to evaluate the overall paraspinal muscle cross-sectional area, muscle density, effects on spinal stability, and changes before and after surgery.

## Limitations

The limitations of our study were that the BMI index of the patients was not known, the measurement parameters that would evaluate lumbar stability other than the cross-sectional muscle areas of the patients were not evaluated, and the cross-sectional area comparison before and after the surgery was not performed.

## CONCLUSION

The study found that atrophy and fatty degeneration of the multifidus muscle due to compression of the spinal root at the spinal canal level were statistically significantly associated, but the cross-sectional area of the psoas muscle did not change at the L3 level in patients with spinal canal stenosis.

*Authors' Contribution*

Study Conception: GÇ; Study Design: GÇ; Supervision: GÇ; Funding: YÇ; Materials: YÇ; Data Collection and/or Processing: YÇ; Statistical Analysis and/or Data Interpretation: GÇ; Literature Review: GÇ; Manuscript Preparation: GÇ and Critical Review: YÇ.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Acceptability of a COVID-19 vaccine and role of knowledge, attitudes and beliefs on vaccination willingness among medical students

Mehmet Onur Kaya[1], Burkay Yakar[2], Esra Pamukçu[3], Erhan Önalan[4], Ramazan Fazıl Akkoç[5], Edibe Pirinçci[6], Mehmet Ferit Gürsu[7]

[1]Department of Biostatistics and Medical Informatics, Fırat University Faculty of Medicine, Elazığ, Turkey
[2]Department of Family Medicine, Fırat University Faculty of Medicine, Elazığ, Turkey
[3]Department of Statistics, Fırat University, Institute of Natural and Applied Sciences, Elazığ, Turkey
[4]Department of Internal Medicine, Fırat University Faculty of Medicine, Elazığ, Turkey
[5]Department of Public Health, Fırat University Faculty of Medicine, Elazığ, Turkey
[6]Department of Anatomy, Fırat University Faculty of Medicine, Elazığ, Turkey
[7]Department of Biochemistry, Fırat University Faculty of Medicine, Elazığ, Turkey

## ABSTRACT

**Objectives:** Vaccination to the population is the most effective way to get rid of the COVID-19 pandemic, which currently has no effective treatment. For the high vaccine acceptance rate, it is important to determine the vaccine willingness of individuals and the barriers to vaccination. We aimed to determine the acceptability of the COVID-19 vaccine and the effect of attitudes, perception and beliefs on vaccination willingness among medical students.
**Methods:** In February 2020, a cross-sectional survey was conducted online among medical student. A total of 734 medical students out of 980 medical students answered the questionnaire forms (response rate: 75.4%). The online questionnaire collected socio-demographic characteristics, attitudes, risk perception and beliefs about the COVID-19 pandemic and vaccine. Vaccine hesitancy, rejection and acceptance were determined by self-report of medical students.
**Results:** Total of 739 medical students included study. Students declared that 60.1% (n = 444) of them to be willing to vaccinated, 14.2% (n = 105) refused and 25.7% (n = 190) were not sure. Covid-19 willingness to be vaccinated was higher in female ($p = 0.005$), clinical students ($p < 0.001$), those who ever tested for COVID-19 ($p = 0.002$), those who had covid-19 infection history in the family ($p = 0.043$), those who had COVID-19 and COVID-19 vaccine education ($p = 0.001$). Positive attitudes skor and higher knowledge scores were related with highly willingness to be vaccinated. High beliefs score (positive beliefs) was found to be associated with a higher willingness to be vaccinated ($p < 0.001$).
**Conclusions:** Although the majority of the study population willingness to be vaccinated, one-third of medical students are hesitant about vaccination. Education, positive attitude and beliefs are related to higher willingness to vaccination.The COVID-19 pandemic immunization programs should be designed to remove barriers to negative vaccine beliefs and perception.
**Keywords:** COVID-19 vaccines, medical students, vaccination awareness, perception, beliefs

Coronavirus diseases 2019 (COVID-19) caused by the new type of coronavirus (SARS-COV2) was first reported in December 2019. It caused a pandemic in a short time and posed a significant threat to the whole world. Despite more than 1 year, humanity still suffers from COVID-19. Although more than 114 million cases have caused more than 2.5 million deaths worldwide, an effective treatment against COVID-19 has still not been found [1, 2].

The COVID-19 pandemic has caused disruptions in almost all areas of life all over the world. Education, especially medical education, is one of the areas negatively affected by the pandemic. Medical schools have been suspended during the pandemic, and as such, many students are stayed at home. This has disrupted the medical education process. Currently, medical education is tried to be given online in Turkey. There have been problems in bedside and practical training with online medical education. Although time is needed to accurately predict the effects of the pandemic on education, it is predicted that it may pose important problems for the learning experience and professional development of medical students [3].

It is known that the most beneficial method for the whole world to get rid of the negative effects of the pandemic is vaccination against COVID-19. It is possible with vaccination on the return of medical education and medical faculty students to normal life [4]. The tickets of medical education and medical faculty students to return to normal life pass from vaccination. Although vaccination seems to be the way out of the pandemic, the willingness of individuals to be vaccinated is also important in combating the pandemic. During the 2009 influenza A (H1N1) pandemic, it has reported that the acceptability of influenza vaccination among adults in the USA was between 50-64%. However, there is not enough information in the literature about the COVID-19 vaccine acceptance and the factors affecting it. Such information which acceptance rate and affecting factors are may be useful for improving COVID-19 vaccine acceptability [5, 6].

The current study was aimed to determine COVID-19 vaccine acceptance and which factors are related vaccination willingness

## METHODS

### Study Design, Population and Sampling

We conducted a prospective cross-sectional online survey study about COVID-19 vaccination with medical students in February 2021. The study population consisted of Fırat University all medical students. Online questionnaire forms were sent to the all medical students by whats-app. A total of 980 medical students who study in the period of 2020-2021 constituted the study population. Online questionnaire forms were sent to all medical students by whats-app, not using the sampling method. A total of 739 medical students who answered the questionnaire forms were included in the study (response rate:75.4%). Before applying the questionnaire, participants were asked to accept a consent form containing statements about the purpose of the study and voluntary participation of the questionnaire, and those who non-volunteers could not answer the questionnaire. The online questionnaire was sent 3 times with one week intervals and students who did not answer were asked to answer. After three weeks, unreturned questionnaires were recorded as missing. Those under the age of 18 years old, foreign students and those who are not active students were excluded from the study.

### Ethical approval, informed consent and permissions

The study was approved by the Firat University Non-Interventional Ethics Committee (Approval code: 23/128/2019), and the World Medical Association Declaration of Helsinki guidance was followed. The consent of the participants was questioned with the first question of the online survey form. The questionnaire was terminated for the participants who did not declare consent to participate in the study. In addition, permission was obtained from the scientific research platform of the Turkish Ministry of Health.

### Measures

The online questionnaire was designed based on previous studies which are assess vaccine acceptance. The contents of the questionnaire included (1) socio-demographic characteristics, such as; age, sex, class, family income, health status, personal and family history of COVID-19 diagnosis (2) perception and attitudes about COVID-19 pandemic; (3) beliefs about COVID-19.

We developed perception, attitude and beliefs survey items on vaccination based on previous literature data and Turkish Ministry of health suggestions for Avoiding and preventing COVID-19. Questionnare

prepared in local language Turkish. First, a group of medical experts provided feedback on the items of the survey, where it was modified as per their comments. Next, the modified draft of the survey went through pilot testing on 30 participants to provide feedback about the clarity and comprehensibility of the items of the survey. The questionnaire was revised one more time, after feedback of the students, and then applied to the study population

### Perception and Attitudes

We assessed participants' perceptions about COVID-19 by calculating the score of three items answered by participants (possible range = 1-4). The perceptions questions are: perceived likelihood of getting a COVID-19 infection in the future (possibility range = 1-4), disease progress in case of covid-19 (severity range = 1-4), how threatening covid-19 is for you (serious range = 1-4). We assessed participants' attitudes about COVID-19 by calculating the score of six items answered by participants (possible range = 0-1). Washing your hands (1 = rarely, 4 = always), Using hand sanitizer or cologne (1 = rarely, 4= always) , Wearing a mask (1 = rarely, 4 = always), Social distancing (1= rarely, 4= always), Staying away from closed area (1 = rarely, 4= always), and follow the recommended guidelines for COVID-19 (1 = rarely, 4 = always). Total score of perception and attitudes include eight questions. Participants could get a minimum of 8 and a maximum of 32 points. We coded each variable so that higher values indicate greater levels of positive perception and attitudes [7-9].

