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Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlEID/cid.htm.

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Original Investigations

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Complications of Cervical Disc Prosthesis Dislocation: A Retrospective Clinical Study

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ABSTRACT

Objective: The most commonly used method for the surgical treatment of cervical disc herniation (CDH) is anterior disc excision with Smith-Robinson's approach. Following the excision of pathological disc space, disc prosthesis is placed if a continuation of dynamic movement in the disc space is desired and a cervical cage is placed for the purpose of fusion. Cervical disc prosthesis seems superior to cervical cage; however, it is not suitable for every patient and can cause serious complications. Our study include data of patients who developed complications following the dislocation of cervical prosthesis and who were referred to our clinic. The aim of our study is to emphasize that the cervical prosthesis is not suitable for every patient and may cause serious complications.

Methods: Data of the patients who were operated due to the diagnosis of CDH in other centers and underwent revision surgery for the development of cervical prosthesis dislocation between 2013 and 2020 were collected.

Results: This study analysed the data of four male and three female patients. The median value of patient ages was 42 (28-53). Neck pain and swallowing difficulty were the most common reasons for admission to the clinic. Dislocation was found to develop after trauma in three patients. Anterior and posterior dislocations were found to develop in five and two patients, respectively. Seven patients underwent revision surgery. All these patients were found to have dislocations at the C5-6 level.

Conclusion: The prosthesis to be placed in the surgical treatment of CDH should be determined based on the patient. Detailed information should be provided to the patient for whom cervical disc prosthesis is to be placed and prosthesis of the most appropriate size for disc space should be placed properly.

Keywords: Cervical disc herniation, cervical prosthesis, prosthesis dislocation

INTRODUCTION

Cervical disc herniation (CDH) is a disease that affects the spinal cord and spinal nerve roots and it most commonly arises at ≥30 years age. It may result in radiculopathy or myelopathy. Anterior cervical discectomy was first described by Smith and Robinson in 1955 and Cloward in 1958. Since then, the anterior approach has become the preferred and frequently used modality for the treatment of CDH (1). The necessity of implant placement in the intervertebral space has been discussed with the widespread use of the anterior approach. Following long-lasting research, it has been found that the implant placed in the intervertebral space provides expansion in the neural foramina and, therefore, it should be used (1-3). Today, research on this subject has mostly focused

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on which implant is more suitable for patients. The placement of cervical prosthesis that allows dynamic movements in the disc space or cervical cage that provides cervical spine fusion. There are studies reporting that cervical prosthesis allows minimal dynamic movement and prevents the development of the adjacent segment disease. Furthermore, cervical prosthesis has several advantages, such as early return to work, no requirement for neck collar and better clinical outcomes, compared to other implants (4,5). However, in literature, the number of studies reporting the complications caused by the use of cervical prostheses is limited. This study aimed to investigate and present data of patients who developed complications following the dislocation of cervical prosthesis and who were referred to our clinic.

METHODS

This study presented the data of seven patients who were operated for cervical prosthesis dislocation in the Neurosurgery Clinic of Hatay Mustafa Kemal University Tayfur Ata Sökmen Medical Faculty Hospital between 2013 and 2020. After the patients' data were evaluated retrospectively, they were found suitable for the purpose of the present study and were included in this study. Preoperative neurological examination information, radiological examinations and operative reports of all patients included in the study were reviewed. Patients who met the study criteria were included in the study. Written consent was obtained from the participants for their records to be included in the study. All data were collected in accordance with the principles of Declaration of Helsinki. This retrospective study was approved by the Non-Interventional Clinical Research Ethics Committee of Hatay Mustafa Kemal University (approval number: 17, date: 03/09/2020).

Statistical Analysis

Basic complementary statistical methods were applied using Microsoft Office Excel 2010. Results were expressed as mean for average or percentage (%) for frequency.

RESULTS

Data of four male and three female patients who were operated due to diagnosis of CDH in other centres and underwent surgery for the placement of a cervical prosthesis in the disc space were analysed (Figure 1). The median value of patients age was 42 (minimum: 28, maximum: 53). Neck pain and swallowing difficulty were the most common reasons for admission in the clinic. Cervical prosthesis dislocation was found to occur after trauma in three patients. One patient developed posterior dislocation and associated spinal shock after trauma, while one patient developed C6 vertebral fracture. Five and two patients developed anterior and posterior dislocations, respectively. Analysis of the early post-operative examinations showed that the prosthesis was closer to the anterior in the sagittal plane in three patients and it was not in the midline in the coronal plane in one patient (Figure 2). All the seven patients underwent revision surgery. After the dislocated cervical prosthesis was removed, cervical cage was placed in six patients and corpectomy cage was placed in one patient, since there was a vertebral fracture (Figure 3A). Dislocation was found to be at the C5-6 cervical disc level in all patients. One patient with anterior dislocation died due to mediastinitis induced by oesophageal perforation (Figure 3B) and another patient with posterior dislocation died due to spinal cord injury. One patient developed cerebrospinal fluid (CSF) fistula (Figure 3C) and one patient had C6 corpus fracture. Dislocation was found to occur in

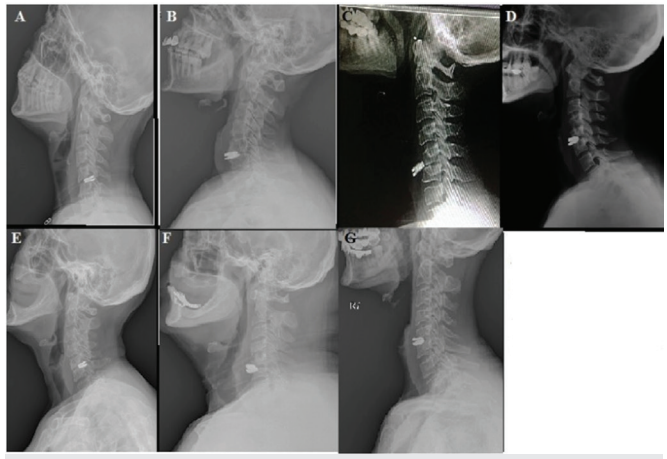

Figure 1. (A-G) Lateral cervical radiographic examination of patients

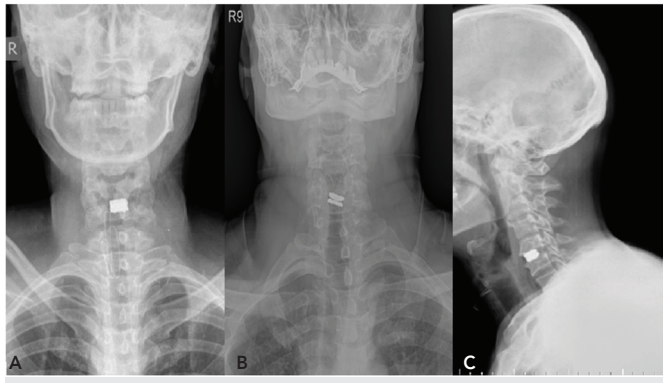

Figure 2. Prosthesis not located in the midline in the coronal plane on anteroposterior radiograph (A), prosthesis migrated anteriorly (B) and early image of post-operative cervical prosthesis is not located in the midline in the sagittal plane (C)

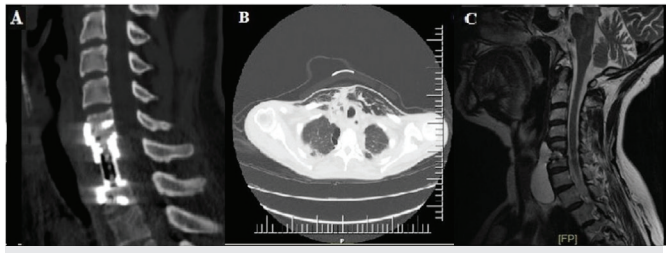

Figure 3. Sagittal computed tomography (CT) image following the development of C6 corpectomy (A), axial thoracic CT showing the development of mediastinitis (B), sagittal T2-weighted magnetic resonance imaging image showing the development of cerebrospinal fluid fistula (C)

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the first post-operative year in five patients and occurred after the first post-operative year in two patients with a history of trauma. Three patients were found to undergo two-level CDH operation and cervical cage was used at the other level.

DISCUSSION

Regardless of the aetiology, pain can be relieved by conservative treatment in patients suffering from neck pain. However, as in the present study, a treatment approach that requires a comprehensive differential diagnosis and adherence to evidence-based instructions are imperative in the presence of conditions accompanying neck pain, such as swallowing difficulty, acute paraparesis and a history of spinal surgery. This is because the implants used today are designed to allow motion at the joints and can dislocate in cases of forceful motion or trauma (5).

The decision on the most appropriate surgical technique for cervical disc diseases has been a controversial issue for a long time. Anterior discectomy is a surgical technique successfully performed for the treatment of radicular and myelopathic cervical disease that causes nerve root and spinal cord compression. Decompression, stabilisation, or both procedures can be performed in the surgical treatment. Following decompression, spinal fusion is performed in the disc space (1). A revision surgery is required for the treatment of complications and adjacent segment disease that develop after the fusion surgery. In light of these data, there have been rapid advances in disc prosthesis implantation following decompression in the cervical region. Cervical disc prosthesis seems to be a more advantageous procedure, since there is no limitation of movement at the level where the prosthesis is placed and due to the fact that complications resulting from fusion surgery are eliminated (4,5).

Surgery for placement of cervical disc prosthesis is also called cervical arthroplasty. Recent studies have focused on cervical arthroplasty. In a study by Yalcin et al. (6) on cervical arthroplasty indications, the prosthesis was reported to be contraindicated for patients with rheumatological diseases, advanced spondylosis, multiple cervical disc pathologies, severe degeneration of cervical lordosis and a history of trauma. In the present study, analysis of the preoperative examinations of the patients showed that three patients underwent two-level CDH surgery. Furthermore, preoperative severe osteodegenerative findings were detected in two elderly patients (aged 51 and 53 years). It was observed that cervical lordosis flattened and that kyphosis started to develop in three patients.

Researches about cervical prosthesis complication increase in literature, as in our study, whether biomechanical studies are sufficient or not has become a matter of debate. Brooke et al. (7) reported that dislocation may also occur in the use of cervical prostheses with carbon fibre technology. Subsequent studies have mostly focused on the need for prostheses with better adhesion to the endplates. Therefore, screwed cervical prostheses have been investigated, but it was found that they increase the operative time and may damage the vertebral bodies. While prostheses that fit between the endplates have been reported to be sufficient in several studies, some studies have shown that porous-coated implants prevent fusion development and mobilisation of prosthesis in the endplates (5,7,8). The prostheses removed in the present study were observed to be implants with a sharp tip attached to the endplates and they were procured from four different medical brands. Furthermore, the physicians who performed the first operations were different.

Post-traumatic dislocations of the cervical prosthesis were observed in three patients included in this study. Cervical prostheses may be dislocated in cases of exposure to excessive vibration or high-energy traumas, since they do not support fusion between the vertebrae (9). Yang et al. (10) demonstrated that the prosthesis was loosened and malposed in the disc space after trauma in some patients. In a case report by Niu et al. (11), the prosthesis dislocated after strain was shown to cause serious complications in a sea sports athlete. Therefore, not only spinal indications, but the patient's profession, or exercises or sports that the patient does should be questioned in cases where surgical treatment for CDH is planned.

In the present study, anterior dislocation was found to develop in five patients. The most common complication accompanying neck pain was observed to be swallowing difficulty in these patients. One patient developed oesophageal injury and mediastinitis. After posterior migration, one patient developed CSF fistula and another patient developed spinal shock. Posterior migration was observed to be more dangerous and was found to have a more mortal course, while serious complications were observed in anterior dislocation.

The present study mostly focused on the dislocation of cervical prosthesis, which is an early complication of cervical prosthesis. Mehren et al. (12) found that fusion developed in the long-term follow-up of patients who underwent cervical arthroplasty and that although it prevented adjacent segment disease pathology in the early period, there was no difference in the later period when compared to the fusion. Our study focuses on early complications caused by cervical prostheses. There is a need for longer-term studies that include larger patient groups.

Study Limitations

This study was not conducted in a clinic where cervical prosthesis operation is conducted and only complicated cases were treated. Therefore, this study does not provide sufficient information about the incidence of complications or other effects.

CONCLUSION

Cervical disc prosthesis seems superior to cervical cage placement; however, it is not suitable for every patient, as it may lead to serious complications. Detailed information should be provided to the patient for whom cervical disc prosthesis is to be placed and prosthesis of the most appropriate size for disc space should be placed properly.

Ethics Committee Approval: This retrospective study was approved by the Non-Interventional Clinical Research Ethics Committee of Hatay Mustafa Kemal University (approval number: 17, date: 03/09/2020).

Informed Consent: Written consent was obtained from the participants for their records to be included in the study. All data were collected in accordance with the principles of Declaration of Helsinki.

Peer-review: Externally and internally peer-reviewed.

Author Contributions: Surgical and Medical Practices - Y.A., B.U. A.M.A.; Concept - Ö.Ö., N.K.; Design - Ö.Ö.; Data Collection and/or Processing - Y.A., B.U.; Analysis and/or Interpretation - Y.A., Ö.Ö.; Literature Search - B.U., A.M.A.; Writing - Y.A., Ö.Ö.

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Clinical Outcomes of Total Hip Arthroplasty in Unilateral Crowe Type IV Hip Dysplasia

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ABSTRACT

Objective: To investigate the functional outcomes of femoral shortening osteotomy concomitant with cementless total hip arthroplasty (THA) in unilateral Crowe type IV hip dysplasia.

Methods: A total of 57 patients that underwent THA with femoral shortening osteotomy having the diagnosis of Crowe type IV developmental dysplasia of the hip between January 2005 and March 2016 were retrospectively reviewed. The Harris hip score and Western Ontario and McMaster Universities Osteoarthritis index were used to evaluate functional results. Abductor function was evaluated with the Trendelenburg sign before and after surgery. Major complications were assessed. The pre-operative and post-operative leg height inequality was also measured for all patients.

Results: The mean follow-up period of patients with a follow-up of at least 2 years was 47.8 months. The Trendelenburg sign, which was positive in all patients before surgery, was positive in three patients (5.2%) during the last follow-up. The mean pre-operative leg inequality was 6.9±2.4 cm, and decreased to 1.3±0.3 cm postoperatively. During the last follow-up, five patients (8.7%) had a complaint of leg length discrepancy due to a longer leg on the THA side. Functional scores of patients during the last follow-up were statistically significant higher compared to the pre-operative period.

Conclusion: Applying cementless THA in patients with unilateral Crowe type IV has satisfactory clinical results. To relieve pain and improve functional scores and quality of life, THA is the best treatment option. The rate of complications is high; however, patient can be managed with a secondary intervention or only observation.

Keywords: Developmental dysplasia, total hip arthroplasty, femoral shortening osteotomy, leg length discrepancy

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INTRODUCTION

Unilateral Crowe type IV developmental dysplasia of the hip (DDH) is one of the most severe hip deformities, resulting to a compensatory scoliosis, leg length discrepancy, walking difficulty and back and hip pain. Total hip arthroplasty (THA) has been performed as the best treatment of choice (1,2); however, this is technically more difficult having higher complication rates when compared to primary THA due to anatomical changes that occur both on the femoral and acetabular sides (3,4). Various methods have been described to prevent complications such as leg length discrepancy and nerve symptoms to facilitate hip reduction in these patients; however, no consensus on the best technique has been reported (5-10). The most common complication after THA in unilateral Crowe type IV hip dysplasia is leg length discrepancy, which significantly reduces patient satisfaction (11,12). The relationship between hip dysplasia and leg length discrepancy depends on various factors and it has been widely investigated in literature (2,5,6,12). Metcalfe et al. (13) reported a post-operatively longer ipsilateral femur length in patients who underwent THA due to unilateral DDH. They argued that this could be an indication for the surgeon and the patient that the shorter side would be longer after surgery. Zhang et al. (14) showed that lengths of the ipsilateral femur and tibia were significantly higher in patients with unilateral DDH. They reported that the pelvic obliquity and leg length discrepancy decreased over time after THA surgery. This current study aimed to evaluate the clinical and functional results of THA with femoral shortening osteotomy in patients with unilateral Crowe type IV hip dysplasia and to compare our results with literature. We hypothesised that patient satisfaction is high in terms of leg length equality.

METHODS

Patient Population

The clinical and radiological data of patients who underwent cementless THA between January 2005 and March 2016 due to unilateral Crowe type IV DDH (15) were retrospectively reviewed. Patients with Crowe type IV hip dysplasia on one side and a healthy hip joint on the other side participated in the study. Patients with unavailable archive records, lost to follow-up and with a history of hip surgery (trauma, infection, osteotomy, etc.) were excluded. A total of 193 consecutive patients with Crowe type IV DDH underwent THA at our hospital between January 2005 and March 2016. Excluded from the study were 67 patients without a healthy hip joint on the other side, 32 patients for whom archive records are unavailable, four patients that were lost to follow-up (mortality) and 33 patients with a history of hip surgery (trauma in seven, infection in three, and pelvic/femoral osteotomies in 23). After exclusions, a total of 57 patients were included in the study. The study was planned after receiving the approval of the University of Health Sciences Turkey, Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital Ethics Committee (approval number: 262, approval date: 29.11.2018). A written informed consent was obtained from each patient.

Preoperatively, detailed physical examination and radiographic imaging (pelvis, hip and lower extremity) were performed in all patients. THA indications include severe pain, trouble walking and difficulty in performing daily life activities. Patients were evaluated clinically and radiologically at the post-operative 1, 3, 6 and 12-month follow-up visits, and then annually. Post-operative complications (non-union of the osteotomy site, superficial/deep infection, nerve damage, leg length discrepancy and dislocation) were assessed.

Clinical Evaluation

The Harris hip score (HHS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) were used to evaluate functional outcomes before and after surgery. Abductor arm function was assessed using the Trendelenburg sign before and after surgery. The use and/or requirement of compensatory insoles to compensate for the post-operative leg length discrepancy were questioned in all patients.

Radiologic Evaluation

Pre-operative planning was performed to determine the size of the implant to be used in the surgery and the required femoral resection length based on direct radiographs. Wooden blocks were placed in all patients under the short extremity until the pelvis was placed parallel to the floor. Routine pelvis anteroposterior (AP), hip AP and lateral and lower extremity orthoroentgenography images were obtained from all patients (Figure 1). The lower extremity length was defined as the length of the leg measured from the inter-teardrop line to the centre of the ankle joint. Leg length discrepancy was defined as the difference in the bilateral leg length (Figure 2). Subtrochanteric transverse femoral osteotomy was performed when operating on hips which necessitated >4 cm femoral lengthening.

Surgical Technique

Surgical operations were performed in a training and research hospital by four experienced arthroplasty surgeons. Templates were used to estimate the size of prosthetic components. General anaesthesia was applied to all patients, using posterolateral incision. The operation started in the femur. Rasping was performed on the femoral medullary canal, starting from the smallest size. Then a subtrochanteric transverse osteotomy was performed 1-2 cm distal to the trochanter minor. The amount

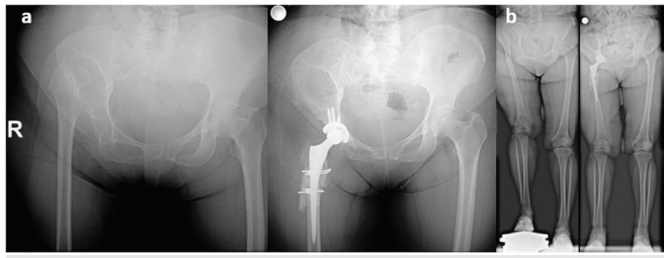

Figure 1. (a) Pre-operative and post-operative pelvis anteroposterior radiograph. (b) Pre-operative and post-operative lower extremity orthoroentgenography

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of resection was determined based on the amount of hip dislocation, and was calculated using the Ranawat method (9), with the following formula: Resection amount (cm) = height (cm) - 3 cm. The true acetabulum became visible after the resection. The acetabular reaming was started with the posterior portion using the smallest reamer in order not to impair the anterior wall with a poorer bone stock. An acetabular cup of 1 or 2 mm larger than the reamer was placed. All patients were ensured that >70% of the acetabular cup was covered with bone, and none required additional acetabular grafting.

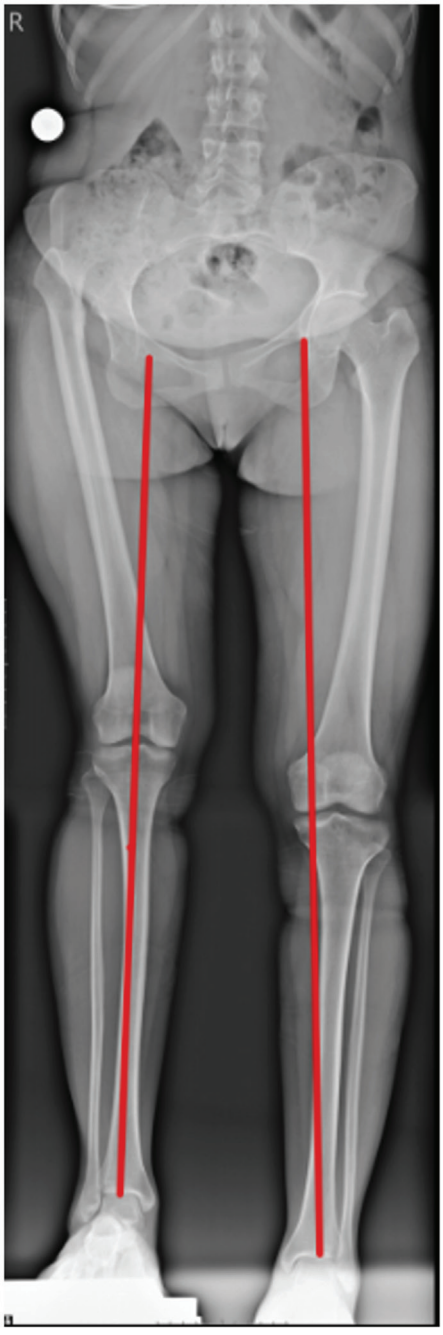

Figure 2. Measurements of leg length on full-length standing anteroposterior radiograph

Shortening osteotomy had been performed on the femur at the beginning of the operation, thus, the cementless femoral stem was strengthened by providing additional fixation with Dall-Miles cables (Stryker, NJ, USA). Contracted soft tissues (gluteus maximus tendon, iliotibial band, hip adductors, tensor fascia latae, flat head of the rectus femoris tendon and iliopsoas tendon) were released in a controlled manner in cases where necessary.

The Trilogy Acetabular Hip System (Zimmer Inc, Warsaw, IN, USA) was used in 37 (65%) patients and Reflection cup (Smith and Nephew, Memphis, TN, USA) in 20 (35%). A highly cross-linked polyethylene liner was used in patients over 65 years old and a Delta ceramic liner in those younger than 65 years. Cementless femoral stems were employed in all patients. The Wagner Cone Prosthesis Stem (Zimmer Inc, Warsaw, IN, USA) was used in 37 (65%) patients and SL-PLUS Stem (Smith & Nephew, London, UK) in 20 (35%).

Post-operative Follow-up

On the first post-operative day, isometric quadriceps exercises were started, with active straight leg lifting and hip and knee exercises on the second post-operative day. Patients were allowed to sit beside their bed on the first post-operative day. All patients were mobilised with the help of a physiotherapist within 24-48 hours post-operatively after drains were removed without placing any load on the operated side. During the follow-up, patients were asked not to bear any load on the operated side for 6-8 weeks. After this period, the partial load was gradually applied, and full weight-bearing load was allowed at the end of the third month.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences v. 22.0 (SPSS Inc., IBM, NY, USA). Categorical variables (gender and affected side) were presented as percentages. Distribution of variables was analysed with the Kolmogorov-Smirnov test. The comparison of continuous data was performed using the Student's t-test. Two-sided, paired Student's t-test was used for the statistical analysis of the preoperative and post-operative WOMAC, HHS and leg length discrepancy value. Statistical differences were considered to be significant when the p-value was <0.05.

RESULTS

Demographics and clinical data of patients are summarised in Table 1. The mean follow-up period was 47.8 months, ranging from 24-122 months. The mean leg length discrepancy values were 6.9±2.4 (4.5-9.8) cm pre-operatively and 1.3±0.3 (0-3) cm post-operatively (Figure 3). All operated legs were shorter than the legs on the contralateral side due to the shortening osteotomy; however, 5 of 57 patients (8.7%) reported a feeling that the operated side was longer than the other.

The mean HHS improved from 41.3±5.2 (28-63) points preoperatively to 85.2±5.2 (70-98) points during the last follow-up (p=0.009) (Table 2). A significant improvement in all WOMAC subscores was observed post-operatively (Table 2).

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Table 1. Demographic and clinical data of patients

	n (%)/median (range)
Number of patients (hips)	57 (57)
Age (years)	46 (22-61)
Gender	
Female	49 (86%)
Male	8 (14%)
Affected side	
Left	35 (51.5%)
Right	22 (38.5%)
Crowe classification	
Grade IV	57 (100%)
Follow-up (months)	47.8 (24-122)
Leg length discrepancy	
Pre-operative (cm)	6.9 (4.5-9.8)
Post-operative (cm)	1.3 (0-3)
Positive Trendelenburg gait	
Pre-operative	57 (100%)
Post-operative	3 (5.2%)

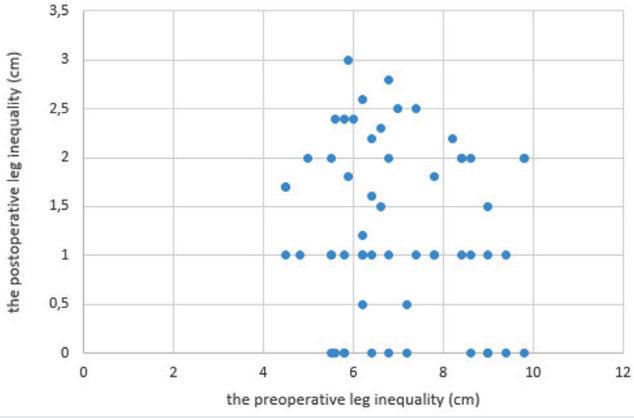

Figure 3. Diagram showing the pre-operative and post-operative leg inequality in patients

Within 1 year after THA, complete union was observed in all osteotomy sites. The mean union time was 6.3±2 (3-10) months. No severe limping during the last follow-up; however, three patients (5.2%) had moderate limping.

Intra-operative periprosthetic femoral fractures (distal femoral fragments) were seen in four cases (7%) that were fixed using titanium cable wires. Early post-operative infections were seen in two patients (3.5%) who were treated over two weeks without the need for a radical intervention other than superficial debridement and intravenous antibiotherapy. Deep vein thrombosis developed in one patient (1.75%), and sciatic nerve injury developed in three patients (5.2%) during the post-operative period. Two patients (3.5%) had complete recovery of the sciatic nerve within 6 months.

Table 2. Pre-operative and post-operative functional outcome scores of patients

	Pre-operative mean value (SD)	Post-operative mean value (SD)	p-value
Harris hip score	41.33 (±11.2)	85.27 (±19.8)	0.009
WOMAC pain	23 (±5.03)	6.24 (±2.12)	0.021
WOMAC stiffness	3.34 (±2.04)	0.62 (±0.18)	0.012
WOMAC function	59.73 (±15.80)	24 (±7.32)	0.001
WOMAC global	76.33 (±17.42)	30.85 (±8.98)	0.001

SD: standard deviation, WOMAC: Western Ontario and McMaster Universities Arthritis Index

Improvement was observed in one patient during the two-year follow-up with the use of ankle-foot orthosis. Early post-operative non-traumatic dislocation developed in four cases (7%), of which two underwent revision surgery within the first 10 days. Wherein, the femoral component anteversion revision was performed with femoral shortening. Closed reduction was undertaken in the remaining two patients, and no dislocation was observed again.

DISCUSSION

The most important finding of the current study was that complication rates remain high despite advances in prosthetic designs. In our patients, the sum of the intra-operative and post-operative complication rates was 24.5%. However, these complications were easily overcome by a second surgical attempt or by observation alone.

Performing THA in Crowe type IV hip dysplasia involves serious difficulties and risks due to anatomical differences (4,5,7,13,16). Femoral shortening osteotomy concomitant with THA is the most preferred technique to overcome these difficulties and minimise risks (14,17,18). In the current literature, a large number of patients that have undergone femoral shortening osteotomy concomitant with cementless THA have been evaluated functionally (11,16,19). Shi et al. (11) reported that THA combined with transverse subtrochanteric osteotomy could be an effective method to achieve equal function leg length with most patients having Crowe type IV. Necas et al. (18) demonstrated good results in the treatment of completely dislocated hips with transverse osteotomy. All acetabular components were implanted into the true acetabulum, and all prostheses were stable at the latest examination. They had identified specific complications in seven hips (25%) in total: Intra-operative femoral fracture in four hips, recurrent dislocation in two hips and aseptic stem loosening in one patient. Rollo used transverse sub-trochanter osteotomy and observed no migrations without requiring revision on implants. No cases of delayed union or non-union were detected. Two patients (11%) showed early symptoms of sciatic nerve palsy which resolved uneventfully in 6 months. Rollo reported that THA with shortening subtrochanteric osteotomy is an effective method in the treatment for patients with Crowe type IV DDH (19). According to our study, the mean HHS and WOMAC scores significantly

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improved after THA similar to previous studies. Post-operative improvement in patients' gait patterns and correction of pre-operative limping were satisfactory. No patient had any complaint of severe limping in the post-operative period. THA is considered to be extensively invasive with high complication rates; however, it is still the gold standard for Crowe type IV dysplasia treatment.

The current literature describes many types of osteotomy (transverse, oblique, double-chevron and step-cut) (20-25). Li et al. (23) reported that transverse femoral osteotomy has a high rate of complications, especially non-union or delayed union, due to the low bone contact area and provision of less rotational stability. In contrast, the transverse osteotomy is technically simple that allows for the correction of torsional deformities (26). In our patients, we did not see any union problems. The osteotomy sites of all patients healed within the first year of surgery. Our results confirm that transverse osteotomy is simple and safe in patients with Crowe type IV with low rate osteotomy site complications.

Ergin et al. (27) concluded that patients with unilateral DDH, pelvic height and femoral and tibial lengths on the affected side might be shorter compared to the unaffected side regardless of the Crowe type. In patients with unilateral hip osteoarthritis, the opposite side joint is intact. Prosthesis is not applied to the healthy hip, thus, the difference in the leg length cannot be overcome by another hip surgery. Therefore, these patients should be carefully evaluated in the pre-operative period to predict post-operative leg length discrepancy. According to our findings, measurements showed shorter operated legs; however, five patients complaint of having a longer lower limb on the operated side. Patients should be made aware of this issue and informed that residual leg length differences can be treated ground sill.

Study Limitations

Certain limitations were encountered in this study. First concerns the retrospective design. Second, patients with a unilateral high hip develop a compensatory low lumbar curvature to decrease the leg length differences; however, the extent of improvement in the lumbar pathology after surgery was not evaluated. Third, our follow-up period was relatively short, and longer-term results are necessary in these patients. Component loosening and revision rates should be evaluated with the Kaplan-Meier survival curve over a long period of time.

CONCLUSION

Applying cementless THA in patients with unilateral Crowe type IV has satisfactory functional results. To relieve pain and improve functional scores and quality of life, THA remains the best treatment option. The rate of associated complications is high; however, they can be usually managed with a secondary intervention or only observation.

Ethics Committee Approval: The study was planned after receiving the approval of the University of Health Sciences Turkey, Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital Ethics Committee (approval number: 262, approval date: 29.11.2018).

Informed Consent: A written informed consent was obtained from each patient.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - M.Ö.B., Y.A., S.B.; Concept - M.Ö.B., S.B., H.B.; Design - M.B.K.; Data Collection and/or Processing - M.B.K., H.B.; Analysis and/or Interpretation - Y.A., H.B.; Literature Search - M.B.K., S.B.; Writing - M.Ö.B., S.B.

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Comparison of the Effects of Topical Ebselen, Propolis and Steroid Applications of Acute Radiodermatitis: Preliminary Results

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ABSTRACT

Objective: Radiodermatitis is one of the early complications of radiotherapy (RT). In an acute setting, skin changes may vary from mild hyperemia to necrosis. This study aimed to compare the effects of vaseline, mometasone furoate (MF), propolis and ebselen on acute radiodermatitis and demonstrate an alternative topical treatment method that could be suggested to reduce adverse effects.

Methods: A total of 40 male Wistar-Hannover rats were separated into 4 groups with 10 members to be applied with vaseline, steroid (0.01% MF), propolis (30%) and ebselen (1%), respectively. All groups received radiation at a dose of 3,000 cGy. Following the application of RT, topical applications were applied to all groups and repeated once daily until the experiment was terminated. Skin biopsies were taken from all subjects and examined in terms of erosion, epidermis atrophy, basal layer degeneration, neovascularisation, polymorphonuclear leukocyte (PMNL) infiltration, loss of hair follicles and collagenisation.

Results: The level of erosion was found to be significantly lower in the MF group. Epidermal atrophy was statistically significant lower in propolis and vaseline groups. Propolis significantly decreased basal layer degeneration compared to other treatments. Neovascularisation rates were significantly higher in vaseline and ebselen groups. No significant difference was found between groups in terms of PMNL, loss of hair follicles and collagenisation.

Conclusion: Different agents with different mechanisms of action may be used according to the physiopathological progress of radiodermatitis. Data obtained in this study suggest that combined use of MF, propolis and ebselen according to erythema phases may mitigate the clinical progression of acute radiodermatitis.

Keywords: Ebselen, propolis, radiation dermatitis, steroid

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INTRODUCTION

One of the early stage complications of radiotherapy (RT) is acute radiodermatitis. As a result of RT, various changes in the skin can be seen both short and long term. In acute period, these changes may be limited to simple hyperemia; however, this may progress to necrosis. In addition to deteriorated skin quality in the acute radiodermatitis, the quality of life of the patient can be seriously negatively affected by redness, pain and itching in this difficult period. Furthermore, the early termination of RT because of unhealthy skin tissue can cause the cancellation or postponement of potentially necessary surgical interventions, leading to treatment delay. There is no way to prevent formation of the acute radiodermatitis; however, various methods are applied to ameliorate the course.

Ebselen is a synthetic seleno-organic compound that shows similar activity to glutathione and thioredoxin peroxidase (1-3). Ebselen was designed to protect the skin from oxidative stress caused by ischaemia-reperfusion damage (3,4). Previous studies have shown that ebselen inhibits radiation-origin apoptosis (3-5).

Propolis is a resinous material collected by bees from plant buds and exudates, mixed with bee enzymes, pollen and beeswax. It has been suggested that in addition to the anti-microbial and antioxidant properties of propolis, it also shows anti-inflammatory, anti-tumoral and immunomodulator activity (6).

This study aimed to evaluate vaseline, steroid [mometasone furoate (MF)], propolis and ebselen in respect of ameliorating or preventing acute radiodermatitis and compare their superiority.

METHODS

This study was approved by the Local Ethics Committee for Animal Studies of University of Health Sciences Turkey, Bağcılar Training and Research Hospital in April 2018 (approval number: 2018/47, approval date: 30.04.2018).

A total of 40 male Wistar-Hannover rats, weighing 200-300 gr each, were divided into 4 groups each to be applied with vaseline (control group), steroid (0.01% MF) propolis (30%) and ebselen (1%), respectively.

On the first day of the experiment, all subjects were anaesthetised with 5 mg/kg xylazine and 50 mg/kg ketamine intraperitoneally, and the caudal dorsal region to which the RT and topical applications were to be applied was shaved.

All groups RT at a dose of 3,000 cGy using a Varian Clinac® IX Linear Accelerator, from a distance of 100 cm, with the use of a 1.5 cm bolus with 6 mV energy as a single fraction in service mode. RT was applied in a total of 5 sessions.

Following the application of RT, topical applications were applied to all groups and repeated once daily until the experiment was terminated on the 12th day.

On day 1 after the application of RT, 2 rats died that were evaluated as anaesthesia-related complications. On days 3-4, rectitis developed in all groups. On day 4, 2 rats died and another 2 on day 6, which was thought to be secondary to the development of severe rectitis despite fluid replacement. On day 12, an additional of 2 rats died, yielding the total losses to 8. The study was terminated although it had been planned to continue for 21 days.

Histopathological Evaluation

Skin and subcutaneous tissue biopsies were taken from all subjects. Following fixation in 10% formaldehyde, samples were embedded in paraffin blocks, and then slices of 4 microns in thickness were obtained. Samples were stained with hematoxylin eosin, Masson trichrome and CD34, then examined under a light microscope and graded by a pathologist in respect of erosion, epidermis atrophy, basal layer degeneration, neovascularisation, polymorphonuclear leukocytes (PMNL) infiltration, loss of hair follicles and collagenisation parameters.

Statistical Analysis

Data obtained in the study were analysed statistically using the Statistical Package for the Social Sciences 24.0 software. Descriptive statistical methods were used, and results were stated as frequency, percentage, mean, standard deviation, median and interquartile range values. To compare erosion measurements between groups, the Kruskal-Wallis test was used. To compare other parameters between groups, the Pearson chi-square test was applied. Results were given in a 95% confidence interval. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Macroscopic Findings

Erythema was observed starting at a significant level in all subjects from the 3rd day onwards. Hyperemia was most evident in the vaseline group (Figure 1). The finding of epilation was not evaluated macroscopically as the area applied with RT was shaved in all subjects so as to prevent topical applications inhibition. During the 12-day follow-up, no erosion, dry/wet desquamation or ulceration was observed macroscopically in any subject.

Microscopic Findings

Erosion

The level of erosion was 0 in all subjects in the steroid group, which was found to be significantly low compared to other groups. The difference between other groups was not determined to be statistically significant ($p>0.05$).

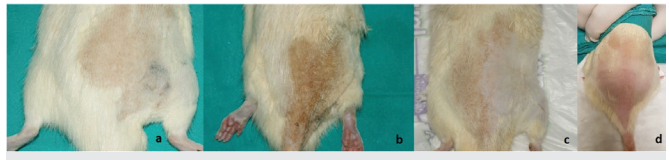

Figure 1. Rats on day 12 post-irradiation a) ebselen, b) propolis, c) steroid and d) vaseline

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Epidermal Atrophy

The presence of epidermis atrophy was determined to be significantly high in ebselen and steroid groups. Whereas, in the propolis and vaseline groups, the rate of epidermis atrophy was significantly low (Figure 2).

Basal Layer Degeneration

The presence of basal layer degeneration was determined at a significantly high rate in ebselen and steroid groups (Figure 3), whereas significantly low in the propolis group. In the vaseline group, basal layer degeneration was observed in 50% of subjects and was not determined in 50%.

Neovascularisation

The rate of neovascularisation was determined to be significantly high in the ebselen and vaseline groups, whereas significantly low in the steroid group (Figure 4).

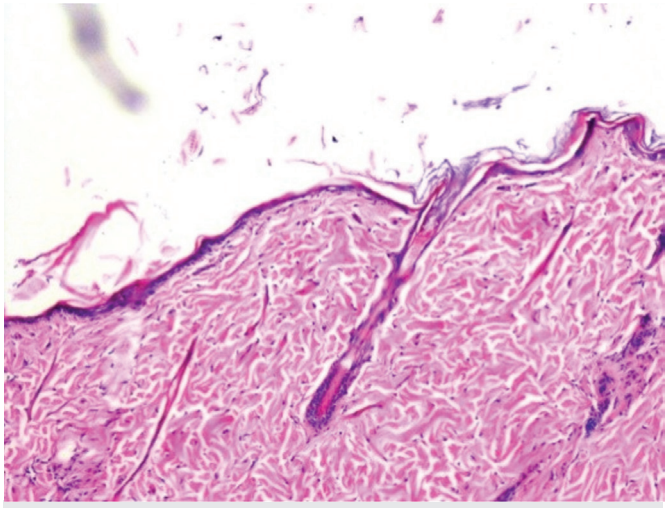


Figure 2. Significant epidermal atrophy in steroid group (hematoxyline and eosine x200)

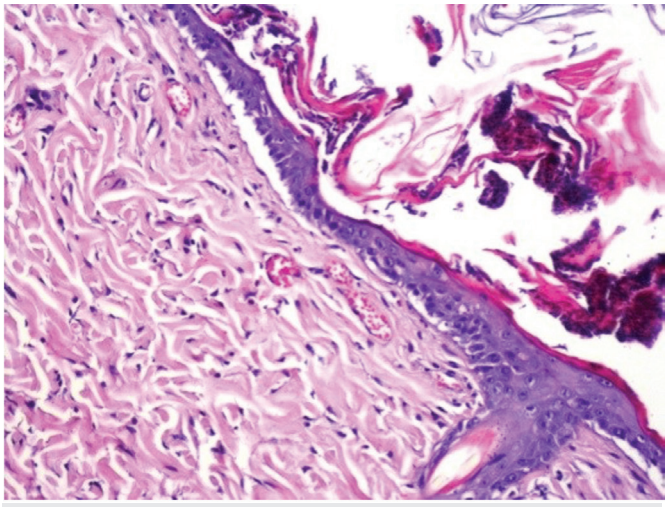


Figure 3. Basal layer degeneration at the epidermal-dermal junction (hematoxyline and eosine x200)

PMNL Infiltration, Hair Follicle Loss and Collagenisation

No statistically significant difference was determined between groups in respect to these 3 parameters (p>0.05).

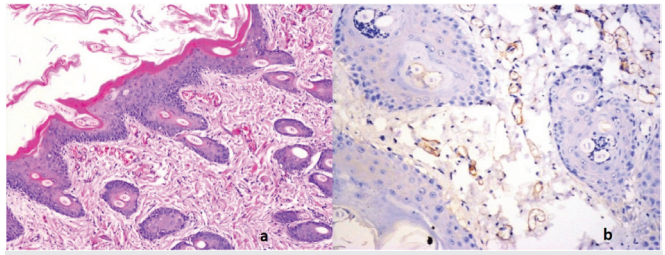


Figure 4. Significant proliferation of capillary structures in dermis in the ebselen group. a) hematoxyline and eosine x200 and b) CD31 x200

DISCUSSION

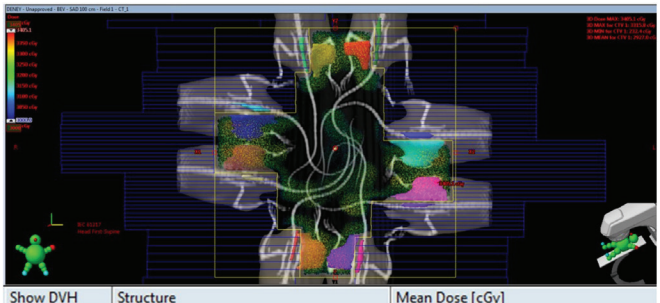
Following the exposure to ionising radiation, double or single chain fractures occur in the nuclear and mitochondrial deoxyribonucleic acid (DNA) structure of cells, and free radicals are formed as a result of water molecules interaction within the cell (7-12). Free radicals inhibit synthesis of DNA causing further DNA damage, leading to structural changes of protein, lipid and carbohydrate molecules (10). The degree of radiation damage shows variations according to the total radiation dose, area of the body irradiated and tissue volume and frequency of application (9,12-14).

The first side-effect seen in the skin associated with RT is erythema, which can be classified in phases of early temporary erythema, primary erythema and late erythema. Early temporary erythema develops within hours of RT and is related to changes in vascular permeability (11,15,16). Within 1-2 days the early temporary erythema recedes, but more intense erythema may develop later (14). In this phase, the skin is hot and has a red and flaky appearance (16). The primary erythema reaction seen on approximately the 10th day originates from the inflammation developing as a result from epithelial basal cell death. Late-stage erythema is seen between the 8th and 10th weeks as a result of dermal ischaemia and may give the skin a slight blue colour (14,15).

Following the loss of basal cells in the epidermis, epidermal hypoplasia starts to develop within 3-5 weeks (15). Epilation and dry skin are observed as a result of reduced mitotic activity in the epidermis germinal cells, hair follicles and sebaceous glands (10,11,16,17). In dry desquamation seen at the end of the 4th week, changes in the skin are characterised by itching, flaking and increased melanin pigment in the basal layer causing a change in skin colour. With increased severity of hypoplasia due to radiation severity, age desquamation starts to be seen. The epidermis is completely lost in irradiated region and the dermis is exposed due to the opening of bullae which develop suprabasally in the irradiated region.

Still, no gold standard treatment method is reported for radiodermatitis, and studies on this subject are on-going. According to the common view, the best method is the moisturisation of the irradiated region to prevent or minimise skin reactions (18). Methods reported in literature include washing with soap, use of deodorant, various lotions, aloe vera, calendula officinalis, biafine cream, hyaluronic acid/sodium hyaluronate cream, corticosteroids, sucralfate, antimicrobials, barrier films, various wound coverings, oral enzymes, pentoxifylline, colony-stimulating factors, Vitamin E, prostaglandins, nitroxides, hyperbaric oxygen, thrombocyte-rich plasma, zinc sulphate, azelastine and captopril (15,16,18-22). Various studies showed that corticosteroids are effective on an acute radiodermatitis table with an anti-inflammatory effect through various mechanisms such as vasoconstriction, capillary permeability and leukocyte proliferation and migration (23-25). Given the fact that moisturisation is the first and indispensable step in radiation dermatitis management, we designed the control group as the vaseline group with the aim of imitating this clinical scenario.

Unlike other rat model studies (21,26-28), the applied dose of radiation in the current study was determined as 30 Gy. As a result of radiation applied with a linear accelerator providing a photon beam, 6 of the 40 rats in the current study were lost within 12 days because of diarrhoea which developed as a result of radiation-related rectitis, thus, the experiment had to be terminated. In the planning of the study, the rectum mean dose of 3.4 Gy did not present any risk in respect to rectitis, hence, no problems were foreseen in using photon energy in the treatment (Figure 5). During the irradiation, some rats were observed to move as the anaesthesia effect had worn off. The inclusion of the rectum region in the radiation field is thought to be the reason for rectitis development. With this study, it was seen that failure of immobilization may lead to increased complication rate. In theory, RT application with photon beam energy was deemed for this study as appropriate. However, due to unforeseen conditions, such as failure of immobilization of the rats in this case, electron beam RT might be a safer option, since it has a lesser depth of penetration.



Show DVH	Structure	Mean Dose [cGy]
☑	R1	342.2
☑	R2	335.9
☑	R3	293.2
☑	R4	346.1
☑	R5	329.2
☑	R6	375.5
☑	R7	320.4
☑	R8	379.7

Figure 5. Distribution of radiation dose shaped with multi-leaf collimator on the irradiated target

According to the study data, MF was seen to be superior in respect to preventing erosion development; however, it was not seen to make any positive contribution to epidermis atrophy, basal layer degeneration or neovascularisation. Steroids exhibit anti-inflammatory properties through various mechanisms such as vasoconstriction, capillary permeability, leukocyte proliferation and migration (23-25). These can be attributed to the effect of MF on erosion.

The only superiority of ebselen, which has antioxidant properties, over the application of propolis, steroid and vaseline, was its increasing neovascularisation effect. However, propolis was determined to have a positive effect on both epidermis atrophy and basal layer degeneration. We believe that ebselen may promote neovascularisation by reducing oxidative stress, vascular damaging factors and inflammation.

Propolis has also provided a biochemically available medium for re-epithelisation through its effects on collagen expression and degradation mechanisms (29). The anti-inflammatory and antioxidant properties of propolis may have played a role in reducing basal layer degeneration (30,31).

No statistically significant difference was determined between groups in respect of PMNL infiltration, loss of hair follicles or collagenisation; however, when scores were examined separately, the collagenisation density was noticeable in the vaseline group (Figure 6).

To make a reliable evaluation of the effect of the agent used, agents applied in previous clinical studies in literature related to radiodermatitis have been used alone. Different effects observed on different agents in this study raised the idea of investigating the effect of combined treatments. In light to the preliminary data obtained, the application of different agents at different time interval and taking stages of radiodermatitis into consideration could be considered as an alternative treatment method in keeping the clinical course of radiodermatitis under control. As early erythema reaction is due to the increase in capillary permeability, corticosteroids may be useful in this phase due to their capillary permeability reducing effect. The primary

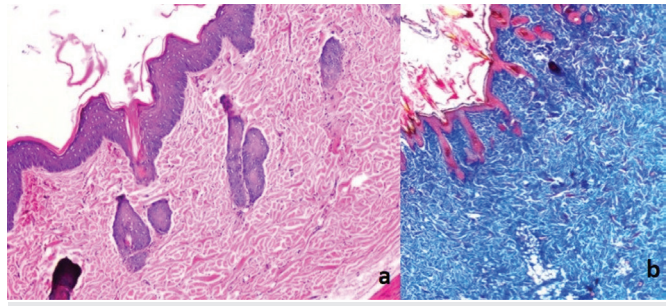


Figure 6. Collagen bundles extending from dermis to subcutaneous fat tissue. a) hematoxyline and eosine x200 and b) Masson Trichrome x200

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erythema reaction that is seen with the loss of epithelial basal cells in the advanced stage can be brought under control by propolis, which has the property of preventing basal layer degeneration. When late-stage erythema is considered to be due to dermal vascular damage, after bringing the primary erythema reaction under control, changing the application to ebselen may be useful in minimising late erythema reaction with the increase in neovascularisation.