### Beliefs about COVID-19

We assessed participants' perceptions about COVID-19 by calculating the score of five items answered by participants (possible range = 1-5). The beliefs questions are: difficulty to get vaccinated (possible range = 1-5); effectiveness of a COVID-19 vaccine personality (possible range = 1-5); effectiveness of a COVID-19 vaccine for your family (possible range = 1-5); effectiveness of a COVID-19 vaccine for public health (possible range = 1-5); potential harms of a COVID-19 vaccine (possible range = 1-5). Total score of beliefs include five questions. Participants could get a minimum of 5 and a maximum of 25 points. We coded each belief variable so that higher values indicate greater levels of positive beliefs [7, 8]

### Statistical Analysis

Statistical analysis of the data was performed by IBM SPSS 22 statistics package program. Kolmogorov-Smirnov test was used to determine whether the data showed normal distribution. Descriptive statistics of the data were expressed as mean ± standard deviation for variables with normal distribution in continuous data and frequency for categorical variables as percentage (n (%)). Pearson Chi-square test was used to analyse categorical data. In comparison of more than two independent groups, One-Way ANOVA and LSD test for post-Hoc test were used for normal distributed continuous data. Significance level was *p* < 0.05. Statistically significant significance values are indicated in bold in the tables.

## RESULTS

Total of 739 medical students included study. The mean age of the students were 21.20 ± 2.68 years. The sample was 57% female, preclinical (65.3%) and clinical (34.7%) students. Students declared that 60.1% (n = 444) of them to be willing to vaccinated, 14.2% (n = 105) refused and 25.7% (n = 190) were not sure. COVID-19 willingness to be vaccinated was higher in female (*p* = 0.005), clinical students (*p* < 0.001), those who ever tested for COVID-19 (*p* = 0.002), those who had covid-19 infection history in the family (*p* = 0.043), those who had COVID-19 and COVID-19 vaccine education (*p* = 0.001) (Table 1).

Covid-19 willingness to be vaccinated was higher in students who declared that "I have a high possibility of getting COVID-19 in the future" (*p* < 0.001), "it can cause high progressive disease" (*p* = 0.008) and "highly threatening for my health" (*p* < 0.001). Positive attitudes skor and higher total knowledge and attitudes scores were related with highly willingness to be vaccinated (Table 2).

The relationship between students' beliefs on the COVID-19 vaccine and their willingness to be vaccinated is presented in Table 3. High beliefs score (positive beliefs) was found to be associated with a higher willingness to be vaccinated (*p* < 0.001) (Table 3).

## DISCUSSION

The current study showed that 60.1% (n = 444) of

**Table 1.** Association of demographic characteristics and COVID-19 vaccine willingness

| Variables | Do you willing to get the COVID-19 vaccine | | | | p value |
|---|---|---|---|---|---|
| | **No** | **Yes** | **Hesidant** | **Total** | |
| **Gender** | | | | | |
| Female | 47 (44.8) | 274 (61.7) | 106 (55.8) | 427 (57.8) | **0.005** |
| Male | 58 (55.2) | 170 (38.3) | 84 (44.2) | 312 (42.2) | |
| **Education level in medical school** | | | | | |
| First year | 37 (35.2) | 89 (20.0) | 69 (36.3) | 195 (26.4) | **< 0.001** |
| Second year | 29 (27.6) | 84 (18.9) | 66 (37.4) | 179 (24.2) | |
| Third year | 12 (11.4) | 61 (13.7) | 36 (18.9) | 109 (14.7) | |
| Fourth year | 18 (17.1) | 70 (15.8) | 7 (3.7) | 95 (12.9) | |
| Fifth year | 3 (2.9) | 71 (16.0) | 11 (5.8) | 85 (11.5) | |
| Internship | 6 (5.7) | 69 (15.5) | 1 (0.5) | 76 (10.3) | |
| **Do you have any chronic disease?** | | | | | |
| No | 95 (90.5) | 410 (92.3) | 171 (90.0) | 676 (91.5) | 0.579 |
| Yes | 10 (9.5) | 34 (7.7) | 19 (10.0) | 63 (8.5) | |
| **How would you describe your health condition?** | | | | | |
| Very bad | 1 (1.0) | 3 (0.7) | 2 (1.1) | 6 (0.8) | |
| Bad | 6 (5.7) | 30 (6.8) | 14 (7.4) | 50 (6.8) | |
| Good | 67 (63.8) | 335 (75.5) | 148 (77.9) | 550 (74.4) | |
| Very good | 31 (29.5) | 76 (17.1) | 26 (13.7) | 133 (18.0) | |
| **Ever tested for COVID-19** | | | | | |
| No | 84(80.0) | 310 (69.8) | 156 (82.1) | 550 (74.4) | **0.002** |
| Yes | 21 (20.0) | 134 (30.2) | 34 (17.9) | 189 (25.6) | |
| **Personal history of COVID-19 diagnosis** | | | | | |
| No | 89 (84.8) | 389 (87.6) | 164 (86.3) | 642 (86.9) | 0.714 |
| Yes | 16 (15.2) | 55 (12.4) | 26 (13.7) | 97 (13.1) | |
| **Family member/friend ever diagnosed with COVID-19** | | | | | |
| No | 72 (68.6) | 256 (57.7) | 102 (53.7) | 430 (58.2) | **0.043** |
| Yes | 33 (31.4) | 188 (42.3) | 88 (46.3) | 309 (41.8) | |
| **Have you ever been quarantined for COVID-19?** | | | | | |
| No | 79(75.2) | 307 (69.1) | 140 (73.7) | 526 (71.2) | 0.313 |
| Yes | 26 (24.8) | 137 (30.9) | 50 (26.3) | 213 (28.8) | |
| **Education for COVID-19** | | | | | |
| No | 42 (40.0) | 139 (31.3) | 88 (46.3) | 269 (36.4) | **0.001** |
| Yes | 63 (60.0) | 305 (68.7) | 102 (53.7) | 470 (63.6) | |
| **Education for COVID-19** | | | | | |
| No | 83 (79.0) | 351 (79.1) | 173 (91.1) | 607 (82.1) | **0.001** |
| Yes | 22 (21.0) | 93 (20.9) | 17 (8.9) | 132 (17.9) | |

Data are shown as n (%).

### Table 2. Relation between perception and attitude score and COVID-19 vaccine willingness

| Perception and Attitudes | Do you willing to get the COVID-19 vaccine | | | |
|---|---|---|---|---|
| | No | Yes | Hesidant | *p* value[*] |
| What are your possibility of getting COVID-19 in the future? | $2.75 \pm 0.69^b$ | $3.60 \pm 0.58^a$ | $3.02 \pm 0.63^a$ | **< 0.001** |
| How do you think the disease will progress if you get COVID-19 in the future? | $2.37 \pm 0.84^b$ | $2.55 \pm 0.70$ | $2.66 \pm 0.84^a$ | **0.008** |
| How threatening is COVID-19 to your health? | $2.48 \pm 0.91^b$ | $2.85 \pm 0.73^a$ | $2.87 \pm 0.76^a$ | **< 0.001** |
| Washing your hands often, especially after being in a public place or after blowing your nose, coughing, or sneezing. | $2.92 \pm 0.98$ | $3.09 \pm 0.89$ | $3.02 \pm 0.89$ | 0.204 |
| Using hand sanitizer or cologne that contains at least 60% alcohol. | $2.81 \pm 1.11^b$ | $3.16 \pm 0.90^a$ | $3.01 \pm 0.95$ | **0.001** |
| Wearing a mask when going out in public. | $3.57 \pm 0.76^b$ | $3.87 \pm 0.38^a$ | $3.74 \pm 0.59^a$ | **< 0.001** |
| Social distancing (keeping about 2 meters between yourself and other people) | $2.52 \pm 0.88$ | $2.62 \pm 0.82$ | $2.61 \pm 0.83$ | 0.584 |
| Staying away from closed area (situations close contact with others) | $2.75 \pm 0.88$ | $2.70 \pm 0.84$ | $2.62 \pm 0.86$ | 0.371 |
| Do you think you follow the recommended guidelines for COVID-19 adequately? | $3.00 \pm 0.76$ | $3.09 \pm 0.68$ | $2.69 \pm 0.79$ | 0.121 |
| **Total score** | $22.18 \pm 4.31^b$ | $23.90 \pm 3.28^a$ | $23.53 \pm 3.72^a$ | **<0.001** |

Data are shown as mean±standard deviation.

Higher scores indicate greater levels of positive attitudes and high kowledge.

[*] One-way Anova test

[a-b] Pairwise comparisons

participants to be willing to vaccinated, 14.2% (n = 105) refused and 25.7% (190) were not sure. The current study showed that more than a third of medical students were undecided about COVID-19 vaccination. Lucia *et al.* [7] reported that 53% of medical students would participate in a COVID-19 vaccine trial and 23% were unwilling to take a COVID-19 vaccine. A previous study reported that 69% of participants were willing to get a COVID-19 vaccine among US adults [8]. Another study reported that 91.3% of the participants would accept vaccination after the vaccine becomes available, among 52.2% of them wanted to get vaccinated as soon as possible, while others 47.8% would delay the vaccination until the vaccine's safety was confirmed [10]. It can be said that individuals still have hesitancy against the vaccine, which is the most important weapon we have to get rid of the pandemic. In the study conducted during the influenza A (H1N1) pandemic period, the vaccination rate was reported as 93.2% in medical students and 84.8% in nursing students [11]. Compared to the high acceptance vaccine

rate in the previous pandemic, the current COVID-19 vaccines hesitancy rate is unacceptable. It can be suggested to remove the obstacles to the willingness to be vaccinated as soon as possible.

The current study showed that sociodemographic factors such as gender, education level, family history of covid-19 diagnosis and having education for COVID-19 and COVID-19 vaccine are related to vaccine willingness and acceptance. Studies examining the relationship between COVID-19 vaccination willingness and sociodemographic characteristics are limited in the literature. A systematic review study reported that age, sex, occupation and educational level are associated with influenza A (H1N1) vaccination during the pandemic [12]. Wang *et al.* [10] reported that being male and believing in the efficacy of COVID-19 vaccination increase the probability of accepting COVID-19 vaccination. The striking finding in our data was that the willingness to be vaccinated was higher in students who declared having COVID-19 education and COVID-19 vaccine education.

**Table 3.** Relation between Beliefs score and COVID-19 vaccine willingness

| Beliefs about COVID-19 vaccination | Do you willing to get the COVID-19 vaccine | | | |
|---|---|---|---|---|
| | No | Yes | Hesidant | *p* value[*] |
| If you have to paid for the vaccine. Does this affect your vaccine acceptance?[1] | $1.86 \pm 0.77^b$ | $4.52 \pm 0.58^a$ | $3.19 \pm 0.49^a$ | **< 0.001** |
| If you decide to get the COVID-19 vaccine, do you think it would be hard to getting the vaccine?[2] | $3.41 \pm 1.12$ | $3.47 \pm 1.14^a$ | $3.12 \pm 0.89^b$ | **0.001** |
| How is important do you think the getting vaccine is to protect your health?[3] | $3.00 \pm 1.04^b$ | $4.41 \pm 0.67^a$ | $3.75 \pm 0.86^a$ | **< 0.001** |
| How important do you think vaccination is in protecting the health of your family members?[4] | $3.09 \pm 1.18^b$ | $4.46 \pm 0.72^a$ | $3.82 \pm 0.91^a$ | **< 0.001** |
| How important vaccination is in protecting public health and combating COVID-19 pandemic.[5] | $3.31 \pm 1.05^b$ | $4.61 \pm 0.57^a$ | $3.93 \pm 0.84^a$ | **< 0.001** |
| Do the possible side effects of the COVID-19 vaccine affect your vaccine acceptance?[6] | $2.16 \pm 0.81^b$ | $2.56 \pm 0.83^a$ | $2.37 \pm 0.77$ | **< 0.001** |
| Total score | $16.83 \pm 3.77^b$ | $24.03 \pm 2.51^a$ | $20.19 \pm 2.69^a$ | **< 0.001** |

Data are shown as mean±standard deviation.

Higher scores indicate greater levels of positive beliefs.

[*] One-way Anova test

[a-b]Pairwise comparisons

[1] 1 item; 5-point response scale ranging from ''absolutely affects'' to ''absolutely does not affect'' (possible range = 1-5)

[2] 1 item; 5-point response scale ranging from "very hard" to "very easy"(possible range=1-5)

[3,4,5] 1 item; 5-point response scale ranging from "unimportant" to "very important" (possible range=1-5)

[6] 1 item; 5-point response scale ranging from ''absolutely affects'' to ''absolutely does not affect'' (possible range = 1-5)

Based on our data, we can say that we should attach importance to education in order to increase the willingness to be vaccinated.

Previous studies showed that risk perception is a central predictor of protection intentions and preventive health behaviours [13]. In addition, it is known that vaccination rates are higher in those who have a positive attitude towards the disease [14]. According to our study, the willingness to be vaccinated was higher in the participants who had a positive attitude and having a high-risk perception. A COVID-19 vaccine acceptance study reported that the COVID-19 vaccine acceptance rates were lower in those with an anti-vaccination attitude and those with low-risk perception. The current study and previous vaccine acceptance studies showed that positive attitude and perception are important to vaccine acceptance. It was thought that we should increase the positive attitudes of individuals for vaccination in the fight against the COVID-19 pandemic and the pandemics that may be experienced from now on.

Health beliefs are associated with adherence to preventive healthcare and treatment. People will not change their health behaviours unless they believe that they are at risk. Previous studies reported taht several health beliefs were also correlated with vaccine acceptability (i.e. perceived likelihood, perceived severity, perceived vaccine effectiveness, perceived potential vaccine harms) [7, 8, 10-14]. In our study, we showed once again that beliefs about the vaccine are associated with vaccination rates. Negative beliefs that especially the vaccine is not protective and the vaccine has side effects and harmful effects were found to be related to the willingness to be vaccinated in our study. Family physicians who are at the forefront of vaccine practice in Turkey, should pay attention to these negative beliefs and they must struggle to change these negative thoughts.

**Implications**

This study showed us that even though we were in the pandemic process, individuals' low knowledge, negative attitudes and beliefs decreased their willingness to be instilled. This study showed that even dur-

ing the pandemic period, which has no current treatment, causes thousands of deaths every day, negatively affects all of life, and we know that vaccination is the only way to escape from the pandemic, but still, individuals experience vaccination hesitation.

## Limitations

We conducted cross-sectional study among medical student so obtained data may not reflect the adult population vaccination willingness. We did not use objective measurement methods for attitudes and beliefs about vaccine willingness. All data are obtained from participants' own declaration. Vaccination against COVID-19 has not yet become widespread in our country, and the lack of sufficient data about the COVID-19 vaccine may have affected the opinions of individuals about the vaccine.

## CONCLUSION

Although the majority of the study population willingness to be vaccinated, one-third of medical students are hesitant about vaccination. Education, positive attitude and beliefs are related to higher willingness to vaccination. Future studies in this area should focus on the effect of vaccine education and attempts to change attitude and belief on vaccine acceptance. The COVID-19 pandemic immunization programs should be designed to remove barriers to negative vaccine beliefs and perception.

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# The use of vitamin C in the intensive care unit during the COVID-19 pandemic

Şeyda Efsun Özgünay[1], İlkay Ceylan[1], Korgün Ökmen[1], Halil Erkan Sayan[1], Şermin Eminoğlu[1], Derya Karasu[1], Şenol Yavuz[2]

*[1]Department of Anesthesiology and Reanimation, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey*
*[2]Department of Cardiovascular Surgery, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey*

## ABSTRACT

**Objectives:** An exaggerated inflammatory immune response is observed in cases of COVİD 19. This study aims to evaluate the effects of vitamin C, as a significant modulator of inflammation, on the inflammatory parameters and mortality in patients followed up in the intensive care unit (ICU) during the pandemic.
**Methods:** This single-center retrospective study included 160 adult patients with confirmed positive nasopharyngeal swab COVID-19 PCR test results, who were followed up in the ICU between 01 March and 01 August 2020. Hospital files were scanned and patients given and not given vitamin C were assigned, to Group 1 and Group 2, respectively. The Acute Physiology and Chronic Health Evaluation (APACHE II) scores, the data entries on demographics, ventilation, laboratory tests, duration of ICU stay, discharge, and mortality were compared. Interventions with antiviral and antibacterial agents. steroids, cytokine apheresis, and renal replacement therapy were evaluated.
**Results:** Vitamin C treatment was started in patients with raised neutrophil/lymphocyte ratio (NLR) at admission to ICU, who made up 20% of the total 160 ICU patients. Mortality incidence was higher among patients with raised NLR. Vitamin C at the doses used did not affect the inflammatory parameters, length of stay or mortality, and 37.5% of the patients recovered and were discharged from the ICU.
**Conclusions:** We think that vitamin C treatment will have positive effects on the initiation of COVID-19 and that the time of intiation of the therapy and the optimal doses used should be determined with prospective studies.
**Keywords:** COVID-19, intensive care, vitamin C, NLR

Given the induction of an exaggerated immune response in coronavirus disease -19 (COVID-19), the use of supplementary therapy with various vitamins has been considered [1-3]. Vitamin C is a water soluble powerful antioxidant agent which is not synthesized and not stored in human body. It has effects on the reduction of gene expression of proinflammatory cytokines and protects the structure and function of DNA, tissue proteins, and lipids [4-7]. It modulates immune response and takes place in lymphocyte proliferation and neutrophil phagocytosis [7]. Vitamin C has been shown to be involved in the control of the immunopathological response augmenting the pathogenesis of severe viral infections of the respiratory

system [1, 8] and also in the prevention of tissue damage in acute respiratory distress syndrome (ARDS) [1, 9]. In ICU patients and other cases of infections, the systemic vitamin C need increases [10, 11] and intravenous (i.v.) replacement with high doses may be required [8].