When optimum skin quality is maintained after taking all phases under control, the possibility of seeing more severe findings such as dry/wet desquamation, ulceration or necrosis can be further minimised. To the best of our knowledge, no previous study has been conducted on radiodermatitis neither with propolis or ebselen nor with photon linear accelerator. Therefore, a need for further experimental and clinical studies on the efficacy of the suggested alternative combined topical treatments to support findings of this study is recommended. The study by Kandaz et al. (32) is such an example.

Study Limitations

The most important limitation includes the termination of the study earlier as planned because the loss of 20% of subjects due to severe rectitis despite sufficient fluid replacement. Consequently, acute stage reactions were not observed over a sufficiently long period, and a suboptimal evaluation was made. Nevertheless, we had the chance to observe the main erythema reaction which occurred on the 10th day following RT. The range of RT models defined in animal studies in literature and complications encountered in those studies demonstrate the need for dosage to be studied on different devices. The short-term follow-up of radiation-related skin changes prevented the scoring of skin damage. In addition, the effect of treatments applied could only be evaluated in the short-term. However, we believe that preliminary results obtained from this study would contribute to the design of future studies.

CONCLUSION

The ability to protect the skin quality at the maximum level after RT is of the greatest importance in respect to patient comfort and treatment compliance, avoiding failure of the treatment process and reducing the complication rate of surgical procedures to be applied. Topical treatments in the prevention or treatment of radiodermatitis are more advantageous in terms of ease of application. Despite many experimental and clinical studies, no consensus has been provided on the point of prevention and treatment of radiodermatitis. Benefit can be gained from different mechanisms of different agents taking the physiopathological course of radiodermatitis into consideration. According to the preliminary data obtained, it can be predicted that according to erythema phases, the combined use of MF, propolis and ebselen could ameliorate the clinical course of acute radiodermatitis.

Ethics Committee Approval: This study was approved by the Local Ethics Committee for Animal Studies of University of Health Sciences Turkey, Bağcılar Training and Research Hospital in April 2018 (approval number: 2018/47, approval date: 30.04.2018).

Informed Consent: This study is an animal experiment.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Concept - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Design - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Data Collection and/or Processing - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Analysis and/or Interpretation - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Literature Search - I.A.D., S.S.Ş., S.H., C.Ö., S.K.; Writing - I.A.D., S.S.Ş., S.H., C.Ö., S.K.

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

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Comparison of Feto-maternal Effects of Twin Pregnancies and Twin Pregnancies Caused By Assisted Reproductive Technology

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ABSTRACT

Objective: Comparison of the effects of twin pregnancies using assisted reproductive technology (ART) and spontaneous twin pregnancies on maternal-fetal parameters.

Methods: A total of 5,670 patients who were deliveried in our clinic between 01.01.2011 and 31.12.2017 were analyzed retrospectively. The feto-maternal results of 94 mothers who deliveried to twins and their infants were compared.

Results: Of the 5,670 patients who gave birth, 94 were twins (1/60) and 8 were triplets (1/709). Twin births account for 92% of multiple births. The incidence of spontaneous twin pregnancy was 1/77 and constituted 79% of all the twin deliveries. The effect of ART on was found to be 21% [17% in vitro fertilization (IVF), 4% ovulation induction].

Mean maternal age was 29.25±6.00 years in twin pregnancy deliveries, 28.50±6.09 years in mothers who had spontaneous twin group, and 32.54±5.09 years in twin births due to ART.

When anemia was excluded, maternal morbidity was 27% in twin pregnancy deliveries. Pre-eclampsia was determined in 19% of the cases and anemia in 63%. Delivery before 37 gestational weeks was recorded in 72.3% of IVF twins and 70.2% of spontaneous twins. The rate of live births was found to be higher in spontaneously conceived twins (p=0.004). The fetal mortality rate was 5.4% in spontaneous twin pregnancies and 20% in twin pregnancies with ART (p=0.003). The perinatal mortality index was determined to be 81 in spontaneously conceived twins, and 250 in the twin pregnancies obtained with ART (p=0.006).

Conclusion: Premature birth and fetal mortality rates are higher in twin pregnancies obtained with ART than in spontaneous twins. The mortality associated with multiple pregnancies may be reduced with preventive measures such as low-dose gonadotropin use, single embryo transfer, and multifetal embryo reduction.

Keywords: Spontaneous pregnancy, assisted reproductive technology, multiple pregnancy

INTRODUCTION

The pregnancy rate using assisted reproductive technology (ART) has increased significantly in the last four decades. Although ART usage rates in infertile patients are reported as 11% in the United States of America (USA) , this rate is estimated to be higher (1). Twin pregnancy rates due to ART have been reported as 18% in the USA (1). After ART applications had their place in reproductive biology clinical practice, the incidence of twin birth increased up to 1-4% (1-6). Although the increase in pregnancy rates was pleasing at first, the significant increase in multiple pregnancy rates due to success-oriented practices such as (i) advanced age, (ii) high dose drug use, (iii) multiple embryo transfer, both

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maternal mortality and maternal mortality. It leads to increased morbidity, as well as fetal morbidity and mortality (6,7). Parallel to the increase in the rates of gestational diabetes, gestational hypertension and preeclampsia in women who conceive after ART, a significant increase in the prevalence of preterm labor, low birth weight infants, respiratory distress syndrome and cerebral palsy draws attention (2,6-11). This study was carried out retrospectively in order to draw attention to the increase in maternal, fetal and perinatal mortality and morbidity rates in twin pregnancies due to ART. Spontaneous twin pregnancies were included in the control group, and maternal, fetal and perinatal morbidity and mortality differences were compared with twins due to ART.

METHODS

This retrospective study was conducted after the approval of the Ethics Committee of Hatay Mustafa Kemal University (approval number: 06, approval date: 05/09/2019). The files of 5,670 pregnant women who admitted to our clinic between 01.01.2011 and 31.12.2017 and gave birth for 20 weeks or later, 500 grams (gr) or more were retrospectively analyzed. Patient consent was obtained in order to use the registered data of the patients. Multiple pregnancy records were made among the pregnant women who gave birth, and the clinical and demographic data of the patients were recorded. Maternal parameters subjected to registration process were; age, form of multiple pregnancy [spontaneous, intrauterine insemination, *in vitro* fertilization (IVF)], gravida, parity, abortion, number of surviving children, last menstrual period. The fetal parameters recorded were; week of birth, birth weight, mode of delivery, 1st and 5th minutes APGAR scores were determined. When it comes to maternal complications; physiological anemia of pregnancy, preeclampsia and gestational diabetes were taken into consideration. As a fetal complication, preterm birth, premature rupture of membranes, oligo-polyhydramnios, low birth weight baby, birth weight incompatibility, presence of fetal anomaly, fetal death were taken into consideration.

Multiple pregnancies with live births were classified as immature, premature and mature according to the following criteria; births between 20th and 28th weeks were recorded as early preterm (immature) births, births between 28th and 37th weeks as preterm birth (premature), and births at 37th weeks and above as term (mature) births. In the classification made according to fetal birth weight; Babies born 2,500 gr and above were considered as normal birth weight, babies born below 2,500 gr low birth weight. In twin pregnancy deliveries, if there is a difference of 25% or more in terms of birth weight between twins, it was recorded as discordance. The perinatal mortality index (PMI) was calculated according to the formula below.

$$PMI=\left(\frac{\text{(Number Of Stillborn Babies+Number of babies dying within the first 7 days after birth)}}{\text{Number of all babies born (alive and dead)}}\right) x1000$$

Statistical Analysis

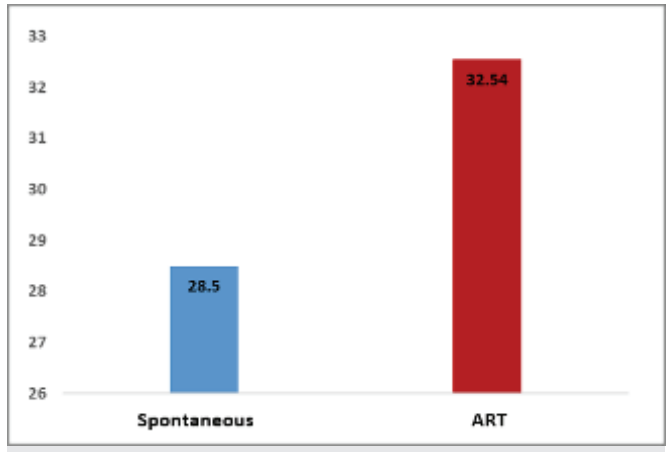
SPSS 21 (SPSS Inc. Chicago, Illinois, USA) package program was used for statistical analysis of the obtained data. Spontaneous and ART twin births were compared with each other in terms of clinical, demographic, maternal and fetal complications. Student t-test was used for analysis of continuous variables. The obtained results were given as mean ± standard deviation or as n (%). Chi-square (χ^2) tests were used for the analysis of non-continuous variables. P<0.05 was considered significant.

RESULTS

Out of 5,670 pregnant women 102 were deliveries due to multiple pregnancy. Of the 102 multiple births, 94 (92.2%) were twin and 8 (7.8%) were triplet births. Seventy-four of the twins (78.7%) were spontaneous twins, 20 of them (21.2%) were twin pregnancies due to ART. Sixteen of ART twins were obtained due to IVF and 4 of them were due to ovulation induction (OI). Among all pregnant women, the incidence of multiple pregnancy was 1/56, twin pregnancy incidence 1/60, and triplet pregnancy incidence 1/709. In our records, there were no quadruplet and quintet pregnancies that occurred either due to ART or spontaneously.

While the spontaneous twin birth incidence was 1/77, the incidence of twin births due to ART was found to be 1/283.5 (Table 1a). Average maternal age was found to be 29.25±6.00 years in general twin pregnancy deliveries, 28.50±6.09 years for mothers who had spontaneous twins, and 32.54±5.09 years for mothers with twins due to ART. The average age of mothers who gave birth to twins due to ART was found to be higher than mothers who gave birth to twins spontaneously (p<0.007) (Table 1a, Graphic 1). While 9.46% of spontaneous twins were over 35 years old, 35% of ART twins were over 35 years old and the difference was found to be significant (p<0.01) (Table 1a, Graphic 2).

Gravida (spontaneous 3.02±1.60; ART 1.64±0.84; p=0.004), parity (1.72±1.30; 0.43±0.76; p=0.001), mean living child (1.49±1.30; 0.36±0.74; p=0.003) in spontaneous twins was found to be



Graphic 1. Average maternal age in spontaneous and ART twin pregnancies
ART: assisted reproductive technology

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Table 1a.Maternal fetal parameters of twin pregnancy deliveries

	Spontaneous twins	ART twins	p*	IVF twins	Ovulation induction	Total
	n (%)			n (%)		
Total birth	74 (78.72)	20 (21.27)	0.001	16 (17)	4 (4)	94 (100)
≤20 years old mothers	9 (12)	0	-	0	0	9 (9.6)
≤35 years	67 (90.54)	13 (65)	0,01	9 (56)	4 (9)	80
Above 35 years old	7 (9.46)	7 (35)		7 (44)	0	14
Twin birth caesarean	63 (85)	16 (80)	0.731	12 (75)	4 (100)	78 (83)
Twin birth vaginal birth	11 (15)	4 (20)		4 (25)	0	16 (17)
Live born baby	140 (95)	32 (80)	0.004	25 (78)	7 (87.5)	172 (91.5)
Number of babies alive	136 (97)	30 (93.75)	0.387	23 (92)	7	166 (96.5)
Incidence	1.3/100	3.52/1,000	0.719	2.82/1,000	7.0/10,000	94 (1.66)
Total sectio						4,297 (75.8)
Total vaginal delivery						1,373 (24.2)
All births						5,670 (100)
Maternal parameter						
Average maternal age	28.50±6.09	32.54±5.09	0.007	33.8±5.14	28.33±1.53	29.25±6
Gravidite average	3.02±1.60	1.64±0.84	0.004	1.45±0.69	2.33±1.155	2.54±1.59
Parity average	1.72±1.30	0.43±0.76	0.001	0.27±0.65	1±1	1.25±1.27
Abortion	0.33±0.76	0.21±0.43	0.51	0.18±0.40	0.33±0.58	0.28±0.69
Living average	1.49±1.30	0.36±0.74	0.003	0.18±0.60	1±1	1.14±1.26
Maternal complications						
Anemia during pregnancy	45 (61)	14 (70)	0.545	10 (62.5)	4 (100)	59 (63)
Preeclampsia-eclampsia	15 (20)	3 (15)	0.845	3 (19)	0	18 (19.15)
Gestational diabetes	1					1
Diabetes mellitus	1					1
Others	4	1	-	-	-	5
EMR	11 (15)	2 (10)	-	2 (12.5)	0	13 (14)
Oligo-anhidroamnios	5 (7)	2 (10)	-	2 (12.5)	0	7 (7.4)
Polyhydramnios	1 (1.35)	1 (5)	-	1 (6.25)	0	2 (2.13)

*Statistics of maternal parameters were given as mean ± standard deviation and Student t-test was used to calculate p. Chi-square tests were used for other statistics. Others: gestational hypertension, gestational diabetes, HELLP syndrome, pregnancy secondary thrombocytopenispontan, gestational thrombocytopenia ART pregnancy.
ART: assisted reproductive technology, EMR: early rupture of membrane, IVF: in vitro fertilization, HELLP syndrome: hemolysis, elevated liver enzymes, and low platelet count syndrome

significantly higher than the ART twins (Table 1a, Graphic 3). The number of babies born alive was found to be significantly higher in spontaneous twin births compared to twin births due to ART (95%, 80%, p=0.004, Table 1a, Graphic 4). No significant difference was found between spontaneous and ART twins in terms of abortion rates (0.33±0.76 vs 0.21±0.43; p=0.51). When anemia was excluded in all twin pregnancies, maternal morbidity was found as 27%. Anemia was detected in 63% of the cases who gave birth to twins (61% spontaneous twin, 70% due to ART), and 19% preeclampsia (20% in spontaneous twins; 15% in twins due to ART).

While the week of delivery was 35.18±3.27 weeks in spontaneous twins, it was found as 34.27±4.20 weeks in ART twins (Table 1b, Graphic 5). When twin births were evaluated in terms of APGAR scores, 1st minute APGAR was found as 6.30±1.87 points in spontaneous twins and 6.21±2.02 points in ART twins. There was no difference between the groups in terms of 1st minute APGAR values (p=0.383), while 5th minute APGAR values were 8.12±1.56 points in spontaneous twins and 7.42±2.11 points in twins with ART (p=0.103, Table 1b, Graphic 5).

While the mean birth weight was 2223.87±603.97 g in spontaneous twin pregnancies, it was found similar as 2270.50±811.02 g in twin pregnancies due to ART (p=0.77). While it was 34% in spontaneous twins who were born 2,500 gr and above, it was found as 62.5% in ART twins (p=0.031; Table 1b). Low birth weight was found as 53.57% in spontaneous twin pregnancy deliveries and 18.75% in

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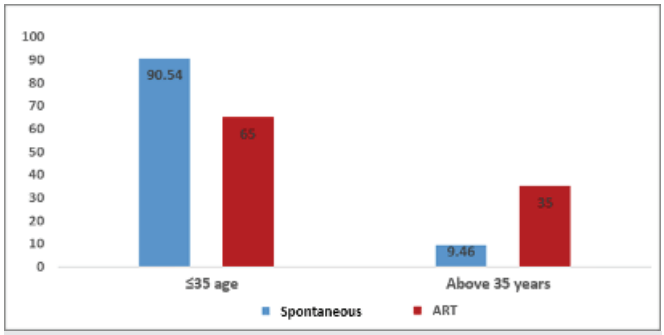


Graphic 2. Distribution ratio of maternal age below and above 35 years in spontaneous and ART twin pregnancies
ART: assisted reproductive technology

ART twins (p=0.056). Discordance between fetuses is shown in Table 1b as SGA, AGA, LGA. Cleft lip, cleft palate, left ventricular cyst in the heart, unilateral polycystic kidney and bilateral ventriculomegaly were found as fetal anomalies. Due to the low number of cases in terms of fetal anomalies, no comparison could be made between the groups.

While both fetuses were stillborn in two of the spontaneous twin pregnancies; In the remaining four deliveries, one of the fetuses was born alive and the other was dead. Both fetuses were stillborn in one of the twin pregnancy deliveries caused by ART. When the gender of stillborn twin babies were also evaluated, the rate of male babies was found to be higher in both spontaneous and ART-induced twins (Table 1b). When all twins were examined, 54% of the babies were female. The frequency of female babies was found to be higher in spontaneous twins compared to ART twins (61%; 30%, respectively; p<0.001; Table 1b).

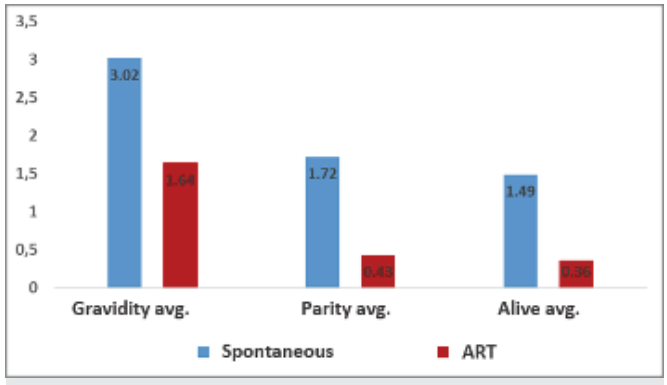
Fetal mortality rate was 5.4% in spontaneous twin births and 20% in ART twins (p=0.003). Neonatal mortality rate was 5.4 in spontaneous twins; and 6.25 in twins connected to ART. PMI was 81 in spontaneous twins and 250 in ART twins (p<0.006), 117 in all twins.

DISCUSSION

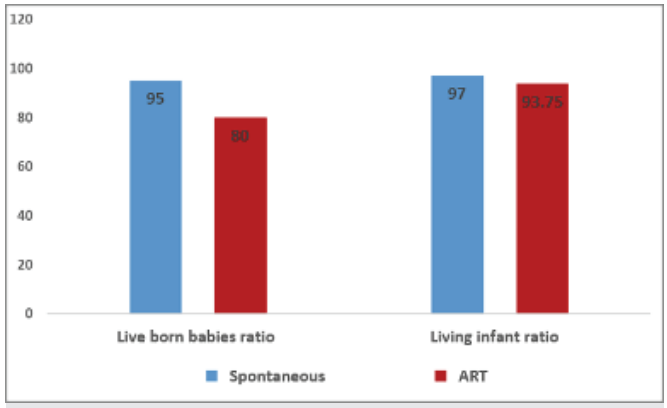
In the USA, 11% of all newborns are reported to occur following the infertility treatment (1). 95% (1) of all multiple pregnancies in the USA, 99% (3) in the United Kingdom (UK), and 91% in Turkey Güler et al. (4) reported to have twin pregnancy births. Of the 102 multiple births in our clinic, 94 (92.2%) were twins and 8 (7.8%) were triplets. Our data are consistent with literature data. It has been reported that 18% of twin births in the USA, 18% in the UK, 23.3% in Australia and 41% in Italy were caused by ART (1,3,12,13). It was found that 74 (78.72%) of the twins born in our clinic were spontaneous and 20 (21.27%) developed with ART, our findings were consistent with the literature. Güler et al. (4) reported that 66.6% of the twins were with ART and 33.4% were spontaneous. We found that 16 (80%) of ART twin births were caused by IVF and 4 (20%) of them were caused by OI. Among all pregnant women, the incidence of multiple pregnancy was 102/5670 (1/56), twin pregnancy incidence 94/5670 (1/60), and triplet pregnancy incidence 8/5670 (1/709) (Table 1a). Twin births constitute 3-4% of all births in the USA (1), Bardis et al. (3) reported that 1.45% of them were twin pregnancy births in Australia. Güler et al. (4) stated the rate of twin birth as 2.7% in their study. Samani et al. (5) reported the rate of multiple births in Iran as 1.48%. Spontaneous twin birth rate has been reported as 0.6% to 2.79% (14), and the rate of twin births among those who underwent ART has been reported as 6.8% to 8.44% (3,5,15).

In studies, the average age of mothers who gave birth to twins with ART was found to be higher than the age of mothers who gave birth spontaneously (3,4,12). In Australia, Wang et al. (12) reported that 25.8% of spontaneous twins are 35 years old and over, and 45.2% of ART twins are over 35 years old. In our study, 9.46% of spontaneous twins were over 35 years old, while 35% of ART twins were over 35 years old (p=0.010, Table 1a, Graphic 2).

In the gravida, parity, living child examination, it is reported in the literature that ART is predominant in mothers who gave birth to primipara twins, and spontaneous twins are dominant in multipara. It has been reported that the number of surviving children of mothers who gave birth to twins spontaneously is higher (7,10,12,13). Gravida, parity, and the mean of living children



Graphic 3. Gravida, parity, living child average in spontaneous and ART twin pregnancies
ART: assisted reproductive technology, avg.: average



Graphic 4. The ratio of live born babies and living babies in spontaneous and ART twin pregnancies
ART: assisted reproductive technology

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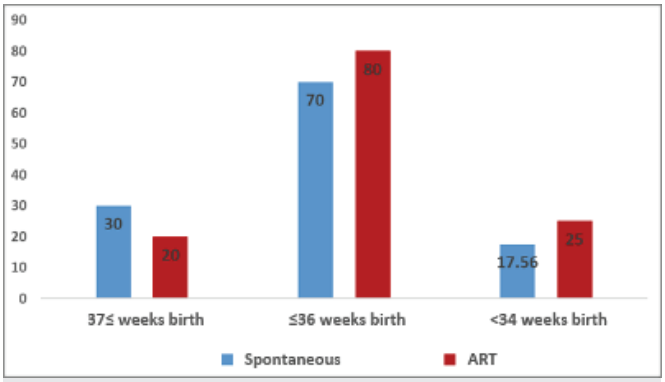
were found to be higher in spontaneous twins compared to ART twins (Table 1a, Graphic 3, 4). In our study, we obtained findings compatible with the literature.

The rate of preterm birth (<37 weeks) in twin pregnancy deliveries ranges between 36.58% and 79% (2,8,9). It has been reported that preterm delivery in ART pregnancies is higher than spontaneous twin pregnancy deliveries due to ART procedure and the underlying reasons (6,7,12,13,16,17). In our study, the week of delivery was 35.18±3.27 weeks in spontaneous twins; 34.27±4.20 weeks in ART twins, similar to the literature. It has been reported that the live birth rate is lower in ART twin pregnancy births than in spontaneous twin pregnancy births (11-13). In our study, the number and rate of babies born alive were found to be higher in spontaneous twins (95%; 80%, respectively; p=0.004, Table 1a, Graphic 4).



Graphic 5. Spontaneous ART birth rates above 37 weeks and below 36 weeks and below 34 weeks in twin pregnancies
ART: assisted reproductive technology

In the literature, it has been reported that maternal morbidity and complications are higher in ART pregnancies than in spontaneous pregnancies (11-13). Preeclampsia rate was reported as 19.6% in ART pregnancies and 13.6% in spontaneous pregnancies (8,11,13). In twin pregnancy deliveries, when anemia was excluded, we found maternal morbidity 27%, anemia 63% and preeclampsia 19%. No significant difference could be found between maternal complications in spontaneous twin pregnancy delivery and ART twin pregnancy deliveries, this may be due to the low number of our cases.

There are publications reporting that baby birth weight is significantly higher than spontaneous twin pregnancy births in ART-induced twin pregnancy deliveries (3,11) and there are also publications reporting that there is no difference (4,6,12,13). There was no significant difference between the groups in terms of birth weight. In spontaneous twins, births of 2,500 gr and above were found to be 34%; It was 62.5% in ART twins (p=0.031; Table 1b). The reason may be that mothers who were pregnant with ART had better perinatal follow-up than mothers who were spontaneously pregnant. There was no significant difference between the groups in terms of the presence of discordance between fetuses. Comparison could not be made in terms of fetal anomalies due to the low number of cases.

There are publications (13) reporting that APGAR 1st-5th minute scores are higher (4) or no difference (12) in spontaneous twin pregnancy deliveries. We also did not find any difference between 1st-5th minutes APGAR values (Table 1b).

There was no significant difference between the groups in terms of the sex of twin babies born alive. When the gender of stillborn twin babies was evaluated, male baby gender was found to be 81.25% (Table 1b). This is because in fetal life, female sex fetuses are more prone to survival than male sex fetuses. Özkan et al. (18) reported male gender as 54% in his study on fetal autopsies. When all twins were examined, 57.5% of the babies were female babies. Yumru et al. (19) reported the male baby gender at birth as 52%. In our study, the frequency of female babies was found to be higher in spontaneous twins compared to ART twins (spontaneous 60.7%; ART 44%).

While neonatal death was reported as 2.1% in ART pregnancies in twins; this rate is 1.8% in spontaneous pregnancies (12). In our study, neonatal mortality was 5.4% in spontaneous twins; 6.25% of twins born with ART; fetal mortality was 5.4% in spontaneous twin pregnancy and 20% in ART twins (p=0.003). In singleton, twin and multiple pregnancy deliveries resulting from ART; it has been reported that perinatal mortality and morbidity are higher than spontaneous pregnancy deliveries (3,6,12,13). We found PMI as 117 in all twins, 81 in spontaneous twins, and 250 in ART twins. Our findings are consistent with the literature data.

Study Limitations

The limitations of our study are the retrospective nature of our study and the low number of our cases as 94. We hope that the studies on a much larger number of cases will contribute significantly to this issue.

CONCLUSION

We can list the measures that can be taken to reduce the maternal and fetal negative effects of ART-related multiple pregnancies as follows: Single embryo transfer should be done in IVF. In cases where 3 or more follicles develop during OI and intrauterin insemination, IVF application or cycle cancellation should be performed. In IVF cycles, ovarian stimulation should be done with low dose gonadotropin. Embryo cryopreservation is required for cycles with hyperstimulation in IVF. While the patient is preparing for IVF, estradiol level should be kept below 3,000 pg/mL as much as possible. We can reduce the occurrence of multiple pregnancies due to ART by using analogues instead of human chorionic gonadotropin and embryo cryopreservation in triggering ovulation in cases where estradiol level is 3,000 pg/mL ≤.

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Table 1b. Fetal parameters of twin pregnancy deliveries

	Spontaneous twins	ART twins	p*	IVF twins	Ovulation induction	Total
	n (%)			n (%)		
Week of birth	35.18±3.27	34.27±4.20	0.3	33.9±4.62	35.50±2.60	34.6±3.99
Birth weight (g)	2223.87±603.97	2270.50±811.02	0.77	2288.50±811.88	2234.50± 885.38	2250.57±586.78
APGAR-1st minute	6.63±1.87	6.21±2.02	0.38	6.13±2.20	6.80±1.30	6±2.57
APGAR-5th minutes	8.12±1.56	7.42±2.11	0.1	7.47±1.92	7.33±2.49	7.49±2.63
For live births						
37≤ weeks birth	22 (30)	4 (20)	0.69	0	4	26 (28)
≤36 weeks birth	52 (70)	16 (80)	0.44	16 (100)	0	68 (72)
≤33 weeks birth	13 (17.56)	5 (25)	0.73	4 (25)	1 (25)	18 (19.15)
2,500 g ≤	48 (34)	20 (62.5)	0.03	15 (60)	5	68 (39.53)
Below 2,500 g	92 (66)	12 (37.5)	0.06	10 (40)	2	104 (60.47)
Low birth weight	75 (53.57)	6 (18.75)	0.1	6	0	81 (47)
Very low birth weight	10 (7.1)	6 (18.75)	0.49	4	2	16 (9.3)
VVLBW	7 (5)	0		0	0	7 (4)
Discordant newborn	13 (9.29)	3 (9.37)		3 (12)	0	16 (9.3)
SGA newborn	45 (32)	6 (18.75)	0.51	4	2	51 (29.65)
AGA newborn	91 (65)	26 (81.25)	0.12	20	6	117 (68)
LGA newborn	4 (2.86)	0		0	0	4 (2.33)
Total	140 (81.4)	32 (18.6)	0	24	8	172
Fetal anomaly	4 (2.7)	0		0	0	4 (2.12)
Fetal mortality	8 (5.4)	8 (20)	0.003	8 (25)	0	16 (8.51)
Neonatal mortality	4 (5.4)	2 (6.25)		1 (4.1)	1 (12.5)	6 (3.49)
PMI	12 (81)	10 (250)	0.006			22 (117)
Live births	140 (94.6)	32 (80)		24 (75)	8 (100)	172 (91.49)
Baby boy	55 (39.3)	18 (56)	0.73	14 (58.33)	4 (50)	73 (42.5)
Baby girl	85 (60.7)	14 (44)	0.74	10 (41.67)	4 (50)	99 (57.5)
IUMF	8 (5.4)	8 (20)	0.003	8 (25)	0	16 (8.51)
Male	6 (75)	7 (87.5)	0.58	8 (100)	0	13 (81.25)
Female	2 (25)	1 (12.5)		0	0	3 (18.75)
All born babies	148	14		32	8	188
Baby boy	58 (39)	2 (70)	0	24 (75)	4 (50)	86 (46)
Baby girl	90 (61)	12 (30)	0	8 (25)	4 (50)	102 (54)

*Student t-test was used to calculate p. Chi-square tests were used for other statistics.
Fetal anomalies: 1) Cleft lip, cleft palate, 2) heart cyst in the left ventricle, 3) polycystic kidney in a single kidney, 4) bilateral ventrculomegaly
APGAR: evaluation index of newborn baby, ART: assisted reproductive technology, IVF: in vitro fertilization,
VVLBW: very very low birth weight, SGA: low birth weight according to gestational week, AGA: normal weight according to gestational week, LGA: larger than gestational week, IUMF: in utero mort fetalis, PMI: perinatal mortality index

Ethics Committee Approval: This retrospective study was conducted after the approval of the Ethics Committee of Hatay Mustafa Kemal University (approval number: 06, approval date: 05/09/2019).

Informed Consent: Patient consent was obtained in order to use the registered data of the patients.

Peer-review: Externally peer-reviewed.

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Psychiatric Comorbidity, Length of Hospital Stays and Readmission Rates in Opiate Addicts Treated in Inpatient Service

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ABSTRACT

Objective: In this study, it was aimed to investigate psychiatric comorbidity, long hospitalization reasons and readmission reasons of opiate addicts treated in inpatient service.

Methods: The study data consisted of patient records treated at Gazi University Hospital Alcohol and Drug Addiction Clinic between 1 January 2005 and 31 December 2017. Four hundred eighty four people with primary diagnosis of opiate addiction were included in the analysis. One hundred of these patients had recurrent hospitalizations.

Results: While the average age of the sample was 29.74±7.05, 89.9% (n=435) were male. The presence of depression as a comorbidity in opiate addiction increased the length of hospitalization 3.3 times [odds ratio (OR): 3.362] and the psychotic symptom 5.4 times (OR: 5.417). In addition to opiate addiction, the diagnosis of anxiety disorder increased the risk of readmission 3.3 times (OR: 3.321), while the diagnosis of personality disorder reduced the risk of readmission 4.58 times (OR: 0.218). It was observed that 5% (n=5) of the patients were re-admitted within the first month and 29% (n=29) within the first three months.

Conclusion: One of every three opiate addicts is admitted in the first three months after discharge. Having anxiety disorder increases recurrent hospitalization 3.3 times, while having personality disorder decreases 4.5 times. Further studies should be conducted on whether short hospitalization periods increase readmission rates.

Keywords: Opiate addiction, length of stay, patient readmission, comorbidity, depression

INTRODUCTION

Opiate addiction is a serious, chronic and recurrent psychiatric disease (1). In the drug report published by the World Health Organization (WHO) in 2018, it was stated that there were 275 million drug users, 34 million of which were opiate users (2). Although opiate use constitutes a relatively small part of all drug use, it accounts for 76% of drug-induced deaths (2). As a result of opiate-containing drug use in 2015, 167,750 people lost their lives (2). Drug use in Turkey is less common than the world average. However, the increase in use over the past years shows that drug addictions are gradually increasing. The Turkey drug report published in 2019 showed that the first drug to be tried is cannabis, and the second is heroin (3). All these data show that drug treatment and health services continue to be inadequate and drug addictions are still a serious public health problem.

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Illegal use of opiates has significant social burden such as frequent reference to emergency services, overdose use, deterioration of family relationships, the increase in violence, criminal activity, work loss, increase in general medical illnesses and adverse effect on the course of psychiatric illnesses (4). In addition to these burdens, patients apply to clinics for treatments often due to the high recurrence rates. Addiction treatment includes a community-oriented treatment and rehabilitation treatment that includes psychosocial variables as well as pharmacological approaches (5). Although these treatments are improved day by day, relapse is still quite common. Even in opiate addicted patients receiving agonist maintenance treatment, it is reported that more than 50% of the treatment discontinues in the first three weeks of treatment (6).

There are many difficulties in addictions, such as the recurrent and chronic nature of the disease, the presence of accompanying psychiatric diseases and the high rate of treatment non-compliance in patients. These difficulties affect the treatment process, changing the duration of hospital stay and may cause recurrent admissions (7). Studies evaluating the duration of hospital stay in opiate use disorders are quite limited in the literature. Studies on the duration of hospital stay generally evaluated substance addiction under a single heading. The general result obtained from these studies is that the duration of hospital stay is shorter in substance use disorders compared to other psychiatric disorders (8). Some studies also show that the shortened length of stay in psychiatric diseases causes recurrent hospitalizations in patients (9). More research is needed on length of stay and recurrent hospitalizations, which are affected by many variables and remain uncertain.

In this study, we hypothesize that the duration of hospital stays of patients treated in the inpatient ward for opiate addiction will be affected by sociodemographic variables such as age and gender, medical comorbidities, and psychiatric disorders accompanying opiate addiction. However, we suggest that these variables are also effective in the readmission of patients to the inpatient service. The results obtained from the study will make a significant contribution to determining the reasons of long-term hospital stay and readmission to the hospital in a short time in opiate addiction. These results can be a guide for reducing treatment costs by determining the variables that increase the cost in the treatment of opiate, the second most commonly used illicit substance in Turkey. In addition, determination of data on the length of hospital stay in opiate addiction and the reasons for readmission in Turkey will provide the opportunity to compare these findings with the international literature.

METHODS

This study is a retrospective descriptive study. The study data consisted of patient records of patients treated between January 1, 2005 and December 31, 2017 at Gazi University Hospital Alcohol and Substance Addiction Clinic. Records between 2005-2016 were provided from the hospital database. The records of 2017 were obtained by scanning the patient files from the archive due to the change in the hospital database system. A total of 789 patients with a diagnosis of opiate addiction, 763 between 2005 and 2016, and 26 in 2017, were hospitalized. Daily treatment records and hospitalization records with missing data were not included in the analysis. Among the remaining 621 hospitalization records, it was seen that patients with a single hospitalization (n=384) had one hospitalization record, and patients with recurrent hospitalizations (n=100) had multiple hospitalization records. Only first hospitalization records of patients with recurrent hospitalizations were evaluated. Thus, the impact that multiple data belonging to a patient can have, was limited. As a result, records of a total of 484 patients, 384 with a single hospitalization and 100 with recurrent hospitalizations, were included in the analysis. The operation flowchart is shown in Figure 1.

Patient diagnoses were evaluated by psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders (DSM), fourth edition, text revision and DSM-5. Since the hospital registry system is based on the International Statistical Classification of Disease and Related Health Problems, 10th revision (ICD-10) diagnostic classification, psychiatry is registered with the most appropriate ICD-code. Patient records with a diagnosis of "Addiction Syndrome (F11.2) Due to Opioid Use" were included in the study.

In the study, average length of hospital stay was found to be 15.19±10.89 days. Since this variable does not show a normal

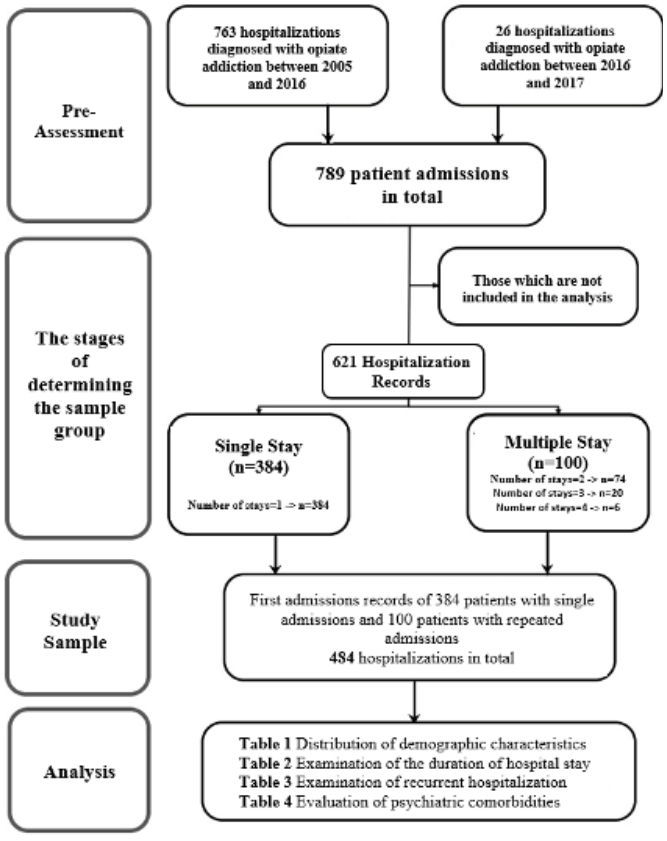

Figure 1. The operation flowchart

distribution, 14 days, which is the median value of the length of stay in the hospital, was taken as the basis for the distinction between long and short length of stay. Short-term hospitalization was evaluated as 14 days or less, while long-term hospitalization was evaluated as 15 days or more.

Admission records included two hospitalization records of 74 patients, three hospitalization records of 20 patients, and four or more hospitalizations for 6 patients. Patients with two or more hospitalizations (n=100) were accepted as recurrent admissions. The time elapsed between the first and second hospitalizations of the patients with recurrent hospitalizations was called the "days between the first two hospitalizations".

The time limit for readmission of patients varies between 1-12 months in the relevant literature (7,10,11). It seems that this time period is usually based on a month. In our study, the rate of patients who were readmitted within the first month after discharge was only 5%. In our study, the readmission time was determined as three months in order to examine the variables related to readmission and the statistical limitation caused by the small sample size in this period. In our study, the rate of readmission in the first three months was 29%.

In addition to the diagnosis of opiate addiction, variables such as "There is a psychiatric comorbidity" in the presence of any coded psychiatric disease (e.g., F32-Depressive seizure, F41-Anxiety disorders, etc.) for the same patient, and "There is a medical comorbidity" in the presence of a coded physical disease (e.g. R94.5-Liver function tests abnormal results, K86.9-Pancreatic disease, etc.) were created. In addition to the diagnosis of F11.2, a new variable was created as "Psychotic symptom" in those with the diagnosis code "Psychotic disorder due to opioid use (F11.5)". Paranoid personality disorder (F60.0), Emotional inactive personality disorder (F60.3), Histrionic personality disorder (F60.4), Anxious personality disorder (F60.6) and Dependent personality disorder (F60.7) were collected under a single heading due to the low frequency of diagnosis and the presence of "Personality disorders, unspecified (F60.9)" diagnosis.

Statistical Analysis

SPSS for Windows 23.0 was used for analysis of research data. Mean, standard deviation, median, minimum value, maximum value, frequency and percentages were used for descriptive statistics. Chi-Square test was used for comparing qualitative data and Fisher's Exact test was used when necessary. Kolmogorov-Smirnov test was used to investigate the compatibility of the data to normal distribution. It was found that the data did not show normal distribution. Spearman correlation test was used to examine the relationship of continuous variables. Logistic regression analysis was used to determine the predictors of length of stay, recurrent hospitalization, and readmission within three months. Short-term hospitalization was evaluated as 14 days and less and was coded as "0". Fifteen days or more was considered as a long-term hospitalization and coded as "1". Similarly, single admission and non-readmission within three months were coded as "0", while recurrent admission and readmission within three months were coded as "1". In the study, the significance value was taken as 0.05.

Ethical Approval

This research was discussed at the Gazi University Ethics Committee meeting dated 04.02.2020 and numbered 02, and received ethical approval with the 2020-110 research code. In addition, permission was obtained from the head physician of the hospital that the patient record data would be used. The study design and management was carried out in accordance with the Declaration of Helsinki.

RESULTS

The demographic data of the sample and some disease characteristics are shown in Table 1. Mean age of the study group was 29.74±7.05 (median: 28), while 89.9% (n=435) were male. Psychotic symptoms were 5.2% (n=25), medical comorbidity 5.2%

Table 1. Demographic data of the sample and some disease characteristics (n=484)

Variables		Mean (n)	SD (%)
Age	Mean ± standard deviation	29.74	7.05
	Median (minimum-maximum)	28	19-61
	18-25	140	28.9
	26-35	263	54.3
	36-45	59	12.2
	≥46	22	4.5
Gender	Female	49	10.1
	Male	435	89.9
Acceptance status	Single	384	79.3
	Recurrent	100	20.7
Medical comorbidity	None	459	94.8
	Exist	25	5.2
Psychiatric comorbidity	None	274	56.6
	Exist	210	43.4
Psychotic symptom	None	459	94.8
	Exist	25	5.2
Duration of hospitalization (days)	Mean ± standard deviation	15.19	10.89
	Median (minimum-maximum)	14	1-60
	≤14	252	52.1
	>14	232	47.9
The days between the first two hospitalizations in patients with recurrent admissions (n=100)	Mean ± standard deviation	274.22	317.10
	Median (minimum-maximum)	148	5-1539

SD: standard deviation

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(n=25), and psychiatric comorbidity 43.4% (n=210). The mean hospitalization period of the patients was 15.19±10.89 (median: 14) days. The average readmission period of those with recurrent hospitalizations was 274.22±317.10 days. 5% of the patients (n=5) were readmitted within the first month, 29% (n=29) within the first three months, 57% (n=57) within the first six months and 78% (n=78) within the first year.

The evaluation of the duration of hospital stay by logistic regression analysis is shown in Table 2. Significant variables predicting the length of hospital stay were found to be age, presence of psychotic symptoms and a diagnosis of depression. It was found that those aged 46 and over stayed in the hospital 3.2 times [odds ratio (OR): 3.204; 95% confidence interval (CI): 1.055-9.730] longer than those in the 18-25 age group. In addition to opiate addiction, it was found that comorbid depression prolonged the length of stay 3.3 times (OR: 3.362; 95% CI: 1.874-6.032) and having psychotic symptoms 5.4 times (OR: 5.417; 95% CI: 1.857-15.798). While the created model was determined significantly, it explained 17% of the total variance. Evaluation of recurrent hospitalization status by logistic regression analysis is shown in Table 3. Significant variables predicting recurrent hospitalization were found to be age, anxiety disorders, and comorbid personality disorders. Those between the ages of 26-35 and 36-45 had 1.88 (OR: 0.531; 95% CI: 0.303-0.932) and 2.81 (OR: 0.355; 95% CI) times lower risk of readmission than those in the 18-25 age group, respectively. A diagnosis of anxiety disorders in addition to opiate addiction increased the risk of readmission by 3.3 times (OR: 3.321; 95% CI: 1.690-6.524), while a diagnosis of personality disorder reduced the risk of readmission 4.58 times (OR: 0.218; 95% CI: 0.049-0.975). While the created model was determined significantly, it explained 15.9% of the total variance. An additional model was created in which the independent variable was the length of hospital stay and the dependent variable was readmission within three months (X^2=8.210; p=0.004; Nagelkerke R^2=0.113).

Accordingly, the risk of re-admission within three months was 3.64 times lower (OR=0.274; 95% CI: 0.111-0.677) in patients with a hospital stay longer than 14 days than those with a hospital stay of 14 days or less.

The relationship between hospital stay and the day of readmission is shown in Figure 2. Accordingly, it was observed that the shortening of the hospitalization period significantly shortened the day of readmission (r=0.307; p=0.002).

The evaluation of psychiatric comorbidities according to age groups and gender is shown in Table 4. A significant difference was found according to age groups in terms of psychiatric comorbidity, anxiety disorders, depression, alcohol dependence, and personality disorder variables (p=0.007; p<0.001; p<0.001; p=0.002; p=0.001, respectively). However, it was observed that

Table 2. Evaluation of length of hospital stay by logistic regression analysis (n=484)

Variables		Length of stay in the hospital			
		≤14 days (n=252)	>14 days (n=232)	p	OR (95% confidence n interval)
		p	n		
Age	18-25	80	60	-	1.000
	26-35	138	125	0.778	0.937 (0.597-1.471)
	36-45	29	30	0.961	0.983 (0.500-1.932)
	46-55	5	17	0.040	3.204 (1.055-9.730)
Gender	Female	27	22	-	1.000
	Male	225	210	0.524	1.237 (0.644-2.377)
Medical comorbidity	None	242	224	-	1.000
	Exist	10	8	0.247	0.522 (0.173-1.570)
Psychotic symptom	None	247	212	-	1.000
	Exist	5	20	0.002	5.417 (1.857-15.798)
Unipolar depression	None	216	136	-	1.000
	Exist	36	96	<0.001	3.362 (1.874-6.032)
Anxiety disorder	None	215	149	-	1.000
	Exist	37	83	0.209	1.473 (0.805-2.695)
Personality disorder	None	233	215	-	1.000
	Exist	19	17	0.659	1.183 (0.560-2.500)
Alcohol addiction	None	245	222	-	1.000
	Exist	7	10	0.943	1.043 (0.332-3.273)

Model analysis results; X^2=66.023; p<0.001; Nagelkerke R square: 0.170. Hosmer and Lemeshow test; X^2: 3.768; df: 6; p=0.708.
OR: odds ratio, df: degrees of freedom

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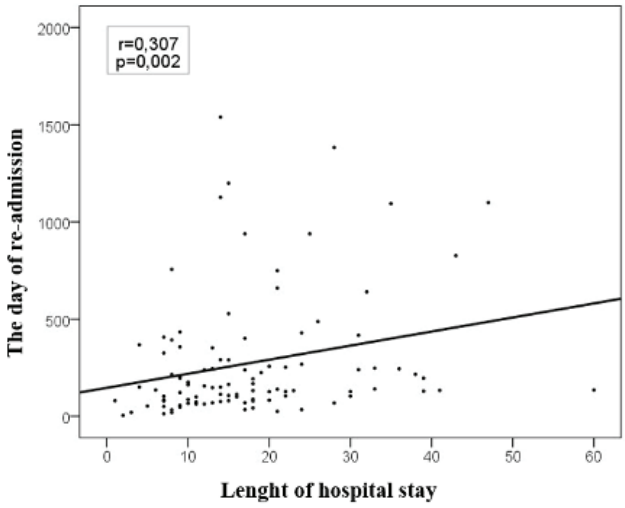

Figure 2. Evaluation of the relationship between the length of hospital stay and the day of re-admission (n=100)

there was no significant difference in terms of these variables according to gender (Table 3). It was found that anxiety disorders, depression and alcohol dependence increased with advancing age. Personality disorder was more common in the age group of 35 and under (19.6% vs. 10.8%). With these results, 1.4% of the whole sample had schizophrenia (n=7), 1% had bipolar affective disorder (n=5), 0.2% had schizoaffective disorder (n=1), 0.8% had cocaine addiction (n=4), 4.5% had cannabinoid addiction (n=22), 1.7% had sedative, hypnotic or anxiolytic addiction (n=8), 0.4% had inhalant addiction (n=2).

DISCUSSION

In our study, the most important reasons for long-term hospitalization were advanced age, presence of psychotic symptoms, and a diagnosis of depression. Approximately one in three patients was readmitted in the first three months, and one in two patients in the first six months. When psychiatric comorbidities of patients with opiate use disorder were evaluated, 43.4% of the patients had a psychiatric diagnosis. Depression and anxiety disorders were the most common psychiatric disorders (27.2% and 24.7%, respectively). While the risk of recurrent hospitalization was 3.3 times higher in those with anxiety disorders, it was 4.5 times lower in those with personality disorders. One of the most important results was that those with a hospital stay of 14 days or less had a 3.6 times greater risk of readmission within three months than those with a long-term (more than 14 days) hospitalization.

The need to control the ever-increasing cost of healthcare is an important issue agreed upon by policy makers all over the world (8). Recurrent admission rate is accepted as important criteria for evaluating the quality of hospital care and discharge planning. Therefore, it has become an important target of health services (10). However, not everything can be explained that easily. Because there are many variables that affect readmission and these variables affect each other in a complex relationship.