The recently increased clinical research on the effect of vitamin C in cases of ARDS and septic shock has produced conflicting reports on the dosages used [1, 2, 5]. Vitamin C has been shown to reduce the severity of multi-organ failure, the levels of circulating biomarkers of tissue damage [2, 10, 12, 13] and the incidence of mortality [14]. Positive effects of 6-24 gm/day i.v. vitamin C have been reported in cases of COVID-19 with serious lung damage [14, 15].

Biomarkers such as D dimer, C-reactive protein (CRP), ferritin, fibrinogen, and IL-6 reach high levels in COVID-19 patients [15, 16]. The effect of vitamin c on lowering the inflammatory production has given hope in the treatment of viral infections [1, 7, 18-20]. It is believed that the timely use of i.v. vitamin C especially inhibits the cytokine storm and improves the outcome in COVID-19 [2]. Research shows that inflammatory parameters neutrophil/lymphocyte ratio (NLR), CRP, D dimer, and troponins are useful to determining the severity of COVID-19 and predicting the prognosis [16]. As an antioxidant and anti-inflammatory nutrient, effects of vitamin C need to be investigated in COVID-19 [7, 17].

Therefore, in this study, we aimed to assess the effects of high dose vitamin c on inflammatory parameters, the duration of ICU stay and mortality in COVID-19 patients admitted to ICU.

## METHODS

The approval for this single center retrospective study was given with the Ethics Committee of the Ministry of Health Scientific Research Council by the document numbered and dated 2011-KAEK-25 2020/07-12. Adult patients who had positive nasopharyngeal swab COVID-19 PCR test results and were admitted to the ICU between 01 March 2020 and 1 August 2020 were included in the study. Exclusion criteria were defined as having unconfirmed PCR and pregnancy was accepted as the exclusion criteria. Treatment protocols in the ICU were designed accord-

ing to the current Ministry of Health guidelines [21]. Vitamin C support was given as 3×2 gm, i.v. Patients were divided into 2 groups as Group 1 and Group 2 on the basis of, receiving and not receiving vitamin C therapy, respectively. Hospital files were scanned for data on demographics including age, gender, blood group, comorbid diseases and also the symptoms at admission to the ICU, the APACHE II scores, invasive ventilation use, oxygen support methods using non breather mask with reservoir, high flow nasal cannula (HFNC), non-invasive ventilation (NIV), duration of hospital stay, and ICU stay, discharge and mortality were recorded. The hematological data on D dimer, procalcitonin, CRP, ferritin, NLR and troponin levels at entry and discharge from the ICU, bacterial cultures, antiviral, antibacterial treatments, use of immune plasma, cytokine apheresis, and renal replacement

**Table 1. The characteristics of the patients**

|                                      | n = 160            |
|--------------------------------------|--------------------|
| Age (year), mean ± SD                | 70.19 ± 14.41      |
| BMI, mean ± SD                       | 22.55 ± 10.53      |
| Gender, n, (%) Female/Male           | 72 (45)/88 (55)    |
| Comorbid disease, n (%)              |                    |
| Non                                  | 14 (8.3)           |
| Hypertension                         | 62 (38.8)          |
| Diabetes mellitus                    | 18 (11.3)          |
| KAD-CHF                              | 20 (12.5)          |
| COPD                                 | 12 (7.5)           |
| Malignancy                           | 7 (4.4)            |
| Others                               | 22 (13.8)          |
| Arrival symptom                      |                    |
| Fever                                | 40 (25)            |
| Cough                                | 6 (3.8)            |
| Respiratory distress                 | 79 (49.4)          |
| Syncope                              | 7 (4.4)            |
| Arrest                               | 3 (1.9)            |
| Others                               | 18 (11.3)          |
| Given Vitamin C, n (%)               | 32 (20)            |
| Exitus, n (%)                        | 92 (57.5)          |

Data are shown as mean ± standard deviation and n (%). KAD-CHF = Coronery arter disease-congestive heart failure, COPD = chronic obstructive pulmonary disease,

therapy (RRT) were evaluated.

**Statistical Analysis**

Statistical evaluations of the data were carried out using the SPSS 21.0 for Windows (Statistical Package for the Social Sciences, Armonk, NY, USA). The Kolmogorov- Smirnov test was used to determine normality of distribution for the variables. Continuous variables were expressed by the mean, the standard deviation, or the median (minimum-maximum) values, and the categorical data were expressed in percentages. Intergroup comparisons were made using the t test. The Pearson chi-square test was used to detect the differences between groups on the basis of categorical variables. For responses at different time points, percent changes above the baseline measurement were calculated. These percent changes were compared using the Mann-Whitney U test and student t test for the two groups. A $p < 0.05$ was accepted to indicate statistical significance..

**RESULTS**

The data of the 160 PCR (+) COVID-19 patients were analyzed. The demographic details of the patients are presented in Table 1. Vitamin C treatment was started on 20% of the patients and mortality incidence was determined as 57.5%. There was no statistically significant difference in the duration of ICU stay in the comparison between the groups (Table 1). The data of the two groups on the APACHE II scores, invasive ventilation, use of HFNC, NIV, and non-breather mask did not differ significantly. The duration of ICU stay, the conditions at discharge and mortality also did not differ significantly between the two groups (Table 2). The NLR value of the patients who were given vitamin C were higher at admission to ICU ($p = 0.028$). The percentage changes at different time points of NLR responses at ICU relative to that at admission did not differ sifnificantly between two groups ($p = 0.18$) (Table 2). The baseline NLR levels of the nonsurviving patients were signifcantly higher as compared to the survivors of ICU treatment ($p = 0.005$). D dimer, procalcitonin, CRP, ferritin, and troponin levels during COVID-19 infection did not differ significantly between both groups (Table 3).

There were no significant differences in terms of steroid use between two groups. Implementations of antiviral, antibacterial, immune plasma treatments, bacterial cultures; the use of cytokine apheresis and RRT, also did not significantly differ between the two groups (Table 4).

**Table 2. Comparison of ventilation method, duration of stay and mortality between groups**

|  | Group 1 (n = 32) | Group 2 (n = 128) | *p* value |
|---|---|---|---|
| APACHE II | 25.46 ± 7.20 | 25.40 ± 6.97 | 0.96 |
| Invasive ventilation | 23 (71.9) | 91 (71.7) | 0.88 |
| HFNC | 11 (34.4) | 40 (31.3) | 0.73 |
| NIV | 6 (18.8) | 14 (10.9) | 0.61 |
| Nonbreather mask with reservoir | 25 (78.1) | 77(60.2) | 0.16 |
| NLR* |  |  |  |
| admission | 24.83 ± 28.70 | 13.26 ± 12.58 | 0.028 |
| discharge | 14.93 ± 47.34 | 14.44 ± 16.47 |  |
| discharge Δ | 0.34 ± 0.93 | -0.54 ± 1.62 | 0.18 |
| Duration of ICU stay** | 8 (2-31) | 7(1-47) | 0.61 |
| Discharge | 12 (37.5) | 34 (26.6) | 0.20 |
| Mortality | 17 (53.1) | 75 (58.6) | 0.57 |

Data are shown as mean± standard deviation and n (%). HFNC = high flow nasal cannula, NIV = non-invasive ventilation, NLR = neutrophil/lymphocyte ratio, Δ percent change between ICU admission and discharge, The chi-square test, *Student t test, ** Man Whitney U, median (minimum-maximum)

**Table 3. Comparison of inflammation markers between groups**

| | Group 1 (n = 32) | | | Group 2 (n = 128) | | | *p* value |
|---|---|---|---|---|---|---|---|
| | Median | Minimum | Maximum | Median | Minimum | Maximum | |
| **D Dimer** | | | | | | | |
| admission | 2.90 | 0.32 | 59.7 | 3.50 | 0.25 | 215.0 | 0.82 |
| discharge | 3.50 | 0.43 | 80.0 | 3.90 | 0.58 | 675.0 | |
| discharge Δ | -0.029 | -0.98 | 90.67 | -0.033 | -0.98 | 162.27 | 0.87 |
| **CRP** | | | | | | | |
| admission | 126.5 | 3.14 | 368.0 | 104.5 | 3.10 | 329.0 | 0.32 |
| discharge | 90.90 | 3.70 | 393.0 | 99.0 | 3.00 | 364.0 | |
| discharge Δ | 0.009 | -0.98 | 101.55 | -0.19 | -0.98 | 101.89 | 0.88 |
| **Ferritin** | | | | | | | |
| admission | 537.30 | 21.40 | 2903.00 | 714.42 | 3.70 | 1245.71 | 0.30 |
| discharge | 293.00 | 99.0 | 2669.0 | 623 | 41.5 | 16.830 | |
| discharge Δ | 0.51 | -0.79 | 8.5 | 0.30 | -0.83 | 226.63 | 0.60 |
| **Procalcitonin** | | | | | | | |
| admission | 4.5 | 0.45 | 29.94 | 1.71 | 0.03 | 86.0 | 0.26 |
| discharge | 4.9 | 0.09 | 36.80 | 0.65 | 0.04 | 41.0 | |
| discharge Δ | -0.82 | -0.99 | 40.72 | -0.35 | -0.99 | 44.56 | 0.88 |
| **Troponin** | | | | | | | |
| admission | 66.8 | 4.12 | 3117 | 103.0 | 4.15 | 1600 | 0.80 |
| discharge | 103.0 | 4.15 | 4609 | 103.0 | 5.82 | 6259 | |
| discharge Δ | 0.48 | -0.79 | 31.86 | 0.62 | -0.83 | 172.43 | 0.46 |

CRP = C-reactive protein, Δ percent change between ICU admission and discharge, Mann-Whitney U test.

**Table 4. Comparison of treatment practices and bacterial culture between groups**

| | Group 1 (n = 32) | Group 2 (n = 128) | *p* value |
|---|---|---|---|
| Antiviral | 25 (78.1) | 84 (65.6) | 0.20 |
| Hydroxychloroquine | 31 (96.9) | 118 (92.2) | 0.34 |
| Antibacterial treatments | 25 (78.1) | 75 (59.1) | 0.46 |
| Steroid | 10 (31.3) | 37 (28.9) | 0.83 |
| Immune plasma | 1 (3.1) | 13 (10.2) | 0.30 |
| RRT | 7 (21.9) | 23 (18.0) | 0.61 |
| Cytokine apheresis | 1 (3.1) | 7 (5.5) | 0.58 |
| Bacterial cultures | 18 (56.3) | 65 (50.8) | 0.69 |

Data are shown as n (%). RRT = Renal replacement therapy, the chi-square test

## DISCUSSION

Although the optimal dosages are not known, high doses of vitamin C have been used for many years for preventing and reducing the severity of respiratory infections without reports regarding serious adverse side effects [1, 7, 14, 18, 19]. The vitamin C levels of COVID-19 patients admitted to ICU were reported by Arvinte *et al*. to be 200% higher in the survivors as compared to the nonsurvivors [22]. In our study, only the NLR values were observed to be significantly higher in the nonsurviving ICU patients in comparison to the survivors. It was seen that the daily use of 6gm vitamin C was started in patients with higher NLR levels at the time of ICU admission. Vitamin C treatment at this dose did not significantly affect the incidence of mortality, length of stay in ICU or the levels of the inflamatuary and biochemical markers such as D dimer, ferritin, troponin and procalcitonin.

In ICU patients and especially in cases of septic shock and renal replacement therapy, vitamin C levels may decrease despite standard nutritional supplementation [10, 11] which needs to be corrected by high (1-4 gm /day) doses of vitamin C [2, 19, 23]. High dose vitamin C support has been shown to reduce the biomarkers of tissue damage in ARDS and sepsis cases [2, 10, 12]. Timely use of this support is believed to inhibit the cytokine storm induced in COVID-19 [2]. The raised levels of the inflammatory parameters such as NLR and D dimer were found to be indicative of the severity and also predictive of the prognosis of COVID-19 [15, 16]. Zang *et al*. [23] showed that the disease severity is increased when CRP levels are > 90 mg/L and D dimer levels are > 1 mg/L by retrospective analysis of the data on 95 COVID-19 patients. In this study, patients started with 6g/day vitamin C dose at admission to ICU and had very high CRP and D dimer levels (113.4 mg/L and 10.9 mg/L, respectively). However, the mean levels of the inflammatory markers did not significantly differ between the two groups of patients in our study.

Raised NLR value is another inflammatory marker used in serious cardiovascular diseases and for mortality prediction in sepsis. It is believed that the abnormal hematopoietic effect of the coronavirus on the bone marrow, peripheral blood cells and the immune response is related to the severity of the COVID-19 infection [16]. In the retrospective study by Yang *et al*. [25] on COVID-19 patients, raised levels of NLR were found to indicate disease severity. The most frequently observed symptoms were fever (83.8%) and cough (70.9%). The most frequently expressed complaints by our patients were respiratory distress (49%) followed by fever (25%). All of our patients had raised NLR and those very high values were started with vitamin C treatment. NLR levels were similar in both groups at discharge from the ICU.

Mortality incidences are increased by the rapid development of COVID-19 pneumonia and ARDS [2]. The World Health Organisation (WHO) does not have a recommendation for the use of vitamin C [26]. In the literature, next to recommendation of daily does of 1-4gm, highest daily dose used was reported as 15 gm [19, 27]. High dose of vitamin C in cases of sepsis and ARDS are believed to reduce the levels of tissue damage markers in the circulation [2, 10, 12]. However, the views on the effect of vitamin C on mortality are variable [12, 28]. In the VITAMINS study on 216 patients, vitamin C (6 gm/day, iv), hydrocortisone (200 mg) and thiamine (400 mg) combination did not show a difference of effect when compared to the use of hydrocortisone alone [28]. In a meta-analysis of the results of 12 studies on a total of 1766 ICU patients, vitamin C treatment was demonstrated to decrease ICU stay by 8% [23]. Another meta-analysis confirmed this result and also showed a reduction of 11% in the use of mechanical ventilation [24]. The multicentred CITRIS-ALI randomised trial on 167 sepsis and ARDS patients were given 50 mg/kg i.v. vitamin C every 6 hours did not change the sequential organ failure assessment (SOFA) score, the levels of inflammatory and vascular damage markers such as CRP and thrombomodulin but reduced the incidence of the 28-day mortality and the days without ventilator use [29]. In the placebo controlled study by Zang *et al*. [30] on 54 patients, with iv. vitamin c at a daily dose of 24 gm for 7 days, the secondary outcome of 28-day mortality dropped from 46.3%.to 29.8%. Our study did not detect any change in mortality rates between two groups. However, it was found that the high NLR level in hospitalization was associated with mortality. In addition, patients who received Vitamin C had higher NLR at hospitalization.

Chiscano-Camón et al. *et al*. [31] found low circulating vitamin C levels in a limited number of patients with ARDS due to COVİD 19. Antiviral agents

were used in 77%, hydroxychloroquine was used in 94% and steroids were used in 55% of the patients and the mean duration of ICU stay was determined as 28.4 days. Vitamin C use was seen to reduce the duration of ICU stay by 7.8 % [29]. In our study, the same treatment protocol was followed for antiviral agents and hydroxychloroquine in both groups. Antiviral and hydroxychloroquine were used in 78.1% and 96.9% of the patient's Group 1, and in 65.6% and 92.2% of the Group 2 patients, respectively. Steroids were used less frequently in our study, being given to 31.3 of group 1 patients and 28.9 of group 2 patients. Steroid treatment had no significant effect on the duration of ICU stay in patients receiving vitamin C therapy.