Table 3. Evaluation of recurrent hospitalization status by logistic regression analysis (n=484)

Variables		Recurrent hospitalization condition			
		None (n=384)	Exist (n=100)	p	OR (95% confidence interval)
		n	n		
Age	18-25	109	31		1.000
	26-35	208	55	0.027	0.531 (0.303-0.932)
	36-45	48	11	0.018	0.355 (0.150-0.838)
	46-55	19	3	0.062	0.267 (0.067–1.069)
Gender	Female	42	7	-	1.000
	Male	342	93	0.210	1.770 (0.724–4.324)
Length of stay	≤14	212	40	-	1.000
	>14	172	60	0.120	1.488 (0.902-2.456)
Medical comorbidity	None	372	96	-	1.000
	Exist	21	4	0.125	2.425 (0.782-7.518)
Psychotic symptom	None	363	96	-	1.000
	Exist	21	4	0.136	0.382 (0.108-1.354)
Unipolar depression	None	298	54	-	1.000
	Exist	86	46	0.397	1.338 (0.682-2.627)
Anxiety disorder	None	311	53	-	1.000
	Exist	73	47	<0.001	3.321 (1.690-6.524)
Personality disorder	None	350	95	-	1.000
	Exist	12	5	0.046	0.218 (0.049-0.975)
Alcohol addiction	None	372	95	-	1.000
	Exist	12	5	0.091	2.861 (0.847-9.665)

Model Analysis Results; X^2=51.866; p<0.001; Nagelkerke R square: 0.159.
Hosmer and Lemeshow test; X^2: 9.166; df: 7; p=0.241.
OR: odds ratio, df: degrees of freedom

Disease characteristics, health policies, social and environmental factors are the main areas that affect the readmission (11). There are many factors associated with readmission. Some of these are infections caused by the use of opiates by injection, additional non-infectious medical diseases, decrease in pain threshold, presence of additional psychiatric diseases, discontinuation of treatment despite medical advice, severity of addiction and a history of sexual abuse (12). In a study conducted in the United States of America (USA), readmission rate is reported as 19% within the first month after discharge in patients with opiate addiction (13). In our study, this rate was found to be 5%. The lower rate of readmission in Turkey may be related with the lower

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frequency of opiate addiction compared to the USA. In addition to having recurrent admissions due to opiate use disorders, it has been found to cause readmission by negatively affecting the course of other medical diseases (7,12,14). In inflammatory bowel diseases, the readmission rate is between 18.9% and 19.4% while it is reported as between 28.9% and 35.1% in patients with accompanying opiate addiction (14). A similar situation was also observed in patients with pancreatitis. While the readmission rate due to pancreatitis picture was 27%, it was found to be 35.3% in patients with accompanying opiate addiction (7). In this study, the presence of any psychiatric disease in patients increased the risk of readmission 1.32 times. In our study, it was observed that the presence of anxiety disorders increased the risk of readmission by 3.3 times, while the diagnosis of personality disorder decreased 4.58 times. Depression and alcohol use disorder had no effect on readmission. Here, the result that a diagnosis of personality disorder reduces repetitive acceptances is thought to be quite important. This conclusion may be the effect of stigmatization developed against the patients with substance abuse. A recent study stated that 37% of Americans see the main cause of addiction in opiate addiction as a weak willpower rather than a medical illness (15). It has been shown that physicians and health policy regulators, who are an important part of the society, also have a similar content of thought (16,17). Negative attitudes and prejudices disrupt communication. Patients' less trust in their physicians, non-compliance with treatment recommendations, and low motivation may distract them from treatment (16). However, the treatment of a patient with personality disorder can be difficult for the physician. As the physician feels helpless and powerless in the face of these difficulties, they may distance themselves from these people. All these may reduce readmission in patients with personality disorders. However, many variables other than these factors may play a role in this process.

Length of hospital stay is another reason for the cost of treatment. Global health policies aim to shorten the length of hospital stay and advocate a community-based rehabilitation process (8). The length of stay of patients with opiate use disorder may vary depending on many variables, especially the characteristics of the treatment clinic and the method of treatment (18,19). In the study evaluating a large sample in the USA, the mean hospitalization period of the patients was determined as 4.4 days (3.8-5.7). In another study, patients with opiate use disorder and those with non-opiate substance use disorder were compared. It was found that patients with opiate use disorder had an average hospitalization of 32.8 days, while the other group had 30.9 days. In our study, the average length of stay was found to be 15.19±10.89 days. The most important factors affecting the length of stay were age of 46 years or older, presence of psychotic symptoms and a diagnosis of depression (Table 2). As the medical and psychiatric comorbidities increase with advancing age, the duration of hospital stay may increase (20). In our study, depression and anxiety disorders were found at a rate of 50% and 36.4% in the 46 years and older group, while these rates were found to be 13.6% and 9.3%, respectively, in the 18-25 age group. In many studies, it has been shown that a diagnosis of depression in patients with opiate addiction increases the length of stay (21,22). Other variables affecting the length of stay may be differences in the treatment modality applied in the clinic, patient demand and early discharge status (18,19). Studies have reported that patients who are discharged despite medical advice naturally have a shorter hospital stay (23). Patients with incomplete hospitalizations have higher readmission rates compared to other patients (19,24). In a study conducted by Gottheil et al. (9), it has been reported that shortened hospitalizations increase repeated hospitalizations. In our study, the risk of readmission of the patients within three months was found to be 3.64 times lower in those with a hospital stay longer than 14 days compared to those with a shorter hospital stay. In other words, it can be interpreted that the short duration of hospitalization causes a high risk of readmission. In Figure 1, it is seen that the day of readmission is earlier for those with shorter hospitalization periods. Length of stay and recurrent admission are two variables that closely affect each other. Recurring admission risks should be comprehensively addressed

Table 4. Evaluation of psychiatric comorbidity according to age groups and gender (n=484)

Variables		Psychiatric comorbidity	Anxiety disorders	Depression	Alcohol addiction	Personality disorder
Age	**n**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
18-25	140	47 (33.6)	13 (9.3)	19 (13.6)	2 (1.4)	21 (15.0)
26-35	263	117 (44.5)	78 (29.7)	79 (30.0)	6 (2.3)	12 (4.6)
36-45	59	35 (59.3)	21 (35.6)	23 (39.0)	6 (10.2)	1 (1.7)
≥46	22	11 (50.0)	8 (36.4)	11 (50.0)	3 (13.6)	2 (9.1)
X^2		12.112	26.624	24.073	13.868	16.097
p		0.007	<0.001	<0.001	0.002	0.001
Gender	**n**					
Female	49	22 (44.9)	12 (24.5)	14 (26.8)	3 (6.1)	5 (10.2)
Male	435	188 (43.2)	108 (24.8)	118 (27.1)	14 (3.2)	31 (7.1)
X^2		0.051	0.003	0.046	0.925	0.554
p		0.822	0.959	0.830	0.336	0.563

in the goals of healthcare services to shorten the length of stay, and health policies should be planned accordingly.

Substance use disorders are strongly associated with other psychiatric disorders. Although lifelong comorbidity with other psychiatric disorders differ significantly according to studies, rates varying between 44-93% have been reported (25). Presence of psychiatric comorbidity has been found to be associated with poor prognosis, more psychosocial impairment, high relapse and high mortality rate (25,26). In a study involving a large sample, psychiatric comorbidities were investigated in inpatients and outpatients with opiate use disorder (27). Psychiatric comorbidity rates in hospitalized patients were found to be 7-22% for alcohol dependence, 15-34% for anxiety disorders and 19-31% for depression. However, in outpatients these rates were reported as 2-15%, 2-10% and 2-8%, respectively. In a study evaluating 652 people receiving methadone treatment for opiate use disorder, it was reported that 78% of the sample had any psychiatric comorbidity, 22% had major depression, 42% had anxiety disorders and 12% had alcohol use disorder (28). In our study, 43.4% of the sample had any psychiatric comorbidity, 27.2% depression, 24.7% anxiety disorders and 3% alcohol use disorder. Except for alcohol addiction, the results of our study seem to be consistent with these studies. In a study conducted by Evren et al. (29) on 70 people with opiate addiction in Turkey, they reported that 67.1% had any Axis 1 diagnosis, 52.9% anxiety disorders, 35.7% major depression and 5.7% alcohol dependence. Similar to the result in our study, alcohol dependence was found at a lower rate in this study compared to the studies of western countries. This result may be related to the amount of alcohol use in Turkey. In the report published by the WHO in 2018, it is reported that per capita alcohol consumption in our country is 2 liters (pure alcohol), while this rate is 9.8 liters in Europe (30).

It is very important to state that the study sample belongs to a non-profit university hospital that provides comprehensive and well-equipped services in the field of alcohol and substance addiction treatment. Although the study is single-centered, it was carried out in an institution where patients from many regions, especially the Central Anatolia Region, were followed. This increases the generalizability of the results across the country. It is very important for our study to include a long period of thirteen years and to consider a serious sample. In addition, the use of ICD-10 diagnosis classification as a result of physician interviews rather than patient feedback in forming the study sample is another strength of our study.

Study Limitations

There are also some limitations in our study due to its long period. Since most of the patient data were accessed from the electronic database, information such as demographic data, social living conditions, doses and patterns of substance use, substance initiation age and treatment modalities were not included in the study. It cannot be denied that part of the sample may be incomplete hospitalization. This can be considered as another limitation of our study. The possibility of patients continuing their treatment in different institutions should not be ignored. This may cause a limitation in the single and repetitive hospitalization variable.

CONCLUSION

Readmission rates are an important indicator of inpatient care quality. The recurrent nature of addictions, especially opiate use disorder, indicates that there is a greater need for studies evaluating readmission rates in addiction areas. In Turkey, data in these areas are very limited. Our study shows that approximately one out of every three patients is readmitted within the first three months after discharge. While the presence of anxiety disorders increases the recurrent hospitalization 3.3 times, the presence of personality disorder decreases it 4.5 times. In our study, considering that comorbidity of personality disorder is more common in the 18-25 age group, it can be concluded that the presence of personality disorder in early adulthood may prevent access to treatment. The result determined that the presence of personality disorder shorten the length of stay should be definitely considered in further studies. It is one of the expected results that the hospitalization period is prolonged with the variables such as advanced age, psychotic symptoms and depression comorbidity. However, the fact that those with a hospitalization period of less than 14 days are 3.6 times more likely to be readmitted within three months than those with a longer stay indicates that health policies in this area should be reconsidered. Another suggestion is to investigate the effect of health policies in multi-centered, higher sampling and prospective studies in terms of the duration of hospital stay affecting hospital readmission.

Ethics Committee Approval: This research was discussed at the Gazi University Ethics Committee meeting dated 04.02.2020 and numbered 02, and received ethical approval with the 2020-110 research code.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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Do INI1 and E-cadherin Expression Loss Have Any Significance in Endometrial Carcinomas?

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ABSTRACT

Objective: Endometrial carcinoma (EC) is a highly heterogeneous malignancy in terms of morphology, clinical course, response to treatment and prognosis. This study aimed to investigate the loss of INI1 (SWI/SNF family member) and E-cadherin expression in type 1 and 2 ECs to elucidate the mechanisms that may elucidate on the differences in pathogenesis and prognosis between low- and high-grade histological types.

Methods: Immunohistochemistry (IHC) was applied for INI1 and E-cadherin in 72 patients who underwent hysterectomy for EC. Loss of INI1 and E-cadherin expression was compared between subjects with low- and high-grade EC.

Results: A total of 63 patients had type 1 (endometrioid) and nine had type 2 (non-endometrioid) tumours. IHC staining revealed loss of INI1 expression in eight cases. While three of these cases were serous carcinoma, three were endometrioid carcinoma with villoglandular pattern and two were grade 3 endometrioid carcinoma. A significant difference was found in the loss of INI1 expression between low-grade (G1-G2) and high-grade (G3 endometrioid carcinoma and non-endometrioid carcinoma) tumours (p=0.004). Loss of expression was observed only in one case of dedifferentiated carcinoma in IHC staining performed for E-cadherin in 72 cases.

Conclusion: A significantly greater loss of INI1 expression was observed in high-grade compared with low-grade endometrial carcinoma. This finding confirms that INI1 loss is a poor prognostic factor in these tumours as in other tumours reported in the literature and sheds light on the different pathogeneses seen in high-grade EC. To the best of our knowledge, this is the first systematic study to investigate INI1 loss in different types of endometrial carcinoma. Our results support the notion that SWI/SNF chromatin remodelling complex plays a role in the pathogenesis of high-grade EC and type 1 endometrioid carcinoma with villoglandular pattern.

Keywords: Endometrial carcinoma, INI1, E-cadherin

INTRODUCTION

Endometrial carcinoma (EC) is the most common malignant tumour of the female genital system, accounting for approximately 4% of all cancers in women with an incidence of 10-20/100,000 individuals (1,2). EC is divided into two main types based on pathogenesis, namely, type 1 and type 2. Type 1 endometrioid carcinomas are oestrogen-dependent low-grade neoplasms with precursor lesions (endometrial hyperplasia/endometrioid intraepithelial neoplasia) that occur in perimenopausal patients.

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Type 2 non-endometrioid (mostly serous or clear-cell) carcinomas are high-grade tumours that occur in postmenopausal older patients in the absence of hyperestrogenism (3). Majority of endometrioid ECs are low-grade tumours (grades 1 and 2) that are associated with a good prognosis when limited to the uterus. Grade 3 endometrioid carcinoma is an aggressive tumour with increased lymph node metastasis. Endometrioid carcinoma grade 3 and serous carcinoma are considered high-grade tumours. Serous carcinoma and grade 3 endometrioid carcinoma constitute 39% and 27% of cancer-related deaths, respectively (4).

Although useful, this traditional classification is different from the histological classification. The increasing information about morphological heterogeneity, clinical symptoms, prognosis, response to treatment and mortality of EC has revealed the limitations of the traditional classification (3). It may be challenging to position certain EC subtypes in one of these two groups. On the contrary, molecular studies have appeared promising to obtain important information about prognosis and establish novel treatment modalities (2,5).

Since serous and endometrioid carcinomas substantially differ in terms of clinical behaviour, it is imperative to distinguish serous carcinoma with papillary morphology from endometrioid carcinoma with villoglandular pattern. Villoglandular endometrioid carcinomas are assumed to behave similar to non-villoglandular endometrioid carcinomas. However, few studies have focused on the villoglandular variant of endometrioid carcinomas (6). While some studies have shown that these tumours exhibit comparable behaviour to non-villoglandular serous carcinomas with vascular invasion, potential lymph node metastasis and a more aggressive course, (7) others have suggested that they behave similar to oestrogen-dependent type 1 endometrioid carcinomas, which are not associated with poor prognosis (6).

The SWI/SNF complex is thought to be involved as a tumour suppressor gene owing to its role in chromatin remodelling and transcriptional regulation (8). Recently, mutations in various genes encoding different subtypes of the SWI/SNF complex have been identified in 20% of different human cancers (9,10). INI1, located on chromosome 22q11.2, is one of the SWI/SNF subtypes and the most well-established member of this pathway (11). Complete loss of the *INI1* gene product resulting from biallelic inactivation of the gene through genetic and epigenetic mechanisms has been reported in various paediatric and soft-tissue sarcomas. Increasing neoplasms with INI1 deficiency include paediatric malignant rhabdoid tumours, epithelioid sarcoma, myxoid chondrosarcoma, medullary carcinoma of the kidney, myoepithelial neoplasms (12,13) and the recently described gastrointestinal, pancreatic and sinonasal system carcinoma variants (13,14). Some studies on these carcinomas have demonstrated that INI1 loss is important not only in establishing a diagnosis but also in correlating with poor prognosis (14). To the best of our knowledge, no studies have investigated INI1 loss in type 1 and type 2 EC to date.

Cell membrane adhesion molecules are responsible for the binding of cells to each other and the extracellular matrix, thereby playing a key role in carcinogenesis (15). E-cadherin is a calcium-dependent transmembrane glycoprotein responsible for maintaining the adhesion between epithelial cells. In cancer cells, metastasis is closely related to the epithelial-mesenchymal transition characterised by downregulation of E-cadherin (16,17). A decrease in E-cadherin expression or loss of function of this protein is a sign of metastasis and poor prognosis in various cancers (18,19). As in other carcinomas, E-cadherin appears to be an indicator of unfavourable clinicopathological factors and poor survival in EC. A number of studies in the literature have explained the more aggressive behaviour of type 2 tumours by detecting loss of E-cadherin expression in type 2 EC compared with type 1 (20,21). However, the exact role of E-cadherin in type 2 EC and the mechanisms responsible for the downregulation of this protein have not been fully clarified. Loss of E-cadherin expression has been associated with dedifferentiation, deep myometrial invasion and increased metastatic potential in EC due to the altered tumour suppressor role of this protein (22). E-cadherin mutations have been recognised as an indicator of poor prognosis and shorter life expectancy in patients with various neoplasms including gastric, breast and colon cancer (17).

This study aimed to investigate loss of INI1 and E-cadherin expression in cases of type 1 and 2 EC, aid in the differential diagnosis by demonstrating the difference in this loss between histological types and to evaluate two markers that can shed light on the carcinogenetic mechanisms that may explain the prognostic difference between these types.

METHODS

A total of 72 patients diagnosed with EC who underwent hysterectomy in 2012-2020 were enrolled in the study. Paraffin-embedded blocks were retrieved from the archive and blocks that contained normal endometrial glands as well as tumour tissue were selected. This is a retrospective study of the archive materials of these patients.

Histopathological Evaluation

The microscope slides (preparations) of all cases were reviewed to confirm the diagnosis. Clinicopathological data of the malignant cases were collected from medical reports and included patients' age, histopathological tumour type, grade and pattern. All specimens were hysterectomy specimens and grading of EC cases was performed only on hysterectomy specimens.

Immunohistochemistry

Sections of 4-micron thickness were obtained from 72 formalin-fixed, paraffin-embedded tissues for immunohistochemical (IHC) analysis and positive-charged microscope slides were used to avoid tissue shedding. The sections were allowed in an incubator at 60 °C for an hour and deparaffinised with xylene for 15 min.

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The samples were hydrated through descending-grade series of alcohol and washed in distilled water. Samples were then introduced to a BenchMark XT device. INI1 (Cell Marque, RTU, MRQ-27, Philadelphia, PA, USA) and E cadherin (Cell Marque, RTU, EP700Y, Rocklin, CA, USA) antibodies were applied, and staining was performed subsequently. The samples stained in the automated staining device were covered using fluid-based covering material. Results were evaluated with an Olympus CX41 microscope.

Immunohistochemistry Evaluation

Clearly observed expression loss in tumour cells was accepted as loss of INI1 expression. Normal mucosal glands, stromal fibroblasts, endothelial cells and inflammatory cells were utilised as internal control.

The intensity of membranous and membranocytoplasmic E-cadherin staining in epithelial cells was graded as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). For E-cadherin, the percentage of positive-stained cells was graded as 0 (0%), 1 (1%-10%), 2 (11%-50%) and 3 (>50%). The final score for E-cadherin (0-6) was determined by combining the intensity and percentage scores (23). An internal positive control, which consisted of endometrial epithelium and the adjacent normal glandular epithelium, was used for quality control as well as an internal negative control of adjacent normal endometrial stroma and myometrium.

Statistical Analysis

Patient demographics and data were analysed using the SPSS 24 programme. Variables were expressed as frequency, percentage, mean (arithmetic mean and median), standard deviation (min-max), tables and graphs. Chi-square test was used to compare patient-related variables in both groups. $P<0.05$ was considered statistically significant.

The study was approved by the Tekirdağ Namık Kemal University Non-interventional Clinical Trials Ethics Committee (approval number: 2020.92.04.16, approval date: 30.04.2020).

RESULTS

The mean age of the 72 patients was 63.6 (37-83) years. Diagnosis distribution of the patients is presented in Table 1. A total of 63 patients had type 1 (endometrioid) tumours and 9 had type 2 (non-endometrioid) tumours. Eight cases with endometrioid carcinoma had squamous differentiation, two had secretory differentiation and five had villoglandular pattern.

Table 1. Diagnosis distribution of the patients

Diagnosis		Number of patients	%
Endometrioid carcinoma	Grade 1	30	87.5
	Grade 2	29	
	Grade 3	4	
Serous carcinoma		7	9.7
Clear-cell carcinoma		1	1.4
Dedifferentiated carcinoma		1	1.4

IHC staining revealed loss of INI1 expression in eight cases. Three of these cases were serous carcinoma, three were endometrioid carcinoma with villoglandular pattern (two cases of grade 1 and one case of grade 2) and two were grade 3 endometrioid carcinoma. The INI1 and E-cadherin staining of the cases are presented in Figure 1.

Loss of INI1 expression was detected as 3/7 in serous carcinoma cases, 3/5 in endometrioid carcinoma with villoglandular pattern and 2/4 in grade 3 endometrioid carcinoma.

A significant difference was observed in the loss of INI1 expression between low-grade (G1-G2) tumours and high-grade (G3 and non-endometrioid carcinoma) tumours ($p=0.004$).

Microscope slides, i.e. preparations of 30 patients, contained tumour tissue as well as atrophic endometrial glands. None of the cases had loss of INI1 and E-cadherin expression in atrophic glands. While atypical endometrial hyperplasia areas as well as carcinoma areas in IHC slides were observed of eight patients, none of these cases had loss of INI1 and E-cadherin expression.

The loss of expression was observed only in one case of dedifferentiated carcinoma in IHC staining performed for E-cadherin in 72 cases. There was no loss of INI1 expression in this case. In all other cases (endometrioid carcinoma grade 1, 2, 3, serous carcinoma and clear-cell carcinoma), E-cadherin staining intensity was 3+, and staining was observed in more than 50% of the cells, resulting in a final score of 6.

DISCUSSION

Aggressive features of high-grade ECs, resistance to chemotherapy, poor prognosis and extremely high rates of recurrence contribute to increased mortality in patients with EC each year. Surgical treatment, together with chemotherapy and radiotherapy, remains in use in high-grade ECs. A clear understanding of the molecular changes in EC subtypes is expected to facilitate accurate diagnosis and prognosis predictions as well as establish targeted therapeutic strategies. In addition to treatments that target somatic hot-point gene mutations, advances in tumour metabolism and immunotherapy further open new doors for patients with high-grade EC. In this regard, emerging molecular studies should help synthesise new therapeutic inhibitors (24).

Bi et al. (25) applied IHC for INI1 and BRG1 in 10 cases of dedifferentiated EC and 16 cases of undifferentiated EC, identifying loss of BRG1 in 12 cases and INI1 loss in 2. The authors found a significant decrease in survival in cases with loss of INI1 and BRG1 expressions (mean, 4.7 and 22.9, $p=0.033$). They showed that BRG1 and INI1 expressions were not associated with age, myometrial invasion, lymph node status and International

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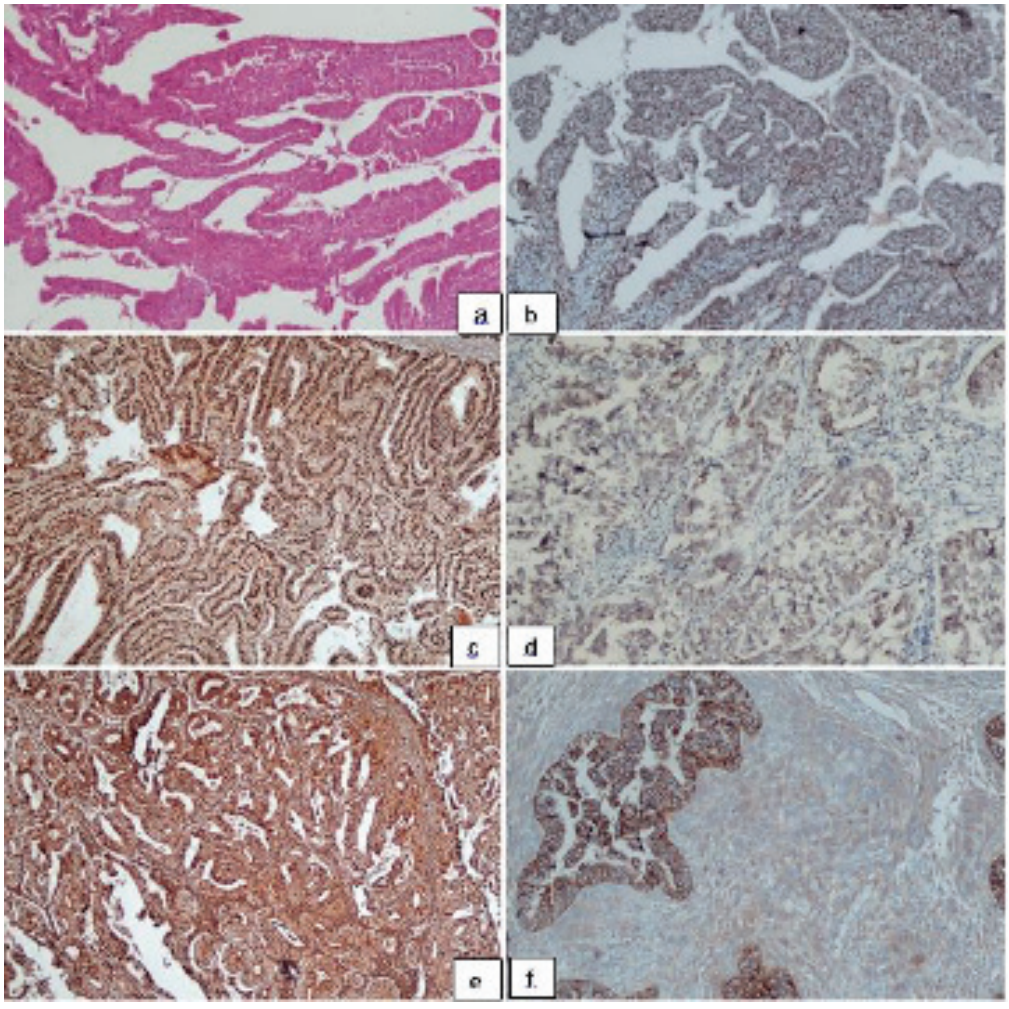

Figure 1. The INI1 and E-cadherin staining of the cases a) endometrioid carcinoma with villoglandular pattern [hematoxylin and eosin staining (H&E), x100]; b) endometrioid carcinoma with villoglandular pattern, INI1 negative (x100); c) endometrioid carcinoma, INI1 positive (x100); d) serous carcinoma, INI1 negative (x100); e) endometrioid carcinoma, E-cadherin positive (x100); and f) dedifferentiated carcinoma, E-cadherin negative (x100)

Federation of Gynecology and Obsterics (FIGO) stage (p=0.437, p=0.672, p=0.242, p=0.348). In light of their findings, they recommended routine IHC staining for BRG1 and INI1 in dedifferentiated and undifferentiated ECs to aid the identification of these tumours, distinguishing them from other carcinomas and predicting their clinical prognosis (25).

Strehl et al. (8) applied IHC for INI1 and BRG1 in 24 cases of grade 3 EC and two cases of undifferentiated EC, showing diffuse INI1 expression in all cases without any loss of expression. They detected loss of BRG1 expression in only one case, again without any loss in INI1 expression. The authors concluded that SWI/SNF may be effective in the pathogenesis of high-grade ECs (8). Apart from these two studies on high-grade ECs, we have not encountered any other systematic study investigating INI1 loss in ECs.

Loss of INI1 has shown importance in establishing diagnosis as well as in predicting poor prognosis in adult soft-tissue sarcomas, paediatric tumours and newly described carcinomas with INI1 loss (14). In addition to being a prognostic factor, some studies have reported PD-L1 positivity in tumours with loss of INI1 expression (13,26). PD-L1-expressing tumours of different histological types are known to show better response to treatment with immune checkpoint inhibitors than PD-L1-negative tumors (13).

In the present study, a significantly greater loss of INI1 expression was observed in high-grade EC compared with low-grade. This finding confirms that INI1 loss is a poor prognostic factor in this malignancy as in tumours reported in the literature and elucidates on the different pathogeneses seen in high-grade EC. On the contrary, cases of EC with villoglandular pattern are generally assumed to behave like type 1 EC. We detected loss of INI1 expression in three out of the five cases of endometrioid carcinoma with villoglandular pattern. We believe that future studies with a greater number of patients may further explore whether the INI1 loss demonstrated in this study is a prognostic

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factor in endometrioid carcinoma with villoglandular pattern and elucidate whether this different morphology stems from the differences in pathogenesis.

In a previous study, patients with atypical endometrial hyperplasia and EC were evaluated for E-cadherin and CD10 by IHC and correlation between loss of E-cadherin expression and tumour grade as well as FIGO stage was found significant despite patients' age, specimen type, tumour pattern or histopathological tumour types (22). Another study investigated type 1 and 2 ECs for E-cadherin and N-cadherin and the loss of E-cadherin expression was statistically significant among type 2 ECs rather than type 1 ECs (p=0.007). Aggressive behaviour was found to be related to loss of E-cadherin in type 2 ECs, but other clinicopathological features were lacking. In addition, decreasing E-cadherin expression in lymphovascular tumour cells were shown be another finding of this study. Myometrial invasion in ECs was suggested to be caused by the epithelial-mesenchymal transition mechanism (21).

Vroobel and Attygalle (27) applied IHC for E-cadherin, EMA and DNA mismatch repair proteins in three cases of undifferentiated and dedifferentiated EC, showing loss of E-cadherin expression in dedifferentiated EC cases and suggested that this could be utilised in routine practice to aid in the differential diagnosis. Consistent with the literature, loss of E-cadherin expression was identified in a dedifferentiated EC case in the study presented herein. We believe this marker may have diagnostic value in dedifferentiated EC. However, further studies with a larger number of patients are required to confirm this notion.

We did not observe loss of E-cadherin expression across EC cases evaluated in this study. Studies have shown that E-cadherin levels are associated with aggressive behaviour, poor survival and increased metastasis (20,21). This may result from the fact that studies in the literature often rely on mRNA levels of E-cadherin and may be due to the differences at clone level in studies utilising IHC. Our findings in this aspect may be associated with the clone we used in the study, which is deemed not appropriate for ECs. We believe this study may be repeated using the clones described in the literature and more robust results may be achieved in the presence of further parameters such as FIGO staging, myometrial invasion rate, survival and metastasis.

Study Limitations

The low number of patients with type 2 EC is one of the limitations of our study. More meaningful results could be obtained with high-grade ECs. Our study only compares the difference in staining of markers between histopathological types. We did not evaluate the relation of INI-1 and E-cadherin loss with prognostic parameters such as myometrial invasion and lymph node metastasis. More significant results can be obtained by comparing the staining of markers with prognostic parameters and by survival analysis.

CONCLUSION

Our results confirm that the SWI/SNF chromatin remodelling complex plays a role in the pathogenesis of high-grade EC and type 1 EC with villoglandular pattern. A few studies have investigated this subject in the literature. We believe studies on INI1 expression in EC cases, together with survival analysis, are warranted in these patients. Furthermore, our study should guide future studies in investigating PD-L1 expression in cases of EC with villoglandular pattern and high-grade ECs, where we identified loss of INI1 expression. This may allow encouraging outcomes with immune checkpoint inhibitors added to treatment of these tumours, especially in high-grade ECs with poor survival.

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University Non-interventional Clinical Trials Ethics Committee (approval number: 2020.92.04.16, approval date: 30.04.2020).

Informed Consent: Retrospective study.

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Evaluation of Oral Anticoagulant Usage Satisfaction in Home Care Patients Using Warfarin

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ABSTRACT

Objective: Warfarin is the most commonly used oral anticoagulant for the prevention and treatment of thromboembolic diseases. However, the difficulty of treatment management adversely affects patient satisfaction. The objective of this study is to measure the satisfaction of using warfarin among home care patients and determine the factors affecting their satisfaction levels.

Methods: This descriptive study was performed with patients who used warfarin and registered at the Home Health Care Services Unit of a tertiary hospital between May 2017 and November 2017. Importantly, the sociodemographic and clinical features of the patients were determined. Patients' satisfaction with drug use was evaluated using the Duke Anticoagulant Satisfaction Scale (DASS).

Results: The ages of 97 patients included in the study ranged from 19 to 92 years; moreover, 71.1% (n=69) of the patients were women. In total, 41.2% (n=40) of the patients have had haemorrhagic events during the use of warfarin. The DASS mean score of the patients was 57.67±14.56, 20.46±5.89 for the limitations with the treatment, 21.54±7.46 for the hassles and burdens, and 15.67±4.87 for the positive impacts. It was determined that the number of additional chronic diseases and bleeding conditions while using warfarin had a significant effect on the total score and subscale scores of DASS (for total score, $p=0.046$; for subscale scores, $p<0.001$) and reduced satisfaction.

Conclusion: This study found that satisfaction with warfarin use was not poor in home care patients, but the history of bleeding and the number of additional chronic diseases worsened the treatment satisfaction. It is believed that their satisfaction can be increase, if counselling interventions are planned according to the needs of patients.

Keywords: Anticoagulants, homecare services, patient satisfaction, warfarin

INTRODUCTION

Home health and care services are defined as a comprehensive care model covering psychological, physiological and medical support services in the living environment of patients. This care model comprises follow-up care, diagnosis and post-treatment care of the elderly; visits of people with chronic disease, disability or recovery; or providing preventive health services to those without a health problem. A regular follow-up of patients using oral anticoagulant (OAC) drugs, especially warfarin, is a crucial part of home health care (1).

OAC drugs are used in both the treatment and prevention of diseases such as arterial and venous thromboembolism, heart

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valve diseases, atrial fibrillation and stroke. As the frequency of such diseases, which are important causes of morbidity and mortality, increases with ageing, there is also an increase in the use of OAC drugs (2,3). These drugs inhibit the coagulation event or reduce the coagulation ability of the blood by disrupting the function or synthesis of coagulation factors (4).

Warfarin, a vitamin K antagonist, is the first-choice OAC drug for the prophylaxis and treatment of thromboembolic diseases (5). Many complications can arise during its usage (6). It holds a vital importance in keeping the international normalised rate (INR) value, which is used in evaluating the drug efficacy at the target limit (7). In warfarin treatment, the lack of standardised dosing, narrow treatment intervals, the need for a strict laboratory follow-up, interaction of multiple drugs and nutrients, and the risk of major and minor side effects negatively impact the benefits and reliability of the drug and decrease the quality of life (8-10).

It was observed that the majority of the patients had insufficient knowledge about anticoagulant treatment management and that the treatment could not be managed well due to the complications arising from bleeding and multiple drug use. Especially with the advancement of age, patients' compliance with the treatment decreased (11-13).

Although it is known that compliance with the treatment is crucial for patient satisfaction, some measurement tools have been developed to determine the treatment satisfaction in patients using anticoagulants (11,14-16). There are a limited number of studies investigating the satisfaction of these patients and effects on treatment (13,17-21).

The objective of this study is to measure the satisfaction of patients who were receiving home care services due to warfarin use and, also, evaluate the relationship between patients' sociodemographic and clinical characteristics along with their satisfaction levels.

METHODS

This prospective study was planned as a single-centre, descriptive study. Ethics committee approval was obtained from the relevant institution on 05.04.2017 (approval number: 36). The study was conducted in compliance with the Declaration of Helsinki's recommendations. The objective of the study was explained to all the participants, and their informed consent was obtained before their participation in the study.

Study Population

The study was conducted between 01.05.2017 and 01.11.2017 with patients who were registered to Home Health Care Services of a tertiary hospital and were receiving warfarin treatment. In total, 97 people aged 18 years and above who did not have any communication problems and agreed to participate in the study were included. Patients aged less than 18 years, those with severe psychiatric diseases, and those who could not communicate verbally were excluded from the study.

Data Collection Tools

Patient Information Form

The sociodemographic and clinical characteristics of the patients were questioned (determined) via the face-to-face interview technique using the patient information form prepared by us. It covers the questions regarding the sociodemographic (age, gender) and clinical features of the patients (the reason for using OAC, duration of use, whether they experience bleeding or thromboembolic events during the use of OAC or additional diseases).

Duke Anticoagulant Satisfaction Scale

Duke Anticoagulant Satisfaction Scale (DASS), developed by Samsa et al. (14) in 2004, and the Turkish validity and reliability study, conducted by Yıldırım and Bayık-Temel (13) in 2014, were used to evaluate patients' satisfaction with drug use. This scale, which allows health care professionals to evaluate the quality of life and satisfaction of patients receiving anticoagulant treatment, shows the patient's perception of treatment, quality of life and the level of disease management. It is a 7-point Likert-type scale comprising 25 items and a 3-factor structure: "Limitations", "burdens and difficulties" and "positive effects". It is measured with a rating of total score of the scale and the average score of each subdimension: "1= none", "2= very little", "3= a little", "4= moderately", "5= a bit more", "6= much" and "7= very much". In the scale, questions 17, 18, 19, 21, 23 and 25 are reverse-coded. The lowest score on the scale is 25, and the highest score is 175 points. High scores indicate a poorer quality of life and satisfaction with anticoagulant treatment (13,14). The alpha coefficient (0.89) determined for the overall scale form and the alpha coefficients (0.78-0.91) determined for the scale and its subdimensions are considered to be highly reliable (13).

Statistical Analysis

IBM SPSS Statistics 22 (IBM SPSS, Turkey) programme was used for the statistical analysis of this study's data. While evaluating the data, the compliance of the parameters to the normal distribution was evaluated with the Shapiro-Wilk test. In addition to descriptive statistical methods (mean, standard deviation, frequency), One-Way ANOVA test was used for intergroup quantitative data comparison with normally distributed parameters. The Tukey honestly significant difference test and Tamhane's T2 test were used to determine the group that caused the difference. The Student's t-test was used for comparing the normally distributed parameters between the two groups. Significance was considered at the level of $p<0.05$.

RESULTS

This study was performed with 97 patients whose ages ranged between 19 and 92 years (mean: 72.52±14.23 years). Most of the participants were aged 65 years and above (n=72, 74.2%), and 22.7% (n=22) of them were aged between 40 and 64 years. In total, 71.1% (n=69) of the patients were women, 95.9% (n=93)

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had an additional chronic disease, and 41.2% (n=40) experienced bleeding under warfarin treatment. Table 1 shows the evaluation of patients according to their sociodemographic and clinical features.

Apart from existing diseases requiring anticoagulant use, the most common additional chronic diseases were hypertension (63.9%, n=62), cerebrovascular diseases (33%, n=32) and chronic ischemic heart disease (27.8%, n=27).

As shown in Table 2, the mean total DASS score of the patients was 57.67±14.56 (minimum: 36, maximum: 103), the subdimension mean score of limitations was 20.46±5.89, the subdimension mean score of burdens and difficulties was 21.54±7.46 and the subdimension mean score of positive effects was 15.67±4.87 (Table 2).

Table 3 shows the distribution of DASS items and responses.

Table 4 presents the evaluation of results according to the sociodemographic and clinical features of participants. It was determined that the number of additional chronic diseases other than diseases requiring the use of OAC and bleeding status while using warfarin had a significant effect on the total score and subscale scores of DASS.

A statistically significant relationship was found between the number of additional chronic diseases and the mean scale score (p=0.046). The mean total score scores of patients with three or more chronic diseases were found to be significantly higher than those with one chronic disease and two chronic diseases (p^1=0.021; p^2=0.038) (Table 4).

A statistically significant relationship was found between the number of chronic diseases and the subdimension mean of burden and difficulties (p=0.022). As a result of the binary comparisons made for the detection of difference, the average burden and difficulties subdimension of patients with three or more chronic diseases were found to be statistically significantly higher than those with a chronic disease (p=0.029) (Table 4).

A statistically significant relationship was found between bleeding history and the total scale score averages (p<0.001). The total scale point averages of those who did not experience bleeding were statistically significantly lower than those who suffered minor bleeding (p=0.002) and those who suffered major bleeding (p=0.001) (Table 4).

A statistically significant difference was found between bleeding status in terms of the subdimension means of limitations and burden and difficulties (p=0.001 and p=0.001, respectively). The subdimension mean scores of limitations and burden and difficulties of those who did not experience bleeding were statistically significantly lower than those who suffered major bleeding (p=0.001 and p=0.007, respectively) (Table 4).

No statistically significant difference was found in terms of subscale mean scores of age, gender, causes of anticoagulant use, duration of anticoagulant use, thromboembolic events and total scale score, restrictions, burdens and difficulties and positive effects (Table 4).

Table 1. Sociodemographic and clinical characteristics of the study population

		n	%
Age groups	<40	3	3.1
	40-64	22	22.7
	≥65	72	74.2
Gender	Female	69	71.1
	Male	28	28.9
Additional chronic disease	No	4	4.1
	1-2	68	70.1
	≥3	25	25.8
Anticoagulant use reason	Atrial fibrillation	63	64.9
	Heart valve replacement	19	19.6
	Deep vein thrombosis	10	10.3
	Pulmonary embolism	5	5.2
Anticoagulant use period	<2 years	20	20.6
	2-5 years	32	33.0
	6-10 years	22	22.7
	≥10 years	23	23.7
History of bleeding	No	57	58.8
	Minor bleeding	30	30.9
	Major bleeding	10	10.3
Thromboembolic events	No	89	91.8
	Yes	8	8.2

Data are presented as number (percentage)

Table 2. Evaluation of Duke Anticoagulant Satisfaction Scale results

Subdimensions of the scale	Min-max	Mean ± SD	Cronbach's alpha
Limitation with treatment	11-42	20.46±5.89	0.756
Burdens and difficulties	11-44	21.54±7.46	0.834
Positive psychological impact	6-28	15.67±4.87	0.739
Total score	36-103	57.67±14.56	0.871

Data are presented as min-max and mean (SD).
SD: standard deviation, Min: minimum, Max: maximum

DISCUSSION

This study found that drug satisfaction was not deficient in home care patients using warfarin, but the history of bleeding during the use of warfarin and the presence of additional chronic disease worsened treatment satisfaction. It was observed that age, gender, the reason for using OAC, duration of using OAC and having a thromboembolic event did not affect the overall satisfaction level and subdimensions of the scale.

As mentioned, our study group comprises people who used warfarin for any reason and needed home care services because

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of additional diseases and/or advanced age, etc. Therefore, it was concluded that even if they are limited in terms of working life, daily work and travel, they are not adversely affected by warfarin use.

Home health and care services are provided to patients of any age group with chronic diseases that restricts their daily life activities, mostly for patients aged 65 and over (22). Karaman et al.'s (23) study determined that 88.1% of the patients were aged 65 years

Table 3. Distribution of Duke Anticoagulant Satisfaction Scale items

	None	Very little	A little	Moderately	A bit more	Much	Very much
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
1. How much does the possibility of experiencing bruising or bleeding restrict your participation in physical activities (e.g. housework, gardening, dancing, doing sports, and other activities)?	38 (39.2)	28 (28.9)	21 (21.6)	7 (7.2)	3 (3.1)	-	-
2. How much does the possibility of experiencing bruising or bleeding restrict your travel?	73 (75.3)	18 (18.6)	6 (6.2)	-	-	-	-
3. How much does the possibility of experiencing bruising or bleeding restrict the medical care you need (e.g. visiting a dentist, massage treatment, or going to another doctor)?	50 (51.5)	19 (19.6)	19 (19.6)	6 (6.2)	3 (3.1)	-	-
4. How much does the possibility of experiencing bruising or bleeding restrict your working life?	84 (86.6)	7 (7.2)	4 (4.1)	1 (1%)	-	1 (1)	-
5. When you consider all these features above, how much does the possibility of bruising or bleeding affect your daily life?	43 (44.3)	34 (35.1)	17 (17.5)	3 (3.1)	-	-	-
6. How much does the anticoagulant treatment restrict your food choices (diet)?	61 (62.9)	10 (10.3)	11 (11.3)	10 (10.3)	2 (2.1)	3 (3.1)	-
7. How much does anticoagulant treatment restrict you from drinking alcoholic beverages when you want?	93 (95.9)	2 (2.1)	2 (2.1)	-	-	-	-
8. How much does the anticoagulant treatment restrict using over-the-counter medicines (aspirin, ibuprofen, vitamins, herbal remedies)?	34 (35.1)	18 (18.6)	23 (23.7)	9 (9.3)	8 (8.2)	3 (3.1)	2 (2.1)
9. When you consider all these features above, how much does anticoagulant treatment affect your daily life?	33 (34)	43 (44.3)	17 (17.5)	3 (3.1)	1 (1)	-	-
10. How much does the anticoagulant treatment make it difficult for you to do your daily responsibilities?	70 (72.2)	17 (17.5)	9 (9.3)	1 (1)	-	-	-
11. How much does the anticoagulant treatment complicate your changing responsibilities when necessary?	72 (74.2)	17 (17.5)	4 (4.1)	4 (4.1)	-	-	-
12. How complex do you find the anticoagulant treatment?	12 (12.4)	16 (16.5)	24 (24.7)	21 (21.6)	19 (19.6)	5 (5.2)	-
13. How much time do you think you lost due to anticoagulant treatment?	19 (19.6)	42 (43.3)	20 (20.6)	5 (5.2)	6 (6.2)	4 (4.1)	1 (1)
14. How much do you think the anticoagulant treatment is frustrating?	28 (28.9)	29 (29.9)	20 (20.6)	8 (8.2)	6 (6.2)	5 (5.2)	1 (1)
15. How much do you think the anticoagulant treatment is demoralising?	21 (21.6)	30 (30.9)	28 (28.9)	8 (8.2)	8 (8.2)	2 (2.1)	-
16. When you consider all these features above, how much difficulty/burden does anticoagulant treatment bring to you?	16 (16.5)	35 (36.1)	29 (29.9)	7 (7.2)	6 (6.2)	3 (3.1)	1 (1)
20. How much do you worry if you experience bruising and bleeding due to your anticoagulant treatment?	4 (4.1)	3 (3.1)	12 (12.4)	15 (15.5)	24 (24.7)	33 (34)	6 (6.2)
22. When you consider all these features above, how much does the anticoagulant treatment have a negative effect on your life?	30 (30.9)	37 (38.1)	21 (21.6)	1 (1)	3 (3.1)	4 (4.1)	1 (1)
24. Compared to the medical treatments you have taken, what is the difficulty of managing the anticoagulant treatment for you?	13 (13.4)	35 (36.1)	24 (24.7)	9 (9.3)	8 (8.2)	6 (6.2)	2 (2.1)

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Table 3. (Cont.) (opposite questions). Distribution of Duke Anticoagulant Satisfaction Scale items

	Very much n (%)	Much n (%)	A bit more n (%)	Moderately n (%)	A little n (%)	Very little n (%)	None n (%)
17. When you consider all these features above, how much do you trust yourself in maintaining anticoagulant treatment?	34 (35.1)	43 (44.3)	12 (12.4)	4 (4.1)	4 (4.1)	-	-
18. How well do you think you understand the medical causes of your anticoagulant treatment?	7 (7.2)	24 (24.7)	16 (16.5)	18 (18.6)	19 (19.6)	12 (12.4)	1 (1)
19. How much do you feel safe due to your anticoagulant treatment?	14 (14.4)	31 (32)	26 (26.8)	5 (5.2)	15 (15.5)	4 (4.1)	2 (2.1)
21. When you consider all these features above, how much does the anticoagulant treatment have a positive effect on your life?	12 (12.4)	37 (38.1)	32 (33)	10 (10.3)	4 (4.1)	2 (2.1)	-
23. When you consider all these features above, how much do you satisfied with the anticoagulant treatment?	12 (12.4)	41 (42.3)	28 (28.9)	11 (11.3)	2 (2.1)	3 (3.1)	-
25. To what extent would you recommend this type of treatment to someone who has to start an anticoagulant treatment due to the disease or treatment?	39 (40.2)	34 (35.1)	17 (17.5)	5 (5.2)	2 (2.1)	-	-

and above, and 61.4% were women. In many studies, the average age of patients receiving anticoagulant treatment was found to be above 55 years (19,20,24). Our study is similar to the studies performed in terms of sociodemographic features. Similar to the literature, in our study, almost all patients (95.9%) had a comorbid disease accompanying their current disease (10,19).

In most studies investigating warfarin use, atrial fibrillation was found as the most common warfarin treatment cause (25). Sjögren et al. (26) found that the most common indication was atrial fibrillation with a ratio of 68%, and Rojas-Fernandez et al. (27) reported this ratio as 63%. In our study, the most common indication for warfarin use was atrial fibrillation, which complies with the literature.

Almeida et al. (18) found that the rate of patients using warfarin for 1 year or more was 79.2%. Connock et al. (7) and Appelboam and Thomas stated that OAC drugs should generally be used for a long time. Our study determined that the majority of patients (79.4%) had been using warfarin for two years and longer, and this finding is in line with the literature.

In the original study wherein DASS was developed by Samsa et al. (14), the total scale mean score was found as 55.0±17.6. This mean score was found as 57.9±16.5 in Pelegrino et al.'s (16) study, as 46.4±8.6 in Oliveira-Kumakura et al.'s (20) study conducted on patients with stroke, and as 67.1±18.2 in another study (18). In a study comparing warfarin with other anticoagulants, warfarin satisfaction was found to be slightly lower than other drugs (28).