### Limitations

The main limitations of our study are that it was retrospective, single-centered, the duration of mechanical ventilation was not determined, and the blood levels of vitamin C were not measured in the patients. The homogeneity of the data on demographic characteristics and distribution of comorbid diseases and the use of the same treatments for COVID-19 are the strengths of the study.

## CONCLUSION

Vitamin C use in COVID-19 patients reduces the oxidative stress and plays an important role in the functioning of immune system. NLR is an important predictor of mortality in these patients, which can be positively affected by vitamin C treatment. Research with varying doses and timings of vitamin C use in the treatment of COVID-19 cases would contribute to the presently limited knowledge on the treatment of this disease and help determine the ICU treatment protocols in any future waves of this viral pandemic and in the cases of sepsis and ARDS.

*Authors' Contribution*

Study Conception: ŞEÖ, İC, KÖ; Study Design: ŞEÖ, İC, ŞE, HES; Supervision: ŞEÖ, ŞY, İC, ŞE, KÖ, HES, DK; Funding: ŞEÖ, ŞE; Materials: ŞEÖ, İC, KÖ; Data Collection and/or Processing: ŞEÖ, İC, KÖ, HES, ŞE, DK, ŞY; Statistical Analysis and/or Data Interpretation: ŞEÖ, İC, KÖ, HES, ŞE, DK, ŞY; Literature Review: ŞEÖ, İC, KÖ, HES, ŞE, DK, ŞY;

Manuscript Preparation: ŞEÖ, İC, KÖ, HES, ŞE, DK, ŞY and Critical Review: ŞEÖ, İC, KÖ, HES, ŞE, DK, ŞY.

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# Importance of prognostic nutritional index in on-pump coronary artery bypass graft surgery

**Arif Gücü**

*Department of Cardiovascular Surgery, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey*

## ABSTRACT

**Objectives:** A simple and appropriate risk index is still required to show the patient's nutritional status undergoing coronary artery bypass graft (CABG) surgery. This study aimed to evaluate the Prognostic Nutritional Index (PNI) value as a predictor of in-hospital mortality in patients undergoing CABG surgery.
**Methods:** In this study, we scanned the medical data of 742 patients' who underwent on-pump CABG surgery retrospectively. Patients' were divided into two groups based on the PNI cut-off value (high-risk group, PNI < 45.85, n = 230; low-risk group, PNI ≥ 45.85, n = 512).
**Results:** To analyze the factors affecting in-hospital mortality in the postoperative period, univariate and multivariate logistic regression analysis was performed. In univariate analysis, advanced age (Odds ratio (OR): 1.219, 95% confidence interval (CI): 1.194-2.669, $p < 0.001$), left ventricular ejection fraction (LVEF) (OR: 3.471, 95% CI: 2.854-6.927, $p < 0.001$), total perfusion time (OR: 0.876, 95% CI: 0.690-0.954, $p = 0.012$), intra-aortic balloon pump (IABP) use (OR: 2.148, 95% CI: 1.394-2.889, p = 0.002), preoperative high creatinine (OR: 1.229, 95% CI: 1.066-2.118, $p = 0.019$), low lymphocyte count (OR: 0.879, 95% CI: 0.789-0.945, $p = 0.017$), low albumin (OR: 1.682, 95% CI: 1.433-2.765, $p = 0.003$), high C-reactive protein (CRP) (OR: 1.0.790, 95% CI: 0.678-0.927, $p = 0.042$) and low PNI (OR: 1.290, 95% CI: 1.119-1.654, $p < 0.001$) were correlated with the postoperative mortality. In multivariate logistic regression analysis, advanced age (OR: 1.145, 95% CI: 1.110-1.938, $p = 0.017$), LVEF (OR: 2.916, 95% CI: 1.768-4.928, $p < 0.001$), IABP use (OR: 1.880, 95% CI: 1.350-2.554, $p = 0.032$) and PNI (OR: 0.932, 95% CI: 0.889-0.978, $p = 0.004$) were independent predictors of mortality.
**Conclusions:** In on-pump CABG surgery, postoperative mortality is associated with low preoperative PNI, and can be a useful and suitable parameter for preoperative risk evaluation.
**Keywords:** Prognostic nutritional index, nutritional status, CABG surgery, cardiopulmonary bypass, risk factors

Coronary artery disease (CAD) is lead to death and disability throughout the world [1]. Coronary artery bypass graft (CABG) surgery is one of the most important treatment methods of CAD. Although new developments in the treatment of CAD have reduced mortality rates, the perioperative and hospital mortality and morbidity rates of patients remain high [2-4]. In CABG surgery, the mortality and mortality rates of patients are affected by many factors [5, 6]. A simple and appropriate risk index is still required to show the

patient's nutritional status undergoing CABG surgery.

Clinical nutritional status is an indirect indicator of patient resistance. This relationship has been demonstrated in patients undergoing gastrointestinal system and malignancy surgery [7, 8]. Various methods such as Mini Nutrition Assessment, Malnutrition Universal Screening Tool and Subjective Global Assessment have been developed to determine the nutritional status of patients undergoing malignancy surgery. The Prognostic Nutritional Index (PNI) is the most widely used method for determining nutritional status, and low PNI has been shown as a predictor of postoperative mortality and morbidity in malignant diseases [9, 10]. The PNI, which was simplified by Onodera et al. [11], was calculated based on the serum albumin concentration and lymphocyte count. Some comprehensive studies evaluate the effects of preoperative nutritional factors on coronary artery surgery outcomes and vascular diseases [12, 13]. However, the importance of PNI in CABG surgery is not yet clear.

This study aims to determine of the value of PNI as a predictor of in-hospital mortality and morbidity in patients undergoing on-pump CABG surgery.

## METHODS

A total of 742 patients were included in this retrospective study that underwent on-pump CABG surgery at our institute between January 2016 and January 2020. In order to avoid the possible effects of the COVID-19 pandemic, the study was terminated in January 2020, when no pandemic patients were detected in our country yet. The research was approved by the institutional ethics committee of the Bursa Yuksek Ihtisas Training and Research Hospital. The study was conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices.

Demographic characteristic and clinical outcomes of the patients were retrospectively collected by searching our hospital database. Other data, body mass index (BMI), cross-clamp (X-clamp) time, cardiopulmonary bypass (CPB) time, preoperative left ventricular ejection fraction (LVEF), intra-aortic balloon pump (IABP) usage, intensive care unit (ICU) stays, postoperative hospital-acquired infection and stroke

were noted. We defined six types of infections after cardiac surgery as hospital-acquired infections based on O'Keefe et al. [14]. These included urinary tract infections, pneumonia, harvest site infections, superficial sternal wound infections, deep sternal wound infections, and sepsis. We also noted that stroke events after surgery.

### Blood parameter analysis

Peripheral blood samples taken on the first day of hospitalization for all patients were noted. The complete blood cell count analysis was performed using automatic blood analyzer (Beckmann Coulter LH 780) and biochemical analyzes were performed using automatic biochemical analyzer (Cobas 6000, Manheim).

### Calculation of PNI

The preoperative nutritional status of the patients was assessed using the PNI classification. PNI was calculated according to the formula: $10 \times$ serum albumin $+ 0.005 \times$ total lymphocyte count [13]. To determine the optimal cut-off value for PNI, a method described by Budczies et al. [15] was used.

### Surgical Technique

All operations were performed under general anaesthesia with a median sternotomy. Standard CPB was used in mild hypothermia (32°C) with aorta-venous two-stage cannulation. Cardioplegic arrest was achieved. Cardiopulmonary bypass was provided by roller pumps at a flow rate of 2-2.4 L/min/m² and a membrane oxygenator (Maquet, Getinge Group, Rastatt, Germany). Arterial filters were used in all operations. All distal anastomoses were performed under aortic cross-clamp (ACC), while proximal anastomoses were performed using the partial clamp technique. All patients received warm potassium-free blood cardioplegia before the ACC was removed. All patients were transferred to the cardiovascular surgery intensive care unit after the operation.

### Statistical Analysis

Continuous data were expressed as the mean ± standard deviation. Categorical data were expressed as the number and percentage. Continuous variables were analyzed with Student's t-test (for normally distributed data) or Mann-Whitney U test (for non-nor-

mally distributed data). The difference in categorical variables was tested using the chi-squared test. Univariate and multivariate logistic regression analysis were performed to determine potential risk factors for postoperative mortality. The predictive value of PNI for mortality was determined with Receiver Operating Characteristic (ROC) curve analysis and Area Under Curve (AUC) was calculated. All statistical analyses were performed using the SPSS package for Windows version 21 (SPSS Inc., Chicago, IL, USA). A $p$ value of < 0.05 was accepted statistically significant.