When the studies conducted in our country are considered, Yıldırım and Bayık-Temel (13) found the total scale mean score as 85.0±25.1 where they adapted the scale to Turkish, whereas Mert et al.'s (17) study of elderly patients found the score as 68.9±22.6 and Uçar's (21) thesis study found the score of 61.71±19.34. The study results of Yıldırım and Bayık-Temel (13) and Mert et al. (17) demonstrate that satisfaction with anticoagulant use is worse in our country.

Our study obtained a total average score of 57.67±14.56; hence, we conclude that patients who received home health care were better satisfied and perceived the problems they experienced less. It is believed that factors such as a regular follow-up of patients receiving home health services, lack of additional burdens such as hospital appointments and transportation costs, and sharing responsibilities with caregivers contribute to the satisfaction perception, thereby improving the perception of the patients' home health services.

Previous studies have shown that patients experience more problems and adverse effects as they get older (12,18). Almeida et al. (18) found that the satisfaction and quality of life perception of patients in the age group of 41-65 years were better than the patients aged 65 years and older. Although no significant relationship was found between the age factor and satisfaction level in our study, this situation is believed to be facilitated by the fact that treatment management getting difficult by age is shared with the caregiver and the health care team in patients receiving home care.

Uçar's (21) study found a significant relationship among gender, scale total score and subdimension mean scores; additionally, the study found that men were having lower satisfaction as compared to women. Moreover, Yıldırım and Bayık-Temel's (13) study found that gender does not affect satisfaction with OAC use. Our study, in which the level of satisfaction between men and women is similar, is compatible with Yıldırım and Bayık-Temel's (13) study.

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Table 4. Evaluation of results according to sociodemographic and clinical features of participants

		Total scale score	Limitations with the treatment	Inconvenient with the treatment	Positive impact
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age	<65	57.21±13.81	20.92±6.36	20.72±7.04	15.48±5.08
	≥65	57.86±14.9	20.31±5.75	21.82±7.62	15.74±4.82
	p[1]	0.828	0.655	0.528	0.822
Gender	Female	57.29±13.8	20.41±5.24	21.39±7.36	15.49±4.86
	Male	58.61±16.5	20.61±7.35	21.89±7.81	16.11±4.95
	p[1]	0.689	0.880	0.766	0.576
Anticoagulant use reason	Atrial fibrillation	59.57±15.01	20.95±6.01	22.21±7.9	16,41±4.62
	Heart valve replacement	53.74±11.53	19.74±5.67	20±4.75	14±5.76
	Deep vein thrombosis	56.1±17.39	19.3±7.04	22±8.11	14.8±4.37
	Pulmonary embolism	51.8±11.65	19.4±2.19	18±9.19	14.4±4.39
	p[2]	0.339	0.740	0.485	0.226
Anticoagulant use period	<2 years	59.2±16.64	20.55±6.13	21.95±7.86	16.7±5.24
	2-5 years	56.25±12.36	20.25±6.48	20.59±6.31	15.41±4.58
	6-10 years	58.55±15.11	20.32±5.66	22.45±7.99	15.77±4.51
	≥10 years	57.48±15.7	20.83±5.36	21.61±8.37	15.04±5.4
	p[2]	0.898	0.986	0.828	0.716
Chronic disease number	1	55.27±14.44	19.63±5.6	20.1±7.23	15.53±5.43
	2	56.58±14.69	20.53±6.71	20.76±6.58	15.29±5.21
	≥3	64.32±13.27	22±4.7	25.24±8.4	17.08±3.45
	p[2]	0.046*	0.332	0.022*	0.335
Bleeding	No	52.68±10.44	18.74±4.82	19.16±5.76	14.79±4.27
	Minor bleeding	63.17±13.98	22.27±5.67	24.13±7.44	16.77±5.06
	Major bleeding	69.6±23.16	24.9±8.33	27.3±10.64	17.4±6.65
	p[2]	0.000*	0.001*	0.000*	0.097
Thromboembolic events	No	58.06±14.83	20.42±6.1	21.75±7.47	15.89±4.87
	Yes	53.38±10.91	21±2.67	19.3±7.41	13.25±4.4
	p[1]	0.386	0.790	0.342	0.143

Data presented as mean (SD). SD: standard deviation, [1]Student's t test, [2]One-Way ANOVA test, *p<0.05
Note: Cases without chronic disease were excluded from the analysis due to the small number of cases

It has been reported that the costs related to disease burden, hospitalisation and access to care services are incurred because of the emergence of complications of each chronic disease (29,30). Yıldırım and Bayık-Temel's (13) study found that burdens and difficulties were experienced more often with an increasing number of chronic diseases. Furthermore, Almeida et al.'s (18) study showed that the presence of additional chronic disease negatively affected the treatment satisfaction, whereas another study found no significant relationship (21). In our study, it is believed that with the increase in the number of chronic diseases, the perception of satisfaction worsens, thereby increasing the burden and difficulties caused by warfarin use. It can be attributed to the burdens caused by chronic diseases and the increasing use of multiple drugs.

Almeida et al. (18) found that patients who received treatment for more than a year had more positive perceptions. Our study found no relationship between treatment time and anticoagulant satisfaction, similar to the study performed by Mert et al. (17).

Previous studies have found that the side effects experienced during warfarin use negatively impacted the satisfaction level of patients (13,18). Furthermore, Sjögren et al.'s (26) study found that the patients had an annual risk of thromboembolic events of 2.65% and a major bleeding risk of 2.24%. Yıldırım and Bayık-Temel's (13) study found that the rate of bleeding was 35.2%. Almeida et al. (18) found the bleeding rate to be 37.5%. Yıldırım and Bayık-Temel (13) found that individuals with a history of bleeding had more problems in the subdimensions of restrictions, burdens

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and difficulties and had worse satisfaction. Our study observed that the state of having thromboembolic events did not affect the perception of satisfaction. However, those who experienced bleeding during treatment were found to have a higher total scale, and all the subdimension mean scores were higher than those who did not experience bleeding. Our study was found to be in compliance with the literature in this aspect. It is believed that the history of bleeding restricts patients more, increases their responsibilities, such as taking the medication regularly and not interrupting their INR follow-ups, thereby worsening their satisfaction.

Study Limitations

The limitations of the study were that the study only included patients using warfarin, and the study had a relatively small sample size. Additionally, it is believed that the answers given to the questions about travel, work life and daily work asked to this group of patients who need home care service due to their presence of DASS may be misleading.

CONCLUSION

As a result, it was found that satisfaction with warfarin use was not poor in patients receiving home care services but a history of bleeding complications while using warfarin and the presence of additional chronic disease worsened treatment satisfaction in our study.

The vast majority of home care patients experience difficulties in the management of medical processes due to advanced age, functional dependencies and chronic diseases. It is believed that consultancy attempts should be planned by health care professionals, and services should be expanded according to the needs of patients, especially those with bleeding problems, multiple morbidities and difficulties in going to control visits. Thus, patients' adherence to treatment and perception of satisfaction can be increased.

Ethics Committee Approval: Ethics committee approval was obtained from the relevant institution on 05.04.2017 (approval no: 36).

Informed Consent: The objective of the study was explained to all the participants, and their informed consent was obtained before their participation in the study.

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Relationship Between Colon Wall Thickness in Computed Tomography Scan and Colon Cancer: A Retrospective Study

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ABSTRACT

Objective: This study aimed to investigate the colonoscopic findings of patients with increased colonic wall thickness (ICWT) detected by abdominal computed tomography (CT) and to define the effectiveness of neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) together with colonic wall thickness in predicting malignancy.

Methods: Data of patients who underwent abdominal CT for any reason between January 2017 and August 2019 were retrospectively reviewed. Records of patients with ICWT in the abdominal CT report were retrospectively analysed. Patients whose colon wall thickness could be measured and who were evaluated with colonoscopy and biopsy within 1-3 months after CT were included in the study. Haemoglobin (hb), albumin, NLR, PLR and MPV values, colon wall thickness and colonoscopy and biopsy results were recorded.

Results: Ninety-seven patients had ICWT data on CT. The colonic wall thickness and presence of positive lymph node were significantly higher in the malignancy group ($p<0.001$). Similarly, values of hb, NLR, PLR and MPV were different in the malignancy group ($p<0.001$). According to the receiver operating characteristics analysis, colon wall thickness over the 8.5 mm threshold value was a significant factor in predicting colon cancer ($p<0.001$).

Conclusion: In patients who do not have a history of gastrointestinal injury or disease, the incidental detection of ICWT >8.5 mm may be an important finding for a possible diagnosis of colon cancer.

Keywords: Colonoscopy, colonic wall thickening, computed tomography, neutrophil lymphocyte ratio, platelet lymphocyte ratio

INTRODUCTION

Computed tomography (CT) is one of the most commonly used radiological imaging methods in the diagnosis of gastrointestinal diseases. Detection of increased colon wall thickness (ICWT) on CT is regarded as an important finding that necessitates additional evaluation (1). Although ICWT may be an imaging finding of colon cancer, it may occur because of several reasons such as peristaltism, insufficient filling of the colon lumen, faecal fragments and inflammatory gastrointestinal diseases. However, it can also be seen in some systemic conditions including cirrhosis, heart failure and hypoalbuminaemia (2).

When ICWT is detected on CT, examination of the lumen by colonoscopy is often requested. However, complications related to endoscopic procedures and an increase in cost are other

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problems. In addition, the presence of a normal colonoscopy in some patients leads to the questioning of CT findings retrospectively. Besides, no guideline stated that colonoscopy should be performed when ICWT is seen during CT (1).

Few studies have focused on the relationship between ICWT and colonic diseases (3). Previous studies have speculated that a relationship exists between the degree of increased wall thickness in CT and colon cancer. Studies have also shown that the possibility of colon cancer increases if ICWT is associated with a mass (4,5). Algorithms on ICWT's association with other findings such as changes in pericolonic fatty tissues and presence of lymph nodes have been studied (6). However, the correlation between isolated colonic wall thickness and colon cancer remains controversial.

Recent studies have extensively investigated the relationship between neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) values with tumoural and inflammatory diseases (7-10). NLR, PLR and MPV are thought to have diagnostic and prognostic roles in patients with colon cancer (7-10). In patients with ICWT, evaluating these laboratory values together with CT findings may be useful to increase diagnostic efficiency.

This study aimed to investigate the colonoscopic findings of patients with ICWT detected by abdominal CT and to determine the effectiveness of NLR, PLR and MPV with colonic wall thickness in predicting malignancy.

METHODS

Patients who underwent abdominal CT for any reason between January 2017 and August 2019 were retrospectively reviewed. The phrase "ICWT" was searched for in CT reports. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all patients. Institutional approval was obtained due to the retrospective design of the study (Surp Pirgiç Armenian Hospital, 2228/2020).

Study Design

Records of patients with "ICWT" in the abdominal CT report were retrospectively analysed. Patients with oral and intravenous contrast-enhanced CT protocols whose colon wall thickness could be measured and who were evaluated with colonoscopy and biopsy within 1-3 months after CT were included in the study. The following conditions that interfered with optimal measurement of the colon wall thickness were excluded: Patients who do not comply with abdominal CT imaging protocols, who do not develop sufficient distension in the colon, who have solid-liquid stool in the colon, who have heart failure, hypoalbuminaemia and nephrotic syndrome that may affect the colon wall thickness, who have a history of abdominal surgery, who were followed up due to any type of bowel diseases, whose CT findings suggested colon cancer, and who had not undergone colonoscopy or had insufficient colonoscopy were excluded from the study. A total of 97 patients who met the study criteria at the last evaluation were included in the study (Figure 1).

Demographic data, haemoglobin [(hb), g/dL], albumin (g/dL), NLR, platelet PLR and MPV (f/L) values, colon wall thickness measurement (mm), colonoscopy and biopsy results of the patients were recorded.

Groups

Abdominal CT images of the cases included in the study were re-evaluated by an experienced radiologist who was unaware of the results of the colonoscopic-histopathological evaluation. ICWT over 3 mm was considered pathological (11). Cases were divided into three groups as normal, benign or malignant according to the results of the colonoscopic evaluation. The first group included patients who had normal colonoscopic findings and did not require further examination, and the second group included benign lesions of the colon that were not considered malignant by colonoscopy. Patients diagnosed with colonic inflammatory causes, diverticula and polyps by endoscopist were included in this group. The third group included patients with lesions diagnosed as malignant colonoscopically.

Cases were divided into two groups as benign and malignant according to their definitive pathological results. In the malignant

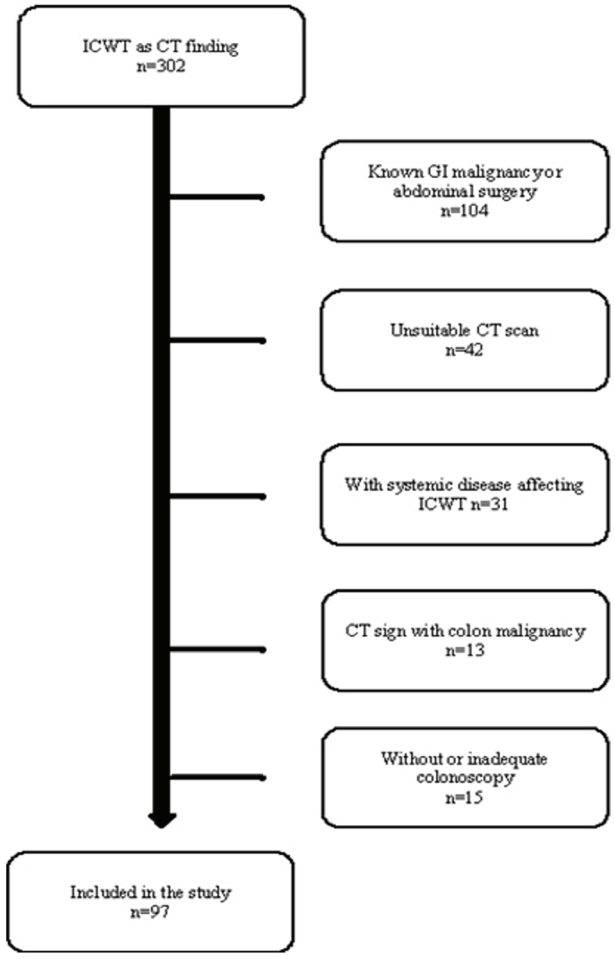

Figure 1. Flowchart of the study
ICWT: increased colonic wall thickness, CT: computed tomography, GI: gastrointestinal system

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group, patients had colon cancer proven pathologically. In the benign group, patients had normal pathology and patients with other benign lesions of the colon other than cancer.

Statistical Analysis

According to the results of colonoscopy and pathological examination, the difference between the groups in terms of the degree of ICWT, age, hb, albumin, NLR, PLR and MPV was investigated with descriptive statistics. Descriptive analysis was made to give information about the general characteristics of the study groups. Continuous variables were presented as mean ± standard deviation or median (25^{th}-75^{th} percentile) depending on their normal distribution pattern. Data on categorical variables were given as frequency with percentage. While comparing the means of quantitative variables between groups, the significance test of the difference between two means and One-Way analysis of variance were used. Tukey honestly significant difference test was used for multiple comparisons to evaluate the groups responsible for the significant difference. Cross tables were created for qualitative variables, and chi-square tests were used for relationships between relevant variables.

The receiver operating curve (ROC) analysis associated with the area under the curve was used to determine optimal threshold values of colonic wall thickness in predicting the presence of pathologically proven colon cancer. Subsequently, patients were analysed as low or high groups according to threshold values to evaluate the relationship between the increase in colon wall thickness and presence of colon cancer. P<0.05 value was considered statistically significant. Statistical analysis was performed with IBM SPSS 19 (IBM Corp., Armonk, NY) software.

RESULTS

Of the 97 patients, 59 (60.8%) were male and 38 (39.2%) were female. The mean age was 62.1±13 years. When classified according to localisation, ICWT was seen mostly in the left colon (63.9%). The median wall thickness of the colon was 9 (7-11) mm. Positive lymph node was observed on CT of 25 (25.7%) patients.

After colonoscopic evaluation, 31 patients (32.0%) were classified into group 1, 39 (40.2%) into group 2 (22 polyps, 9 colitis, 8 diverticular diseases) and 27 (27.8%) (adenocarcinoma) into group 3. No significant difference was found between the groups in terms of age and sex (p>0.05). The median wall thickness of the colon were 9 (7-10) mm in group 1, 8 (7-9.5) mm in group 2 and 10 (9-14.5) mm in group 3 (Table 1). While no significant difference was noted between groups 1 and 2 in terms of the degree of ICWT, the wall thickness in group 3 was significantly higher than that of group 1 and group 2 (p=0.001).

When the three groups were compared based on the colonoscopic diagnosis, two (6.5%) and five patients (12.8%) in groups 1 and 2 had positive lymph nodes, respectively, whereas positive lymph nodes were detected in 18 patients (66.7%) in group 3. The rate of positive lymph node was significantly higher in group 2 than in other groups (p=0.001).

According to the pathology results, when malignant and benign groups were compared in terms of colon wall thickness, the ICWT value was significantly higher in the malignancy group (p=0.001).

The values of hb NLR, PLR and MPV were significantly different in group 3 than in groups 1 and 2 (p<0.001). However, no difference was noted between the groups in terms of the serum albumin value.

ROC analysis using sensitivity and specificity to determine threshold values based on pathological colon cancer diagnosis revealed that the optimal threshold value for colon wall thickness

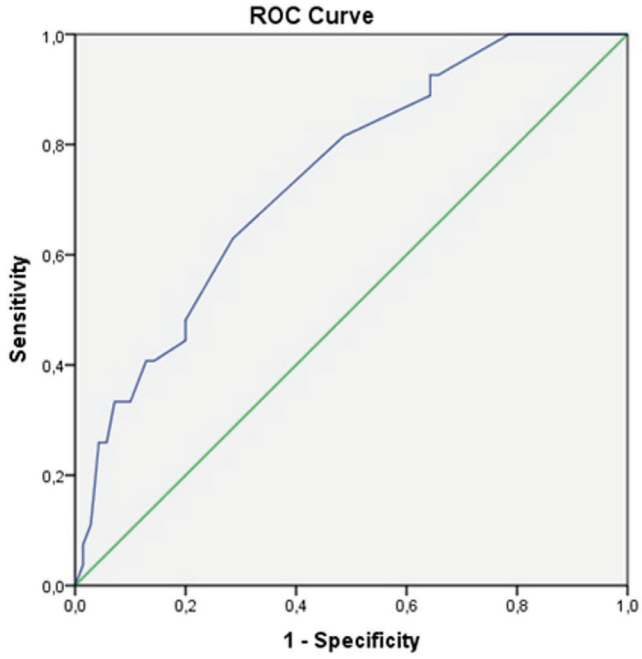

Figure 2. Receiver operating characteristics curve
ROC: receiver operating characteristics

Table 1. Comparison between groups

	Group 1 (n=31)	Group 2 (n=39)	Group 3 (n=27)
CWT (mm) (25^{th}-75^{th} percentile)	9 (7-10)	9 (7-9.5)	10 (9-14.5)
Pathologic LAP, n (%)	2 (6.5%)	5 (12.8%)	18 (66.7%)

CWT: colonic wall thickness, LAP: lymphadenopathy

Table 2. Comparison of groups according to the threshold value determined according to the result of the ROC analysis

	CWT <8.5 mm (n=41)	CWT ≥8.5 mm (n=56)
CWT (mm) (25^{th}-75^{th} percentile)	7 (6-8)	10 (9-13.7)
Pathologic LAP, n (%)	5	20
Patient with malignancy (n)	5	22

CWT: colonic wall thickness, LAP: lymphadenopathy, ROC: receiver operating characteristics

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was 8.5 mm. Optimal threshold sensitivity and specificity values are shown in Figure 2.

In the grouping according to the 8.5 mm threshold value, 56 patients (57.7%) had ICWT. In these patients, 22 (39.2%) had colon cancer and 20 (35.7%) had positive lymph nodes. The presence of colon cancer and positive lymph nodes on CT were significantly higher in patients with ICWT (p=0.001) (Table 2).

DISCUSSION

This study shows that ICWT of >8.5 mm as an abdominal CT finding in patients who had not had gastrointestinal system disease or surgery before could be associated with a possible diagnosis of colonic malignancy.

Although ICWT in CT is not a specific finding, it may be a symptom of colon diseases, including colon cancer. The importance of the incidentally detected ICWT is not completely clear (4). There is no algorithm for the management of these patients. If an appropriate colonic lumen expansion can be achieved during CT, normal colonic wall thickness should not be >3 mm (12).

According to the literature, colonoscopy is regarded as normal at the rates varying between 12% and 28% of patients with ICWT (3,4,11,13-16). In this study, colonoscopy results were normal in 31.9% of the patients with ICWT. This means the additional cost and complication risk of colonoscopy in nearly one-third of the patients. On the contrary, the same studies have observed that the rate of colon cancer in patients with ICWT ranges from 14% to 46.5% (4,11,13-16). In our study, similar to the literature, the rate of colonic malignancy was 27.8%.

Another CT finding of colon cancer is the presence of enlarged lymph nodes in the region adjacent to the related colon loops. In the colon cancer group, 66.7% of the patients had positive lymph nodes, which was significantly higher than those in the groups with normal and benign diseases.

Few studies have examined the amount of ICWT and colonoscopy findings (3,14). According to Ergul and Filik (3), the average ICWT values were 8 mm and 15 mm in the normal and malignant groups, respectively. The mean values were 9.4 and 16.2 mm in Akbas et al.'s (14) study. In both studies, the thickness measurement was significantly higher in the malignancy group. In our study, ICWT values were 8.6 mm and 11.5 mm in the normal and malignancy groups, respectively. To distinguish patients with benign pathologies and to avoid unnecessary colonoscopy, a threshold value of 8.5 mm was determined for the wall thickness in patients with ICWT by ROC analysis. When grouping according to this threshold value, the rate of normal colonoscopy results decreased to 28.5%. We believe that such threshold values are necessary in prospective larger groups in association with additional CT findings to decrease the need for colonoscopy.

Some parameters known to increase in inflammatory processes are thought to be linked to colon cancer (7-10). If inflammatory markers such as NLR, PLR, hb and MPV are associated with ICWT, they may be associated with colon cancer. In our study, these laboratory markers were significantly different in the malignancy group. A significant relationship was found between their association with ICWT and suspicion of colon cancer. This suggests that colonoscopy request can be determined more effectively using these tests.

Study Limitations

The limitations of our study were the small number of patients and the retrospective study design. More efficient results can be obtained with multicentre prospective studies with a large number of patients.

CONCLUSION

As a result, in patients who do not have a history of gastrointestinal surgery or disease, an incidental detection of ICWT >8.5 mm may be important for a possible diagnosis of colon cancer. Besides, positive lymph nodes in association with ICWT might be a more predictive finding for the detection of colonic cancer. Colonoscopic examinations should be taken into consideration in patients with these findings.

Ethics Committee Approval: Institutional approval was obtained due to the retrospective design of the study (Surp Pirgic Armenian Hospital, 2228/2020).

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Vitamin D Deficiency is Associated with Depression, Anxiety and Sleep Disturbance in Pregnant Women

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ABSTRACT

Objective: Vitamin D deficiency is prevalent in pregnancy and has been associated with psychological symptoms in non-pregnant patient populations. This study aimed to evaluate the relationship between depression, anxiety, sleep quality and vitamin D deficiency in pregnant women.

Methods: In this prospective cross-sectional study, data from a total of 153 pregnant women including demographic, pregnancy and laboratory were obtained. Participants were divided into two groups with low (<20 ng/mL) and normal (≥20 ng/mL) serum 25 (OH) vitamin D levels. Pregnant women were screened for symptoms of depression and anxiety using the Beck depression inventory (BDI) and Beck anxiety inventory (BAI). Pittsburgh sleep quality index (PSQI) was utilised to investigate sleep quality. Groups were compared statistically.

Results: BDI, BAI and PSQI scores were significantly higher in patients with low vitamin D levels. Overall, 22.2% of pregnant women had depression symptoms and 37.8% had moderate-severe anxiety symptoms in vitamin D deficient group.

Conclusion: Depression, anxiety and poor quality sleep during pregnancy were associated to vitamin D deficiency. This study emphasises the importance of screening for vitamin D deficiency among pregnant women.

Keywords: Vitamin D, pregnancy, depression, anxiety, sleep quality

INTRODUCTION

The high prevalence of depression and anxiety in pregnancy has been attributed to pregnancy-related anatomic, hormonal and physiologic changes. Women are at high risk for depression during pregnancy (1,2). 10-15% of women report depressive symptoms, whereas 4-7% are diagnosed with major depression during pregnancy (2,3).

Many essential nutrients are required for a healthy nervous system and mood regulation (4,5). Vitamin D is one of those nutritional factors that may have neuroregulatory activity and act as a neuroactive hormone (6). This unique neurosteroid hormone may be involved in the anterior pituitary lobe functions, and its receptors are abundant across the human brain (7). Additionally, cytokines and inflammatory markers are enhanced by deficiencies of vitamin D via elevated concentrations of neuronal calcium. Literature

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suggests the role of vitamin D in neuro-immunomodulation and neuro-plasticity with various mechanisms (hypothalamic-pituitary-adrenal axis, insulin or serotonin mediated pathways, etc.) (8-10). It has been postulated that its deficiency alters neuro-transmitters such as serotonin, dopamine and noradrenaline, which are implicated to cause depressive symptoms (11). High prevalence of Vitamin D deficiency during pregnancy makes it a significant clinical problem.

Emerging lines of evidence suggest that vitamin D also regulates immune system and decreases the release of inflammatory substances, including those that regulate sleep such as tumour necrosis factor-alpha and interleukin 1 (12). Vitamin D receptors that expressed in many brain areas may influence the daily sleep-wake cycle. Association between inadequate vitamin D and sleep disorders is not well-understood; however, some observational researches suggest the connection of vitamin D deficiency and poor sleep quality among non-pregnant populations (13,14). Similarly, the protective effect of vitamin D on mood disorders, mostly depression, in non-pregnant people has been reported previously in literature. However, results have not been entirely consistent due to the complexity of this connection. In addition, this research area was rarely conducted among pregnant women, and the relationship of vitamin D and anxiety symptoms is under-investigated in pregnancy. In this study, we investigated the vitamin D effect on depression and anxiety symptoms and examine the frequency of sleep disturbances with the goal of clarifying the association between inadequate vitamin D and sleep disorders risk in pregnant women.

METHODS

This study was designed as a prospective cross-sectional study. The study population for this report is from the first 200 pregnant women who attended an outpatient obstetric clinic for routine exam for pregnancy in each trimester. Pregnant women were interviewed in the period of April 2018 and May 2018. Inclusion criteria includes the following: 18 years or older, no schizophrenia or other psychiatric history, restless legs syndrome, drug addiction, psychiatric drug use, any chronic musculoskeletal disorder leading to poor sleep quality, sleep disturbance history before pregnancy and comorbidities such as diabetes and hypertension. Consequently, 153 pregnant women were available for the analysis.

Pregnant women were screened for depression symptoms using the Beck depression inventory (BDI) and Beck anxiety inventory (BAI) for anxiety symptoms (15,16).

BDI is a 21-item self-report scale used to assess the current severity of depression symptoms. Each item is rated in a four-point scale (0-3) with possible total scores ranging from 0 to 63. A cutting score of 17 or over represents depression. Turkish validity and reliability analysis of this scale was reported by Hisli (17). The BAI consists of 21 self-reported items (four-point scale) to assess the intensity of physical and cognitive anxiety symptoms during the past week. Scores may range from 0 to 63 evaluated as follows: Mild anxiety (8-15), moderate anxiety (16-25) and severe anxiety (26-63). The Turkish version of this inventory was devised by Ulusoy (18). The Pittsburgh sleep quality index (PSQI) was used to assess sleep quality over the previous month. In this scale, a total sum of ≥5 indicates low sleep quality (19). The PSQI was demonstrated to have good validity and reliability among Turkish population (20).

The immune chemiluminometric assay (Architect i2000, Abbott, Germany) was used to determine vitamin D levels in patients. It has been accepted that serum 25-hydroxyl vitamin D [25 (OH) D] of <20 ng/mL is the level of vitamin D deficiency (21). Participants were divided into two groups according to serum 25 (OH) D levels: 90 patients with serum 25 (OH) D levels of <20 ng/mL comprising the vitamin D deficient group (group 1) and 63 women with serum 25(OH) D levels of ≥20 ng/mL comprising the vitamin D sufficient group (group 2).

This study was approved and initiated by the Clinical Research Ethics Committee of Karadeniz Technical University Faculty of Medicine (date: 02.10.2017, protocol no: 142). All participants were informed about the research concept, and written informed consent was obtained from all participants.

Statistical Analysis

Data were analysed using Statistical Package for the Social Sciences 23.0 statistics programme. Chi-square test was used for categorical variables. Descriptive statistics of evaluation results were as follows: Number and percentage for categorical variables, mean and standard deviation for numerical variables. The normality of variables was assessed using Kolmogorov-Smirnov test. Mann-Whitney U test was used for comparison of groups, and correlation analysis was done by Spearman test. Interpreting the correlation coefficients was based on following points: Values between 0.3 and 0.5 indicate a low positive linear relationship, values between 0.5 and 0.7 indicate a moderate positive linear relationship, values between 0.7 and 0.9 indicate a high positive linear relationship and values between 0.9 and 1 indicate a very high positive linear relationship (22). Cronbach's alpha values were evaluated as follows: $0.5 \leq \alpha < 0.6$ indicating a poor internal consistency, $0.6 \leq \alpha < 0.7$ indicating a fair internal consistency, $0.7 \leq \alpha < 0.9$ indicating a good internal consistency and ≥0.9 indicating an excellent/strong internal consistency (23). A probability level of <0.05 was considered as statistically significant.

RESULTS

Socio-demographic and Laboratory Parameters

Data from a total of 90 participants in group 1 and 63 participants in group 2 were reviewed. No significant differences were determined for age, number of previous pregnancies, gestational week, pre-pregnancy smoking history and concomitant diseases of pregnant women in both groups. Mean values for serum haemoglobin, ferritin, thyroxin, thyroid-stimulating hormone and calcium in both groups were similar. The socio-demographic and laboratory parameters are summarised in Table 1.

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Evaluation of Depression and Anxiety

Among all participants with vitamin D deficiency (group 1), mean score of BDI was 14.8±10.3, whereas mean BAI score was 15.3±8.7. BDI and BAI scores were significantly higher in group 1 (p<0.001) (Table 1). In group 1, 22.2% (n=20) had high BDI values, whereas 0% in group 2. In group 1, 6.2% had mild, 25.6% had moderate and 12.2% had severe anxiety symptoms. In group 2, 96.8% had mild and 3.2% had moderate level of anxiety symptoms (p<0.001) (Table 2).

Sleep Quality

According to PSQI screening, the mean score was 9.4±5.6 in group 1 indicating low sleep quality, whereas 2.6±3.1 in group 2. PSQI points were significantly worse in the vitamin D deficient group compared to that of the vitamin D sufficient group (p<0.001) (Table 2). In group 1, 85.6% had sleep impairment, whereas 12.7% in group 2.

A high positive correlation was found between BDI and PSQI scores of pregnant women (r=0.755) (p<0.001). Correlation between BAI and PSQI scores of pregnant women was also examined, and a statistically significant high positive correlation was found (Table 3). The Cronbach's alpha values for scales used in this study indicated a strong internal consistency (0.945 for BDI, 0.913 for BAI and 0.951 for PSQI).

Table 1. Socio-demographic and clinical characteristics of study groups

	Group 1 (n=90) mean±SD	Group 2 (n=63) mean±SD	p
Age (year)	27.4±5.7	27.9±5.9	0.467
Number of previous pregnancies	1.8±1.0	1.6±0.8	0.145
Gestational week	27.6±10.8	28.1±6.9	0.340
Pre-pregnancy smoking history	7.7% (n=7)	9.5% (n=6)	0.931
Haemoglobin (g/dL)	11.5±1.4	11.7±1.1	0.544
Ferritin (ng/mL)	26.0±7.6	25.2±10.0	0.359
T4	1.2±0.5	1.2±0.3	0.753
TSH	2.3±2.1	2.2±1.4	0.838
Calcium (mg/dL)	8.9±0.5	8.9±0.7	0.780
BDI	14.8±10.3	3.6±3.0	<0.001
BAI	15.3±8.7	4.1±3.8	<0.001
PSQI	9.4±5.6	2.6±3.1	<0.001

BAI: Beck anxiety inventory, BDI: Beck depression inventory, SD: standard deviation, PSQI: Pittsburgh sleep quality index, T4: thyroxine hormone, TSH: thyroid-stimulating hormone

Table 2. Comparison of BDI, BAI and PSQI between groups

	Group 1, n (%)	Group 2, n (%)	p	Total, n (%)
BDI: 0-17	70 (77.8)	63 (100.0)	<0.001	133 (86.9)
BDI ≥17	20 (22.2)	0 (0.0)	<0.001	20 (13.1)
BAI: 8-15	56 (6.2)	61 (96.8)	<0.001	117 (76.5)
BAI: 16-25	23 (25.6)	2 (3.2)	<0.001	25 (16.3)
BAI ≥26	11 (12.2)	0 (0.0)	<0.001	11 (7.2)
PSQI: 0-4	13 (14.4)	55 (87.3)	<0.001	68 (44.4)
PSQI ≥5	77 (85.6)	8 (12.7)	<0.001	85 (55.6)

BAI: Beck anxiety inventory, BDI: Beck depression inventory, PSQI: Pitt burgh sleep quality index

Table 3. The correlations between PSQI, BDI and BAI scores

	BAI	BDI
PSQI	r=0.724 p<0.001	r=0.755 p<0.001

BAI: Beck anxiety inventory, BDI: Beck depression inventory, PSQI: Pitt burgh sleep quality index

DISCUSSION

Depression and anxiety were reported as the most common psychiatric disorders that affect up to 15% of pregnant women and are the leading causes of disease-related disability among women. Several studies have investigated the evidence for a causal link between low vitamin D levels and mood disorders in pregnancy. Results remain controversial; however, vitamin D deficiency has been associated with an increased risk of depression in some studies (24-27). In this study, the incidence of depression, anxiety and sleep disturbance were found to be higher in vitamin D deficient pregnant women compared to that of the vitamin D sufficient pregnant women. Limited data was found regarding the relationship between low vitamin D levels and presence of anxiety (28), thus, our study revealed that vitamin D deficiency is connected with elevated anxiety symptoms as well as depressive symptoms. This result proves the fact that insufficient level of vitamin D in pregnant women is linked to psychosocial stress and mood disorders.

A systematic review examined protective function of vitamin D on perinatal depression, and authors indicated a significant association between inadequate vitamin D and depression in pregnancy. A total of ten studies on vitamin D were included, eight were prospective cohort studies, and six of them reported vitamin D deficiency as predictive for increased risk of depression during pregnancy. The remaining four studies could not show any significant relationship between vitamin D levels and depression (29). Another study on this topic systematically reviewed literature, mostly observational studies, to show whether vitamin D status might influence the possibility of perinatal depression. Significant associations were found between vitamin D deficiency and depressive symptoms among pregnant women in Caucasian, Chinese, Iranian and Turkish populations (30). A cross-sectional cohort investigated the association of vitamin D levels and depression and anxiety symptoms in early pregnancy with 498

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women. Participants were screened by depression, anxiety, and stress scales (DASS-21) and patient health questionnaire depression module (PHQ-9) instruments. This study suggested that low vitamin D status is related to self-reported symptoms of depression in early pregnancy. Results show that 12% of women had moderate anxiety and depression. A 1 ng/mL reduction in serum 25 (OH) vitamin D concentrations was associated with 0.043 and 0.040 higher DASS-21 anxiety and PHQ-9 scores, respectively. Since vitamin D deficiency was not prevalent in patients in this study, the mean 25 (OH) vitamin D level for this study was 34.4 ng/mL (28). Nielsen et al. (25) measured serum concentrations of 25 (OH) vitamin D in 605 women with postpartum depression and 875 in control. In contrast to the above mentioned studies, this case-control study revealed that an increase in vitamin D levels enhances the prevalence of postnatal depression. This unexpected result was explained with genetic differences.

For the present study, tools for determining depression and anxiety symptoms were different from previous studies which mainly used Edinburgh postnatal depression scale (EPDS). We measured the mood disorder symptoms of pregnant women using the screening instruments of BDI and BAI, which are widely used scales, similar with EPDS, although not diagnostic; however, highly correlated with physicians diagnostic opinion. A cut-off point of 17 was used to identify depression using BDI, and 22.2% of vitamin D deficient group was defined to have depressive symptoms. Williams et al. (26) observed patients with BDI according to the same vitamin D threshold level with our study and found inverse association between vitamin D status and depression scores in early and late pregnancy but not postpartum. Mean BDI scores at 12-20 gestational weeks for vitamin D deficient group (n=98) and vitamin D sufficient group (n=19) were 7.4.2±4.9 and 10.2±7.1, respectively. In this present study, BAI scores of pregnant women were also investigated and classified in three sections. Anxiety symptoms were found to be associated with vitamin D deficiency. Results demonstrated that; 6.2% of vitamin D deficient group had mild, 25.6% had moderate and 12.2% had severe anxiety symptoms.

Sleep deprivation enhances the risk of gestational complications; however, few studies focused on sleep quality across pregnancy. An internet based survey demonstrated that 76% of women suffers from poor sleep quality across all months of pregnancy (31). In the current study, a significant relationship was observed between low vitamin D status and sleep disturbances with the average gestational week of 27.6±10.8 (group 1) and 28.1±6.9 (group 2). Notably, the prevalence of unhealthy sleep among vitamin D deficient group was 85.6% as defined by PSQI score of >5. Similar to our findings, vitamin D deficiency was found to be a risk factor of unhealthy sleep in a meta-analysis by Gao et al. (32) that reported vitamin D deficiency (cut-off value: 20 ng/mL) increases the incidence of sleep disorders by approximately 60%. The underlying mechanism of this relationship is currently unknown; however, the possible hypothesis is that vitamin D plays a role in circadian rhythms (33). Furthermore, vitamin D receptors are common in human brain, such as the hypothalamus, prefrontal cortex, substantia nigra and raphe nuclei, which are known to execute important roles in sleep (34). Cheng et al. (35) and colleagues provided evidence for vitamin D deficiency related to poor sleep quality at mid-pregnancy, which is consistent with our findings. They examined sleep quality by PSQI, which yielded scores of 7.04±3.47 for vitamin D deficient group indicating poor sleep quality. Vitamin D deficient group in our study had mean PSQI of 9.4±5.6 regarding sleep disturbances, whereas the control group had lower PSQI scores. Another related study was conducted among women in the last trimester of pregnancy. In contrast to our study, this study showed no statistical significant difference in PSQI scores between the vitamin D deficient group and vitamin D sufficient groups (36).

Strengths of our research include the following: A substantial study population of vitamin D deficient participants making comparable analyses possible, 58.8% of participants had severe vitamin D deficiency. Moreover, psychological status was assessed with well validated, internationally and widely used questionnaires. Study population was limited after eliminating participants with previous psychiatric diseases and chronic musculoskeletal disorders. In addition, an important limitation includes the fact that depression and anxiety were not systematically evaluated by psychiatrists. Furthermore, no power analysis was performed to determine the optimal sample size before the initiation of the study.

Further interventional researches should also address depression and anxiety given the impact of vitamin D on the psychological well-being in pregnancy. This is one of the few studies that have comprehensively evaluated the association of depression and anxiety and low vitamin D levels across pregnancy. Significant relationships were observed between BDI and BAI scores and vitamin D deficiency. Results of this study provided additional evidence that a strong relationship between poor sleep quality and low vitamin D levels is present, whereas BAI and BDI scores were correlated with PSQI scores as expected. Previous studies have not examined psychological symptoms and sleep quality among pregnant women beside the fact that depression is linked with sleep disorders. Risk for mood disorders was determined to be higher in vitamin D deficient group and this may influence the association of vitamin D levels and sleep quality; however, this study suggest the relationship between inadequate vitamin D and sleep disorders in pregnant women.

CONCLUSION

Vitamin D deficiency can have a negative impact on pregnant woman as it may induce depressive and anxiety symptoms and worsen sleep quality. High-quality studies in deficient populations with large sample sizes should be carried out to verify this relationship and determine the effect of vitamin D supplementation in sleep disorders among pregnant women and strengthen the evidence base on this topic.

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Informed Consent: All participants were informed about the research concept, and written informed consent was obtained from all participants.

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Evaluation of the Relationship Between Metabolic Syndrome, Visceral Adiposity Index and Lipid Accumulation Product in Patients with Obesity

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ABSTRACT

Objective: Visceral adiposity index (VAI) and lipid accumulation product index (LAPI) are the new methods to determine the visceral adiposity and to predict the cardiometabolic risks in patients with. In this study, it was aimed to determine whether VAI or LAPI could be a predictor for metabolic syndrome (MS) in obesity, and to evaluate their relationship with other biochemical and anthropometric parameters.

Methods: All patients who were admitted to the obesity outpatient clinic for the first time in January-February 2020 were included in the study. Age, gender, height, weight, body mass index, waist circumference (WC), hip circumference, waist/hip (W/H) ratio, biochemical parameters, and degree of hepatosteatosis were recorded. The presence of MS was determined according to the National Cholesterol Education Program Adult Treatment Panel-III criteria. VAI and LAPI were calculated according to fixed formulations. Results were evaluated by SPSS.

Results: A total of 49 subjects, (48 females), with obesity were included in the study. Thirty-two patients (65.3%) had MS. In the MS (+) group, fasting blood glucose (FBG), insulin resistance (HOMA-IR) and triglyceride (TG) levels, VAI, LAPI, diabetes mellitus and hypertension ratios were higher than the group with MS (-). A positive correlation was observed between VAI and LAPI. There was a positive correlation between the VAI and the TG value, and a negative correlation between the VAI and high density lipoprotein value. A positive correlation was observed between LBU and TG, FBG, HOMA-IR, WC, W/H ratios.

Conclusion: It is important to determine the comorbidities in obesity on a timely manner and to make the necessary interventions. With a simple formulation, VAI and LAPI can predict important health risks accompanying obesity.

Keywords: Obesity, metabolic syndrome, visceral adiposity index, lipid accumulation product index

INTRODUCTION

Obesity prevalence is increasing day-by-day in our country. It is a serious cause of morbidity and mortality, and puts a heavy burden on the health system. Therefore, it is vital to reveal the effects of obesity at an early stage and to take necessary precautions against the pathologies accompanying the obesity, as well as the treatment of it (1,2).

Perhaps the most important parameter to be evaluated in obesity is the visceral adiposity. Visceral adipose tissue has been shown to cause cardio-metabolic pathologies, and the visceral adiposity index (VAI) is valuable in determining the risk for these diseases. VAI can now be measured using methods such as bioelectrical impedance analysis, dual energy X-ray absorptiometry, computed tomography, and magnetic resonance. However, since these

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methods are not practical, they bring costs and some of them are not available in every hospital/obesity center, new methods are needed to determine visceral adiposity (1-3).

VAI and lipid deposition product index (LAPI) are also new methods used in this subject. Both are essentially mathematical models calculated by anthropometric data, and are used to show visceral adiposity, adipose dysfunction, insulin resistance (HOMA-IR), metabolic dysfunction, and cardio metabolic risk. Thus, with a simple formulation, they can predict the significant accompanying health risks and help take early action (4,5).

In this study, it was aimed to evaluate the relationship between VAI and LAP in patients with and without metabolic syndrome (MS) in obesity, and to determine whether they could be a predictor of MS, as well as to evaluate their relationship with other biochemical parameters, anthropometric measurements, and hepatosteatosis.

METHODS

All patients who were admitted to the obesity outpatient clinic for the first time in January-February 2020 were included in the study. Age, gender, height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist/hip ratio (WHR), biochemical parameters [fasting blood glucose (FBG), triglyceride (TG), high density lipoprotein (HDL), HOMA-IR], degree of hepatosteatosis (detected by abdominal ultrasonography), blood pressure arterial (TA) value, presence of diabetes mellitus (DM) and hypertension (HT) were recorded. Presence of MS was determined according to National Cholesterol Education Program Adult Treatment Panel-III (NCEP-ATP-III) criteria. VAI and LAPI were calculated according to the fixed formulations (Table 1, 2). The results were analyzed on SPSS.

The study was conducted in accordance with the 1964 Helsinki Declaration. Ethics committee approval was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (approval number: 2254, tarih: 27.04.2020) and informed consent was obtained from the patients.

Table 1. VAI Formulation for male and female patients

- Male VAI : $\left[\frac{WC\ (cm)}{\left\{39.68+(1.88\times BMI\left(\frac{kg}{m^2}\right))\right\}}\right] \times \left[\frac{TG(mmol/l)}{1.03}\right] \times \left[\frac{1.31}{HDL(mmol/l)}\right]$
- Female VAI : $\left[\frac{WC\ (cm)}{\left\{36.58+(1.89\times BMI\left(\frac{kg}{m^2}\right))\right\}}\right] \times \left[\frac{TG(mmol/l)}{0.81}\right] \times \left[\frac{1.52}{HDL(mmol/l)}\right]$

VAI: Visceral adiposity index, WC: waist circumference, BMI: body mass index, TG: triglyceride, HDL: high density lipoprotein

Table 2. LAPI Formulation for male and female patients

Male LAPI = [waist (cm)-65] × TG concentration (mmol/L)
Female LAPI = [waist (cm)-58] × TG concentration (mmol/L)

LAP: lipid accumulation product index, TG: triglyceride

Statistical Analysis

In the descriptive statistics of the data; mean, standard deviation, median lowest, highest, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov-Simonov test. Mann-Whitney U test and Independent Sample test were used in the analysis of quantitative independent data. Chi-square test was used in the analysis of categorical data, and Fisher's exact test was used when chi-square test conditions were not met. SPSS 26.0 program was used in the analysis.

RESULTS

A total of 49 people, 48 females and 1 male, with obesity were included in the study. Thirty-two patients (65.3%) had MS. The mean and median values of the data for the parameters are shown on Table 3.

Age, gender distribution, height, weight, WC, HC, WHR, BMI values of the patients with and without MS did not show a significant difference ($p>0.05$). In the group with MS (+), FBG, HOMA-IR and TG levels, VAI, LAPI, DM and HT ratios were significantly higher ($p<0.05$) than the group with MS (-). HDL values and hepatosteatosis degrees of the patients in the group with and without MS did not show a significant difference ($p>0.05$) (Table 4, Figure 1).

Significant positive correlation ($p<0.05$) was observed between the visceral adipocyte index and lipid deposition product. There was a significant positive correlation ($p<0.05$) between visceral adipocyte index and TG value, and a significant negative correlation ($p<0.05$) between visceral adipocyte index HDL value. There was no significant correlation ($p>0.05$) between VAI and age, FBG, HOMA-IR, BMI, WC, HC, WHR (Table 5).

A significant positive correlation ($p<0.05$) was observed between lipid deposition product and TG, FBG, HOMA-IR, WC, WHR values. There was no significant correlation ($p<0.05$) with age, HDL, BMI, and HC (Table 5).

The age, gender distribution, height and weight values of the patients in the group with hepatosteatosis grade (0-1) and grade (2-3) did not differ significantly ($p>0.05$). The BMI of the patients in the group with hepatosteatosis grade (2-3) was significantly higher ($p<0.05$) than the patients in the hepatosteatosis grade (0-1) group. WC, HC, WHR, FBG, HOMA-IR, TG, HDL, VAI, LAPI values, DM, HT, MS ratios values did not differ significantly ($p>0.05$) in the group with hepatosteatosis grade (0-1) and grade (2-3) (Table 6).

DISCUSSION

Obesity is the abnormal accumulation of fat in the body that poses a health risk (6). Most of the adipose tissue consists of white adipocytes, although there are also beige/brown adipocytes in humans. The white adipose tissue responsible for energy storage is mainly located under the skin. However, when visceral steatosis causes ectopic steatosis in the liver, heart and muscles, it causes low-level chronic inflammation, HOMA-IR, and consequently metabolic complications, in addition to cardiovascular diseases (7,8).

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Table 3. General data of the parameters investigated

		Min-Max	Median	Mean ± SD/ (n, %)
Age		20.0-64.0	51.0	49.1±10.8
Gender	Female	-	-	48 (98.0%)
	Male	-	-	1 (2.0%)
Height		117.0-177.0	160.0	160.0±8.9
Weight		67.0-134.0	97.0	99.6±18.3
Body mass index		28.8-53.3	39.1	38.7±6.4
Waist circumference		90.0-132.0	113.0	113.0±9.5
Hip circumference		106.0-167.0	130.0	130.1±12.2
Waist/hip ratio		0.7-1.0	0.9	0.9±0.1
Fasting blood glucose		79.0-187.0	99.0	105.0±21.5
HOMA-IR		0.1-7.6	2.5	2.6±1.5
Visceral adipocyte index		1.7-19.0	5.3	6.0±3.2
Lipid accumulation product		1792.0-18148	8250.0	8382.6±3563.4
Triglyceride		56.0-349.0	150.0	152.0±59.5
HDL		38.0-102.0	50.0	53.0±12.2
Diabetes	(-)	-	-	38 (77.6%)
	(+)	-	-	11 (22.4%)
HT	(-)	-	-	39 (79.6%)
	(+)	-	-	10 (20.4%)
Hepatosteatosis	0-1	-	-	17 (34.7%)
	2-3	-	-	32 (65.3%)
Hepatosteatosis grade	0	-	-	4 (8.2%)
	1	-	-	13 (26.5%)
	2	-	-	28 (57.1%)
	3	-	-	4 (8.2%)
Metabolic syndrome	(-)	-	-	17 (34.7%)
	(+)	-	-	32 (65.3%)

Min: minimum, Max: maximum, SD: standard deviation, HOMA-IR: insulin resistance, HDL: high density lipoprotein, HT: hypertension

MS is one of the diseases accompanying obesity. NCEP-ATP-III criteria are commonly used for identification purposes. Increase in WC, being diagnosed with TA or under HT treatment, high BG or being under DM treatment, high TG and low HDL are the diagnostic criteria. It is important to screen these criteria in individuals with obesity and to take the necessary precautions in the presence of MS in order to prevent obesity-related morbidity and mortality (8,9). In our study, MS is present in two-thirds of the patients, and FBG, HOMA-IR, TG level, VAI and LAPI were found to be high in this group.

VAI and LAPI are also new methods that have been used to determine visceral adiposity. It is a formulation that uses simple anthropometric measurements such as WC and BMI and biochemical parameters such as TG and HDL. Fixed values vary for men and women (5).

VAI was positively correlated with peripheral glucose use in euglycemic and hyperinsulinemic clamp studies. Many studies have been published showing that it is associated with type 2 DM, MS, cardiovascular diseases and polycystic ovary syndrome (10). In our study, in the group with MS, VAI was found to be higher than the group without MS and correlated with atherogenic dyslipidemia profile. However, no relationship was found between HOMA-IR and BMI. It has led to the thought that VAI has a stronger relationship with the diagnostic combination of the parameters that constitute the MS, rather than a single effect. As a matter of fact, different studies have shown the relationship between VAI and MS in support of this idea (11,12).

The LAPI is based on a formulation using WC and TG level. It was found to be more effective than BMI and WC in reflecting HOMA-IR and predicting cardiovascular disease risk (4,13). It is associated with glucose/insulin homeostasis and dietary pattern as well as anthropometric data and is an inexpensive alternative to indirect visceral adiposity measurement method (14). In the study conducted by Chiang and Koo (15), LAPI was seen to predict MS. Similarly, in our study, LAPI was found to be high in patients with MS.

VAI is usually high in hepatosteatosis, but there are studies showing that it is not directly related to steatosis and has poor diagnostic power in this regard. WC is seen as a stronger predictor for liver fattening (16,17). Although the degree of hepatosteatosis was found to be associated with BMI in our study, the MS was not found to be associated with VAI or LAPI in parallel with these data. However, it is thought that VAI may be a marker for adipose tissue dysfunction, especially in the absence of MS (18).

Study Limitations

The limited number of patients and the fact that the same parameters were not examined in individuals without obesity are the limitations of this study.

CONCLUSION

In individuals with obesity, VAI and LAPI are associated with MS and the parameters that constitute MS. It can be calculated with a simple formulation and can be used as a practical tool in determining the level of inflammation, adipocyte dysfunction, and the metabolic and cardiovascular risks.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital, Clinic Research Ethics Committee (approval number: 2254, date: 27.04.2020).

Informed Consent: Informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - F.A.; Concept - F.A., H.U.A., Ş.D.; Design - F.A., H.U.A., Ş.D.; Data Collection and/or Processing - F.A., Ş.D.; Analysis and/or Interpretation - F.A., Ş.D.; Literature Search - F.A., H.U.A., Ş.D.; Writing Manuscript - F.A., H.U.A.

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Table 4. Parameters investigated according to the presence of metabolic syndrome

		Metabolic syndrome (+)		Metabolic syndrome (-)		p	
		Mean ± SD/(n, %)	Median	Mean ± SD/(n, %)	Median		
Age		50.0±10.0	51.5	47.2±12.5	48.5	0.518	m
Gender	Female	31 (96.9%)	-	17 (100.0%)	-	1.000	X²
	Male	1 (3.1%)	-	0 (0.0%)	-		
Height		159.8±9.8	160.0	160.4±7.3	160.0	0.591	m
Weight		99.8±20.0	93.6	99.3±15.0	102.0	0.934	t
Body mass index		38.7±7.0	38.2	38.6±5.2	39.9	0.964	t
Waist circumference		113.6±10.4	113.5	111.9±7.9	113.0	0.556	t
Hip circumference		128.7±12.6	126.0	132.8±11.2	130.0	0.260	t
Waist/hip ratio		0.9±0.1	0.9	0.8±0.1	0.9	0.059	t
Fasting blood glucose		110.8±24.3	100.0	94.1±6.8	95.0	0.012	m
HOMA-IR		3.0±1.5	3.0	1.7±1.1	1.4	0.003	t
Visceral adipocyte index		6.8±3.4	6.3	4.6±2.4	3.8	0.026	t
Lipid accumulation product		9329.1±3448.3	8970.0	6600.9±3145.1	5170.0	0.009	t
Triglyceride		168.9±56.9	173.0	120.2±51.8	109.0	0.005	t
HDL		53.1±13.0	49.5	52.6±10.7	51.0	0.897	t
Diabetes	(-)	21 (65.6%)	-	17 (100.0%)	-	0.006	X²
	(+)	11 (34.4%)	-	0 (0.0%)	-		
HT	(-)	22 (68.8%)	-	17 (100.0%)	-	0.010	X²
	(+)	10 (31.3%)	-	0 (0.0%)	-		
Hepatosteatosis grade	0	2 (6.3%)	-	2 (11.8%)	-	0.487	X²
	1	8 (25.0%)	-	5 (29.4%)	-		
	2	20 (62.5%)	-	8 (47.1%)	-		
	3	2 (6.3%)	-	2 (11.8%)	-		

m: Mann-Whitney U test, X²: chi-square test, t: Independent Sample t-test, HOMA-IR: insulin resistance, HDL: high density lipoprotein, HT: hypertension, SD: standard deviation

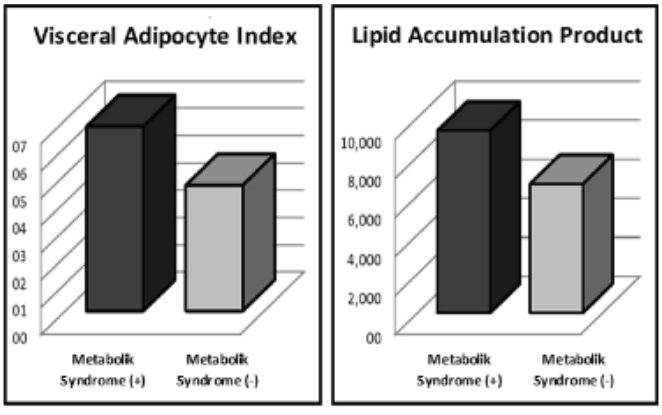

Figure 1. MS and VAI-LAPI box plot
MS: metabolic syndrome, VAİ: visceral adiposity index, LAPI: lipid accumulation product index

Table 5. VAI, LAP and research parameters

	Visceral adipocyte index		Lipid accumulation product	
	r	p	r	p
Lipid accumulation product	0.835	0.000	-	-
Age	0.046	0.755	0.137	0.355
Triglyceride	0.924	0.000	0.915	0.000
HDL	-0.603	0.000	-0.222	0.125
Fasting blood sugar	0.268	0.063	0.339	0.017
HOMA-IR	0.182	0.211	0.290	0.044
Body mass index	-0.208	0.152	0.116	0.429
Waist circumference	-0.037	0.800	0.372	0.009
Hip circumference	-0.160	0.274	0.069	0.636
Waist/hip ratio	0.227	0.117	0.389	0.006

Spearman Correlation.
HDL: high density lipoprotein, HOMA-IR: insulin resistance

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Table 6. Hepatosteatosis and research parameters

		Hepatosteatosis grade (0-1)		Hepatosteatosis grade (2-3)		p	
		Mean±SD (n, %)	Median	Mean ± SD/(n, %)	Median		
Age		48.7±10.4	50.0	49.3±11.2	51.0	0.746	m
Gender	Female	16 (94.1%)	-	32 (100.0%)	-	0.347	X^2
	Male	1 (5.9%)	-	0 (0.0%)	-		
Height		161.6±8.1	160.0	159.2±9.4	160.0	0.916	m
Weight		94.5±14.7	93.0	102.4±19.6	101.5	0.153	t
Body mass index		36.1±4.7	35.1	40.0±6.8	40.0	0.038	t
Waist circumference		110.4±7.1	112.0	114.4±10.4	113.0	0.159	t
Hip circumference		128.9±12.4	126.0	130.7±12.3	131.0	0.633	t
Waist/hip ratio		0.9±0.1	0.9	0.9±0.1	0.9	0.415	t
Fasting blood glucose		111.8±29.8	101.0	101.4±14.8	98.0	0.333	m
HOMA-IR		2.5±1.3	2.4	2.6±1.6	2.6	0.934	t
Visceral adipocyte index		6.1±4.0	4.6	6.0±2.8	6.0	0.903	t
Lipid accumulation product		7509.0±3576.1	7198.0	8846.7±3524.2	9247.0	0.214	t
Triglyceride		145.4±68.3	131.0	155.6±55.1	157.0	0.572	t
HDL		50.6±10.0	50.0	54.2±13.1	51.0	0.337	t
Diabetes	(-)	11 (64.7%)	-	27 (84.4%)	-	0.116	X^2
	(+)	6 (35.3%)	-	5 (15.6%)	-		
HT	(-)	12 (70.6%)	-	27 (84.4%)	-	0.254	X^2
	(+)	5 (29.4%)	-	5 (15.6%)	-		
Metabolic syndrome	(-)	7 (41.2%)	-	10 (31.3%)	-	0.487	X^2
	(+)	10 (58.8%)	-	22 (68.8%)	-		
Hepatosteatosis grade	0	4 (23.5%)	-	0 (0.0%)	-	0.000	X^2
	1	13 (76.5%)	-	0 (0.0%)	-		
	2	0 (0.0%)	-	28 (87.5%)	-		
	3	0 (0.0%)	-	4 (12.5%)	-		
	ı	-	-	-	-		

m: Mann-Whitney U test, X^2: chi-square test, t Independent Sample t-test, SD: standard deviation, HOMA-IR: insulin resistance, HDL: high density lipoprotein, HT: hypertension

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Could Chest Computed Tomography Scores Assess the Inflammatory Markers and Disease Severity of Coronavirus Disease-2019 Patients?

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ABSTRACT

Objective: This study aimed to investigate the relationship between chest computed tomography (CT) scores and inflammatory markers in patients with coronavirus disease-2019 (COVID-19).

Methods: A total of 259 patients who were confirmed to be COVID-19 positive with polymerase chain reaction test and CT findings, between 20 March and 30 May 2020, were included in our study. The patients were divided into two groups as critical and moderate according to their clinical conditions. CT findings, complete blood account and C-reactive protein (CRP) were recorded and statistically analysed. Receiver operating characteristic (ROC) curves were plotted in terms of CT scores, white blood cells, neutrophil/lymphocyte ratio (NLR) and CRP for the patients in the moderate and critical groups.

Results: CT scores ($p<0.001$), neutrophil counts ($p<0.001$), NLR ($p<0.001$) and CRP values ($p<0.001$) were significantly higher in the critical group than in the moderate group, whereas the lymphocyte count ($p<0.001$) and haemoglobin values ($p=0.003$) were significantly lower. In the ROC curve analysis, when the cut-off value was set at 6.5 to differentiate the moderate and critical groups, the CT score sensitivity was 0.86, specificity 0.809, area under the curve 0.899 and accuracy 0.81.

Conclusion: There is a correlation between CT scores and NLR. It is crucial to differentiate patients in critical and moderate conditions for the treatment planning of COVID-19. In addition to clinical findings and inflammatory markers, radiological imaging is an effective method to identify patients and determine the prognosis.

Keywords: Chest computed tomography, COVID-19, neutrophil/lymphocyte ratio, CT score

INTRODUCTION

The World Health Organization (WHO) China Country Office first reported cases of pneumonia with unknown origin in Wuhan, China, on 31 December 2020. In the following days, it was announced that the cause of pneumonia is a new coronavirus that has not been previously detected in humans, and the disease was named as coronavirus disease-2019 (COVID-19) (1-3). Because of its rapid transmission from person to person, COVID-19 has

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crossed the borders of China and caused a pandemic, and according to WHO's COVID-19 epidemiologic update in January 2021, it has infected 96 million people worldwide and caused 2 million deaths.

The factor of COVID-19 mostly affects the respiratory system in humans, and the spectrum of the illness severity ranges from a simple cold to severe respiratory failure (2,4-6). It has been reported that it may involve enteric, hepatic, nephrotic and neurological systems as well as the respiratory system (4-6).

The diagnostic reference of COVID-19 is the examination of the obtained respiratory tract samples by reverse-transcription polymerase chain reaction (PCR) (2,3). However, insufficient sampling, sampling too early or in the late phase of infection and sampling with inappropriate method may cause false-negative results (6). In possible/definite COVID-19 cases, chest computed tomography (CT) is used to support diagnosis, demonstrate lung involvement and evaluate the extent of lung infection (7-10). The most frequent CT findings of patients are peripherally located ground-glass density and vascular thickening, and their CT findings may be positive, although the PCR test results are negative (4,8,10,11). In a recent study, the sensitivity of chest CT (98%) was higher than that of the PCR test (10).

There are four clinical stages of the disease [early stage (days: 0-4)], progression period (days: 5-8), peak stage (days: 9-13) and resolution phase (after day: 14). Radiological findings may vary according to these stages (11-13). The distinction of the critical group from the non-critical group according to the patients' clinical manifestations is important for treatment choice and prognosis (14-16). There are publications examining the relationship between CT findings of the disease and its clinical manifestations and prognosis (10,13-15,17). However, studies comparing radiological findings with laboratory findings are limited (17,18). This study aimed to investigate the role of patients' lung involvement score to distinguish between the moderate and critical groups of patients and to assess which laboratory markers are correlated with the chest CT involvement score.

METHODS

This retrospective study was approved by the Local Ethics Committee University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital (approval number: 89, approval date: 28.05.2020) and the Republic of Turkey Ministry of Health, COVID-19 Scientific Research Committee. Written informed consent was obtained from the patients enrolled. Between 20 March and 30 May 2020, 330 patients between 18 and 90 years old who were admitted to our hospital with suspicion of COVID-19 pneumonia and underwent chest CT were retrospectively reviewed using picture archiving communication systems. Of these patients, those with positive COVID-19 PCR test results and positive COVID-19 CT images with appropriate imaging protocol (n=259) were included in the study. Those who had negative PCR test results and had no evidence of typical COVID-19 pneumonia (n=71) were excluded from the study. A total of 259 patients were included in the study. Laboratory findings, complete blood count (number/μL) and CRP (mg/dL), presence of comorbidities [hypertension, diabetes mellitus (DM), heart failure, renal failure, malignancy history and chronic respiratory diseases] at the first hospital admission and clinical findings of the patients were obtained from the hospital archive. Care was taken to ensure that the laboratory data and CT images were acquired on the same day at the time of admission.

In terms of their clinical features at the time of hospital admission, patients were divided into two groups: moderate and critical [according to the guideline on COVID-19 (trial version 5) issued by the China National Health Commission] (19). The moderate group was composed of patients with fever, cough or shortness of breath only, whereas the critical group comprised those with Tachypnea (>30 breaths per minute), with less than 93% oxygen saturation at room environment or with PaO_2/FiO_2 <300 mmHg and with mechanical ventilation and intensive care requirement.

All images were acquired using a multidetector scanner (GE Optima CT660, 64 slices; General Electric, USA, 5 mm collimation) with the following parameters: 120 kVp, 40 mm beam collimation, 5 mm image thickness, 500 mm reconstruction field of view, 500 mA (maximum), 10 mA (minimum) and 1 s rotation time. Images were evaluated by two radiologists, "Yasemin Kayadibi and Neşe Uçar" with 9 and 11 years of chest radiology experiences, respectively. Image examination parameters such as ground-glass opacity, consolidation, vascular thickening, interlobular septal thickening, mediastinal lymphadenopathy, pleural effusion, subpleural bands and pericardial effusion were evaluated. CT images were scored from 1 to 4 (1: 0%-25%; 2: 26%-50%; 3: 51%-75%; 4: 76%-100%) for each lobe according to the semiquantitative scoring system described in the literature (9,10,14,15). Scores determined for all lobes were summed, and the total CT score (from 0 to 20) was determined by the consensus of the two radiologists (Yasemin Kayadibi and Neşe Uçar).

Statistical Analysis

The statistical software SPSS version 22.0 IBM was used for data analysis. As descriptive analyses, frequency rates and percentages were described for the categorical variables and means and standard deviations for the continuous variables. Normality was checked using the Shapiro-Wilk test. The Mann-Whitney U and Student's t-tests were used for non-normally and normally distributed variables, respectively. Receiver operating characteristic (ROC) was used to determine the cut-off values, sensitivity and specificity. The chi-square test was used to test relationships between categorical variables. A p-value of <0.05 was considered statistically significant. For the correlation analysis, the Spearman test was used as a non-parametric test.

RESULTS

Patients Analysis

Table 1 presents patient analysis details. A total of 259 patients were included in the study. Patients whose clinical and laboratory

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Table 1. Demographic features of patients

Parameters	Moderate (n=173)	Critical (n=86)	Total (n=259)
Mean age (years)	53.2	63.18	56.52
Sex (n, %)			
Men	80 (46.2)	57 (66.3)	122 (47)
Women	93 (53.8)	29 (33.7)	137 (53)
Comorbidities (n, %)	117 (67.6)	86 (100)	208 (80)
Hypertension	50 (43)	33 (38.4)	85 (41)
Diabetes mellitus	30 (25.6)	21 (24.4)	53 (25.5)
Heart disease	11 (9.4)	12 (14)	24 (11.5)
Respiratory disorders	19 (16)	8 (9.3)	27 (13)
Renal dysfunction	5 (4.3)	9 (10.4)	14 (6.7)
Malignancy	2 (1.7)	3 (3.5)	5 (0.9)

findings could not be reached, who had negative PCR results and those with tomography images having artefacts were excluded in the study. Of patients, 122 (80 moderate and 57 critical) were men and 137 (93 moderate and 29 critical) women. Men dominated the critical group (p=0.02), whereas no gender dominated the moderate group. The mean ages of patients were 53.2 and 63.18 years in moderate and critical groups, respectively. Comorbidity was present in 208 of 259 patients.

Radiological Findings

All CT images were obtained at the time of patients' first hospital admission. The summary of radiological and laboratory findings of the patients is presented in Table 2. Considering the radiological findings, a comparison of the two groups revealed that the lower lobes were statistically significantly higher in the severe group in which the disease affected more than one lobe. There was no significant difference in terms of right and left lung involvement in both groups (p=0.684). The right lower lobe was the most commonly involved lobe (93%). There was no significant difference between the number of lobes involved in both groups (p=0.152). The detection rate of ground-glass opacity was similar in both groups (p=0.792); however, the prevalence of consolidation and crazy paving were higher in the critical group (p<0.001 for both). Central involvement between the two groups was significantly higher in the critical group (p<0.001). Pleural effusion and subpleural band formation were observed significantly higher in the critical group (p<0.001 and p=0.005, respectively). The total mean CT score of the moderate and critical groups were 4.8 and 10.8, respectively. The CT score in the critical group was significantly higher than in the moderate group according to the Mann-Whitney U test (p<0.001). ROC curve analysis revealed that for a cut-off value of 6.5, the CT score had a sensitivity of 0.86, specificity 0.809, area under the curve (AUC) 0.899 and accuracy 0.81 (with 95% confidence interval: 0.854-0.944; Figure 1, 2).

Laboratory Findings

Table 3 presents the curves obtained from laboratory findings. The mean white blood cell (WBC) value was 6,845 in the total patient group, 6,335 in the moderate group and 8,335 in the critical group. WBC values were statistically significant (p<0.001) between the critical and moderate groups according to the Mann-Whitney U test after normality evaluation using the Shapiro-Wilk test (p<0.001). Increased neutrophil count, decreased lymphocyte count and increased CRP amount were statistically significantly higher in the critical group (p<0.001) according to the chi-square test. There was no significant difference in haemoglobin and platelet levels between the groups (p=0.003 and 0.265, respectively). Neutrophil/lymphocyte ratio (NLR) was statistically significantly higher in the critical group than in the moderate group (p<0.001) according to the Mann-Whitney U test.

Evaluation of the Relation between CT Scores and Laboratory Findings

When the correlation between CT scores and laboratory findings was compared, it was found that there was a positive correlation between WBC (r=0.341), neutrophil count (r=0.457), NLR (r=496), CRP (r=0.750) and CT scores, whereas there was a negative correlation between CT scores and lymphocyte counts (r=-0.315). The highest correlation was found between CRP and CT scores (Figure 3, Table 4). Spearman test was used for correlation analysis because of the value of p<0.001 obtained as a result of the normality calculation performed using the Shapiro-Wilk test.

DISCUSSION

Chest CT plays an important role in the diagnosis and follow-up of COVID-19 treatment (10). Many studies examine the relationship between the CT findings of the disease and its clinical manifestation (7,12,16,20,21). In the literature, areas of ground-glass density, crazy-paving pattern and vascular congestion, which are the most common findings observed in COVID-19 pneumonia, are predicted to reflect the virus-related septal and alveolar damage, the less frequently observed consolidations reflect the exudate in alveoli and hyaline membrane formation. A limited number of autopsy studies also support these findings (13,20-22). Similarly, in our study, the most common finding observed in the whole patient population was ground-glass density areas involving more than one lobe, but consolidations were statistically significantly higher in the critical group.

In COVID-19 pneumonia, a better prognosis is expected in the moderate group, whereas worse prognosis is likely in the critical group. It has been shown that early distinction between the two groups and early treatment has increased the survival rates significantly. CT scoring systems were developed to calculate the radiological involvement of the disease, and a significant correlation was found between the high score, clinical conditions and laboratory findings (8,12,15,17,18,20). In our study, we aimed to investigate the relationship between lung involvement severity and inflammatory markers in patients diagnosed with COVID-19 and the role of CT score in differentiating clinical severity. Li et al. (20), Liu et al. (15) and Francone et al. (17) reported the cut-off values for CT scores at 7, 4 and 18, respectively, to distinguish the moderate and critical groups. In our study (n=259), this value was

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Table 2. Summary of chest CT and laboratory findings

	Moderate (n=173)	Critical (n=86)	Total (n=259)	p
Chest CT findings				
Right upper lobe (n, %)	131 (76)	82 (95)	213 (82)	<0.001**
Right middle lobe (n, %)	109 (63)	81 (94)	190 (73)	<0.001**
Right lower lobe (n, %)	160 (93)	82 (95)	242 (93)	0.439
Left upper lobe (n, %)	132 (76)	79 (92)	211 (82)	0.002**
Left lower lobe (n, %)	145 (84)	84 (98)	229 (88.4)	0.001**
Single lobe (n, %)	12 (6.9)	2 (2)	14 (5.4)	0.152
Multiple lobes (n, %)	161 (93)	84 (98)	245 (95)	0.152
Total CT score (mean ± SD)	4.8±0.17)	10.8±0.45)	6.8±4.12)	<0.001**
GGD (n, %)	168 (97)	84 (98)	252 (97)	0.792
Consolidation (n, %)	65 (38)	57(66)	122 (47)	<0.001**
Crazy-paving pattern (n, %)	39 (23)	46 (54)	85 (33)	<0.001**
Round (n, %)	131 (76)	64 (74)	195 (75)	0.879
Patchy (n, %)	104 (60)	76 (88)	180 (70)	0.001**
Peripheric distribution (n, %)	153 (88.4)	86 (100)	239 (92)	0.001**
Central distribution (n, %)	64 (37)	67 (78)	131 (51)	0.001**
Peripheral + central (n (%))	60 (35)	67 (78)	127 (49)	0.001**
Single focus (n, %)	12 (7)	0 (0)	12 (4.6)	0.01*
Multiple foci (n, %)	161 (93)	86 (100)	247 (95.4)	0.01*
Halo sign (n, %)	38 (22)	19 (22)	57 (22)	0.981
Reverse halo sign (n, %)	14 (8)	9 (10.5)	23 (9)	0.643
Vascular thickening (n, %)	51 (29.5)	34 (40)	85 (34)	0.123
Pleural effusion (n, %)	1 (0.6)	12 (14)	13 (5)	0.001**
Lymph node enlargements (n, %)	7 (4)	7 (8)	14 (5.4)	0.241
Subpleural bants (n, %)	37 (21.4)	33 (38)	70 (27)	0.005*
Three in bud appearance (n, %)	7 (4)	6 (7)	13 (5)	0.367
Dilatation of peripheral airways (n, %)	20 (12)	15 (17.4)	35 (14)	0.246
Fibrous stripe (n, %)	31 (18)	22 (25.6)	53 (21)	0.190
Laboratory findings				
WBC count (mean ± SD)	6.1±0.21)	8.3±0.39)	6.85±3.22)	<0.001**
Increased WBC (n, %)	7 (4)	19 (22)	26 (10)	<0.001**
Neutrophil count (mean ± SD)	4.14±0.18)	6.84±0.38)	5.04±3.09)	<0.001**
Increased neutrophil (n, %)	8 (4.6)	25 (29)	33 (13)	<0.001**
Lymp count (mean ± SD)	1.41±0.05)	1.6±0.39)	1.5±2.1)	<0.001**
Decreased lymp (n, %)	45 (26)	47 (55)	92 (36)	<0.001**
NLR	3.53±0.21)	8.74±0.94)	5.3±6.02)	<0.001**
Platelet count (mean ± SD)	197.59±5.47)	212.57±9.99)	202.56±79.39)	0.256
Increased platelet (n, %)	2 (1.2%)	2 (2%)	4 (1.5%)	0.602
Hb (mean ± SD)	133.65±1.32)	126.93±1.82)	131.42±17.48)	0.003*
Decreased Hb (n, %)	12 (7)	12 (14)	24 (9)	0.073
CRP (mean ± SD)	58.74±10.97)	135±13.49)	84.07±142.53)	<0.001**
İncreased CRP (n, %)	146 (84.4)	86 (100)	232 (90)	<0.001**

The p-values for CT scores and WBC were calculated using the Mann-Whitney U test. The p-values for other parameters were calculated using the Pearson chi-square test. *P<0.05, **p<0.001.
CT: computed tomography, GGD: ground-glass appearance, WBC: white blood cells, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, Lymp: lymphocyte, Hb: haemoglobin, SD: standard deviation

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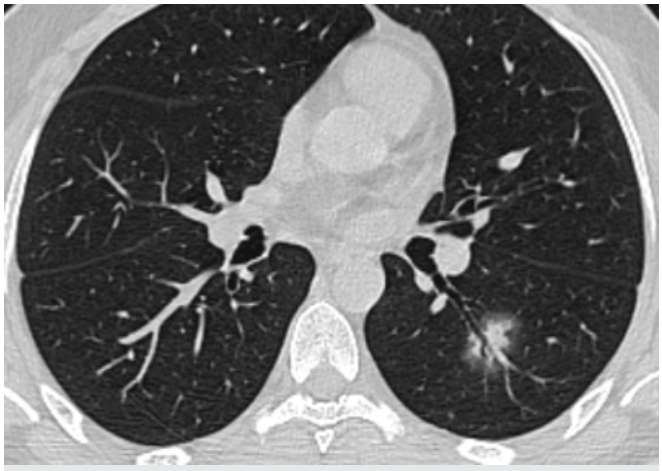

Figure 1. Chest computed tomography (CT) example of the moderate group: a 30-year-old man (CT score: 1, neutrophil/lymphocyte ratio: 2.5, C-reactive protein: 3.5). The axial CT image revealed a single focus of ground-glass opacity with adjacent dilated bronchus

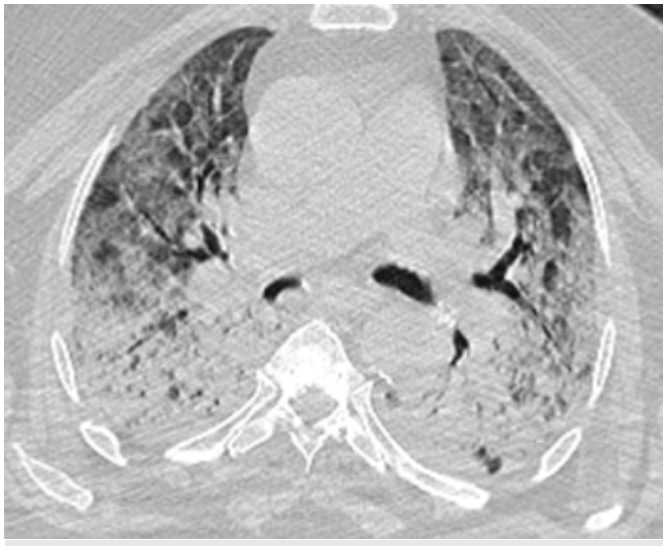

Figure 2. Chest computed tomography (CT) example of the critical group: A 79-year-old man (CT score: 19, neutrophil/lymphocyte ratio: 12.5, C-reactive protein: 190). Axial CT image revealed bilateral diffuse ground-glass opacities and consolidations with dilated bronchi and air bubbles

6.5 (AUC: 0.899, specificity: 0.809, sensitivity: 0.86 and accuracy: 0.81). In addition to the clinical and laboratory data of the patient, this study has demonstrated that chest CT findings are also useful in the distinction of disease severity.

Many alterations occur in the immune and haematopoietic systems of patients diagnosed with COVID-19. In the literature, various laboratory findings including lymphopenia and elevated liver enzymes, lactate dehydrogenase, inflammatory markers (e.g. CRP and ferritin), D-dimer (>1 mcg/mL), prothrombin time, troponin and creatine phosphokinase have been associated with poor prognosis in COVID-19 (14,15,17,20). Immune cell destruction, impaired immune cell function, decreased lymphocyte level as a result of bone marrow involvement and simultaneous neutrophil dominance are the most striking findings in the haematological system in COVID-19 (22-25). NLR, as a marker of inflammatory response, has been associated with poor prognosis in sepsis, cardiovascular diseases and malignancies (4,14,23-25). Fu et al. (26) found a significant difference between the mild/moderate and severe groups in terms of WBC, NLR, lymphocyte counts, D-dimer levels and fibrinogen levels. Among these parameters, the highest AUC was 0.88, which was the most significant parameter for NLR. They also argued that the change in haematological parameters is an earlier and more reliable change than inflammatory markers (26). The study by Lagunas-Rangel (27) also supported the statistically significant increase in NLR in severe patients.

In our study, the CRP level, neutrophil count and NLR in the critical group increased statistically significantly than in the moderate group ($p<0.001$ for all), whereas the lymphocyte count decreased statistically significantly ($p<0.001$). In the ROC curve analysis, it was calculated as 47.9 mg/dL (AUC: 0.812) for CRP, 6.13 n/μL (AUC: 0.777) for WBC, 4.38 n/μL (AUC: 0.757) for neutrophil count, 1.04 n/μL (AUC: 0.683) for lymphocyte count and 3.75 (AUC: 0.792) for NLR. In addition, a positive correlation was noted between CT scores and CRP, WBC, neutrophil count and NLR and a negative correlation with lymphocyte count (correlation coefficients: 0.496, 0.341, 0,457, 0.496 and -0.315, respectively). Our study showed that the CT score in patients' initial tomography was significantly related to the patient's inflammatory response and NLR. Our study is also compatible with studies in the literature that reveal the correlation between CRP, D-dimer, NLR and decreased lymphocyte count and CT scores (17,18,25-27).

Table 3. Cut-off values and statistical analysis in distinguishing the moderate and critical groups

	Cut-off value	AUC (CI: lower-upper)	Specificity	Sensitivity	Accuracy
Total CT Score	6.5	0.899 (0.854-0.944)	0.809	0.86	0.81
WBC (n/μL)	6.13	0.712 (0.646-0.779)	0.601	0.686	0.629
Neu (n/μL)	4.38	0.773 (0.712-0.834)	0.659	0.709	0.675
Lymp (n/μL)	1.04	0.683 (0.613-0.752)	0.593	0.699	0.664
NLR	3.74	0.792 (0.729-0.855)	0.74	0.802	0.76
CRP	47.9	0.812 (0.758-0.866)	0.682	0.802	0.722

CT: computed tomography, WBC: white blood cells, Neu: neutrophil, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, Lymp: lymphocyte, AUC: areas under the curve, CI: confidence interval

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Table 4. Statistical analysis of the correlation between laboratory findings and CT scores

	CT scores	
	r	p
WBC	0.341	<0.001
Neu	0.457	<0.001
NLR	0.496	<0.001
CRP	0.750	<0.001
Lymp	-0.315	<0.001

Spearman test (r) was used for correlation analysis.
CT: computed tomography, WBC: white blood cells, Neu: neutrophil, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, Lymp: lymphocyte

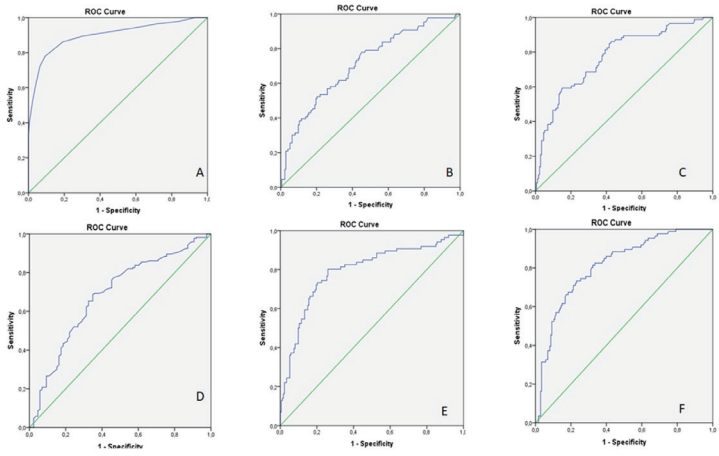

Figure 3. Receiver operating characteristic analysis for (a) computed tomography score, (b) white blood cells, (c) neutrophil count, (d) lymphocyte count, (e) neutrophil/lymphocyte ratio and (f) C-reactive protein
ROC: Receiver operating characteristic

Although COVID-19 is known to cause infection in completely healthy adults, more serious involvement is observed in adults with advanced age and comorbidities such as cardiovascular diseases, DM, hypertension, chronic lung diseases, cancer and chronic kidney disease (15,17,18,21,23). In our study, the comorbidity rate was higher in the critical group than in the moderate group, and the most common comorbidity was hypertension, followed by DM.

Study Limitations

Our study has some limitations. Our study was single-centred and retrospective. There are four clinical stages of the disease [early stage (days: 0-4), progression period (days: 5-8), peak stage (days: 9-13) and resolution phase (after day: 14)], and radiological findings may vary according to these stages. However, in our study, it was impossible to obtain the information from the patients' records about which date the patients were admitted to the hospital after the onset of the disease and at which stage of the disease the chest CT was obtained. Except for patients who did not worsen, control follow-up CT was not requested, and follow-up was generally conducted by chest radiography. Patients who have positive PCR with no radiological findings, have negative PCR with positive radiological findings and did not require hospitalisation were excluded in the study, and therefore, we may have caused bias in patient selection in our study.

CONCLUSION

The demographic data of our study are compatible with other studies investigating the correlation between CT scores and NLR among the studies conducted to date are limited in number. Many clinical parameters are used to determine the critical patient group in COVID-19 pneumonia. In our study, we showed that the findings observed in chest CT reflect the severity of the disease at the time of admission. We found a positive correlation between CT scores and NLR, which has a prognostic side, and a determined cut-off score of 6.5 to distinguish the moderate and critical groups. We think that it can guide clinicians to determine the severity of involvement and choose an effective treatment according to the severity of the disease.

Ethics Committee Approval: This retrospective study was approved by the Local Ethics Committee University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital (approval number: 89, approval date: 28.05.2020).

Informed Consent: Written informed consent was obtained from the patients enrolled.

Peer-review: Externally peer-reviwed.

Author Contributions: Surgical and Medical Practices - B.D.; Concept - N.U.; Design - Y.K.; Data Collection and/or Processing - N.U., M.F.K., O.G.; Analysis and/or Interpretation - Y.K.; Literature Search - Y.N.A.; Writing Manuscript - Y.K.

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

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The Risk Factors of Unplanned Hospital Readmission Following Percutaneous Nephrolithotomy

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ABSTRACT

Objective: Percutaneous nephrolithotomy (PNL) is currently recommended as the first treatment option for complex kidney stones larger than 2 cm. Although it is a safe and effective treatment, emergency room admissions and hospitalisations occur because of PNL complications. This study aimed to examine patient-related independent risk factors that may cause unplanned hospital readmission after PNL.

Methods: Data of patients who were admitted to our clinic due to upper urinary system calculi and underwent conventional PNL operation between January 2015 and December 2019 were evaluated retrospectively. Hospital readmission was defined as unscheduled rehospitalisation within 30 days after discharge. The study cohort was divided into two groups: the readmission (group A) and non-readmission groups (group B). Post-operative complication scoring was performed according to the Clavien-Dindo classification. Multivariate logistic regression analysis was used to evaluate independent prognostic risk factors on readmission after PNL.

Results: A total of 390 patients were included in our study. Of patients, 24 (6.1%) were readmitted to our clinic in the post-operative period. Gender, age, body mass index and stone volume were statistically similar between the groups. Based on multivariate analysis, the presence of Clavien 3a and 3b complications, post-operative blood transfusion, presence of preoperative hydronephrosis, American Society of Anaesthesiologist score and low stone density (<859 Hounsfield units) were determined as significant independent risk factors for readmission.

Conclusion: Determination of preoperative risk factors will reduce hospital readmissions rates, thus reducing the potential burden on the health system and increasing patient comfort.

Keywords: Percutaneous nephrolithotomy, patient readmission, risk factors

INTRODUCTION

Percutaneous nephrolithotomy (PNL) is currently recommended as the first treatment option for complex kidney stones larger than 2 cm (1). It is also used for extracorporeal shockwave lithotripsy treatment-resistant lower pole stones bigger than 1 cm and patients with anatomical variation (2). Since the first use of PNL in 1976, a comprehensive modification has been performed to decrease post-operative complications, pain, hospital stay length and hospital readmission rate (3). In addition to technological improvements in endoscopic devices (flexible pyeloscope, flexible urethroscope, etc.), improvements in lithotripsy techniques increased the success of PNL by up to 90% (4).

With its high success and low complication rates, PNL is regarded as a safe treatment method. Complications associated with PNL are generally related to perioperative neighbouring organ

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injuries (lung, pleura, colon, spleen, etc.), post-operative bleeding and urinary system infection (5). Because of unpredictable complications, emergency room (ER) admissions and unplanned hospital readmissions may occur in the post-operative period (6). In many countries, as an objective measure of the quality of health care, unplanned post-surgery readmission is considered. This situation causes both a decrease in the quality of life of patients and a severe financial burden on the healthcare system (7). More studies have been found in the literature on readmissions after urological surgeries and their causes in recent years (8,9). These studies include general urological surgical procedures, and there are limited studies examining potential predictive factors for endourology and stone surgery (10,11). This study aimed to examine patient-related independent risk factors that may increase the unplanned hospital readmission possibility after PNL.

METHODS

Data of 485 patients over the age of 18 years who were admitted to our clinic with upper urinary system calculi and underwent PNL operation between January 2015 and December 2019 were analysed retrospectively. The study protocol was approved by the local ethics committee of the Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee, and the study was conducted according to the Declaration of Helsinki (approval number: 2020-17, approval date: 24.08.2020). Written informed consent, approved by our institutional review board, was obtained from all patients.

Hospital readmission was defined as unscheduled rehospitalisation within 30 days after discharge. Patients with a history of malignancy, concomitant surgery or readmitted to the hospital for other reasons were excluded from the study. Post-operative complication scoring was conducted according to the Clavien-Dindo classification (12).

Patients were evaluated using contrast-enhanced computed tomography (CT) and/or intravenous pyelography, which were taken before the operation. The preoperative total volume of stone (cm^3) was calculated from axial images with coronal reconstructions of non-contrast CT scan with length×height×width×π×1/6 formula (13). Urine cultures of all patients were sterile preoperatively. Second-generation cephalosporin prophylaxis was given to all patients before the operation. All procedures were performed under general anaesthesia in the prone or supine position by three different surgeons with similar PNL experiences (Y.O.D., M.G.Y. and F.A.A.). Calculi were fragmented through pneumatic lithotripter (Vibrolith®, Elmed, Ankara, Turkey) using 24 Fr nephroscope (Karl Storz GmbH & Co. KG, Tuttlingen, Germany) following ultrasonography-guided fluoroscopic access and extracted with the help of forceps. In all cases, a 14 Fr nephrostomy tube was placed in the renal pelvis or the involved calyx. If there were no contraindications, they were removed at the 24th hour postoperatively. A routine ureteral catheter is not placed unless there are indications such as pelvic or ureter injury, high volume residual fragment or obstruction. In cases where they are required, ureteral catheters are removed 2-3 weeks later. Stone-free status of the patients was evaluated using direct urinary system graphy in the post-operative early period and by low dose non-contrast abdominal CT in the third month.

Statistical Analysis

Categorical data were presented as numbers and percentages. Data on continuous variables are presented as mean and standard deviation. Dependent t-test and Mann-Whitney U tests were used to compare the mean differences of normally and non-normally distributed data, respectively. Frequencies of categorical variables were compared using the Pearson chi-square test. A p-value of <0.05 was considered statistically significant. Logistic regression analysis was performed to determine readmission predictors after PNL. Multivariate logistic regression analysis was used to evaluate independent prognostic risk factors on readmission after PNL. Statistical analysis was performed using the Statistical Package of Social Sciences version 21 (IBM SPSS Statistics; IBM Corp., Armonk, USA).

RESULTS

A total of 390 patients were included in the study. The characteristics and demographic data of the patients are presented in Table 1. Of patients, 24 (6.1%) were readmitted to our clinic in the post-operative period. The reasons for readmission after surgery were renal colic (n=7, 29.1%), urinary tract infection (n=6, 25%), haematuria (n=6, 25%), leakage (n=2, 8.3%), ureteral stent displacement (n=1, 4.1%), urinary retention (n=1, 4.1%) and urinothorax (n=1, 4.1%). The study cohort was divided into two groups: The readmission group (group A; n=24) and non-readmission group (group B; n=366).

Group A consisted of 20 (83.3%) male and 4 (16.7%) female patients with a mean age of 44.8±17 years and group B of 238 (65%) male and 128 (35%) female patients with a mean age of 44±13.8 years. The mean stone volumes were 2,630±1,171 and 2,311±1,036 mm^3 in groups A and B, respectively. No significant differences were noted between the two groups in gender, age, body mass index and stone volume ($p>0.05$). In the preoperative evaluation, the mean stone density was 710.7±451.2 Hounsfield units (HU) in group A and 1004.8±324.7 HU in group B ($p=0.001$).

In all PNL procedures, 103 (26.4%) were performed in the supine position and 287 (73.6%) in the prone position. A total of 21 (27.2%) and 56 (72.8%) complications were observed in patients who underwent supine and prone PNL, respectively. In group A, PNL was performed in 7 (29.1%) and 17 (70.9%) patients in the supine and prone positions, respectively. The positions in the procedures have no statistically significant difference in complication and rehospitalisation rates ($p=0.8$ and 0.752, respectively). The rate of patients with Clavien 3a and 3b complications was statistically higher in group A than in group B (25% vs 3.3%; $p<0.001$). In both groups, the American Society of Anaesthesiologists (ASA) score was significantly higher in group A ($p=0.047$). The proportion of patients with ASA III score was 62.5% in group A and 6.6% in

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Table 1. Comparison of patients' characteristics according to readmission

Variables	Group A (readmission)	Group B (no readmission)	p-value
Number of patients	24	366	
Sex, n (%)			
Male	20 (83.3)	238 (65.0)	0.066[&]
Female	4 (16.7)	128 (35.0)	
Mean age ± SD (years)	44.8±17	44±13.8	0.802*
Mean BMI ± SD (kg/m^2)	25.6±1.4	26.8±3.2	0.076*
Mean stone volume (mm^3)	2,630±1,171	2,311±1,036	0.148*
Mean stone density ± SD (HU)	771.1±290.8	1004.8±324.7	0.001*
Mean HD ± SD (day)	4.1±2.1	3.6±1.5	0.373*
Mean operation time ± SD (min)	75.7±12.8	80.6±18.2	0.143**
ASA score, n (%)			<0.001[&]
1	4 (16.7)	136 (36.4)	ASA 3 vs ASA
2	5 (20.8)	214 (57.2)	1-2
3	15 (62.5)	24 (6.4)	<0.001[&]
Post-operative complication, n (%)			
Clavien 0	3 (12.5)	310 (84.7)	<0.001
Clavien 1	5 (20.8)	34 (9.3)	
Clavien 2	10 (41.7)	10 (2.7)	
Clavien 3a and 3b	6 (25)	12 (3.3)	
Post-operative blood transfusion, n (%)			
Positive	14 (58.3)	20 (5.5)	<0.001[&]
Negative	10 (41.7)	346 (94.5)	
Preoperative hydronephrosis, n (%)			
Positive	15 (62.5)	132 (36.1)	<0.001[&]
Negative	9 (37.5)	234 (63.9)	
Residual stone, n (%)			
Positive	7 (29.2)	76 (20.8)	0.330[&]
Negative	17 (70.8)	290 (79.2)	
Staghorn calculi, n (%)			
Positive	4 (16.7)	44 (12.0)	0.502[&]
Negative	20 (83.3)	322 (88.0)	
Preoperative culture positive, n (%)			
Positive	6 (25.0)	20 (5.6)	<0.001[&]
Negative	18 (75.0)	338 (94.4)	
Post-operative fever, n (%)			
Positive	2 (8.3)	50 (13.8)	0.446[&]
Negative	22 (91.7)	312 (86.2)	
Preoperative nephrostomy catheter, n (%)			
Positive	2 (8.3)	18 (5.0)	0.472[&]
Negative	22 (91.7)	344 (95.0)	
Preoperative ureteral stent, n (%)			
Positive	4 (16.7)	50 (13.8)	0.446[&]
Negative	20 (91.7)	312 (86.2)	
Open stone surgery history, n (%)			
Positive	0 (0)	48 (13.1)	0.058[&]
Negative	24 (100)	318 (86.9)	
Endourologic surgery history, n (%)			
Positive	12 (50)	114 (31.1)	0.056[&]
Negative	12 (50)	252 (68.9)	
Surgical method, n (%)			
Supine position	7 (29.2)	96 (26.2)	0.752[&]
Prone position	17 (70.8)	270 (73.8)	

SD: standard deviation, BMI: body mass index, HD: hospitalisation duration, HU: hounsfield unit, ASA: American Society of Anaesthesiologists, *Independent t-test, **Mann-Whitney U test, [&]Pearson chi-square test

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the other group (p<0.001). Post-operative blood transfusion was applied to 58.3% of patients in group A and 5.5% of patients in group B (p<0.001). Preoperative hydroureteronephrosis (HUN) was present in 62.5% and 36.1% of the patients in groups A and B, respectively (p<0.001). When the patients who received antibiotherapy due to positive preoperative urine culture were examined, there were statistically significant differences between the groups (group A: 25%; group B: 5.6%; p<0.001).

According to multivariate logistic regression analysis, ASA score, presence of Clavien 3a and 3b complications, post-operative blood transfusion requirement, presence of preoperative HUN, preoperative urine culture growth and presence of low stone density were determined as significant independent risk factors for readmission (Table 2). According to the receiver operating characteristic (ROC) curve analysis, the threshold value for stone density was 859 HU, with a sensitivity of 66.7% and specificity of 65.7%, and values under this threshold were regarded as low stone density. In predicting readmission, the area under the curve (AUC) values for preoperative HUN and low stone density (<859 HU) were 0.714 [95% confidence interval (CI) 0.557-0.872; p=0.007] and 0.768 (95% CI 0.677-0.858; p=0.001), respectively (Figure 1 and Table 3).