## RESULTS

The overall study consisted of 742 patients. In-hospital mortality occurred in 43 (5.4%) patients. The mean and median levels of the PNI were 49.92-7.44 and 49.81 (45.58-54.27). The cut-off point was set at 45.85. When the cut-off point was 45.85, the specificity was 58%, the sensitivity was 70.7% satisfactory, and patients were divided into two groups according to this cut-off point (high-risk group; PNI < 45.85, n = 230; low-risk group; PNI ≥ 45.85, n = 512).

The low-risk group and the high-risk group patients were compared, and there was a significant difference in age (low-risk group: 66 ± 0.75 vs. high-risk group: 62.62 ± 0.43, $p = 0.001$). The BMI was similar between the groups (26.97 ± 0.19 vs. 26.51 ± 0.13; $p = 0.179$). Preoperative LVEF was statistically lower in the high-risk group (49.67 ± 0.62 vs. 51.20 ± 0.43; $p = 0.018$). There was no difference between two groups in terms of hypertension, DM and COPD rates. Comparisons of the groups regarding the laboratory data revealed that the high-risk group had significantly lower hemoglobin and albumin levels ($p = 0.001$). Preoperative serum creatinine levels were differences between groups and the high-risk group had significantly higher creatinine levels (1.25 ± 0.08 vs. 0.94 ± 0.02; $p = 0.001$). Comparison of demographic features and basic blood values of patients in groups are demonstrated in Table 1.

Operative X-clamp time ($p = 0.661$), CPB time ($p = 0.143$) was similar between the groups, but IABP usage ($p = 0.001$), and ICU stay ($p = 0.011$) significantly higher in the high-risk group. Since the infection was broadly categorized in our study, we compared hospital-acquired infection between groups.

**Table 1. Comparison of demographic features and basic blood values of patients in groups**

| Features | High-risk group | Low-risk group | $p$ values |
|---|---|---|---|
| Male, n (%) | 185 (80.5) | 409 (79.9) | 0.527 |
| Age (year) | 66 ± 0.75 | 62.62 ± 0.43 | **0.001** |
| Preoperative LVEF (%) | 49.67 ± 0.62 | 51.20 ± 0.43 | **0.018** |
| BMI (m²/kg) | 26.97 ± 0.19 | 26.51 ± 0.13 | 0.179 |
| Hypertension, n (%) | 132 (57.3) | 277 (54.1) | 0.108 |
| Diabetes mellitus, n (%) | 48 (20.8) | 93 (18.1) | 0.227 |
| COPD, n (%) | 42 (18.2) | 79 (15.4) | 0.090 |
| Hemoglobin (gr/dL) | 12.15 ± 0.14 | 13.66 ± 0.08 | **0.001** |
| WBC (x10³) | 9.36 ± 0.26 | 9.22 ± 0.11 | 0.431 |
| Neu (x10³) | 7.11 ± 0.22 | 6.05 ± 0.07 | **0.001** |
| Lym (x10³) | 1.42 ± 0.04 | 2.32 ± 0.04 | **0.001** |
| Platelets (x10³) | 240.24 ± 0.54 | 247.30 ± 2.96 | **0.011** |
| CRP (mg/L) | 22.17 ± 2.19 | 9.31 ± 0.71 | **0.001** |
| Cre (mg/dL) | 1.25 ± 0.08 | 0.94 ± 0.02 | **0.001** |
| Total protein (g/dl) | 6.47 ± 0.04 | 7.07 ± 0.03 | **0.001** |
| Albumin (g/dL) | 3.39 ± 0.03 | 4.04 ± 0.02 | **0.001** |

Data are shown as mean±standard deviation or n (%). WBC = White blood cell, CRP = C-reactive protein, Cre = Creatinine, Neu = Neutrophil, Lym = Lymphocyte, LVEF = Left ventricular ejection fraction, BMI = Body Mass Index

**Table 2. Operative and postoperative features of patients**

| Characteristic | High-risk group | Low-risk group | *p* values |
|---|---|---|---|
| X-clamp time (min) | 66.41 ± 1.58 | 64.32 ± 0.9 | 0.661 |
| CPB time (min) | 95.20 ± 2.05 | 90.54 ± 1.22 | 0.143 |
| IABP usage, n (%) | 29 (12.6%) | 24 (4.6) | **0.001** |
| ICU stay (days) | 3.71 ± 0.16 | 3.09 ± 0.13 | **0.011** |
| Mortality, n (%) | 25 (10.8) | 18 (3.5) | **< 0.001** |
| Morbidity | | | |
| Hospital-acquired infection, n (%) | 59 (25.6) | 62 (12.1) | **0.001** |
| Stroke, n (%) | 26 (3.7) | 36 (7) | 0.052 |

Data are shown as mean ± standard deviation or n (%). X- clamp = Cross clamp, CPB = Cardiopulmonary bypass, IABP = Intra-aortic balloon pump, ICU = Intensive care unit

There was a significantly higher tendency of hospital-acquired infection in the high-risk group (59; 25.6% vs. 62; 12.1%; *p* = 0.001). Postoperative stroke was similar between the groups (26; 11.3% vs. 36; 7%; *p* = 0.052) (Table 2).

To analyze the factors affecting in-hospital mortality in the postoperative period, univariate and multivariate logistic regression analysis was performed (Table 3). In univariate analysis, advanced age (OR [odds ratio]: 1.219, 95% CI [confidence interval]: 1.194-2.669, *p* < 0.001), LVEF (OR: 3.471, 95% CI: 2.854-6.927, *p* < 0.001), total perfusion time (OR: 0.876, 95% CI: 0.690-0.954, *p* = 0.012), IABP use (OR: 2.148, 95% CI: 1.394-2.889, p = 0.002), preoperative high creatinine (OR: 1.229, 95% CI: 1.066-2.118, *p* = 0.019), low lymphocyte count (OR: 0.879, 95% CI: 0.789-0.945, *p* = 0.017), low albumin (OR: 1.682, 95% CI: 1.433-2.765, *p* = 0.003), high CRP (OR: 0.790, 95% CI: 0.678-0.927, *p* = 0.042) and low PNI (OR: 1.290, 95% CI: 1.119-1.654, *p* < 0.001) were correlated with the postoperative mortality. In multivariate logistic regression analysis, advanced age (OR: 1.145, 95% CI: 1.110-1.938, *p* = 0.017), LVEF (OR: 2.916, 95% CI: 1.768-4.928, *p* < 0.001), IABP

**Table 3. Logistic regression analysis to identify factors affecting postoperative mortality**

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | *p* value | Exp (B) Odds Ratio | 95% CI Lower-Upper | *p* value | Exp (B) Odds Ratio | 95% CI Lower-Upper |
| Age | **< 0.001** | 1.219 | 1.194- 2.669 | **0.017** | 1.145 | 1.110- 1.938 |
| Hypertension | 0.221 | 1.317 | 0.975- 1.786 | -- | -- | -- |
| COPD | 0.190 | 0.895 | 0.667- 1.110 | -- | -- | -- |
| LVEF | **< 0.001** | 3.471 | 2.854- 6.927 | **< 0.001** | 2.916 | 1.768- 4.928 |
| Total perfusion time | **0.012** | 0.876 | 0.690- 0.954 | 0.345 | 0.790 | 0.690- 1.134 |
| IABP use | **0.002** | 2.148 | 1.394- 2.889 | 0.032 | 1.880 | 1.350- 2.554 |
| Creatinin | **0.019** | 1.229 | 1.066- 2.118 | 0.314 | 1.110 | 0.890- 1.440 |
| Lymphocyte count | **0.017** | 0.879 | 0.789- 0.945 | -- | -- | -- |
| Albumin | **0.003** | 1.682 | 1.433- 2.765 | -- | -- | -- |
| CRP | **0.042** | 0.790 | 0.678- 0.927 | 0.410 | 0.914 | 0.776- 1.190 |
| PNI | **< 0.001** | 1.290 | 1.119- 1.654 | **0.004** | 0.932 | 0.889- 0.978 |