Table 2. Multivariate analysis of potential predictors for readmission after PNL

	OR	95% CI	p-value
Variables			
ASA score	1.990	0.866-4.573	<0.001
Clavien 3a and 3b complications	2.998	1.864-4.789	0.042
Post-operative blood transfusion	2.450	0.594-10.112	<0.001
Preoperative hydronephrosis	3.190	1.022-9.957	0.046
Preoperative urine culture positive	1.120	1.100-1.160	0.215
Hounsfield unit <859	3.669	1.176-11.449	0.025

OR: odds ratio, CI: confidence interval, ASA: American Society of Anaesthesiologists, PNL: percutaneous nephrolithotomy

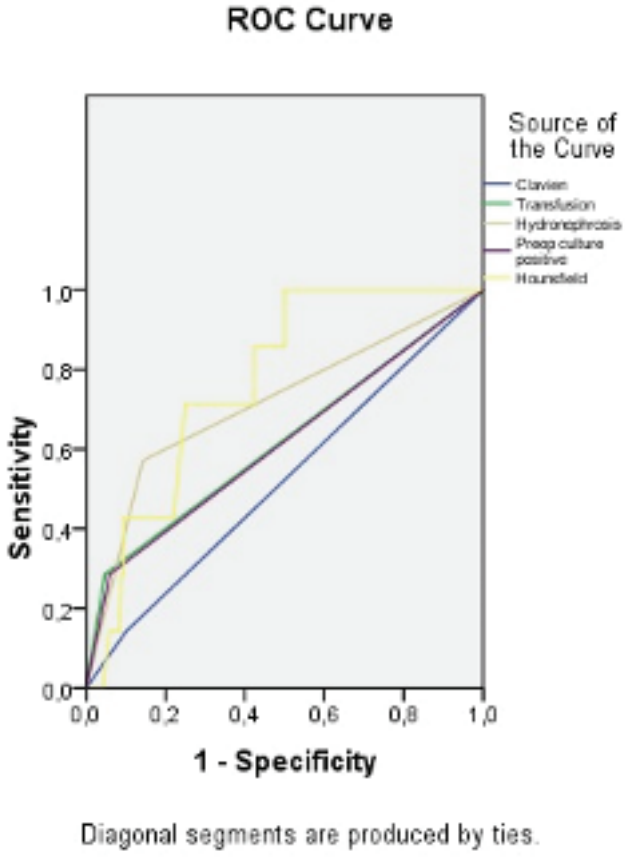

Figure 1. Receiver operating characteristic curves of readmission predicting factors of patients
ROC: receiver operating characteristic

Table 3. Receiver operating characteristic curve analysis for predictive factors for readmission

	AUC	95% CI	p-value
Variables			
Clavien 3a and 3b complication	0.521	0.362-0.680	0.792
Post-operative blood transfusion	0.619	0.447-0.791	0.131
Preoperative hydronephrosis	0.714	0.557-0.872	0.007
Preoperative urine culture positive	0.613	0.442-0.784	0.152
Low Hounsfield score	0.768	0.677-0.858	0.001

AUC: area under the curve, CI: confidence interval, the cut-off value for HU was 859, with a sensitivity of 66.7% and specificity of 65.7%

DISCUSSION

The readmission rate of patients after PNL operation, which is frequently applied for treating kidney stones, and the predictive factors causing this were determined in our study. Studies in the literature provide us with an idea about the reasons and incidence of recurrence of general urological procedures. However, studies on readmission rates and predictive factors, especially in endourological stone surgeries, are limited in the literature. Unplanned hospital readmissions in the early post-operative period can also impair the quality of life of patients and the current functioning of urology clinics (14). Post-operative readmissions to the ER are negative indicators of the quality of healthcare services and cause significant economic burdens. In the United States of America, only in 2010, insurance companies were paid $17.5 billion for referrals to the hospital, and it was decided to impose sanctions on centres with high readmission rates (8). Unplanned hospital readmissions after surgery constitute a source of concern for all endourologists working in the field of stone surgery (5). When cystoscopy, transurethral bladder tumour resection, transurethral prostate resection, hydrocele excision and urethral sling surgeries, as five surgical procedures most applied in urology were examined in a study, the hospital readmission rate was 3.7%. Cancer history, male gender, bleeding disorders and ASA score > III were detected as significant risk factors (8). In a similar study by

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Kumar et al. (14), male gender, smoking, chronic obstructive lung disease, diabetes mellitus (DM), coronary artery disease, bleeding disorders and ASA score >III were risk factors for readmission. Consistent with the literature, a significantly higher readmission rate was detected in patients with an ASA score of III, and this was an independent predictive factor in our study. In the literature, the readmission rates after PNL were between 1.7% and 9% (6,14,15). In two different studies conducted in Turkey, readmission rates were 5.27% and 27.1% (10,11). In our study, the rate of hospital readmission was 6.5%, which is consistent with the literature.

Advanced age was related to post-operative morbidity in urological surgeries (16). There is evidence in the literature that advanced age increases the readmission rate (8,9,14). Johnston et al. (17) determined that the readmission rates after PNL were 18.7% and 7.1% in patients aged over 75 and under 18 years, respectively. Considering that our data exclude patients younger than 18 years, the mean age between both groups was statistically similar.

In endourological procedures such as PNL, perioperative and post-operative complications related to urinary infections are common problems. The severity of these infections ranges from simple bacteriuria to severe sepsis, which can cause multiple organ failure (1). In a systematic review investigating PNL complications including 12,000 patients, fever and sepsis were observed at a rate of 10.1% and 0.5%, respectively (18). The most common reason for hospital readmissions in the post-operative period is urosepsis (14). Risk factors for post-PNL sepsis include positive urine culture, stone size, infection stone, neurogenic bladder dysfunction, abnormal kidney anatomy, long operation duration, presence of nephrostomy tube and/or urethral catheter (19). In our current study, no significant difference was found between the two groups in terms of post-operative fever. Although a significant difference was observed among the two groups in preoperative urine culture positivity, it was not identified as an independent risk factor in multivariate analysis.

Perioperative and post-operative bleeding is a complication of PNL that may cause mortality. It can be seen in different stages of the operation such as percutaneous access, tract dilatation, stone fragmentation and post-operative period (5). The presence of DM, perioperative complications, operation duration, imaging method used during access (ultrasonic or fluoroscopic), tract dilatation technique, tract diameter and renal parenchymal thickness were significant risk factors for bleeding and the need for transfusion (20). In a multicentre prospective study, the blood transfusion rate after PNL was 5.7% (21). In the study by Armitage et al. (6), readmission was observed because of haematuria or haemorrhage with a rate of 1.7% after PNL. Although haematuria and bleeding have not been examined separately, we concluded that blood transfusion, which may have a consequence, is an independent risk factor for hospital readmission.

Renal parenchymal thickness and stone density are factors that may affect PNL outcome (20). In the study examining complication rates, the operation time and blood loss were stated to increase in patients with a stone density of <1000 HU (22). Although data such as perioperative haemorrhage and decrease in haematocrit are indirectly related to readmission, there are no data in the literature examining the direct relationship between HU and readmission rates. Thus, this study is the first to determine HU as a risk factor for unplanned readmission within post-operative early period following PNL. In the ROC analysis, the threshold value for stone density was 859 HU, and a stone density lower than 859 HU was a risk factor for patients' readmission. According to the ROC analysis, it was determined to have the highest AUC value. Although the composition of stones was not determined in the patients in our study, low HU determined in the readmission group constitutes the possibility of these patients having infection stones (1).

Complications due to residual stone fragments may be a marker for readmission (23). In the study in which a high stone burden was found to be a significant risk factor for readmission, it was concluded that this condition was associated with prolonged operation time and more residual stone fragments. It has been reported that if surgery is planned when patients are asymptomatic and the stone burden is not high, it may be possible to decrease the readmission rate (10). Although we did not find a significant difference between the two groups in terms of stone volume, the presence of preoperative HUN is an independent risk factor for readmission. Based on these data, surgeries performed with less stone burden in the early period and before HUN development may have a significant effect on readmission rates.

Complication rates reaching 83% were reported after PNL (5). In the study by Tepeler et al. (11), Clavien grades 1, 2, 3a+3b and 4 complication rates were 1.8%, 3.2%, 1.3% and 0.1%, respectively. In our study, the post-operative complication rates in group A were significantly higher than in group B.

Study Limitations

One of the main limiting factors was the retrospective nature of our study. In addition, the comorbidities of the patients were not evaluated separately, and data on stone analysis, time until readmission and intraoperative complications were lacking in our study. The absence of stone localisation can be considered a limitation, but most of the patients who applied to our clinic have staghorn and semistaghorn stones. Thus, stone localisation of the patients could not be included in the study.

CONCLUSION

Hospital readmissions after PNL affect patient comfort and constitute a burden on the healthcare system. According to our study, ASA scores, the presence of Clavien 3a and 3b complications, the need for post-operative blood transfusion and the presence of preoperative HUN, preoperative urine culture growth and low stone density were risk factors for hospital readmissions. Choosing the patient and the surgical method according to these risk factors will reduce both the complication and readmission rates. Our study may help surgeons take constructive precautions

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in the treatment planning of patients with risk factors and may enlighten the way of future studies on this subject.

Ethics Committee Approval: The study protocol was approved by the local ethics committee of the Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee, and the study was conducted according to the Declaration of Helsinki (approval number: 2020-17, approval date: 24.08.2020).

Informed Consent: Written informed consent, approved by our institutional review board, was obtained from all patients.

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Does Hair Strand Cause Failure of Sterilization? A Controlled Experimental Study

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ABSTRACT

Objective: Besides the standard applications of surgical aseptic techniques, it is known that different teams display different approaches in the presence of a hair strand in sterile packs. Few of the teams prefer not to use the instruments and postpone the surgery, whereas others may decide to remove the hair and the instruments in contact and continue using the remaining part. Evidence is required to determine a standard approach in such practices, which leads to negative consequences.

Methods: Overall, 108 surgical clamps were sterilised using autoclave (n=36), hydrogen peroxide (n=36), and ethylene oxide (n=36). One third of the instruments in each group were packed along with a free hair strand, another third with a strangulated hair strand, and the last third were packed alone as the control group. Microbiological specimens of the instruments were collected with swabs. Hair samples were inoculated on thioglycolate broth. Growth was evaluated after 24 and 48 hours.

Results: No growth was observed among the groups after 24 and 48 hours. Thus, all the instruments were considered sterile.

Conclusion: Hair was shown to have no significant effect as a biological burden on bacterial contamination risk.

Keywords: Infection, sterilization, disinfection, hair, asepsis

INTRODUCTION

Despite advances in diagnosis, treatment and surgical intervention methods in surgical diseases, surgical infections remain to be the most common surgical complication. In addition to preoperative patient preparation, full and complete application of sterilization and surgical aseptic technical principles is the most significant basic element in preventing surgical infections (1-4).

Creating and maintaining the surgical aseptic area throughout the procedure is crucial in patient safety in the operating room. Surgical instruments to be used in the operation should be decontaminated, washed and disinfected with the correct methods before sterilization. In the presence of macroscopic remnants or inappropriate results, the processes should be repeated. Packaging should be done with appropriate materials. Appropriate sterilization, transfer and storage of the

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sterile materials should be used to provide the sterilization cycle (1,5-7).

Following the arrival of the surgical instruments and equipment to the operation room after a complete application of the sterilization cycle, it is crucial for the scrub and circulating nurse to create and maintain the aseptic area according to patient safety practices (8). Besides standard applications of surgical aseptic techniques, different teams exhibit different approaches in the presence of a hair strand in sterile packs, sets or bundles, a problem faced primarily by operating room nurses.

This situation might be a result of the incompatibility of working conditions during the packaging stage; however, the most significant issue to be sure about before making a decision is to determine whether the hair falls after opening of the package or not. In case of doubt, in accordance with the surgical aseptic technical standards, any suspicious occurrence should be considered as an impairment of sterilization (9,10).

Few teams prefer not to use the instruments, sets or bundles and even postpone cases that have no set alternative even though they know the hair was placed in the packaging stage. Some other teams may choose to continue using the set after removal of the hair strand. In cases with no set alternatives (e.g. the orthopaedic kit supplied by the company), evidence is required in determining a standard approach in such practices, which leads to significant consequences with adverse effects on the patient, the workflows of the teams and corporate functioning. This study aimed to evaluate the effect of a hair strand on sterilization of surgical instruments sterilised with autoclave, hydrogen peroxide and ethylene oxide techniques.

METHODS

The experimental protocol for the study was approved by the İstanbul Yeni Yüzyıl University, Local Ethics Committee (approval number: 2020/06-453, approval date: 08.06.2020). This non-randomised post-test-controlled study was designed to provide evidence in the case of presence of a hair strand in packages sterilised in autoclave, hydrogen peroxide and ethylene oxide. There was no contact with the patient within the scope of the study; hence, patient consent was waived.

Cleaning of Surgical Instruments

Overall, 108 surgical instruments (surgical clamps) were cleaned by washing and rinsing at 60 °C (LK/QX-500, Laoken Medical Technology Co., Ltd.). No additional treatment such as prewash, drying, or disinfection was applied.

Classifying of Surgical Instruments Into Groups and Subgroups

The instruments were divided into three groups (n=36 each) and were to be sterilised using autoclave, hydrogen peroxide and ethylene oxide. Each group was divided into three subgroups (n=12 each). Twelve instruments in each group were packed separately to create the control groups. Twelve instruments in each group were packed with a free hair strand to create experimental group 1, and 12 in each group were packed with a strangulated hair strand on them to create experimental group 2. All packs were assigned a descriptive number (Figure 1).

Packing of Surgical Instruments

All instruments were packed separately in double layers, using paper-film packaging (Sterintech, SP Medikal Co., Ltd.) of 75x250 mm in size as the inner layer and 100x300 mm in size as the outer layer. Chemical indicators suitable for sterilization method were placed on the first layer of the packages (Attest Rapid Readout 1292, 3M; Attest Rapid Readout 1295, 3M; Attest 1264, 3M); all packages were sealed using the same device at 80 °C (Rebi Evo, Gandus Saldatrici Srl).

Sterilisation of Surgical Instruments

Thirty-six of the packs were sterilised in autoclave at 134 °C and press steamed for 7 minutes (V-1263, Steris), 36 in hydrogen peroxide at 55 °C for 70 minutes (HRF3000, Teknomar) and 36 in ethylene oxide at 55 °C for 180 minutes (ETO C 1445, Teknomar), all at the same stage and without any delay. Device performances were followed by daily rapid test biological markers and weekly applied air leak test packs. All instruments were unpacked 12 hours after sterilization in sterile conditions.

Microbiological Cultivation

The instruments were unpacked one by one in sterile conditions placed close to the spirit stove. Swab samples of the instruments were taken using cotton swab sticks dampened with saline solution. Microbiological swab cultivation was performed on 5% sheep blood agar (GBL/Gül Biology Laboratory Industry and Trade Limited Company, rrf. no: 0854), which is suitable for the growth of several microorganisms with rich nutrient content and ensures that hemolysis is evident (Figure 2). Incubation was performed for 24-48 hours at 37 °C.

Free or strangulated hair samples were plated in the thioglycolate broth (GBL/Gül Biology Laboratory Industry and Trade Limited

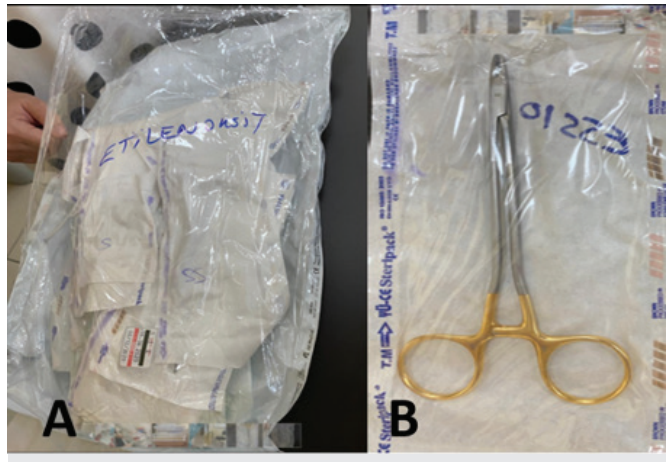

Figure 1. A) Stack of surgical instruments sterilised in ethylene oxide. B) A pack of instrument with the descriptive number

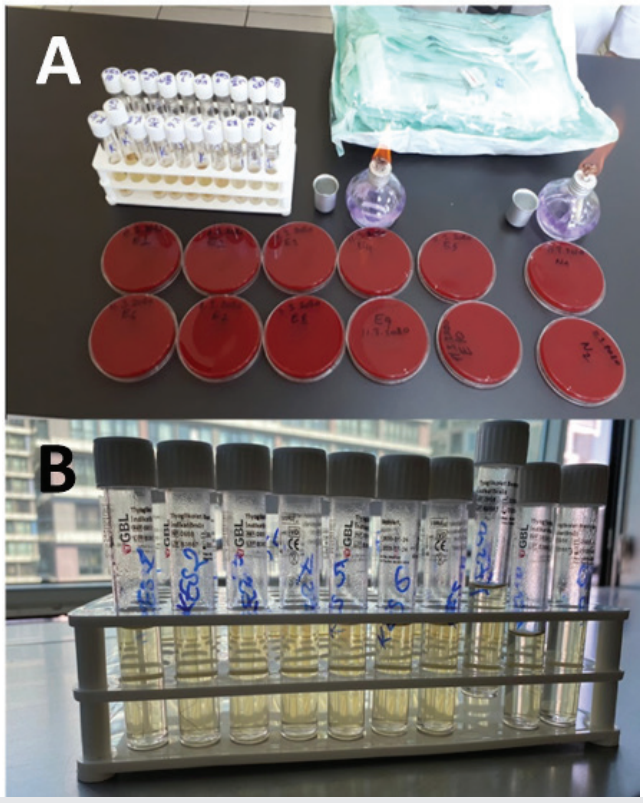

Figure 2. A) Cultivated blood agar mediums. B) Cultivated thioglycolate mediums

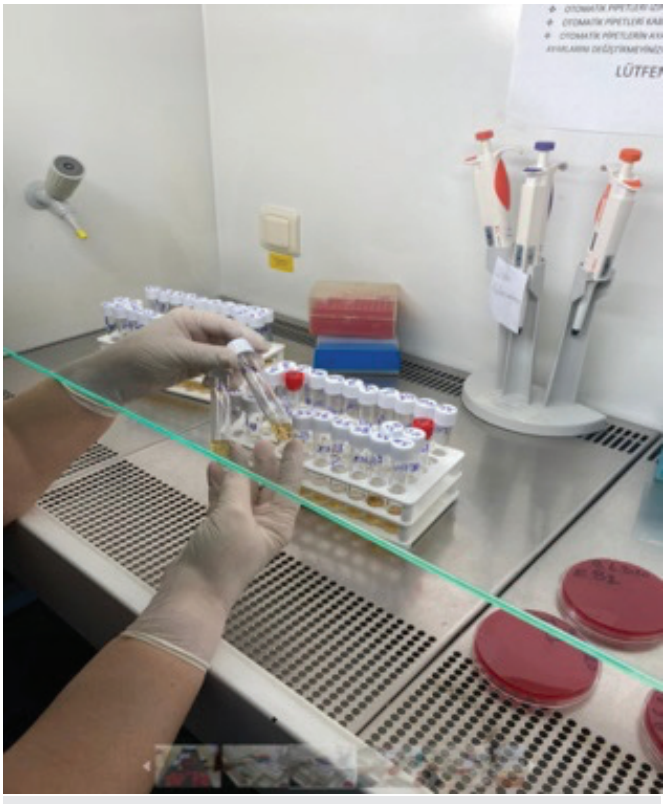

Figure 3. Plating of the hair samples to the blood agar and thioglycolate media in the laminar flow cabinet

Company, rrf. no: 0658) which is a general-purpose medium used for the cultivation of anaerobes and microaerophiles and recommended for tests of biologic materials. This process was performed in a laminar flow cabinet (Figure 3). Incubation was performed for 24-48 hours at 37 °C in aerobic incubator. The growth in all mediums was evaluated after 24 and 48 hours (Figure 4) (11).

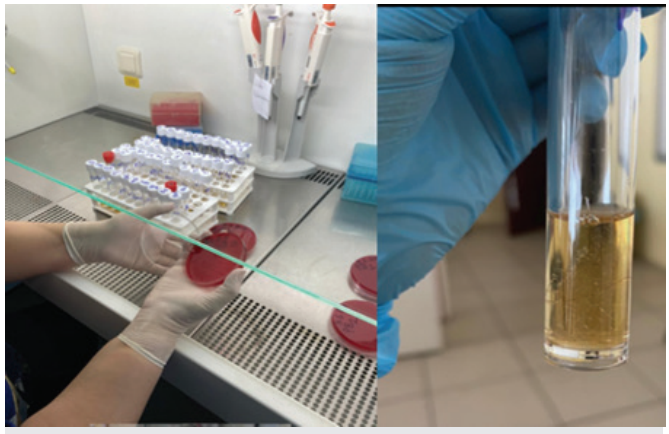

Figure 4. Growth evaluation of plated samples on the blood agar and thioglycolate media in the laminar flow cabinet

Statistical Analysis

Statistical analysis was not required as no growth was detected in all sterilization methods and packages.

RESULTS

Evaluations of the cultivation of the swab cultures in the experimental groups and control group on the blood agar medium after 24 and 48 hours revealed no growth. Moreover, observations after 24 and 48 hours of thioglycolate broth cultivation of the hair strands in the experimental groups 1 and 2 showed no growth (Table 1).

DISCUSSION

Presence of a hair strand in the sterile set is considered to ruin the sterilization by increasing the biological load (9,10). Surgical instruments are considered crucial materials since they penetrate the sterile tissue. Critical materials should be sterile while in use to prevent the risk of infection (12).

Rutala et al. (12), in their study to evaluate the microbial load on surgical instruments before sterilization, showed that the microbial load on surgical instruments after standard cleaning was low. It has been reported that 72% of devices had 0-10 colony-forming units (CFUs), and only 4% exceeded 425 CFUs. Furthermore, it has been reported that clean, clean contaminated, contaminated, or dirty nature of operations does not significantly affect microbial load (12). Even if the washing process is applied on the instruments kept without using any sterilization method, there may be microbial load; it should be regarded as non-sterile

Table 1. Surgical instrument microbiological swab and hair cultivation results

Pack No.	Autoclave sterilization						Hydrogen peroxide sterilization						Ethylene oxide sterilization					
	Control group		Experimental group 1		Experimental group 2		Control group		Experimental group 1		Experimental group 2		Control group		Experimental group 1		Experimental group 2	
	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48
IO 1	N	N					N	N					N	N				
IO 2	N	N					N	N					N	N				
IO 3	N	N					N	N					N	N				
IO 4	N	N					N	N					N	N				
IO 5	N	N					N	N					N	N				
IO 6	N	N					N	N					N	N				
IO 7	N	N					N	N					N	N				
IO 8	N	N					N	N					N	N				
IO 9	N	N					N	N					N	N				
IO 10	N	N					N	N					N	N				
IO 11	N	N					N	N					N	N				
IO 12	N	N					N	N					N	N				
FH 1	N	N	N	N			N	N	N	N			N	N	N	N		
FH 2	N	N	N	N			N	N	N	N			N	N	N	N		
FH 3	N	N	N	N			N	N	N	N			N	N	N	N		
FH 4	N	N	N	N			N	N	N	N			N	N	N	N		
FH 5	N	N	N	N			N	N	N	N			N	N	N	N		
FH 6	N	N	N	N			N	N	N	N			N	N	N	N		
FH 7	N	N	N	N			N	N	N	N			N	N	N	N		
FH 8	N	N	N	N			N	N	N	N			N	N	N	N		
FH 9	N	N	N	N			N	N	N	N			N	N	N	N		
FH 10	N	N	N	N			N	N	N	N			N	N	N	N		
FH 11	N	N	N	N			N	N	N	N			N	N	N	N		
FH 12	N	N	N	N			N	N	N	N			N	N	N	N		
SH 1	N	N			N	N	N	N			N	N	N	N			N	N
SH 2	N	N			N	N	N	N			N	N	N	N			N	N
SH 3	N	N			N	N	N	N			N	N	N	N			N	N
SH 4	N	N			N	N	N	N			N	N	N	N			N	N
SH 5	N	N			N	N	N	N			N	N	N	N			N	N
SH 6	N	N			N	N	N	N			N	N	N	N			N	N
SH 7	N	N			N	N	N	N			N	N	N	N			N	N
SH 8	N	N			N	N	N	N			N	N	N	N			N	N
SH 9	N	N			N	N	N	N			N	N	N	N			N	N
SH 10	N	N			N	N	N	N			N	N	N	N			N	N
SH 11	N	N			N	N	N	N			N	N	N	N			N	N
SH 12	N	N			N	N	N	N			N	N	N	N			N	N

IO: packages including instruments only without a hair strand, FH: packages including a free hair strand, SH: packages including a hair strand strangulated to the instrument, N: negative

and must be subjected to a sterilization process before use. In this study, all surgical instruments were washed in a washing device at programme 1 at 60 °C for 22 minutes, passing through the stages of washing, initial rinsing 1 and second rinsing. Microbiological load assessment was not performed before sterilization; hence, all instruments were considered non-sterile.

In this study, paper + film packaging method was used and surgical instruments were placed in individual packages. The packs that

contained instrument only were used as the control group and the packs containing a hair strand, free or strangulated, were the study groups that contained biologic load. Resendiz et al. (13) studied the risk of bacterial survival and contamination in surgical instruments in the presence of dried blood inoculation. Although it was not statistically significant, wrapped sets were found to be in higher risk of bacterial reproduction in presence of blood (13). Additionally, in this study, it was clearly shown that steam sterilization remains inadequate in the presence of biological debris and contaminated instruments that cause a risk for other clean instruments in the set as well. In this study, a hair strand was used as biological burden and unlike blood residue, it did not cause a higher risk of contamination.

Regardless of the sterilization method, with the presence of hair in the sterile package, especially in cases with no alternative sets, the surgery needs to be cancelled. Karahan et al. (14) reported that 14% of the delays or cancellations of surgical operations was due to operating room problems. Moreover, they found the mean continuous anxiety scores of the patients who had delayed surgery as significantly higher (45.28±5.67) (14).

In their letter, Gillespie et al. (15) reported that their operation was cancelled due to a 7 cm hair strand found in the surgical set opened in the operative table preparation at Southern Health Hospital. It was stated that this cancelled surgery caused an additional cost of 5,000 Australian Dollars and increased surgical stress for the patient, since there were no spare surgical instruments. Although the recommendations of The Australian College of Operating Room Nurses (16) were followed, an experimental study was conducted due to the material and moral damages mentioned. For this purpose, two 5 cm hair strands, two 5 cm nylon sutures, and two 5 cm silk sutures were first dipped in 0.5 McFarland (10^8 CFUs per millilitre) *Staphylococcus aureus* (ATCC 25923) solution, and each sample was inoculated in tryptic soy broth at 35 °C without sterilization as the control group. The other half of samples were left in the surgical set as the experimental group, and the sterilization of the surgical set was achieved in the pre-vacuum steam steriliser. Growth was detected within 24 hours in each of the sample cultivated in the control group. In the experimental group, all samples were cultivated in tryptic soy broth at 35 °C under aseptic conditions after sterilization, and no growth was reported in the control group after 24 and 48 hours and 1 week. The results of this study supported that the use of the surgical instruments in the presence of a hair strand may be possible in cases where cancellation of the surgery carries a high risk (e.g. when there is no spare surgical set); however, these results should be supported with comparative studies on larger sample groups. The results of our study supported the conclusion of Gillespie's study.

No growth was observed from the samples taken from the instruments or the hair in any of the groups for all of the techniques used in our study. Thus, current practices should be revised in the light of the results obtained from our study.

This study was conducted using single instruments in paper and film packages. Multiple instruments in large containers should be tested in further studies before applying the principle in daily practice. Nonetheless, the results in this study are thought to be guiding.

In our study, no statistically significant growth was observed in any of the groups sterilised with all three methods which are the most commonly used ones in our country.

Study Limitations

The limitations of the study are that surgical instruments are not packaged as a set and that a single surgical instrument is packaged in a double-layer package and subjected to sterilization.

CONCLUSION

The presence of hair, free or strangulated in the instrument, in the sterile package has no effect on bacterial contamination risk. Further experiments are warranted to explore the effect on larger surgical sets before clinical application.

Ethics Committee Approval: The experimental protocol for the study was approved by the İstanbul Yeni Yüzyıl University, Local Ethics Committee (approval number: 2020/06-453, approval date: 08.06.2020).

Informed Consent: Patient consent was waived.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - N.A., H.B.K., A.D., F.A.; Concept - N.A., S.Y., H.B.K., A.D.; Design - N.A., A.C., H.B.K., S.G., A.D.; Data Collection and/or Processing - N.A., S.Y., A.C., H.B.K., A.D., F.A.; Analysis and/or Interpretation - N.A., S.Y., A.C., H.B.K., S.G., A.D.; Literature Search - N.A., H.B.K., S.G., A.D.; Writing - N.A., S.Y., A.C., H.B.K., S.G., A.D., F.A.

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The Diagnostic Value of Free Androgen Index in Obese Adolescent Females with Idiopathic Hirsutism and Polycystic Ovary Syndrome

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ABSTRACT

Objective: The free androgen index (FAI) is the ratio between total testosterone and sex hormone binding globulin (SHBG), and it has been proposed as a marker. FAI is increasingly used in the definitive diagnosis of patients with hyperandrogenism. Although the reference range of FAI in adult females has been identified, there are no adequate studies on the reference range for adolescent girls. We determined the diagnostic value of FAI in adolescent girls diagnosed with obesity, idiopathic hirsutism (IH) and polycystic ovary syndrome (PCOS).

Methods: Patients aged 12 to 21 years and diagnosed with obesity, IH and PCOS were included in the study. FAI was found by calculating the ratio of total testosterone to SHBG.

Results: According to receiver-operating characteristic (ROC) analysis results, FAI level under 3.45 indicated that the participants were healthy. ROC analysis was also used to determine the usefulness of FAI in distinguishingbetween healthy participants and those diagnosed withPCOS, obesity, IH. It was determined that patients with FAI above 6.15 should be evaluated for PCOS.

Conclusion: We concluded that FAI is a reliable marker to identify and followup patients with hyperandrogenism and the reference values we found in our study can be used in clinical practice.

Keywords: Free androgen index, obesity, idiopatic hirsutism, PCOS

INTRODUCTION

Androgen excess is one of the most common endocrine disorders of reproductive-aged women, affecting approximately 7% of this population (1-3). Androgen excess results in the development of androgenic features in the affected women with the development of hirsutism, androgenic alopecia, acne and ovulatory dysfunction, and if it is extreme and prolonged, it could even lead to virilisation and masculinisation (4). Androgen excess is the cardinal underlying phenomenon in various disorders in females, particularly polycystic ovary syndrome (PCOS), idiopathic

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hirsutism (IH), congenital adrenal hyperplasia and ovarian/adrenal neoplasms, as well as insulin resistance/obesity. Increased insulin in obese patients stimulates ovarian androgen production driven by increased luteinising hormone secretion and contributes to hyperandrogenism. Free or bioactive testosterone is responsible for the pathogenesis of androgen excess status. Nevertheless, it is difficult to identify hyperandrogenism in the laboratory in children and adolescents and associate it with clinical findings. However, there is no consensus regarding what serum level is significantly higher and when treatment should be started (5,6). Simple and practical criteria/methods, therefore, need to be developed.

The free androgen index (FAI) is the ratio between total testosterone and sex hormone binding globulin (SHBG), and it has been proposed as a marker. FAI is increasingly used in the definitive diagnosis of patients with hyperandrogenism (5). Although the reference range of the FAI in adult females has been identified, there are no adequate studies on the reference range for adolescent girls (7). In our study, we determined the diagnostic value of FAI in adolescent girls diagnosed with obesity, IH and PCOS.

METHODS

Patients aged 12 to 21 years and diagnosed with obesity, IH or PCOS in the pediatric endocrinology clinic of our hospital were included in the study.

Definition of the groups:

The obesity group consisted of patients with a body mass index (BMI) of >2 standard deviation score (SDS).

IH group consisted of patients with IH who had a Ferriman-Gallwey score 8 or more and no other reason for the hirsutism.

The PCOS group consisted ofpatients who met all of the PCOS Amsterdam 2013 diagnostic criteria for adolescents, which are as follows (8):

1- Oligomenorrhea or primary amenorrhea present 2 years after menarche,

2- At least one ovary of a volume >10 mL,

3- Presence of a clinical and biochemical hyperandrogenism (diagnosis criteria for clinical hyperandrogenism: Ferriman-Gallwey score of 8 or more; diagnostic criteria for biochemical hyperandrogenism: Total testosterone level >51 ng/dL).

The control group consisted of healthy adolescent females with a normal weight, without any clinical or laboratory signs of hyperandrogenism, and not using any medication affecting the androgen metabolism.

Laboratory studies:

Blood samples were drawn in the first week of the menstrual cycle. Total testosterone and SHBG were studied from the sera. All tests were performed at the biochemistry department of our hospital. Total testosterone was measured by solid-phase competitive chemiluminescent enzyme immunoassay, using the ADVIa Centaur-XP instrument (Siemens, Germany), and SHBG was measured by radioimmunoassay, using Beckman Coulter (USA).

FAI and homeostasis model assessment-estimated insulin resistance (HOMA-IR) calculation:

FAI was calculated according to the formula: [total testosterone (nmol/L)/SHBG (nmol/L)]×100 (5). Total testosterone was measured in ng/dL, so ng/dL was converted to nmol/L (ng/dL×0.03470: nmol/L). HOMA-IR was calculated according to the following formula: fasting blood glucose (mg/dL)×fasting blood insulin (IU/mL)/405 (9).

Statistical Analysis

Descriptive statistics for the study variables were presented as median, mean, standard deviation and minimum and maximum values. The Kruskal-Wallis test was used to compare the control and patient groups. The cut-off value for the hormone was determined by receiver-operating characteristic (ROC) analysis. Statistical significance levels were considered at 5%. The SPSS (ver. 13) statistical programme was used for all statistical computations.

The study was approved by the Ethical Committee of Dr. Zekai Tahir Burak Women's Health Training and Research Hospital, and conducted according to the principles of the Declaration of Helsinki. The approval number is 23/2014 (approval date: 15.04.2014). All parents/guardians of the children provided written informed consent before the children were included in the study.

RESULTS

The patients' ages, BMI, BMI-SDS, HOMA-IR, total testosterone, SHBG and FAI values are shown in Table 1. There were differences in ages between the four groups. The PCOS group consisted of patients older than those in the obesity and control groups. The ages of patients in IH group were similar to those in other groups. As expected, BMI and BMI-SDS were higher in the obesity group than the other groups. In addition, BMI and BMI-SDS were found to be higher in the PCOS and IH groups than the control group. While HOMA-IR was similar in obesity, IH and PCOS groups, it was higher in these groups than the control group. While serum total testosterone levels were the highest in the PCOS group (total testosterone: 84.3±22.9 ng/dL), serum total testosterone levels were 68.5±16.3, 60.5±10.6 and 37.1±12.6 ng/dL in IH, obesity and control groups, respectively. There was no difference in testosterone levels between IH and obesity groups. Testosterone levels were lower in the control group than the other groups. While serum SHBG levels were similar in obesity, IH and PCOS groups, it was higher in the control group.

ROC analysis was used to determine the usefulness of FAI in distinguishing healthy adolescents (control group) from other groups. According to ROC analysis, the area under the curve (AUC) was found to be 0.930±0.22. The sensitivity and specificity values for FAI, whose cut-off value was 3.45, were found to be 90% and 84%,respectively. Accordingly, FAI under 3.45 indicated that the participants were healthy (Figure 1).

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Table 1. Patients' clinical features, anthropometric measurements and laboratory values

	Obesity n=26	IH n=26	PCOS n=20	Control n=72	p
Age (years)	14.6±2.02[b] 12-18	15.6±1.56[ab] 12-18	16±1.58[a] 13.5-19	14.9±1.72[b] 12-18	0.040
BMI (kg/m²)	31.7±4.1[a] 26-40.68	24.5±3.88[b] 17.3-31.60	25.4±4.96[b] 18.2-34.9	21±2.82[c] 15.7-27.34	0.01
BMI-SDS	2.8±0.69[a] 2-4.33	1.1±1.1[b] -1.20-3.5	1.2±1.4[b] -1.60-3.98	-0.1±1.2[c] -1.80-1.91	0.01
HOMA-IR*	3.89±1.7[a] 1.44-8.48	3.06±2.2[a] 0.80-11.20	3.19±1.3[a] 1.16-6.60	1.7±0.56[b] 0.90-3.5	0.01
Total testosterone (ng/dL)	60.5±10.6[b] 51-89.10	68.5±16.3[b] 52.97-112.66	84.3±22.9[a] 53.10-142	37.1±12.6[c] 10-50	0.01
SHBG (nmol/L)	23.9±14.8[b] 6.71-66.71	34.8±16[b] 7.35-77.40	28.6±18.1[b] 9.54-75.68	56.1±22.6[a] 17.44-10.2	0.01
FAI (%)	9.9±6.4[a] 1.70-27.30	7.7±6[a] 1.80-29.70	14.4±9.6[a] 3.40-43.0	2.4±1.3[b] 0.20-6.40	0.01

*HOMA-IR was calculated according to the following formula: fasting blood glucose (mg/dL)×fasting blood insulin (IU/mL)/405 (9).
[a,b,c] Statistically different groups represented by different letters ($p<0.05$). IH: idiopathic hirsutism, PCOS: polycystic ovary syndrome, BMI: body mass index, SDS: standard deviation score, HOMA-IR: homeostasis model assessment-estimated insulin resistance, SHBG: sex hormone binding globulin, FAI: free androgen index

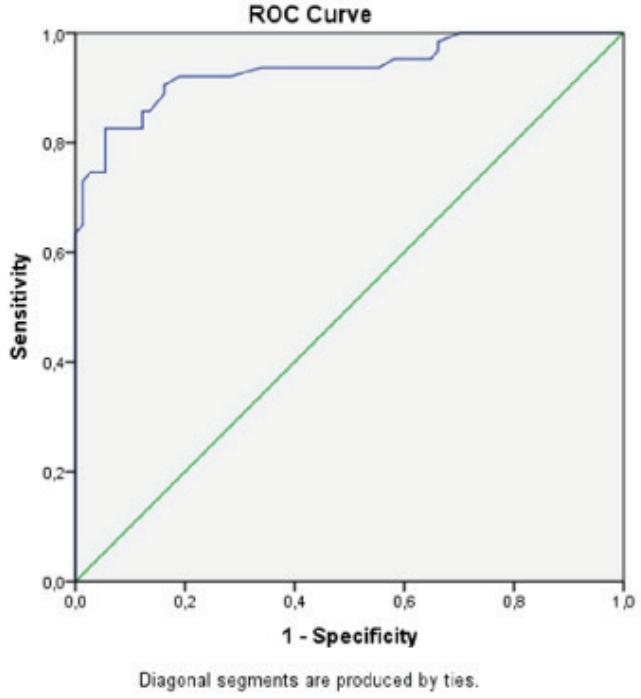

Figure 1. ROC analysis curve detecting the usefulness of free androgen index in distinguishing between the control group and other groups
ROC: receiver-operating characteristic

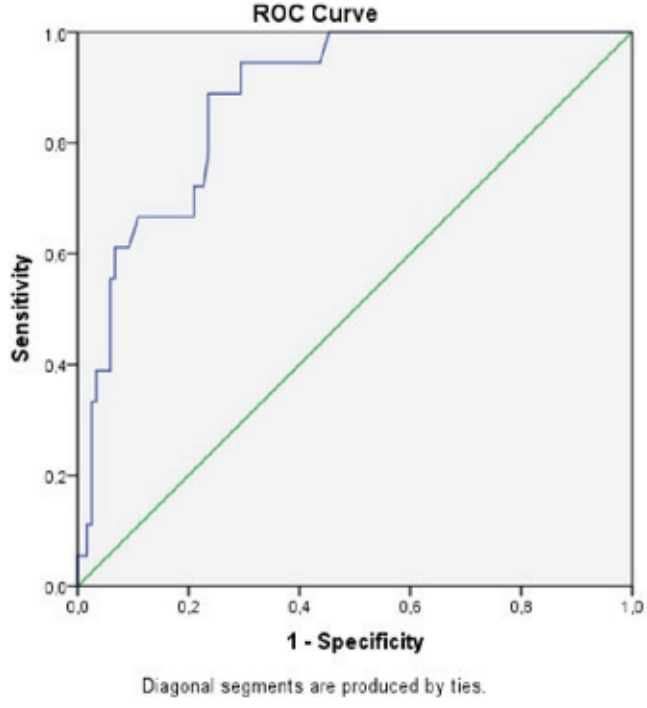

Figure 2. ROC analysis curve detecting the usefulness of free androgen index in distinguishing between polycystic ovary syndrome group and other groups
ROC: Receiver-operating characteristic

ROC analysis was also used to determine the usefulness of FAI in distinguishing between patients in PCOS group and those in obesity and IH groups. According to ROC analysis, AUC was found to be 0.88±0.35. Sensitivity and specificity values for FAI with a cut-off value under 6.15 were found to be 89% and 77%, respectively. Therefore, it was determined that patients whose FAI was above 6.15 should be evaluated for PCOS (Figure 2).

In the obesity group, there was a positive correlation between FAI and BMI (r=0.426; p=0.048) and HOMA-IR (r=0.530; p=0.011).

DISCUSSION

Androgen excess is the cardinal underlying phenomenon in various disorders in females. Free or bioactive testosterone is responsible for the pathogenesis of androgen excess status. It is

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difficult to identify hyperandrogenism in the laboratory in children and adolescents and associate it with clinical findings.

In literature, studies on FAI were commonly conducted on adult females. In a study, Pinola et al. (7) found that FAI was 2.1±1.3 in adult females, whereas it was 4.4±3.8 in females with PCOS. In females aged 18-24 years old, healthy females had an FAI of 1.9±1.1, while females diagnosed with PCOS had an FAI of 4.9±3.2. In addition, it was cited that FAI could be used as an important parameter to diagnose PCOS in all age groups. Our study evaluated adolescentsin the age range of 12-21 years, and it was found that FAI with a cut-off value <3.45 indicated that the participants were healthy and a cut-off value >6.15 indicated that participants should be evaluated for PCOS. These values had a high sensitivity and specificity.

A study from China reported FAI reference range of 0.7-6.4 in 444 patients aged 20-46 years selected from a group of 1,526 females after excluding disorders influencing the androgen level. The 5th percentile FAI value was 0.8 and the 95th percentile value was 6.7 for patients aged 20-28 years. The same study found FAI to be negatively correlated with age. They stated that FAI was higher in patients diagnosed with hirsutism and PCOS than in the healthy population, and BMI and FAI had a positive correlation (10). Moreover, FAI increased in insulin excess (11,12).

In our study, similar to previous studies, a positive correlation was found between FAI and HOMA-IR in the obesity group. In a previous study reported in Mexico, FAI of 83 healthy nonobese females was 5.3±3.8, while that of 238 obese females was 8.5±5.3 (13).

Few studies on FAI in adolescents are available. A study by Ibanez et al. (14) reported that normal FAI in healthy adolescents aged 14-18 years was <5. A Canadian study by Raizman and Quinn (15) investigated FAI in 66 females aged 9-14 years. The lowest value was 0.12 and the highest value was 2.63. In healthy females aged 14-19 years, the lowest and highest values were 0.59 and 6.5, respectively.

The age range of our control group was 12-18 years. The lowest and highest FAI values were 0.2 and 6.4, respectively. These values are close to the values found by Raizman and Quinn (15). In a study by Liimatta et al. (16), FAI was 0.38 (0.32-0.53) in 97 healthy females younger than eight years old and 2.93 (2.33-5.94) in 16 healthy adolescent females.

Few studies investigating FAI values in adolescents with PCOS are available. A study by Yetim et al. (17) reported that FAI was 6.75 (0.97-23.66) in the PCOS group consisting of 53 adolescent females and 3 (0.22-36.65) in the control group. These values are consistent with ours, with a cut-off value of 6.15, indicating a diagnosis of PCOS, and 3.45 in healthy adolescents.

Study Limitations

The limitation of this study is the small number of participants.

CONCLUSION

In our study, we determined that an FAI with a cut-off value of 6.15 indicated a diagnosis of PCOS and 3.45 in healthy adolescents and these valuescan be used in practice. We concluded that FAI is a reliable marker to identify and follow-up patients with hyperandrogenism in clinical situations with elevated androgen levels and that the reference values we found in our study can be used in clinical practice.

Ethics Committee Approval: The study was approved by the Ethical Committee of Dr. Zekai Tahir Burak Women's Health Training and Research Hospital (approval number: 23/2014, approval date: 15.04.2014).

Informed Consent: All parents/guardians of the children provided written informed consent before the children were included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - E.S., Z.A.; Design - E.S., S.Ç., M.K., Ş.S.E.; Data Collection or Processing - E.S., M.K.; Analysis or Interpretation - E.S., Ş.S.E., S.Ç., Z.A.; Literature Search - E.S., M.K.; Writing - E.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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SMAD4 Gene Methylation May Be Effective in Adenocarcinoma Lung Cancers

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ABSTRACT

Objective: In this study, we aimed to investigate the effect of promoter region methylation of *small mothers against decapentaplegic 4* (*SMAD4*) gene in adenoma type lung cancer cases. Adenocarcinoma and squamous type carcinomas are the most common types of lung cancer. *SMAD4* gene is an intracellular signal protein. The protein of this gene, which is one of the transcription factors, functions in tissue homeostasis during embryonic development and has effects in the cancer process.

Methods: In this retrospective study, a total of 40 samples including 20 paraffin-embedded tumor tissues of 20 patients with adenocarcinoma lung cancer and normal lung tissue of the same patients were included. After DNA isolation from this paraffin-embedded adenocarcinoma lung tumor tissue and its normal counterparts, methylation specific polymerase chain reaction followed by agarose gel imaging methods were applied to investigate the SMAD4 promoter methylation after bisulfite modification.

Results: As a result of our study, an increased presence of methylation in the promoter region of the *SMAD4* gene in the tumor tissue of a total of 12 (60%) of 20 adenocarcinoma cases compared to normal tissue was detected. A statistically significant increase in methylation rate of approximately 25-45% was found in tumor tissues of these cases compared to normal tissues ($p<0.05$).

Conclusion: As a result of our study, we suggested that *SMAD4* gene methylation may be a tumor marker for lung cancers and may contribute to the development of cancer by inhibiting SMAD4 protein expression by gene methylation and disrupting the intracellular signal pathway.

Keywords: SMAD4, DNA methylation, lung cancer, adenocarcinoma

INTRODUCTION

Lung cancer is the first cancer to cause death in men and the second cancer in women among all types of cancer. Approximately 1.3 million people die from lung cancer each year in the world. However, with the newly developed lung cancer treatment methods, the average life span and quality have started to increase relatively (1). Approximately 80% of lung cancers with a high mortality rate worldwide are reported as non-small-cell lung cancer (NSCLC). NSCLC have two subtypes, adenocarcinoma and squamous, and are classified histopathologically as adenocarcinoma and squamous cellular carcinoma. Adenocarcinomas and squamous carcinomas represent approximately 50% of all NSCLC (1,2).

Small mothers against decapentaplegic 4 (SMAD4) is a gene that synthesizes a protein product that carries chemical signals from the cell surface towards the nucleus. This signaling pathway works through the transforming growth factor (TGF)-pathway and can be affected by the cell's peripheral environment. Signal formation begins with the binding of TGF-beta (TGF-β) protein to the corresponding receptor on the cell surface, and this event provides

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the activation of SMAD group proteins (3). SMAD proteins bind to SMAD4 to form a protein complex, and this new complex that is formed transmits the signal by moving towards the cell nucleus. It also controls the growth and activation of tumor suppressor genes in the nucleus (4).

Methylation is a process characterized by the addition of a methyl group to the 5-carbon cytosine in the CpG nucleotide sequences, particularly in the promoter regions of genes. Studies have shown that a significant portion of mammalian genomes are methylated (5,6). Methylation reactions in genes affect gene expression and therefore genes can be inactivated or activated. In this study, in order to determine the contribution of the *SMAD4* gene to the formation of lung cancers, we aimed to show the changes in the stage of transformation from normal tissue to tumor by determining the potential cellular methylation in the promoter region of normal and tumor tissues in adenoma type lung cancers with methylation specific polymerase chain reaction (MSP) method. In this respect, our study is the first study in the literature.

METHODS

Our study is a cross-sectional retrospective study and patient consent was not obtained. For this study, 10 histologically confirmed advanced stages (stage 3); 5 sections from early stage (stage 1) and middle stage (stage 2) lung tumor tissues with adenocarcinoma and 5 sections from the outermost parts of the surgical margins as normal lung tissue of the same cases, (10 pieces per case) a total of 200 slice slide tissue samples belonging to 20 cases were prepared in formalin-fixed paraffin-embedded tissue blocks (7). The clinical characteristics of the patients are shown in Table 1. The number of samples to be studied was determined by power analysis, and all cases used in this study were used in the paraffin tissue archive of patients diagnosed with lung cancer between 2007-2018 in Trakya University Pathology Department. Pathological evaluation of all tissues was made in pathology department. This study was conducted with the permission of the Trakya University Local Ethics Committee (approval number: 05/21, approval date: 27.02.2013).

Genomic DNA Isolation and Bisulfite Modification from Paraffin Embedded Tissues

As stated before, sections were obtained from selected tissues and were scraped from the slides into tubes and their DNAs were isolated as stated in the literature (8). These DNAs were then analyzed by spectrophotometric method after determining the amount of DNA at 280 nm and 260 nm wavelengths; and samples were stored at +4 °C for further analysis. Bisulfite modifications of DNAs were made in accordance with the EpiJET Bisulfite Conversion Kit and Protocol (Thermo Fisher Scientific-USA) (7,8).

Methylation Analysis of the SMAD4 Promoter

With the sodium bisulfite modification of genomic DNA, all unmethylated cytosines in DNA are converted to thymine, but this reaction does not affect cytosines in methylated state, creating a potential sequence difference. After this chemical modification of DNA, DNA samples were used for methylation analysis of *SMAD4* gene. The MethPrimer V1.1 beta program was used (available at www.urogene.org) to identify potential methylation sites and methylation-specific primer sequences for the *SMAD4* gene sequence (9).

Methylation Specific Polymerase Chain Reaction Method

The methylation specific primers for the promoter of the *SMAD4* gene region are as follows: Forward 5′: GTAATAATACGGTTTTGGTCGTC-3′, Reverse: 5′-TCCCACCCCC TAAACGACCGCG-3′, product size: 164 base pair (bp), Tm: 77.4 °C, *SMAD4* gene specific primers of unmethylated used for the promoter region were as follows: Forward: 5′- GTAATAATATGGTTTTGGTTGTT-3′, reverse: 5′-CTCCCACCCCCTAAACAACCACA-3′, product size: 163 bp, Tm: 72.3 °C. The polymerase chain reaction (PCR) conditions have been created from 95 °C 45′, 55 °C 30′, 72 °C 30′x35 cycles following initiation for 10′ at 95 °C and finally 5′ termination at 72 °C. Methylated and unmethylated PCR media; PCR buffer 1x, $MgCl_2$: 2 mM, DMSO: 5% (v/v), dNTP: 12.5 mM, primer forward: 10 nM, primer reverse: 10 nM, taq polymerase: 1U (5U/µL). Template DNA 100 ng was completed with up to 50 µL of dH_2O. Methylated and unmethylated human DNAs were used as positive and negative control DNAs (S8001 | CpGenome ™ Human Methylated and Unmethylated DNA Standard Set). These DNAs were bisulfite modified before PCR. PCR with these modified DNA samples was performed using methylation-specific and non-methylation-specific primers. Later, PCR products were evaluated for 2% agarose gel under ultraviolet light (9), tumors and normal tissues were compared for methylation, and methylation rates were determined.

Statistical Analysis

Statistical analysis of the study was performed using the SPSS 20 program (IBM Corp, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation and qualitative variables as percentages. Statistical analysis of methylation specific PCR results was performed and the results of the experimental and control groups were compared with the χ^2 test, $p<0.05$ was considered statistically significant (10).

RESULTS

1-3 µL of bisulfite modified DNA was used for each methylated and un-MSP. MSP and un-MSP products were stained with ethidium bromide and evaluated on a 2 percent agarose gel (Figure 1 A-B). The expected band size in the promoter of the *SMAD4* gene was 163-164 bp for MSP and un-MSP (Figure 1) (11). As a result, SMAD4 promoter methylation was present in approximately 12 (60%) of adenocarcinoma tumors and un-methylation was 3-10%, whereas in normal lung tissues, 70% un-methylation and approximately 20% methylation were observed ($p<0.05$). When evaluated according to tumor stages, 40% of the first stage tumors (in 4 samples), 87.5% of the second stage tumors (6 samples) and 66%

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of the third stage tumors (in 2 samples) were observed to increase in methylation (Table 1).

DISCUSSION

The *SMAD4* gene, located on chromosome 18q21, is known somatically as a candidate tumor suppressor gene in many pancreatic and colorectal tumors. It has been shown that changes in the *SMAD4* gene that cause it to inactivate by mutation mechanisms such as deletion have been shown to be effective in pancreatic and colorectal cancers (11). For these reasons, SMAD4 methylation has been studied primarily in colorectal and pancreatic cancers. MSP is the main method used to investigate gene methylations and has been used successfully in many cancer and other disease groups (12). Since there is no study in the literature showing the SMAD4 methylation status in lung cancers, we examined the SMAD4 promoter region using the MSP method. SMAD4 may be a good chemotherapeutic option, especially because of its role in transcriptional function (13). There is a relationship between SMAD4 expression and associated TGF-β and increased functional gene expression and activation of oncogenesis signaling pathways, especially in cancer development (10,11). Our research has shown that SMAD4 methylations can be seen in lung adenocarcinoma. Perhaps in this way, it could be one of the reasons for *SMAD4* gene inactivation and, in conjunction with other active genes, reduced life expectancy in lung cancers. There is limited information on this subject in the literature. In the light of this information, it shows the potential importance of activating or inactivating oncogenesis pathways in cancer treatments. Studies examining both epigenetic properties of this gene and explaining epigenetic inhibition mechanisms may be interesting in the search for new cancer chemotherapy agents (10,11,14).

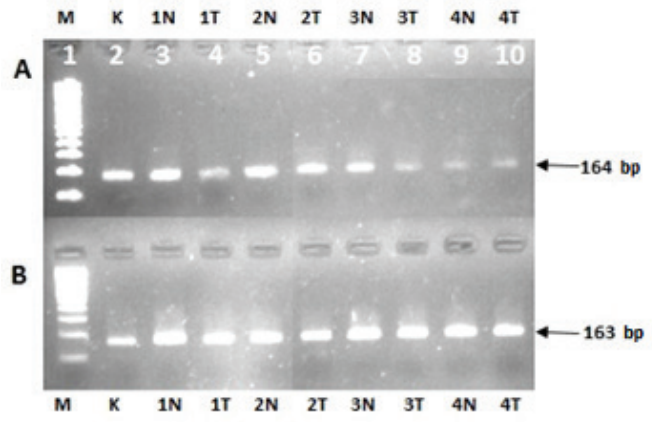

Figure 1. A. Un-methylation specific polymerase chain reaction (PCR) result for *SMAD4* gene, line 1: M: 100 bp marker, line 2: (+) control DNA, normal and tumor pairs for each case line 3-11. B. Specific PCR results for *SMAD4* gene methylation, line 1: M: 100 bp marker, line 2: (+) control DNA, normal and tumor pairs for each case line 3-11
N: normal, T: tumor

Table 1. Clinical characteristics of patients with adenocarcinoma

	Normal case (n=20)	Methylation normal tissue	Tumor case (n=20)		Methylation tumor tissue
	n (%)	n (%)	n (%)	%	n (%)
Age, year (Mean ± SD)	58±7	-	-	-	-
≤53	9 (45)	-	9 (45)	45	-
>59	11 (55)	-	11 (55)	55	-
Gender					
Female	5 (25)	-	5 (25)	25	-
Male	15 (75)	-	15 (75)	75	-
Phase					
1	-	-	10	50	4 (40)
2	-	-	7	35	6 (85.7)
3	-	-	3	15	2 (66.6)
SD: standard deviation					

However, even from this aspect alone, our study showed that SMAD4 methylation occurs in one of the most common lung cancer types, namely adenocarcinoma lung cancers. Our study is a rare study showing that SMAD4 promoter methylation may be associated with adenocarcinoma lung cancers.

Study Limitations

The fact that *SMAD4* gene methylation and *SMAD4* gene expression in RNA and protein levels could not be studied in tumor and normal lung tissues in our study is a limitation.

CONCLUSION

More research is needed on gene expression and pathways for SMAD4 in lung cancers. In the near future, the methylation sites of SMAD4 promoters promise to be therapeutic targets in lung cancers and other types of cancer where this gene is known to play a role. We believe that this may increase the effectiveness of available cancer treatments and have positive effects on life expectancy/quality of life for cancer patients.

Acknowledgement: I thanked to Dr. Ömer Yalçın for pathology examinations.

Ethics Committee Approval: This study was conducted with the permission of the Trakya University Local Ethics Committee (approval no: 05/21, approval date: 27.02.2013).

Informed Consent: Our study is a cross-sectional retrospective study and patient consent was not obtained.

Peer-review: Externally peer-reviewed.

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Retrospective Evaluation of the Neonatal Cholestasis Cases

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ABSTRACT

Objective: Neonatal cholestasis is a condition that begins in the first months of life and is accompanied by a direct increase in bilirubin and jaundice as a result of deterioration in bile production or excavation. Early and accurate diagnosis is important for treatment success and prognosis. In this study, we aimed to examine the demographic characteristics, etiological factors, clinical signs, treatment and final conditions of patients monitored for neonatal cholestasis and to determine the etiological factors of liver transplant patients.

Methods: Patients who were diagnosed with cholestasis in the neonatal period (<6 months) and followed up in our clinic for at least six months between January 2005 and January 2018 were included in the study. The clinical course and final status of the patients were recorded retrospectively.

Results: The median age of onset of jaundice in 131 patients (61.1% male) enrolled in the study was 6 days (range: 1-180 days). Ninety-nine (75.6%) patients were in the intrahepatic cholestasis group, and 32 (24.4%) were in the extrahepatic cholestasis group. In the intrahepatic cholestasis group, total parenteral nutrition-related cholestasis (27.3%) was the most common, and biliary atresia (71.9%) was the most common in the extrahepatic cholestasis group. Other main reasons were systemic (19.1%), metabolic (12.2%), hereditary cholestatic diseases (9.9%) and infectious (7.6%) causes. The median time of Kasai portoenterostomy in patients with biliary atresia was 64 days (range: 28-180 days). The highest (44%) mortality rate was in the patients with systemic disease-related cholestasis. Liver transplantation (n=21, 16%) was the most frequently performed in patients with biliary atresia.

Conclusion: Early diagnosis and timely treatment are very important for the optimal prognosis in neonatal cholestasis. The presence of acholic stools, maturity, early onset of jaundice and high gamma-glutamyl transferase levels should suggest biliary atresia. Early surgical treatment is warranted once the diagnoses was made, and liver transplantation is a treatment method that increases survival rate in these patient groups.

Keywords: Biliary atresia, transplant, neonatal cholestasis

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INTRODUCTION

Neonatal cholestasis is a condition that starts in the first months of life and progresses with direct (conjugated) bilirubin increase and jaundice as a result of impaired bile production or excretion (1). Its incidence is known as 1 in 2,500 live births (2). As a result of increased conjugated bilirubin and bile acids, toxin components cause liver damage and cause hepatobiliary dysfunction. Therefore, in a patient presenting with jaundice, it is critical to distinguish whether the jaundice is cholestatic or not.

Newborn babies, especially premature ones, are prone to cholestasis. The reasons of this are because of the irregularity in canalicular structure and function, the increase in hypomotility and paracellular permeability, and the decrease in hepatic immaturity and bile flow. Hepatic maturation is complete around the end of the first year. Therefore, there is no consensus regarding the age range of neonatal cholestasis. In most studies, it was considered to be about the first six months (3).

The most common cause of neonatal cholestasis is intrahepatic cholestasis (60-70%), and most of these cases are idiopathic neonatal hepatitis (INH) (4). INH covers a group of patients whose etiology cannot be found or whose specific diagnosis cannot be made by existing examinations rather than a diagnosis. Although it has been determined in studies that more than half of the cholestatic infants are patients with INH, the rate of INH diagnosis has decreased in recent years thanks to clinical and molecular developments (5). Infections [toxoplasmosis, rubella, cytomegalovirus (CMV), herpes virus, TORCH and syphilis], endocrinopathies (hypothyroidism, hypopituitarism, adrenal insufficiency), systemic diseases, genetic-chromosomal disorders, metabolism diseases, total parenteral nutrition (TPN), hereditary cholestatic diseases and anatomical disorders are other causes of intrahepatic cholestasis (6). Most patients with extrahepatic cholestasis are diagnosed with biliary atresia (BA). In etiological studies reported from different centers, BA accounted for about 25-35% of all neonatal cholestasis cases, while other major causes were genetic disorders (25%), metabolic diseases (20%) and alpha-1 antitrypsin deficiency (10%) (7). In recent years, the frequency of TPN-related neonatal cholestasis cases has also increased as a result of increased life rates of premature and low birth-weighted newborns.

Early and accurate diagnosis of neonatal cholestasis patients is very important for treatment success and prognosis. Evaluation of these patients is difficult due to the variety of cholestatic syndromes and the lack of clinical manifestations specific to diseases. But thanks to clinical and molecular advances, differential diagnosis can be made more easily in recent years.

In this study, it was planned to examine the neonatal cholestasis cases followed in our clinic with their demographic characteristics, etiological factors, clinical signs, treatment and final status and to compare these data with other studies in the literature.

METHODS

Patients diagnosed with neonatal cholestasis (0-6 months old) and followed for at least six months in the Karadeniz Technical University Faculty of Medicine Pediatric Gastroenterology, Hepatology and Nutrition Department between January 2005 and January 2018 were included in this study. Cholestasis was considered to be conjugated bilirubin levels above 1 mg/dL when serum total bilirubin levels were below 5 mg/dL, or conjugated bilirubin was more than 20% of total when total bilirubin levels were above 5 mg/dL (5,8). Demographic characteristics, etiological factors, clinical signs, treatment and final status of patients were examined and etiological factors were determined in the liver transplant cases.

Causes of cholestasis were grouped as extrahepatic and intrahepatic cholestasis. Intrahepatic cholestasis; i) INH, ii) metabolic diseases, iii) hereditary cholestatic syndromes, iv) infectious diseases, v) systemic diseases, vi) toxins (TPN, drugs), vii) anatomical disorders of the biliary tract, viii) undiagnosed extrahepatic cholestasis was classified as i) BA, ii) choledochal cysts, iii) biliary hypoplasia, iv) cholelithiasis (9).

Ethics committee approval was obtained from the Karadeniz Teknik University Faculty of Medicine Scientific Research Ethics Committee for the study (approval number: 2016/128, approval date: 21.09.2016). Our study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Consent was obtained from the patients.

Statistical Analysis

Statistical analysis was performed using "SPSS® for Windows version 22.0" (IBM Corp., Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Descriptive statistics of evaluation results; number and percentage for categorical variables, mean ± standard deviation for continuous variables were given as minimum, maximum, and median. Chi-square test was used to analyze the differences between the ratios of categorical variables in independent groups. Statistical significance level of alpha was accepted as $p<0.05$.

RESULTS

A total of 131 patients were enrolled in the study [61.1% male, median age; 35 days (range: 1-180 days)]. Patients' median age of onset of jaundice; it was 6 days (range: 1-180 days). Forty-three patients (32.8%) had prematurity and parents of 43 patients (32.8%) had a consanguineous marriage. The findings other than jaundice were hepatomegaly (n=102, 77.9%), splenomegaly (n=48, 36.6%), acholic stool (n=46, 35.1%), umbilical hernia (n=9, 6.9%), limb anomaly (n=7, 5.3%) and atypical facial appearance (n=7, 5.3%). The demographic and clinical features of the patients at the time of application are shown on Table 1.

Patients were divided into two groups according to their diagnosis. Extrahepatic cholestasis was detected in 32 (24.4%) patients and intrahepatic cholestasis was detected in 99 (75.6%) patients. Recent diagnoses of patients are shown in Table 2.

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Table 1. Demographic characteristics of the patients at the time of admission

Demographic characteristics at the time of admission	Number of patients (n=131) n (%)
Age of admission, days	
Mean ± SD	45.2±41.3
Median	35
30≤ days, n (%)	52 (39.7)
31-90 days, n (%)	63 (48.1)
91-150 days, n (%)	9 (6.9)
151-180 days, n (%)	7 (5.3)
Gender, n (%)	
Female	51 (38.9)
Male	80 (61.1)
Age of onset of jaundice, day	
Mean ± SD	22.7±35.0
Median	6
15< days, n (%)	81 (61.8)
15≥ days, n (%)	50 (38.2)
Prematurity, n (%)	43 (32.8)
34-37 weeks	23 (53.5)
30-33 weeks	12 (27.9)
30< week	8 (18.6)
Close kinship, n (%)	43 (32.8)

Table 2. Distribution of patients by diagnosis

Localization	Etiology	Number of patients; n	(%)	Total
Extrahepatic cholestasis	Biliary atresia	23	17.6	24.4%
	Choledoc cyst	5	3.8	
	Cholelithiasis	2	1,5	
	Biliary hypoplasia	2	1.5	
Intrahepatic cholestasis	TPN-associated cholestase	27	20.7	75.6%
	Associated with systemic disease	25	19.1	
	Metabolic causes	16	12.2	
	Hereditary cholestatic diseases	13	9.9	
	Associated with infection	10	7.6	
	Undiagnosed	4	3.1	
	Idiopathic neonatal hepatitis	2	1.5	
	Anatomical disorder	2	1.5	

TPN: total parenteral nütrisyon

BA (n=23) constituted 71.9% of patients with extrahepatic cholestasis and 17.6% of all patients with cholestasis. Kasai portoentrostomy was performed in 22 (95.7%) of the patients with BA after the diagnosis was made by intraoperative cholangiography. The mean age of Kasai portoentrostomy was 68.6±33.5 (median: 64 days, range: 28-180) days. Since one patient (4.3%) presented late (225th day), liver transplantation was performed in the follow-up before Kasai operation could be performed. Liver transplantation was performed in 10 (43.5%) of 22 patients who underwent Kasai operation (all living donors). All of the transplant patients (n=11, 47.8%) are alive and their median age at present is 6.8 years (range: 0.9-13.5 years). Three (25%) of the 12 patients who did not have a transplant, died due to decompensated cirrhosis while waiting on the transplant list. When liver function was evaluated in nine living patients (39.1%), seven patients (30.4%) had compensated cirrhosis, and two patients (8.7%) had decompensated cirrhosis. Among all BA patients, there were three (13%) patients with excitus, and 20 (87%) patients who survived. Other causes of extrahepatic cholestasis (n=9) were biliary cyst (n=5), biliary hypoplasia (n=2), and cholelithiasis (n=2). Cystectomy was performed in five patients with biliary cyst, external drainage was performed on a patient with biliary hypoplasia, cholecystectomy was performed on a patient with cholelithiasis, and other patients were followed up with medical treatment.

Most patients in the intrahepatic cholestasis group had TPN-associated cholestasis (n=27, 27.3%). The cases of 21 (77.8%) and premature indications for TPN; necrotizing enterocolitis (NEC) (n=13, 48.1%), short bowel syndrome (n=7, 36%), sepsis (n=5, 18.5%), Pierre-Robin variant (n=1, 3.7%) and anatomical problems (n=1, 3.7%). Patients were given cyclic fat support (n=27, lipid 1 g/kg/day three days a week), ursodeoxycholic acid (n=22, 15 mg/kg/day), N-acetylcysteine (n=4, 5 mg/kg/hour-5 days), omega-3 (n=1, 1-2 mL/kg) therapy as a treatment protocol. In follow-up, five patients had died due to sepsis (two with fungal sepsis, three with catheter-related sepsis). The average follow-up period of living patients (n=22, 77.8%) was 5.6 years (range: 1-11.5 years) and liver function tests were normal in their last applications.

In the metabolic diseases group (n=16, 16.2%); four patients (25%) galactosemia (homozygous Q188R mutation), four patients (25%) tyrosinemia, two patients (12.5%) cystic fibrosis (*CFTR* gene; p.F508del homozygous mutation), one patient (6.3%) was diagnosed with Wollman's disease. Five patients (31.3%) were described as possible metabolic diseases due to non-specific changes in their metabolic tests, a history of close kinship, a history of infant death, coagulopathy, and ascites. A lactose-free diet for patients with galactosemia, a diet for patients with tyrosinemia and NTBC (1 mg/kg), and medical treatment for patients with cystic fibrosis (pancreatic enzyme replacement, medium-chain triglyceride fat support and vitamin A, D, E, K) were initiated. In follow-up, 10 patients underwent medical treatment as well as a new diet, and three patients (n=4, 25%) who had Wollman's disease and were considered a possible metabolic

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disease died. One of the two patients with tyrosinemia had a liver transplant due to hepatocellular carcinoma and the other due to decompensated cirrhosis (one from a cadaver, one from a live donor from the mother).

Patients with hereditary cholestatic syndrome (n=13, 13.1%); seven (53.8%) progressive familial intrahepatic cholestasis (PFIC, *ABCB4* gene in three patients; p.Ala953Asp homozygous mutation-PFIC-3 and *ABCB11* gene; p.E1302 homozygous mutation-PFIC-2, *ATP8B1* gene in one patient; 18q21.31 homozygous mutation -PFIC-1), six of them (46.2%) were diagnosed with Alagille syndrome. A total of five patients had liver transplantation. The median age of transplant PFIC patients (n=4, 57.1%) at the time of transplant was 29.5 months (range: 5-66 months), all alive, and their current median age of 8.8 years (range: 3.7-13.5 years). One of the patients with Alagille syndrome (16.7%) underwent liver transplantation (living donor) at the age of two months, but died eight months after transplantation due to organ rejection. Two of the five patients (83.3%) who were not transplanted died while waiting on the list for transplant. The median follow-up period of surviving patients (n=3) is 9.7 years (range: 9.2-10.2 years).

Patients in the infectious group (n=10, 10.1%) accounted for 7.6% of all patients with cholestasis [congenital CMV (n=4, 40%), toxoplasma infection (n=1, 10%) and sepsis-associated cholestasis (n=5, 50%)]. Four of the patients with sepsis had pyelonephritis *Escherichia coli* (*E.coli*), and one had nosocomial sepsis. Two of these patients died [20%, CMV (n=1), nosocomial sepsis (n=1)].

Patients in the cholestasis group associated with systemic disease (n=25, 25.3%) constituted the 19.1% of all patients with cholestasis. Eight of these patients (32%) had genetic-chromosomal disease [arthrogryposis-renal dysfunction-cholestasis (ARC) (n=3), Down syndrome (n=1), trisomy 13 (n=1) and genetic syndrome (n=3, multiple congenital anomalies)], endocrinopathy in seven (28%) [congenital hypothyroidism (n=5), hypopituitarism (n=1), adrenal insufficiency (n=1)], in nine (36%) hematologic-immunological diseases [chronic hemolytic disease (n=4), neonatal hemochromatosis (n=2), hemophagocytic lymphohistiocytosis (HLH, n=1), common variable immunodeficiency (n=1), autoimmune giant cell hepatitis and autoimmune hemolytic anemia (n=1)] and in one (4%) had congenital heart disease (left ventricular hypertrophy, aortic valve dysplasia, pulmonary stenosis). In general, the highest mortality (n=11, 44%) was in this group, and genetic-chromosomal disease (three patients with genetic syndrome and ARC) in six patients, endocrinopathy (congenital hypothyroidism) in one, hematological-immunological disease in three patients (one was HLH, one had neonatal hemochromatosis, the other was immunodeficiency) and one had congenital heart disease. In this group, only one patient (autoimmune giant cell hepatitis + autoimmune hemolytic anemia) received liver transplantation (living donor) at the age of four months due to decompensated cirrhosis. The transplanted patient is alive and the average follow-up period is 6 years.

Patients in the cholestasis group associated with anatomical disorders (n=2, 2%) constituted 1.5% of all patients with cholestasis. The median onset of jaundice in these patients was 35 days (range: 1-68 days). One of the patients died five days after being diagnosed with Caroli syndrome. While the other patient was being followed up with the diagnosis of congenital hepatic fibrosis and autosomal recessive (AR) polycystic kidney disease, liver transplantation (live donor) was performed due to decompensated cirrhosis, and the patient died due to sepsis after transplantation.

INH group (n=2, 2%) comprised 1.5% of all patients with cholestasis. Patients with unfitting clinical and laboratory findings to a specific disease group and with undetermined cholestasis etiology were evaluated as INH. All patients had hepatomegaly and splenomegaly on physical examination. One (50%) of INH patients received liver transplantation at the age of seven months (living donor). The liver functions of the other patient are normal and follow-up continues.

Four patients (4%) who could not be diagnosed constituted 3.1% of all patients with cholestasis. One of these patients (25%) died at the age of two months and the etiology was not found. The median follow-up period of the surviving patients was 5.8 years (range: 0.9-10.7 years). Liver functions of three patients who were living in the follow-up were completely normalized.

When BA and other diseases are compared; while there was no significant difference in terms of gender and kinship, a significant difference was found in the age of onset of acholic defecation, jaundice, gestational week and gamma-glutamyl transferase (GGT) ($p<0.001$, $p=0.028$, $p=0.001$ and $p=0.001$, respectively) (Table 3).

When the last conditions of the patients are evaluated; liver transplantation was performed in 21 (16%) of 131 patients (20 living, one cadaver donor) (Table 4). 99 (75.6%) of all patients were alive (61.1% with their own liver, 14.5% after transplantation) 32 of them (24.4%) were exitus (22.9% with their own liver and 1.5% after transplantation). Both five-year and 10-year survival rates of BA patients were 86%. Five-year and 10-year survival rates of systemic disease-related cholestasis cases with the highest mortality were determined as 56%. The final status of the patients is shown in Table 5.

Table 3. Comparison of biliary atresia and other diseases

	Biliary atresia (n=23)	Other cholestatic diseases (n=108)	p
Gender, n (%), M	11 (47.8)	69 (63.9)	0.231
Age of onset of jaundice, day, mean ± SD	3.4±22.8	24.7±36.9	0.028
Prematurity, n (%)	0	43 (39.8)	0.001
Week of birth, mean ± SD	38.9±0.7	36.3±3.8	0.001
Acolic defecation, n (%)	21 (91.3)	25 (23.1)	<0.001
Close kinship, n (%)	6 (26.1)	37 (34.3)	0.608
GGT values (U/L)	477.9±336.4	274.7±317.2	0.001

GGT: gamma-glutamyl transferase, SD: standard deviation

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Table 4. Demographic characteristics, recent diagnoses and recent status of liver transplant patients

	Number	Transplant age (median)	Gender	Donor	Final situation	Current age (median)
Biliary atresia	11	13 months (5-152 month)	Female (n=7) Male (n=4)	Living donor	Live	6.8 years (0.9-13.5 years)
PFIC	4	29.5 ay (5-66 month)	Female (n=2) Male (n=2)	Living donor	Live	8.8 years (3.7-13.5 years)
Alagille syndrome	1	2 month	Female	Living donor	Exitus after 8 months	-
INH	1	7 month	Female	Living donor	Live	5 years 7 months
OIHA+OIH	1	4 month	Male	Living donor	Live	7 years
Tyrosinemia	1	32 month	Male	Cadaver donor	Live	8 years 1 month
Tirozinemi-HCC	1	14 month	Female	Living donor	Live	9 years 5 months
ORPCKD+CHF	1	6 month	Female	Living donor	Exitus after 2 months	-

HCC: hepatocellular carcinoma, INH: idiopathic neonatal hepatitis, OIHA+OIH: giant cell autoimmune hepatitis and autoimmune hemolytic anemia, ORPCK-D+CHF: autosomal recessive polycystic kidney disease + congenital hepatic fibrosis, PFIC: progressive familial intrahepatic cholestasis

Table 5. Assessment of recent conditions of patients with cholestasis

Feature	n	Living with his own liver n (%)	After living liver tx n (%)	Total living n (%)	Exitus with his own liver n (%)	Exitıs after living liver tx n (%)	Total exitus n (%)
Biliary atresia	23	9 (39.2)	11 (47.8)	20 (87)	3 (13)	-	3 (13)
Other extrahepatic causes	9	8 (88.9)	-	8 (88.9)	1 (11.1)	-	1 (11.1)
TPN associated	27	22 (81.5)	-	22 (81.5)	5 (18.5)	-	5 (18.5)
Systemic diseases	25	13 (52)	1 (4)	14 (56)	11 (44)	-	11 (44)
Metabolic causes	16	10 (62.5)	2 (12.5)	12 (75)	4 (25)	-	4 (25)
Hereditary cholestatic diseases	13	6 (46.2)	4 (30.8)	10 (77)	2 (15.4)	1 (7.6)	3 (23)
Associated infection	10	8 (80)	-	8 (80)	2 (20)	-	2 (20)
Other	8	4 (50)	1 (12.5)	5 (62.5)	2 (25)	1 (12.5)	3 (37.5)
Total	131	80 (61.1)	19 (14.5)	99 (75.6)	30 (22.9)	2 (1.5)	32 (24.4)

TPN: total parenteral nutrition

DISCUSSION

Evaluation of patients with cholestasis is difficult due to the variety of cholestatic syndromes and nonspecific clinical findings. With a clearer understanding of the mechanisms of the hepatobiliary system, differential diagnosis can be made better recently. Considering the etiology of 131 patients in our study; (i) intrahepatic cholestasis was detected in the majority of cases (75.6%) and most of this was TPN-related cholestasis (27.3%), (ii) the majority of cases with extrahepatic cholestasis had BA (71.9%), (iii) Kasai portoenterostomy in BA patients, it was observed that the median time was 64 days, (iv) the majority of the cases undergoing liver transplantation were BA (52.4%), (v) the mortality of cholestasis cases associated with systemic disease was high (44%).

In our study, the etiology of neonatal cholestasis was determined to be 60-70% intrahepatic cholestasis, similar to other studies in the literature. The majority of intrahepatic cholestasis cases are INH and TPN-related cholestasis, depending on the period during which the study was conducted (4). In recent years, the rate of INH diagnosis has decreased as a result of clinical and molecular developments (5). As in our study, the frequency of TPN cholestasis is increasing due to better neonatal care of premature and low birth weight babies and better treatment of complications. Most of the risk factors in the studies are NEC, sepsis and major intestinal surgeries (10). The best treatment method is to start enteral nutrition as soon as possible and stop parenteral nutrition, and omega-3 supplementation and lipid reduction are other recommended applications (11). Although organ transplantation is required for long-term TPN cholestasis due to liver failure and cirrhosis, in our cases, there was no need for liver transplantation. The cause of death of the vast majority of patients who have died is sepsis associated with catheter or blood flow.

The most common and most important cause of extrahepatic cholestasis is BA (12). Although the centers vary depending on the

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patient density, BA accounts for 20-35% of all cholestasis. A study conducted in our country found that 16.8% of all cholestasis cases constitute extrahepatic cholestasis, while 75% of extrahepatic cholestasis is BA (13). In another study, the BA rate was found to be 25.9% (438/1,692) in all neonatal cholestasis patients (5). Early diagnosis and treatment in BA is very important for prognosis. In cases where early diagnosis was made and Kasai portoenterostomy was performed at an early age, it was observed that the need for liver transplantation was less (12). The median time for Kasai portoenterostomy in our center was 64 days. Compared to other studies, our center found that the age of operation Kasai is similar to that of many centers. It was reported as 63 days in an organ transplant center in America and 66 days in another study based in London (14,15).

Although acholic stools are specific for BA, the bile ducts must be visualized by intraoperative cholangiography for definitive diagnosis. Until now, various objective scoring systems have been created for BA to reduce the need for unnecessary intraoperative cholangiography, and a diagnosis has been attempted non-invasively. In a study conducted in America; acholic stools, high GGT (>204 U/L) and normal weight Z score were found to be associated with BA (12). In a study conducted in our country; it has been reported that ultrasonography findings, high GGT (>197 U/L) and acholic stools are associated with BA (16). Our study also found that acholic stool and GGT height were significant for BA, similar to these studies in the literature.

The liver is one of the most important target organs of metabolic diseases. In the neonatal period, patients with galactosemia, tyrosinemia and cystic fibrosis can apply with signs of acute liver failure. In a study, 36.5% (27/74) of 74 patients with metabolic disease and cholestasis were diagnosed with galactosemia, 8.1% (6/74) of them were diagnosed with tyrosinemia. In the same study, the diagnosis of cystic fibrosis was found at a rate of 0.89% (15/1,692) in the general screening (4). In our study, the rate of metabolic disease was determined as 12.1% and galactosemia and tyrosinemia were most commonly detected. In most AR inherited diseases, detailed questioning of family history and close kinship is important, and early diagnosis and treatment are important.

Especially in recent years, an increase in the diagnosis of hereditary cholestatic syndromes has been observed with the increase in molecular studies. In addition to jaundice, signs such as itching, diarrhea, growth retardation in patients are significant in terms of PFIC; skeletal abnormalities, typical facial appearance, pulmonary stenosis, and eye signs are significant in terms of Alagille syndrome (17). In these patients, both due to liver failure and due to complications (itching, growth retardation, especially in patients diagnosed with PFIC, etc.) indication of liver transplantation may occur. Similar to the studies in the literature, in our study, liver transplantation was performed most often to these patients after BA.

When the literature is examined, cholestasis due to infectious diseases was observed with a rate of 2.6% (5/190) in a study conducted in our country, while it was seen at a rate of 7.7% in our study (13). In the large-scale screening conducted in the United States, cholestasis due to infection was observed at a rate of 11.5% (194/1692), and in the infectious group, 33.5% of the patients were CMV infection, 3.6% toxoplasma infection, 24.7% sepsis and 9.8% urinary tract infections (UTI) (4). Cholestasis may also accompany bacterial sepsis. Mediators caused by bacterial products and endotoxins cause cholestasis, leading to changes in hepatic circulation and a decrease in bile flow. In a study conducted by Tiker et al. (18), the most common cause of cholestasis is sepsis (*E.coli*-UTI-related) and cholestasis findings developed on the 10th day on average.

Even if neonatal cholestasis is a sign of liver disease, other systemic diseases involving the liver may also present with cholestasis. Therefore, when a cholestatic patient admits, it is recommended to investigate cardiac, renal and, if there are neurological findings, in terms of central nervous system malformations that may accompany. Similar to our study, in the study of Sarı et al. (13); echocardiography (echo) results mostly found atrial septal defect, patent foramen ovale, and peripheral pulmonary stenosis. These echo findings may sometimes accompany the disease incidentally and sometimes give clues about the etiology. In particular, peripheral pulmonary stenosis is significant in patients with Alagille syndrome. If we examine the renal findings, polycystic kidney findings are important for Caroli's disease, and and renal parenchymal echo findings are important for metabolic diseases.

Neonatal cholestasis accounts for more than half of the causes of liver transplantation in children, and the most common cause is known as BA (40%-50%). McDiarmid et al. (19) found that 33.5% of 1,187 liver transplant patients were under 1 year of age and 65.6% of these patients were BA. In another study conducted by Haberal et al. (20) in children, 101 liver transplants were performed and the most common indication was BA (n=24, 23.7%). In another liver transplant center, Zeytunlu et al. (21) examined 1,001 liver transplant patients (183 pediatric patients) and found the most common transplant indication in pediatric patients as cholestatic diseases (69%) and the most common BA and PFIC in cholestatic diseases. Similarly in our study, the most common indications for transplantation are BA and hereditary cholestatic diseases.

Study Limitations

Our study is retrospective and based on patient file records. In addition, there are patients with idiopathic cholestasis who have not been diagnosed as a result of technical impossibilities (lack of metabolic and genetic examination methods) in the first years of the study.

CONCLUSION

The causes of cholestasis vary in the neonatal period. Early diagnosis and timely treatment are crucial for mortality, morbidity and optimal prognosis. Presence of acholic stools, maturity, early onset of jaundice and high GGT levels should primarily suggest BA. In particular, the diagnosis of BA and metabolic diseases that

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require early surgery is important, and liver transplantation is a treatment method that increases the survival rate in these groups of patients.

Ethics Committee Approval: Ethics committee approval was obtained from the Karadeniz Teknik University Faculty of Medicine Scientific Research Ethics Committee for the study (approval number: 2016/128, approval date: 21.09.2016).

Informed Consent: Consent form was obtained from the patients.

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Targeted Follow-up of Incidental Lung Nodules: Will the New Nodules in Unscanned Regions Be Missed?

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ABSTRACT

Objective: Targeted computed tomography (TCT) scans a limited part or those parts of the lung with nodules that require follow-up. In order to apply targeted tomography safely, it is important to know the frequency of newly appearing nodules in unscanned regions of lung. We aimed to evaluate the frequency and importance of new pulmonary nodules that appear in patients followed-up for nodules according to Fleischner society guidelines.

Methods: A total of 117 patients (women: 54; men: 63; mean age: 55±14 years; range: 30-88 years) who were followed-up for 265 lung nodules were included in this study. The inclusion criteria was presence of at least 1 nodule that was followed-up for at least 6 months with CT. Patients with calcified nodules or known malignancy during initial CT were excluded.

Results: The median follow-up time was 22 months (range: 6-80 months) and the median number of follow-up CT scans was 2 (range: 1-5). New nodules appeared only in 6% (7/117) of the patients, of which 5 had nodules that disappeared or decreased in size during follow-up.

Conclusion: For patients who were followed-up for pulmonary nodules, the frequency of new nodule formation was low. This should encourage the use of TCT for nodule follow-up. With an appropriate TCT follow-up schedule, patients will receive reasonably low radiation levels without affecting their management.

Keywords: Lung nodules follow-up, chest CT, targeted computed tomography, low-dose CT

INTRODUCTION

Lung nodules are commonly detected on chest computed tomography (CT) scans. Although most of these nodules are benign, there is currently no safe method to predict the malignant potential of the nodules unless they are followed-up (1). Many patients with solid lung nodules require follow-up with CT for 2 years (2,3). Subsolid lung nodules may even require follow-up for as long as 3-5 years (4). After initial CT, depending on the risk factors of the patient, as well as the size and configuration of the nodules, patients may require 2-6 control CT examinations (2).

Repeated CT examinations increase the radiation exposure of patients. Particularly, for younger individuals, the potential harms of radiation exposure via repeated diagnostic imaging may exceed its benefits. A number of studies have attempted to lower CT radiation doses (5-7). Targeted CT (TCT) scans a limited part or those parts of the lung with nodules that require follow-up. In addition to low-dose CT techniques, TCT for lung nodules may reduce the dose delivered to a patient by as much as 80% (8).

However, before TCT for lung nodule follow-up can gain wide acceptance, the potential of new nodule formation in unscanned lung regions must be defined. Thus, this study aimed to evaluate

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the frequency and importance of new pulmonary nodules in patients followed-up for incidental lung nodules.

METHODS

Patient Selection and Imaging

The study protocol was reviewed and approved by the Koç University Ethics Committee (approval number: 2014.073.IRB2.019, approval date: 29.04.2014), which waived the requirement of informed consent. All lung CT medical reports in our radiology database between January 2008 and December 2010 were searched retrospectively.

We included a total of 117 patients who had at least 1 nodule and control CT imaging (no sooner than 6 months). Patients with calcified nodules or a known malignancy during initial CT were excluded. Of the 117 patients, 62 had a smoking history of over 20 pack/year, 41 had a smoking history between 10 and 19 pack/year, and 12 had a smoking history between 4 and 9 pack/year.

For all patients, CT was performed using 2×64 Somatom Definition and 2×128 Somatom Definition Flash Multidetector CT (Siemens Healthcare, Erlangen, Germany). The entire chest starting from the lung apices down to posterior costophrenic sulci was scanned with 0.625 mm collimation, 80-120 kVp and 40-120 mAs. Images were reconstructed with 1.5 mm section thickness.

All nodules identified from previous reports were re-evaluated using a picture archiving and communication system (Centricity PACS, General Electric Medical Systems, Milwaukee, WI) by two experienced radiologist who have over 10 years of experience in interpreting CT scans of the chest. All discrepancies were resolved by consensus. The number and mean dimensions of the nodules were determined. Follow-up duration was determined according to Fleischner society guidelines (2). Location of the nodules was defined on the basis of pulmonary lobes. The margins of the nodules were classified as well- or ill-defined with a smooth or spiculated border. The nodules were also classified as solid or subsolid. New nodules were recorded and evaluated on the basis of size, location, margin characteristics and density. Changes in new nodules were evaluated when available. In addition, all patients mediastinum and bones were also evaluated for potential pathologies.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, Version 22 (SPSS Inc., Chicago, Illinois, USA). Results were expressed as medians (ranges).

RESULTS

We included a total of 117 patients (women: 54; men: 63; mean age: 55±14 years; range: 30-88 years) with 265 nodules on initial CT scans (Table 1). Median follow-up time was 22 months (range: 6-80 months) (Table 2) and median number of follow-up CT scans was 2 (range: 1-5).

Of the 265 nodules, 246 (93%) were solid, 19 (7%) were subsolid; 254 (96%) nodules had smooth borders, while 11 (4%) had irregular borders. Of the 117 patients, 57 (49%) had a single nodule, 22 (19%) had 2 nodules, 14 (12%) had 3 nodules and 24 (21%) had >3 nodules.

Table 1. Selected baseline characteristics of the study group

Age	
30-39	19 (16.2%)
40-49	24 (20.5%)
50-59	25 (21.4%)
60-69	25 (21.4%)
70-79	18 (15.4%)
≥80	6 (5%)
Gender	
Female	54 (46%)
Male	63 (54%)

Table 2. Follow-up time for initial nodules

Follow-up time	
6-12 months	34 (13.3%)
13-18 months	60 (23.5%)
19-24 months	58 (22.6%)
>24 months	104 (40.6%)

Regarding the location of the nodules, 46 (17.4%) were in the right upper lobe, 26 (10%) were in the middle lobe, 89 (33%) were in the right lower lobe, 36 (14%) were in the left upper lobe and 68 (26%) were in the left lower lobe.

Among the 265 nodules, 252 (95%) were stable, 5 (2%) increased in size and 8 (3%) decreased in size on follow-up. Two of the 5 nodules that increased in size were proven malignant by histopathological evaluation and removed by lobectomy. One of them decreased in size on subsequent follow-up and the other two maintained a stable size.

A total of 16 new nodules were detected in 7 (6%) patients. Subgroup analysis for these new nodules revealed the following: In 3 patients, the nodules disappeared and, in 2 patients, the nodules decreased in size (from 7 mm to 3 mm in 1 patient and from 12 mm to 9 mm in the other patient). Two patients had been lost to follow-up: One patient had new nodules of 2 mm and 3 mm and the other had new nodules of 3 mm and 5 mm and could not be followed-up (Figure 1). All patients with new nodules are summarised in Table 3. During follow-up in none of the patients, new lymphadenopathies or bone lesions were detected.

DISCUSSION

Follow-up of lung nodules and screening for lung cancer are routine procedures in many countries. Although many studies have attempted to reduce patient doses during lung CT, TCT for nodule follow-up has not been extensively discussed. After initial CT for patients with fewer than 3 nodules or nodules that

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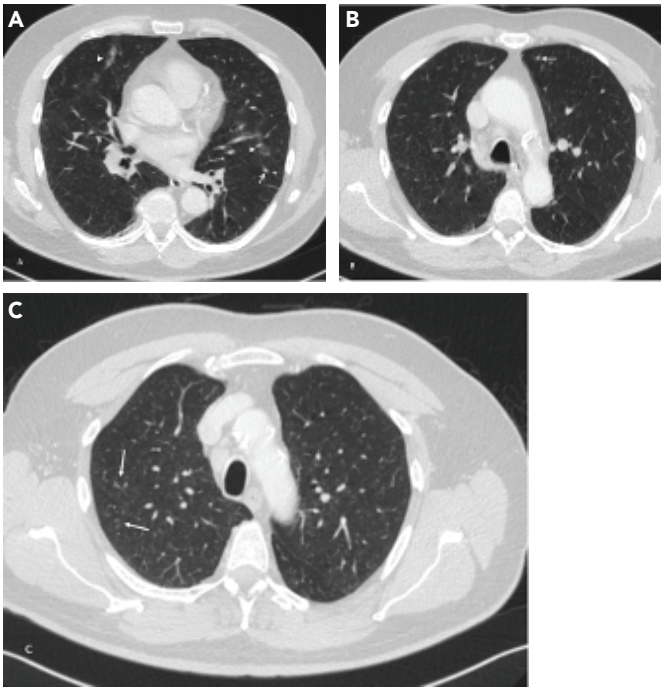

Figure 1. A 55-year-old male patient who has been followed-up for 3 mm and 5 mm nodules underwent computed tomography (CT) imaging for 22th month control. Non-contrast CT image shows (A) 5 mm and (B) 3 mm new appearing nodules (arrows) in the left upper lobe. Note that there is focal ground glass infiltration (arrowhead) in both upper lobes. (C) Right upper lobe image shows centrilobular nodules, which were absent in previous CT. Unless we lost contact with patient, these secondary findings may be suggestive of an infectious origin of these two nodules

are in the same lobe, TCT may be the most effective method for reducing the radiation dose delivered to a patient.

In this study, we determined the frequency of new nodules and their outcomes, which may be a major drawback for using TCT. Assuming that TCT was used for our study group instead of conventional whole lung scanning, only 7 of the 117 patients were found to have new nodules. Five patients' nodules disappeared or decreased in size during follow-up. Only 2 (1.7%) of the 117 patients would have nodules that may have been missed with TCT. These nodules were smaller than 5 mm and had smooth contours. None of these nodules were proven malignant.

Our median follow-up period was nearly 2 years, which should have been sufficient to detect new nodule potential in a population that was being followed-up for incidental lung nodules. This showed that at least 97.3% patients who were followed-up for incidental lung nodules had unnecessary whole lung CT scanning. We lost contact with 2 patients who had new nodules. For patients who had fewer than 4 nodules, follow-up CT within a year using TCT appears feasible. However, this technique still requires validation prospectively in larger groups.

These findings are important because patients may be followed-up with significantly reduced radiation exposure. TCT will also save the scanner tube life, which will reduce the expenses of clinics. In addition, using TCT would shorten interpretation and report times due to the decreased anatomical coverage.

Follow-up with TCT may be impractical for multiple nodules; nevertheless, it may be practical to use TCT for less than four nodules. Among our patients, 79.5% had fewer than 4 nodules and 48.7% had only 1 nodule. These findings suggest that a large

Table 3. Characteristics of new nodules

Patients	Nodule	Gender	Age	Location	Size (mm)	Density	Margin	Follow-up
Patient 1	Nodule 1	Female	75	right lower lobe	3	subsolid	smooth	na
Patient 1	Nodule 2			left upper lobe	2	solid	smooth	na
Patient 2	Nodule 1	Female	40	right upper lob	2	solid	smooth	disappeared
Patient 3	Nodule 1	Male	55	left upper lobe	5	solid	smooth	na
Patient 3	Nodule 2			left upper lobe	3	solid	smooth	na
Patient 4	Nodule 1	Female	51	right upper lob	3	subsolid	smooth	disappeared
Patient 4	Nodule 2			left upper lobe	6	subsolid	smooth	disappeared
Patient 4	Nodule 3			left upper lobe	3	solid	smooth	disappeared
Patient 4	Nodule 4			left upper lobe	3	solid	smooth	disappeared
Patient 4	Nodule 5			right upper lob	4	subsolid	smooth	disappeared
Patient 4	Nodule 6			right upper lob	3	solid	smooth	disappeared
Patient 4	Nodule 7			right lower lobe	4	solid	smooth	disappeared
Patient 4	Nodule 8			left upper lobe	3	solid	smooth	disappeared
Patient 5	Nodule 1	Female	61	right upper lob	5	solid	irregular	disappeared
Patient 6	Nodule 1	Male	35	left upper lobe	12	solid	smooth	decreased in size
Patient 7	Nodule 1	Female	71	right middle lobe	7	solid	irregular	decreased in size

na: not available

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number of patients who are followed-up for incidental nodules will experience the benefits of TCT.

There have been discussions regarding the lowering of the age for lung nodule screening. Screening younger age groups may increase the chance of early lung cancer detection, which should contribute to improvement in survival rates (9,10). The major drawback of lowering the population age for lung screening is the possible harmful effects of ionising radiation, since younger people are more sensitive to the effects of ionising radiation (11). When TCT is used for follow-up, high-risk populations may be screened at a younger age. After an initial low dose of whole lung CT scan, patients with fewer than 4 nodules may be followed-up using TCT at very low doses. Further studies must be conducted to determine the age groups and risk factors for screening people who are younger than 55 years. Using TCT for nodule follow-up may make earlier screening safe and applicable.

In this study, all the 16 new nodules detected were sub-centimetric, except one. Most of these nodules probably had an infectious origin and had decreased in size or disappeared on follow-up. One patient showed 8 new nodules on her second follow-up CT; however, 6 months later during her next control CT, all those nodules had disappeared. One patient had a 5-mm nodule that disappeared within 15 months (Figure 2) and another patient had a 2-mm nodule that disappeared within 12 months. One patient was being followed-up for left upper lobe nodules that were most likely of granulomatous origin and a new 12-mm nodule was found in the same lobe. This new nodule decreased to 10 mm within 20 months, which suggests that it is from a benign origin (Figure 3). One patient was being followed-up for 6 nodules (size range: 4-6 mm) and a new 7 mm nodule was found, which decreased to 5 mm within 9 months (Figure 4).