COPD = Chronic obstructive pulmonary disease, LVEF = Left ventricular ejection fraction, IABP = Intraaortic balloon pump, CRP = C reactive protein, PNI = Prognostic nutritional index
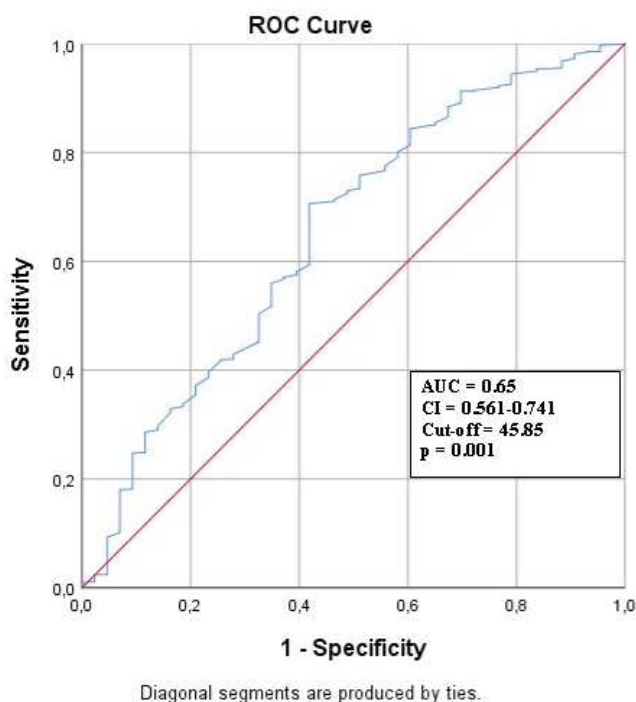
**Fig. 1. Data figure of the area under the curve (AUC), confidence interval (CI), and cut-off values in receiver-operating characteristic (ROC) curve analysis for prognostic nutritional index.**

use (OR: 1.880, 95% CI: 1.350-2.554, $p = 0.032$) and PNI (OR: 0.932, 95% CI: 0.889-0.978, $p = 0.004$) were independent predictors of mortality.

In the receiver-operating characteristic analysis, PNI lower than 45.85 predicted in-hospital mortality with 70.7% sensitivity and 58% specificity [area under the curve: 0.651, 95% confidence interval: 0.561-0.741; $p = 0.001$] (Fig. 1). In-hospital outcomes were significantly worse in low-risk group (Fig. 2).
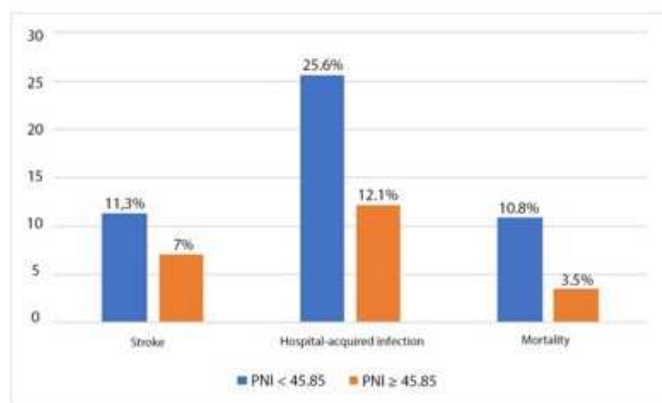


**Fig. 2. In-hospital outcomes were significantly worse when the PNI was lower.**

## DISCUSSION

CABG surgery is one of the most important treatment methods of CAD. Operation success is related to individual risk factors as well as technical skills. In this current study, we showed that PNI value, which is an important indicator of malnutrition status, is an independent predictor of postoperative mortality in addition to known risk factors such as age and low LVEF value.

Patients with poor nutritional status before cardiac surgery have been higher postoperative morbidity and mortality rates [16]. In the literature some studies have demonstrated the importance of energy and protein metabolism in the early period after heart surgery and documented significant post-operative consumption of macronutrients and micronutrients [16, 18]. Sufficient nutritional therapy has been proposed to improve patient outcomes by maintaining energy metabolism and promoting improved wound healing after surgery [19]. Lomivorotov *et al*. found that depending on the used nutrition screening tool, the percentage of malnourished cardiac surgery patients before surgery ranged between 4.6-19.1% [18]. The present findings point to high malnutrition rates in cardiac patients, and it is very important to consider the nutritional profiles of these patients before surgery and simultaneously, and to draw more attention to the concept of individual diet for pre-operative optimization in these patients [20, 22]. Therefore, preoperative evaluation of nutritional status in patients at high risk of developing postoperative complications may guide to consider postoperative nutritional interventions. Studies evaluating the relationship of BMI, albumin, and prealbumin levels have demonstrated that they are independent predictors of mortality and morbidity after coronary artery and valve surgery [23, 24].

The PNI, calculated based on the serum albumin concentration and lymphocyte count of peripheral blood was designed by Buzby *et al*. [7] in 1980 and simplified by Onodera *et al*. [11] in 1984. Lymphocytes are significant part of the immune system, and the prognostic role of lymphocyte count has been previously investigated in cardiovascular diseases [25, 27]. Lymphopenia is an important mortality predictor in patients undergoing CABG surgery [28]. Albumin is a serum protein that is a good indicator of a patient's nutritional status, and it makes up the majority of the

serum total protein and is mainly responsible for the serum osmotic pressure. Also albumin has antioxidant and anti-inflammatory properties in scavenging reactive oxygen radicals and limiting their production [29]. In particular, postoperative hypoalbuminemia is an independent risk factor for postoperative outcome in patients undergoing CABG surgery [30].

PNI was originally designed to determine immunonutrition status and has been widely used to assess surgical risk, particularly in patients with malignancy and gastrointestinal operations [7, 8, 31]. Several studies have reported that lower PNI levels are significantly associated with higher mortality in patients with cardiovascular disease [32]. PNI can be used to predict patients' outcomes before cardiac surgery and to choose an appropriate surgical strategy. Given all these data, the management of patients with low PNI may require a "Cardio-metabolic Team" approach to optimized patient care prior to surgery. In the cardio-metabolic team consisting of cardiologist, internal medicine, dietician, cardiovascular surgeon and needed additional branches. It is clear that pre-operative metabolic optimal support is particularly important in this low- and middle-income patient. On the other hand, in patients with low PNI, cardiac surgery plan can be re-evaluated by the cardio-metabolic team. Less invasive surgery may be appropriate if possible. There are various studies in the literature investigating the effect of PNI value on clinical outcomes after cardiac operations. In a study conducted by Lee *et al*. [33], the prognostic role of PNI value in predicting early clinical outcomes after cardiac surgery was investigated. In this study, the authors divided the patients into two groups according to the value they determined as cut-off (46.13). At the end of the study, early mortality rates were found to be higher in the group with low PNI values. In addition, the mean length of stay on mechanical ventilation and length of stay in the intensive care unit were found to be significantly higher in this group [33]. In our study patients' were seperated into two groups based on the PNI cut-off value (high-risk group, PNI < 45.85; low-risk group, PNI ≥ 45.85). There was a significantly higher tendency of hospital-acquired infection in the high-risk group (59; 25.6% vs. 62; 12.1%; $p = 0.001$) and postoperative stroke was similar between the groups (26; 11.3% vs. 36; 7%; $p = 0.052$). In multivariate analysis PNI value was found as an independent pre-dictor of postoperative mortality.

An increasing number of elderly people are accepted for elective CABG surgery. When low preoperative PNI is detected in elderly patients undergoing elective coronary artery surgery, postoperative methods such as serum albumin supplementation, dietary maintenance or nutritional support should be considered to improve the nutritional status of patients [34].

Our research demonstrated that a low PNI value affects postoperative mortality and morbidity. There was significantly different a higher tendency for postoperative hospital-acquired infection in the high-risk group. PNI may be used to predict patients' outcomes before coronary artery surgery and select an adequate surgical strategy. In the case of patients with a low PNI, less invasive surgery may be suitable if possible.

### Limitations

Our study does not include the effects of preoperative nutritional support on postoperative mortality and morbidity in patients with low PNI values. The fact that this is a single-center study with a retrospective design is the most important limitation. Further studies are needed to show the results of preoperative nutritional support in elective cases.

### CONCLUSION

Preoperative low PNI level was statistically significantly associated with postoperative mortality and morbidity in cardiac surgery. According to the results we obtained in our study, we firmly believe that PNI is a useful and suitable parameter for preoperative assessment of nutritional status and should be regarded in managing the indication and strategy in on-pump CABG surgery.

*Authors' Contribution*

Study Conception: AG; Study Design: AG; Supervision: AG; Funding: AG; Materials: AG; Data Collection and/or Processing: AG; Statistical Analysis and/or Data Interpretation: AG; Literature Review: AG; Manuscript Preparation: AG and Critical Review: AG.

*Conflict of interest*

The author disclosed no conflict of interest during

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