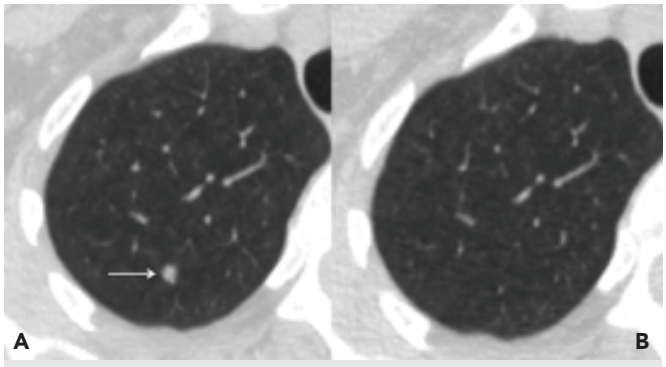

Figure 2. A 61-year-old female who has been followed-up for a 6 mm nodule in the left lower lobe underwent non-contrast control computed tomography imaging, (A) which showed a 5 mm new nodule (arrow) in the right upper lobe. (B) 15 months later, this 5 mm nodule disappeared, thus revealing its benign nature

Study Limitations

The limitations of our study included the retrospective nature of the study. Also, we could not follow-up 2 patients with new

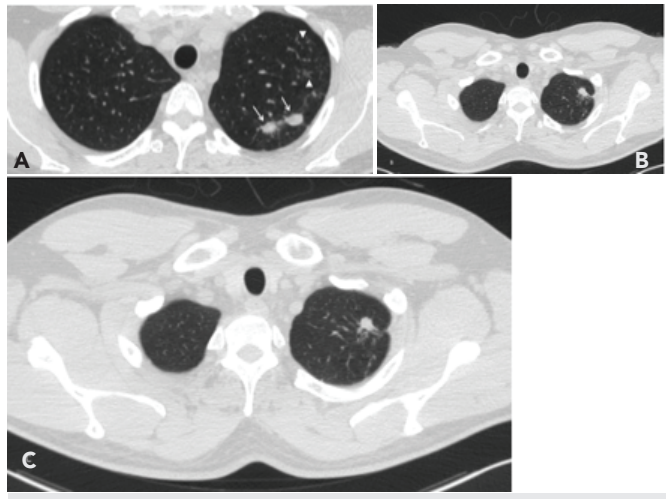

Figure 3. A 35-year-old male. (A) Non-contrast computed tomography (CT) image shows two nodules (arrows) that were decided to be followed-up. Anteriorly centrilobular nodules (arrowhead) may suggest an active infectious process. (B) 6th month control CT of left upper lobe shows new nodule in the area where centrilobular nodules were previously seen. (C) Follow-up CT after 20 months shows a decrease in size of the nodule

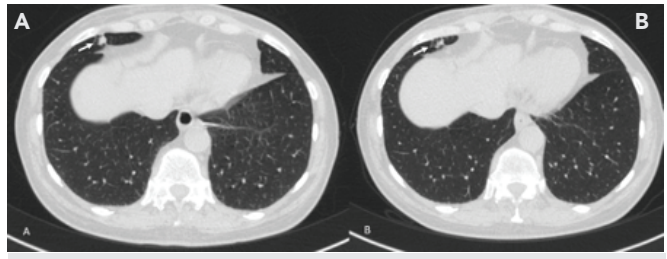

Figure 4. A 71-year-old female who has been followed-up for subcentimetric six nodules underwent non-contrast control computed tomography imaging and (A) showed new 7 mm nodule (arrow) in middle lobe. (B) 9 months later, the nodule decreased in size

nodules because we lost contact with them. In our study group, with 256 nodules, the malignancy rate was low (0.78%). Most of the patients who have nodules with malign CT features undergo biopsy or surgery after initial CT instead to be followed-up. This could explain the low malignancy rate seen in our cohort.

In this study, we did not define whether TCT was practical for covering the targeted nodule or nodules. One technique proposed for TCT for nodule follow-up claimed that all nodules during follow-up were scanned in their entirety (8). In addition, we could not compare the radiation doses between TCT and conventional whole lung CT. Our primary aim was to provide information regarding new nodule frequency in a population that was followed-up for lung nodules. The low frequency of new nodule formation should instigate the need for further studies.

Determination of the application method and patient selection criteria for TCT were beyond the scope of this study. To resolve these issues, prospective studies will be required for TCT for lung nodule follow-up.

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CONCLUSION

In patients who were followed-up for incidental nodules, the frequency of new nodule formation seems to be low. This should encourage the use of TCT for nodule follow-up. With an appropriate TCT follow-up schedule, patients will administered reasonably low radiation levels without affecting their management.

Ethics Committee Approval: The study protocol was reviewed and approved by the Koç University Ethics Committee (approval number: 2014.073.IRB2.019, approval date: 29.04.2014).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.G., G.U.; Design - T.G.; Data Collection and/or Processing - T.G., G.U.; Analysis and/or Interpretation - T.G., G.U.; Literature Search - G.U.; Writing - T.G.

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

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An Approach to Perianastomotic Pouches due to Anastomotic Leakage After Rectal Resection

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ABSTRACT

Objective: Conservative treatment of perianastomotic pouch due to low anastomosis in rectal surgery is possible in patients without generalized peritonitis. This report describes the treatment of this complication using Endo-SPONGE® and transrectal endoscopic lavage.

Methods: Sixteen patients with abscess resulting from anastomotic leakage after rectal resections were retrospectively reviewed; nine of them were treated with transrectal endoscopic lavage and the other seven patients were treated with endoscopic vacuum therapy.

Results: During the initial operation, 13 patients underwent loop ileostomy. In three patients, diverting stoma was created after anastomotic leakage was observed. The mean volume of the abscess cavity was 82.6 cc (24.7-128) for those treated with EndoVAC (vacuum-assisted closure) and 33.3 cc (10.5-61.1) for those treated with endoscopic lavage. The number of sponges exchanged was 13.8 (5-25), and the time required for pouch closure was 74.3 days (20-136) for negative aspiration therapy and 66.1 days (30-210) for transrectal endoscopic lavage. As a late anastomotic complication, we recorded stricture in only one of seven patients (14.2%) treated with Endo-SPONGE®. Four of nine patients (44.4%) that underwent endoscopic lavage developed strictures, which needed reoperative procedures.

Conclusion: According to our experience, the sponge placement and negative pressure aspiration can be helpful in the treatment of anastomotic leakage after low anterior resections for rectal cancer. The results of time until cavity closure are not inferior to those of the conventional treatment, and a functional advantage over the conventional approach was observed. Patients with Endo-SPONGE® placement had less stricture and defecation problems.

Keywords: Anastomotic leakage, vacuum-assisted closure, colorectal surgery, endoscopically transrectal lavage, endo sponge

INTRODUCTION

Leakage of low colorectal anastomosis continues to be the most important complication of colon surgery as it can lead to generalized peritonitis, sepsis and multiple-organ failure (1-3). Treatments range from conservative measures, such as broad antibiotics and diverting ostomy, to endoscopical abscess drainage, daily transrectal pouch lavage, or Hartmann's procedure and abdominoperineal resection as a final option (1-5).

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Recently, perianastomotic pouches without peritoneal irritation are treated by endoluminally placed Endo-SPONGE®. This method provides continuous drainage of the perianastomotic abscess, control of infections, reduction of the size of the abscess cavity, increased blood flow and stimulation of granulation tissue (6-8). The drawback of this method is as follows: Endo-SPONGE® (B-Braun Medical®, Braun Melsungen AG, Germany) is the only product in the market, and it has high costs as it is used every two to four days until the abscess regresses. This study described our experience with handmade Endo-SPONGE® treatment and compared this modality with the traditional procedure, transanal endoscopic lavage.

METHODS

This retrospective study was approved by the Ethical Committee of Kafkas University (approval number: 279, approval date: 04.11.2020). All subjects had given a written informed consent before the endoscopic procedures. From 2014 to 2019, all patients with clinical features of anastomotic leakage after rectal resections were evaluated. Nine patients were treated by the conservative approach (daily endoscopic transanal debridement and lavage), and seven patients were treated by transanal Endo-SPONGE®. Endo-SPONGE® and transanal endoscopic lavage were started in patients without peritoneal irritation and persistent severe sepsis and after an evaluation of the perianastomotic abscess cavity by a computerized tomography of the lower abdomen.

For patients who cannot be treated with endoscopic vacuum-assisted closure (Endo-SPONGE®), the pouch was irrigated every one to two days and endoscopic debridement was performed if needed. In patients who underwent endoscopic vacuum-assisted closure, a "handmade" polyurethane sponge (Figures 1a and 1b) was inserted transanally by hand or through the anastomotic defect by endoscopy after irrigation and debridement of the perirectal abscess cavity. This procedure was performed after a light sedation with midazolam (2.5-5 mg IV) (Figure 2). The polyurethane sponge dressing was made from an open-cell polyurethane sponge used for large open wounds, appropriate for the size of the abscess cavity and connected to an evacuation tube (nasogastric tube CH 12). The end of the tube was connected to an intermittent vacuum drainage system (KCI Acelity, San Antonio, Texas, USA). Pressure levels were kept between -70 mmHg and -90 mmHg,

Figure 1a. Original Endo-SPONGE® (Braun Medical®, Braun Melsungen AG, Germany)

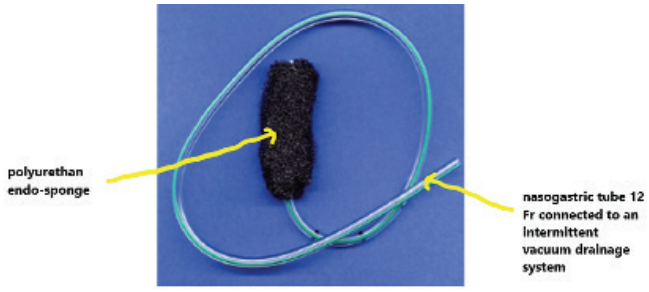

Figure 1b. "Handmade" Endo-SPONGE® from polyurethane sponge

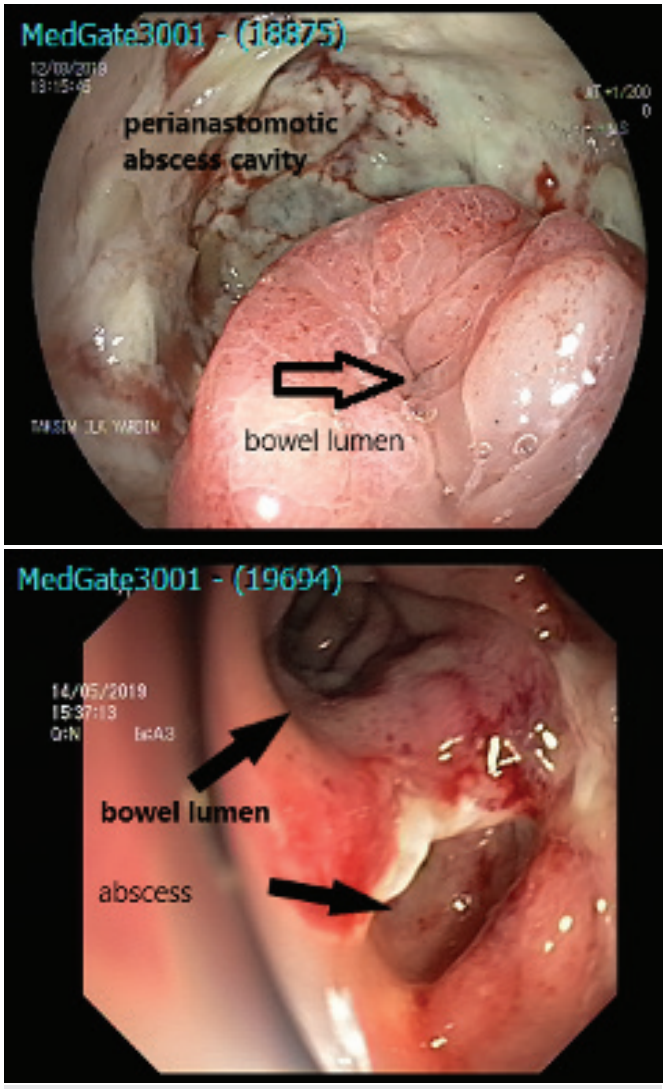

Figure 2. Taken from the archive of Dr. Doğan Gönüllü

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and the sponge was replaced every three days to prevent the growth of granulation tissue inside it. Patients were discharged when their vital signs improved. Endo-SPONGE® was stopped when the cavity shrank, and stoma resulting from ileostomy was closed when the cavity was resolved. Traditionally, before it was known that Endo-SPONGE® is connected to negative aspiration, transanal endoscopic exploration, lavage and debridement of the perianastomotic pouch, this procedure was repeated daily or every other day.

Due to the small sample size, we could not make any "statistical analysis" in this study.

RESULTS

Between 2014 and 2019, 16 patients (12 males and 4 females) with anastomotic leakage after low anterior resection for rectal cancer (double stapler) were treated with transanal endoscopic lavage (n=9) or Endo-SPONGE® (endoscopic vacuum therapy) (n=7) (Figure 3). The median age was 61.4 years (46-73 years). Thirteen patients received chemoradiotherapy preoperatively, and three patients with rectum malignant tumour underwent an operation directly. During the initial operation on 13 patients, loop ileostomy was performed. In three patients, the diverting stoma was created after anastomotic leakage was observed. Anastomotic leakage was diagnosed at a mean of 6.6 (range: 3-24) days; a mean of 7.1 (3-10) days for the endoscopic lavage group; 8.3 (4-24) days for the group treated with Endo-SPONGE®. Endo-SPONGE® was stopped in one patient at post-operative 48th day with nearly complete anastomotic disruption, and a terminal colostomy was performed. The results of the use of Endo-SPONGE® and transanal lavage are shown in Table 1.

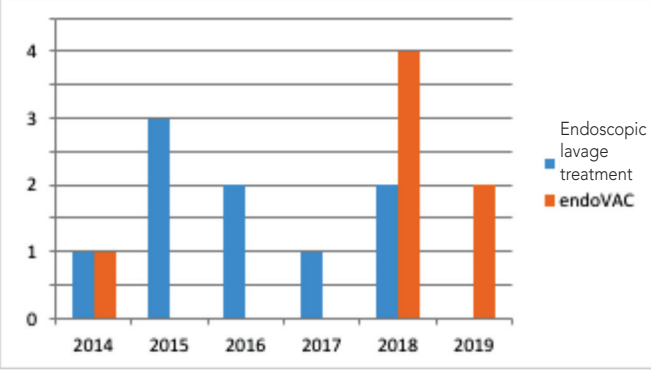

Figure 3. The distribution of anastomotic leakage treated conservatively per year

Table 1. Variables studied

Variable	Endo-SPONGE® treatment	Endoscopic lavage treatment
Volume of the abscess cavity (cc)* (CT, MRI)	82.6 (24.7-128)	33.3 (10.5-61.1)
Dimensions of initial abscess cavities (mm)*	53.3x30.1x100.6	35.9x29.3x56.4
Time until closure of pouches*	74.3 (20-136) days	66.1 (30-210) days
No. of sponges changed*	13.8 (5-25)	
Diverting stoma closure*	146 (105-195) days	86.4 (60-145) days
Definitive stoma	2	1
Outcome	1 stricture	4 strictures
	(endoscopic dilatation)	2 defecation problems**

*Values are median (range), **Inability to evacuate the bowel completely, faecal urgency
CT: computed tomography, MRI: magnetic resonance imaging

DISCUSSION

Anastomotic leaks after low anterior resection operations for rectal cancer continue to be a feared complication. It prolongs the duration of the illness, and sometimes recovery happens with fibrosis of the anastomotic line and perianastomotic tissue. This leads to stenosis, perturbation of defecation and permanent stoma (1,2,9).

Relaparotomy and lavage and stoma creation can effectively decrease the mortality in post-operative leakage by reducing the generalized peritonitis and sepsis. Alternative treatment options have been introduced through developing endoscopic interventions (9-11). Transanal endoscopic debridement and lavage, negative pressure drainage application and fibrin sealant application have been used in limited case series. Until today, there are no studies about the treatment desired to be used in anastomosis leakage (5,9,12).

In the last decade, the application of endoscopic negative pressure (Endo-SPONGE®, Braun Aesculap, Germany), which is a minimally invasive procedure for low colorectal anastomotic leakage, has been shown to be an effective way for reducing pelvic sepsis (4,5,12). After endoscopic debridement and lavage, the application of Endo-SPONGE® connected to a negative aspiration device allows a continuous drainage and cleaning of presacral septic pouch by increasing tissue perfusion and formation of granulation tissue that will close the cavity in a short time (13).

Despite the limited number of patients, we investigated the effects of Endo-SPONGE® treatments and compared the outcomes with endoscopic drainage and lavage treatment.

Chopra et al. (12) compared the results of repeated endoscopic debridement combined with stent, endoluminal vacuum device and endoscopic fibrin injection. They mentioned that vacuum-assisted therapy seems to be suitable for leaks with large perirectal abscess. The median size of the initial abscess was 53.3x30.1x100.6 mm for the Endo-SPONGE® group and 35.9x29.3x56.4 mm for the repeated endoscopic lavage group. These values are superior

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to the size reported in the literature. Weidenhagen et al. (14) reported that the mean length of the cavity at the beginning of the treatment was 7.4±5.1 (2-20) cm. von Berstorff et al. (15) reported that, in a series of 26 patients, the initial size of cavities ranged from 2x2 cm^2 4 cm^2 to 10x12 cm^2/120 cm^2. They reported that patients who underwent radiochemotherapy previously had significantly larger cavities than those who did not undergo neoadjuvant therapy. In a systematic review by Shalaby et al. (16), the median size of the defect was 6 (4.7-34.9) cm.

The timing of Endo-SPONGE® can influence the success of the procedure. Weidenhagen et al. (14) reported a high success rate when negative aspiration was initiated within six weeks postoperatively. A similar rate was reported by van Koperen et al. (17), where success rate was 75% if Endo-SPONGE® was started within six weeks and 38% if patients underwent endoscopic negative pressure therapy. In our study, the anastomotic leakages were diagnosed after a median of 8.3 (4-24) days, and negative pressure therapy with Endo-SPONGE® was started after one or two days. The sponge was changed every two to four days, and the median number of sponges used was 15.1 (range: 5-25). A review reported that sponges were changed every two to three days in nine studies and every three to four days in eight studies. The median number of sponges used was 7 (range: 3.4-13) (16).

In our study, the closure of the abscess cavity is achieved in 13 patients (81.2%). Two patients underwent Hartman's procedure after applying Endo-SPONGE® three to four times because of the progressive dehiscence and complete disruption of the anastomosis. One patient developed chronic presacral sinus despite a transrectal lavage for 27 days. The stoma of this patient closed four months after the operation, because of incomplete closure of the presacral sinus. The mean time of cavity closure for patients treated with Endo-SPONGE® was 74.3 (20-136) days and for the patients treated with only transanal lavage was 66.1 (30-210) days. Nagell and Holte (8) investigated the cavity closure times of five patients treated with negative pressure aspiration and 10 patients treated with the conventional ways. The mean time of EndoVAC group was 96.3 (43-195) days and that of the control group was 336 (52-1,464) days. There have been insufficient data to determine whether Endo-SPONGE® or endorectal lavage or observation is the best treatment. However, we believe the transanal lavage might be preferable, according to the different sizes of the cavities treated: 53.3x30.1x100.6 mm vs. 35.9x29.3x56x4 mm.

Glitsch et al. (18) reported an efficient treatment with transanal vacuum rectal drainage in 94.1% of their patients. They concluded that the cavity closure time depended on the cavity size, distance of anastomosis to the anal verge and patient's age. In a systematic study (16), variables that were significantly associated with failure were reported as preoperative radiotherapy and presence or absence of a protective stoma. In our study, all patients treated with Endo-SPONGE® had a protective ileostomy in addition to the first operation, and the other three stomas were done after the formation of anastomosis leakage. All patients who were treated with Endo-SPONGE® underwent preoperative long-term radiochemotherapy. Only three patients treated with endoscopic lavage underwent an operation directly. We did not observe any significant difference in terms of the size of the perirectal abscess between patients treated with neoadjuvant radiotherapy and those who did not receive neoadjuvant therapy.

Some authors have reported recurrence of fistula or abscess pouch. A multicentre study by Stefan et al. (19) reported that 25% of patients who were treated successfully developed recurrent abscesses.

The recovery of bowel continuity after successful eradication of the abscess cavity was achieved after a median time of 146 (105-195) days for patients treated with Endo-SPONGE® and 86.4 (60-145) days for patients treated with endoscopic lavage. Two patients in the Endo-SPONGE® group had a definitive ileostomy, and one patient in the conventionally treated group had a definitive ostomy. Weidenhagen et al. (14) reported that stoma reversal was possible in 22 of their 25 patients (88%) after an average of 169 days. During their follow-up, 10 patients (35%) had stenosis treated successfully by balloon dilatation. Srinivasamurthy et al. (20) reported a 62.5% recovery rate.

As a late anastomotic complication, we recorded only one stricture (1/7, 14.2%) resolved by endoscopic dilatation in patients treated with Endo-SPONGE®. Four patients who had endoscopic lavage developed strictures, which needed reoperative procedures (4/9, 44.4%). Two patients in this group developed defecation problems such as an inability to evacuate the bowel completely and faecal urgency. During follow-up of the 11 patients treated with Endo-SPONGE®, Mussetto et al. (21) observed that two patients had anastomotic stricture. One of them was treated with endoscopic dilatation and the other was treated with placement of a covered stent that was removed after five weeks.

Study Limitations

This study had some limitations. The rarity of anastomotic leakage makes the randomization difficult. The study was of a retrospective nature and included selected patients in the period of 2018-2019. Patients with larger cavities were treated predominantly with Endo-SPONGE® placements. In addition, the small number of patients in each group makes statistical validation difficult. To sum it up, more multicentre studies are needed to continue this preliminary design by increasing the number of these patients.

CONCLUSION

According to our experience, the sponge placement and negative pressure aspiration can be helpful in the treatment of anastomotic leakage after low anterior resections for rectal cancer. The results of time until cavity closure are not inferior to those of the conventional treatment, and a functional advantage over the conventional approach was observed. Patients with Endo-SPONGE® placement had less stricture and defecation problems.

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Ethics Committee Approval: This retrospective study was approved by the Ethical Committee of Kafkas University (approval number: 279, approval date: 04.11.2020).

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Analysis of Pregnancy and Lactation-related Expressions in the Summary of Product Characteristics and Patient Information Leaflets of the Drugs Used for the Treatment of Nausea and Vomiting

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ABSTRACT

Objective: Concerns and limited data on drug use in pregnancy and lactation raise the importance of relevant information in summary of product characteristics (SmPC) and patient information leaflets (PIL). This study aimed to examine the consistency of the information related to pregnancy/lactation periods in SmPCs/PILs of drugs used for the treatment of nausea/vomiting.

Methods: Details of the statements regarding pregnancy and lactation periods included in the current SmPCs/PILs of a total of 118 preparations, 21 of which were original, belonging to 12 drugs with nausea/vomiting indication, were examined. SmPCs/PILs of the generic drugs was compared with that of the original drugs to identify any "minor" or "major" difference.

Results: Any of SmPCs or PILs did not contain pregnancy indication or related posology information. Pregnancy was contraindicated in all tropisetron preparations; pregnancy in 38.5% and lactation in 46.2% of metoclopramide preparations; and lactation in 66.7% of dimenhydrinate preparations. It is stated that drug is passed in milk and placenta in 60.2% and 35.6% of SmPCs respectively. The presence of the expression regarding the placental passage showed inconsistency only among metoclopramide preparations. The presence of "pregnancy" and "lactation" in the "cautions before use" section of the PILs showed intra-drug variations for dimenhydrinate, metoclopramide, ondansetron, and domperidone. Except aprepitant, at least one major difference was detected between the SmPCs/PILs of original and generic preparations. Major differences were most commonly (15.0%) seen in the lactation section of the PILs.

Conclusion: It was determined that presence of specific expressions that may be critical for clinical practice, like the management of nausea/vomiting in pregnancy, might differ in the informative documents of medicinal products. It is noteworthy that there is at least one major difference in the documents of six of the seven drugs and that the warnings/precautions in the SmPCs/PILs of the originals of the drugs vary considerably from those of the generics. These findings indicate the need for new approaches in terms of both standardization and usefulness in clinical practice when developing informative content in SmPCs/PILs.

Keywords: Pregnancy, nausea, vomiting, generic, summary of product characteristics, patient information leaflet

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INTRODUCTION

Many medical conditions that are directly related to pregnancy or not may require the mother to take a medication (1). Physiological changes experienced during pregnancy, lactation and the process of placental development affect some pharmacokinetic and pharmacodynamic properties of the drugs in these individuals (2). On the other hand, it was revealed that the use of drugs in this period should not be ignored in terms of teratogenicity, as a result of the phocomelia cases seen in the children of pregnant women who used thalidomide in the early 1960s (3). There is limited information to adequately reflect the differences in substance, especially in new drugs. Conducting phase studies in pregnant and reproductive women was banned by the American Food and Drug Administration (FDA) in 1977, and in the mid-nineties, it was decided that women in this group were included in the studies on the condition of proving that they were not pregnant and on the condition of contraception. For this reason, information on the effects of drugs during pregnancy in humans can only be collected in line with limited data obtained from pregnant women who have already used the drug (4). In a study conducted in the United States of America (USA), it was reported that the mean time until the safety status of a new drug with an "uncertain" risk of teratogenicity to be included in a specific category was 27 years (5). This situation indicates the difficulty of processing and transferring the data obtained regarding a sensitive process such as pregnancy. New information gained from the routine use of the drug in these special populations over the years after the drug is licensed is reflected in drug information resources.

Among the reference sources for pharmaceutical preparations, it is expected that the content in the summary of product characteristics (SmPC) and patient information leaflets (PIL) for healthcare professionals should consist of standard information based on the current literature and compatible between each preparation of a drug as much as possible. Health authorities make various regulations in order to protect the standards and convey the necessary information to their interlocutors, provide available sources and make the necessary corrections and carry out the related audit and follow-up procedures. In Turkey, while standard information on drugs was presented under the name of "prospectus" in the medicine box in the past, it was rearranged as to consist of two documents, SmPC and PIL as of 2005 (6). There may be difficulties in reflecting the limited information that can be obtained regarding the gestational period to SmPCs/PILs. Various inconsistencies can be encountered in terms of compliance, following up-to-date literature, and indications in the information contained in the aforementioned documents (7,8). For example, factors such as teratogenicity information, are taken more cautiously while obtaining a license, and they may change with the experience of use in pregnancy over the years, and that the generic preparations that are later on the market can find this information more when they are licensed, which may disrupt the compatibility between the information of the same active substance in the preparations (7).

Nausea and vomiting are common clinical conditions (60-70%) during pregnancy, especially in the first trimester (9). It has been reported that 60% of pregnant women used medication for nausea and vomiting at least once during pregnancy (10). Due to teratogenicity and potential adverse effects concerns, both physicians, pharmacists and patients experience various reservations about drug use during pregnancy and lactation (11). SmPCs and PILs are among the basic sources of information that healthcare professionals and patients are recommended to apply to obtain reliable practical information about these special periods, especially indications and posology. The accuracy, coverage, standardization and usefulness of the information on pregnancy and lactation in these resources are of great importance.

In this study, it was aimed to examine the compatibility of the expressions related to pregnancy and lactation in the SmPCs and PILs of nausea and vomiting drugs for the original and generic preparations.

METHODS

Drugs with nausea or vomiting indication in the SmPCs were determined. Among them, there were twelve active substances of which at least one product registered by Turkish Medicines and Medical Devices Agency (TMMDA) (dimenhydrinate, trifluoperazine, lorazepam, ondansetron, granisetron, tropisetron, palonosetron, aprepitant, trimethobenzamide, metoclopramide, itoprid and domperidone) included in the study (Table 1). Cannabinoid and scopolamine, which are known to be used frequently in nausea and vomiting, were not included in the study because they did not have their preparations registered in TMMDA, as well as H_2 receptor blockers and pyridoxine which were not directly declared as indicated for nausea or vomiting. In

Table 1. List of antiemetic drugs included in the study

Drug groups examined (ATC)	Drugs included		
	Active ingredients	ATC-5 code	Number of preparations
Antihistamines (R06)	Dimenhydrinate	R06AA02	3
Antipsychotics (N05A)	Trifluoperazine	N05AB06	4
Benzodiazepines (N05BA)	Lorazepam	N05BA06	2
Serotonin 5-HT3 antagonists (A04AA)	Ondansetron	A04AA01	37
	Granisetron	A04AA02	25
	Tropisetron	A04AA03	2
	Palonosetron	A04AA05	18
Other antiemetics (A04AD)	Aprepitant	A04AD12	2
	Trimethobenzamide	A04AD	4
Prokinetics (A03F)	Metoclopramide	A03FA01	13
	Itopride	A03FA07	1
	Domperidone	A03FA03	7

ATC: anatomical therapeutic chemical classification system

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addition, corticosteroids with many indications and combination preparations containing more than one active ingredient were not included in the study.

The SmPCs/PILs information of a total of 118 preparations (97 generic and 21 original) consisting of different strength and forms of the twelve drugs included in the study were examined. The expressions related to pregnancy and lactation period in the current SmPCs and PILs of original and generic preparations of these drugs were evaluated in detail. It was analyzed whether there was any statement regarding pregnancy and lactation under "therapeutic indications", "posology and method of administration" and "contraindications" headings in the SmPCs and the "cautions before use" heading in the PILs of the drugs and also whether there was any statements regarding the passage of the drug to milk or placenta in the SmPCs.

Statements under the "women with childbearing potential", "pregnancy period", "lactation period", "reproductive ability", "undesirable effects" headings in SmPCs and "pregnancy" and "lactation" headings in PILs were gradually evaluated by a total of five pharmacologists, including two experts and three residents. It was determined how many original expressions were used under each heading in the SmPCs/PILs for each drug examined.

The comparison of the expressions in the generic and original preparations was made specifically for seven drugs (dimenhydrinate, ondansetron, granisetron, palonosetron, aprepitant, trimetobenzamide, domperidone), each of which has at least one original and generic preparation registered in TMMDA. The expressions in the related titles in the SmPCs/PILs of the generic preparations of these drugs were analyzed using the method we used in our previous study (7). Accordingly, the original preparations were compared with reference and the expressions were examined in three groups as those with "similar", "minor difference" or "major difference". When comparing with its generic, if different pharmaceutical forms of the original drug of an active substance were found, the information of the original preparation to which the drug was the same/closest as the pharmaceutical form was used. Phrases were considered "similar phrases" if they were exactly the same or contained several different words giving the same meaning. The existence of fundamental differences such as "discontinuation of the drug", "not recommending its use", "not advising its use", the amount of data being different, or the presence of different/missing statements regarding the drug-related adverse effect risk are considered as "major difference" in the relevant statement. The characteristics of the major differences detected were examined for each drug. Smaller scale differences that did not fit the definition of major difference were accepted as "minor difference". In the SmPCs/PILs of the seven drugs that were compared, whether the expressions about "pregnancy" and "lactation" were included under the above-mentioned headings were also evaluated in terms of generics and originals. The investigations in the study were carried out using the most up-to-date SmPCs/PILs registered in TMMDA in September 2019. This study did not require patient consent because it did not contain any patient data.

Statistical Analysis

In this descriptive study, the data were expressed as numbers and percentages for categorical variables. Statistical analysis was performed with GraphPad Prism 5.0 program.

RESULTS

Indication, Contraindication and Warning Signs

In the study, it was determined that there were a total of 97 generic preparations in different "strength and forms" of 21 original products in different "strength and forms" with licence in Turkey of 12 active substances whose SmPCs/PILs information were examined, and that there were 48 different companies with licenses for these products.

Pregnancy categories of the preparations were B (83.1%), C (15.2%) and D (1.7%). It was observed that none of the examined 118 preparations had pregnancy and hyperemesis gravidarum indications in their SmPCs, and there was no statement regarding pregnancy in the "posology" section of any drug. Pregnancy in seven preparations [metoclopramide (n=5; 38.5%), tropisetron (n=2; 100%)], lactation in eight preparations [dimenhydrinate (n=2; 66.7%), metoclopramide (n=6; 46.2%).)] were among the contraindicated situations. Under the heading of "cautions before use" in PILs, "pregnancy" was included in all preparations of itopride and tropisetron, 33.3% of dimenhydrinate, 24.3% of ondansetron and 38.5% of metoclopramide. Again, under the same heading, "lactation" was found in 24.3% of ondansetron, 69.2% of metoclopramide and 28.6% of domperidone in all preparations of dimenhydrinate and itopride (Table 2).

Findings of Transition to Placenta and Milk

Expressions related to the transition to the placenta were found in the SmPC of 35.6% (n=42) of the preparations included in the study. The presence of this statement was inconsistent only in metoclopramide preparations, and this statement was included in 76.9% (n=10) of the preparations. Other drugs for which information on placental transfer was indicated were palonosetron, tropisetron, dimenhydrinate, lorazepam, and domperidone. In the SmPC of 60.2% (n=71) of the preparations examined, it was found that the drug was transferred to milk. While there is information that the drug passes into breast milk in animal studies in 31 (26.3%) preparations belonging to domperidone, dimenhydrinate, trifluoperazine, lorazepam, aprepitant and metoclopramide, it is stated that tropisetron, ondansetron and itopridine in total 40 (33.9%) preparations are passed into milk in all preparations was taking place. In the preparations of granisetron, palonosetron and trimethobenzamide (n=47), it was stated that it was not known whether the drug passed into the milk (Table 2).

Findings of Compatibility Between Preparations

It was observed that the number of unique statements under each heading examined was the highest (n=27) in the section

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Table 2. The number of original and generic preparations for each active substance, pregnancy category, and the percentage of preparations in which the relevant expression is included in the parameters evaluated as "exists/does not exist"

Active ingredient	Number of preparations			Pregnancy category	The preparation in which the relevant expression "exists", n (%*)								
					SmPC							PIL	
					Presence of pregnancy indication	Presence of expression regarding pregnancy in posology	Presence of expression regarding teratogenicity in "undesirable effects"	Presence of a statement that it is passed into placenta	The presence of the statement that it is passed into milk	Contraindications		Cautions before use	
	Original	Generic	Total							The presence of expression regarding pregnancy	The presence of expression regarding lactation	Pregnancy-related statement presence	The presence of expression regarding lactation
Dimenhydrinate	2	1	3	B	0	0	0	3 (100.0)	3 (100.0)	0	2 (66.7)	1 (33.3)	3 (100.0)
Trifluoperazine	0	4	4	C	0	0	4 (100.0)	0	4 (100.0)	0	0	0	0
Lorazepam	2	0	2	D	0	0	0	2 (100.0)	2 (100.0)	0	0	0	0
Ondansetron	6	31	37	B	0	0	0	0	37 (100.0)	0	0	9 (24.3)	9 (24.3)
Granisetron	3	22	25	B	0	0	0	0	0	0	0	0	0
Tropisetron	2	0	2	C	0	0	0	2 (100.0)	2 (100.0)	2 (100.0)	0	2 (100.0)	0
Palonosetron	1	17	18	B	0	0	0	18 (100.0)	0	0	0	0	0
Aprepitant	1	1	2	B	0	0	0	0	2 (100.0)	0	0	0	0
Trimethobenzamide	1	3	4	C	0	0	0	0	0	0	0	0	0
Metoclopramide	0	13	13	B	0	0	0	10 (76.9)	13 (100.0)	5 (38.5)	6 (46.2)	5 (38.5)	9 (69.2)
Itopride	0	1	1	C	0	0	0	0	1 (100.0)	0	0	1 (100.0)	1 (100.0)
Domperidone	3	4	7	C	0	0	0	7 (100.0)	7 (100.0)	0	0	0	2 (28.6)
Total	21	97	118	-	0	0	4 (3.4)	42 (35.6)	71 (60.2)	7 (5.9)	8 (6.8)	18 (15.3)	24 (20.3)

%*: row percentages were calculated according to the total number of preparations. PIL: patient information leaflet, SmPC: summary of product characteristics

on pregnancy in the SmPCs, followed by the statement about pregnancy (n=22) and lactation (n=21) in the PILs. Ondansetron, which has 37 preparations, was the drug that contained the most unique expressions for each title. It was determined that only trifluoperazine preparations included a statement regarding teratogenicity ("neonatal withdrawal syndrome seen in pregnancy, post-pregnancy and perinatal conditions") under the title of "undesirable effects" and this expression was the same in all preparations of the drug. It was observed that the preparations of lorazepam and tropisetron used a single expression in each of the titles examined, and there was consistence between the preparations (Table 3).

The expressions in the SmPCs/PILs of the generic preparations of seven drugs with at least one original and generic preparation registered in the TMMDA were compared with the original preparations. It was observed that there were more major differences in the related titles in the PILs than those in the SmPC. A major difference was detected in 15% of the preparations in the statement part about lactation in PILs, and in 13.7% in the expression part about pregnancy. In the SmPC, all of the differences detected in the ondansetron and trimethobenzamide preparations in the expressions for women of childbearing age and in the preparations of the drugs other than granisetron and palonosetron in the expressions about pregnancy were minor. It was determined that there was a difference in the expression related to lactation in the SmPC for other drugs except palonosetron and this difference was at a major level in granisetron, trimethobenzamide and domperidone. The differences in the expression regarding fertility were minor in ondansetron and aprepitant preparations, and major in palonosetron and trimethobenzamide preparations. It was determined that there is a difference between the preparations of

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Table 3. The distribution of the specific expression diversity used in the parameters examined for pregnancy, lactation, fertility and teratogenicity in the subtitles of the summary of product characteristics and patient information leaflets for each drug

Active ingredients	Number of preparations	Number of original expressions used						
		SmPC					PILs	
		Statement for women of childbearing age	Pregnancy-oriented statement	Lactation related statement	Fertility related statement	Statement about teratogenicity	Pregnancy-oriented statement	Lactation related statement
Dimenhydrinate	3	1	3	2	1	-	2	2
Trifluoperazine	4	2	2	1	2	1	1	1
Lorazepam	2	1	1	1	1	-	1	1
Ondansetron	37	3	4	3	2	-	3	3
Granisetron	25	1	2	3	1	-	1	2
Tropisetron	2	1	1	1	1	-	1	1
Palonosetron	18	1	1	1	2	-	2	2
Aprepitant	2	1	2	2	2	-	1	2
Trimethobenzamide	4	2	2	2	2	-	2	2
Metoclopramide	13	1	4	3	1	-	3	3
Itopride	1	1	1	1	1	-	1	1
Domperidone	7	2	4	2	1	-	2	3
All medications studied	118	16	27	20	17	1	21	22

SmPC: summary of product characteristics, PILs: patient information leaflet

all drugs except granisetron in the expression part for pregnancy in PIL and this difference is major in ondansetron preparations. All of the differences between the original and generic preparations of other drugs except granisetron and aprepitant were found to be major in the expression section regarding lactation in the PILs (Table 4).

The major differences determined were in the statements about lactation and fertility in the SmPCs, and in the statements about pregnancy and lactation in the PILs. In the SMPC, the most common reason for the major difference related to lactation was "the lack/presence of the statement that the use of the drug is not recommended" in the lactation part, while in the PILs, the "lack of the statement about not using/not leaving the drug" in the lactation part. In terms of domperidone, the lack of expression regarding the cardiac adverse effects that will arise due to the drug in the baby in the lactation section in both SmPCs and PILs was determined as the reason for the major difference (Table 5).

Whether or not a statement about pregnancy or lactation was included in certain titles examined in the SmPCs/PILs of seven drugs with at least one generic preparation was compared with the original preparation. Accordingly, although the original preparation of dimenhydrinate has a statement regarding lactation in contraindications in the SmPC, such a statement is not included in one generic preparation available in the market; in addition, while there was no statement regarding pregnancy in the "cautions before use" section in the PIL of the original preparation, it was determined that this expression was included in the generic preparation. Under the same heading, "lactation" was present in one (25.0%) of the generic preparations of domperidone and nine (29.0%) of ondansetron, although it was not included in their original preparations. Unlike the original preparation of ondansetron, "pregnancy" was found in nine generic preparations (29.0%) in the "cautions before use" section. The generic preparations of trimethobenzamide, aprepitant, granisetron and palonosetron were consistent with the original preparations in these titles examined in terms of whether they included the terms "lactation" or "pregnancy".

DISCUSSION

In this study, important findings were made that there were various differences in the expressions related to pregnancy and lactation periods in SmPCs/PILs of the drugs used for nausea and vomiting. It is noteworthy that none of the preparations of the active substances included in the study are among the indications of pregnancy-related conditions in SmPCs and PILs, and the posiology is not specifically specified. The fact that this information is not clearly mentioned in these documents may be associated with the inadequacy of the literature on the use of these drugs during pregnancy and lactation. In addition, although none of the drugs examined were in the category "X", which is considered teratogen, the fact that pregnancy in about one of every 16 preparations and lactation in one of every 14 preparations as being counted among contradictions indicates that there are inconsistencies within the documents themselves. The fact that the presence of the information about the passage to placenta and

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Table 4. The number of generic preparations that differ from the expressions in the SmPCs/PILs of the original preparations of drugs, and the percentage distribution of generic preparations with a major difference in each of the expressions

Drugs (Number of generic preparations)	SmPC						PIL			
	Statement for women of childbearing age*	Pregnancy oriented expression *	Lactation related statement		Fertility related statement		Pregnancy oriented expression		Lactation related statement	
	Differences generics n (%)	Differences generics n (%)	Differences generics n (%)	Major difference n (%)	Differences generics n (%)	Major difference n (%)	Differences generics n (%)	Major difference n (%)	Differences generics n (%)	Major difference n (%)
Dimenhydrinate (n=1)	0 (0.0)	1 (100.0)	1 (100.0)	-	0 (0.0)	-	1 (100.0)	-	1 (100.0)	1 (100.0)
Ondansetron (n=31)	11 (35.5)	13 (41.9)	2 (6.5)	-	24 (77.4)	-	11 (35.5)	11 (35.5)	2 (6.5)	2 (6.5)
Granisetron (n=22)	0 (0.0)	0 (0.0)	3 (13.6)	3 (13.6)	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
Palonosetron (n=17)	0 (0.0)	0 (0.0)	0 (0.0)	-	2 (11.8)	2 (11.8)	5 (29.4)	-	4 (23.5)	4 (23.5)
Aprepitant (n=1)	0 (0.0)	100 (100.0)	1 (100.0)	-	1 (100.0)	-	1 (100.0)	-	0 (0.0)	-
Trimethobenzamide (n=3)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	-	3 (100.0)	3 (100.0)
Domperidone (n=4)	0 (0.0)	2 (50.0)	2 (50.0)	2 (50.0)	0 (0.0)	-	2 (50.0)	-	2 (50.0)	2 (50.0)
Total (n=80)	15 (18.8)	18 (22.5)	12 (15.0)	8 (10.0)	30 (37.5)	5 (6.3)	23 (28.8)	11 (13.7)	13 (16.3)	12 (15.0)

*All differences were minor. PIL: patient information leaflet, SmPC: summary of product characteristics

Table 5. Differences characteristics of the expressions written in the SmPCs/PILs of the drugs with a major difference

Features of the expressions written in the SmPCs/PILs with major differences			
Drug	**SmPCs/PILs**	**The section of the statement**	**The reason for the major difference**
Dimenhydrinate	PIL	Lactation	Lack of drug withdrawal directive (n=1)
Ondansetron	PIL	Pregnancy	Lack of statement stating that the use of the drug is not recommended (n=11)
	PIL	Lactation	Lack of the directive not to breastfeed the baby during the use of the drug (n=2)
Granisetron	SmPC	Lactation	Lack of the statement that the use of the drug is not recommended (n=3)
Palonosetron	SmPC	Fertility	The difference in the data level regarding the effect of the drug (n=2)
	PIL	Lactation	Lack of drug discontinuation directive (n=4)
Trimethobenzamide	SmPC	Lactation	Lack of the statement that the use of the drug is not recommended (n=3)
	SmPC	Fertility	The difference in the data level regarding the effect of the drug (n=3)
	PIL	Lactation	Lack of directive not to use the drug (n=3)
Domperidone	SmPC	Lactation	Lack of statement stating the risk of cardiac adverse effects that may occur in the infant with the use of the drug (n=1) Presence of a statement that the use of the drug is not recommended (n=1)
	PIL	Lactation	Lack of statement stating the risk of cardiac adverse effects that may occur in the infant with the use of the drug (n=1) Presence of a statement that the use of the drug is not recommended (n=1)

PIL: patient information leaflet, SmPC: summary of product characteristics

milk varies in different preparations of the same active substance also suggests an inconsistency between the preparations. It can be said that these differences are more common in PILs and are also observed prominently when the original and generic preparations are compared. These inconsistencies suggest that the standardization of the expressions regarding pregnancy and lactation period in SmPCs/PILs is not fully ensured and updated information is not reflected in the relevant documents of some

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drugs. On the other hand, although there has been a change in the FDA's risk assessment category for drug use in pregnancy in recent years, in some countries, including Turkey, documents such as SmPCs still use this classification (12). Partial uncertainty and different approaches of the authorities may have contributed to the development of this inconsistency.

It has been reported that 90% of women are exposed to at least one drug during pregnancy and take an average of 2.6 drugs (13). Between 2000 and 2010, it was reported that more than 97% of the 172 drugs approved by the FDA had no safety data on teratogenity in humans, and 73% had no information about their use during pregnancy (5). It is known that 2-3% of congenital anomalies occur due to drug-related causes, and reducing the risks associated with drug use is among the priorities for protecting public health (14). In our study, the fact that none of the nausea/vomiting drugs known to be used frequently during pregnancy had no indication for use in SmPCs or PILs can be considered as one of the most striking findings. This indicates that the drugs examined are often used out of indication.

In a study examining the SmPCs of 534 preparations in the drug groups frequently used during pregnancy and lactation, registered on the official website of the European Medicines Agency (EMA), it was reported that 89.3% of the drugs did not contain information on the passage of the drug to the placenta and 61.4% did not contain information on whether the drug was transferred to breast milk (8). In our study, it was determined that the information regarding whether approximately two-thirds of the drugs passed through the placenta and whether approximately three-fourths of them passed into breast milk was not included in the relevant SmPCs of the drugs. The fact that more space was given to placental passage in SmPCs/PILs examined in our study seems positive for the content of the documents in our country, while the picture is in the opposite direction in passage to milk, reflecting the need to pay more attention to the information on passage to breast milk in the relevant documents. On the other hand, it has been seen that this information is not included in some preparations of metoclopramide, which have been reported to have passed to the placenta, and even not included in any of the preparations of trifluoperazine, ondansetron and granisetron. (15-18). These findings point to problems with standardization and up-to-dateness in terms of information on drug exposure of the fetus in the preparations of some drugs. Unless this information in SmPCs is updated, it can lead to increased incompatibilities with the literature and, as a result, physicians who use these documents when arranging treatment may be misled. As a matter of fact, it has been reported that significant differences in drug use in pregnancy between these documents and drug information systems based on current literature can complicate clinical decisions (19). In order to address the lack of information regarding drug exposure during pregnancy and lactation, post-licensing data must be actively collected, kept up-to-date and included in official sources of information to help make decisions (8).

PILs are one of the important documents that can meet the need for getting information about drug use of pregnant women and nursing mothers. In our study, it was noted that pregnancy was present in 15.3% of drugs and lactation in 20.3% under the heading "things to consider before use" in the PILs of the drugs, but the presence of these expressions did not show consistency in all preparations of some drugs. For example, pregnancy was under the relevant heading in about a third of dimenhydrinate and metoclopramide preparations, and about a quarter of ondansetron preparations. Similarly, lactation was under the relevant heading in about a quarter of ondansetron and domperidone preparations and 69% of metoclopramide preparations. These data suggest that the standard information presented by consensus on a large accumulation of literature on drugs is not sufficiently compatible with the approaches of reflecting this to SmPCs/PILs or specifying this if there is a lack of data. On the other hand, when comparing the documents of the original and generic preparations, there was a greater difference in PILs in both pregnancy and lactation compared to SmPCs. Considering that the target audience of PILs is people with limited health literacy, situations like the information contained in such documents is inconsistent with the treatment planned by the physician or incompatible with the current literature, etc. may cause preparations with the same active substance to give different impressions to the patient. For example, one of the preparations whose documents have been updated at different times may be perceived as more reliable or more risky than the other. It can be considered that this may reduce the patient's compliance with treatment. Whether the variability between PILs containing the same active substance will make a difference in the continuation/discontinuation of the use of the drug by medical illiterate patients should be tested with standardized tests such as readability tests and examined with detailed studies.

It is expected that the information in the SmPCs/PILs of generic drugs will be similar to the originals and consistent in all respects. (20-22). However, similarity or consistency in SmPCs/PILs of drugs may be affected by technical situations such as differences in translations from different languages in the transfer of information of the original preparation to its corresponding documents, or the inability to apply new data/developments simultaneously to the documents of all preparations belonging to the same drug. In our study, when the original and generic preparations of the active substances are compared with each other in terms of expression differences, it can be considered as striking that all but one of the active substances evaluated (aprepitant) have a major difference in their SmPC and/or PIL under at least one heading, and when viewed in detail, a major difference is found in approximately one in every 11 SmPCs and approximately one in every 6 PILs. The fact that major differences are only seen in ondansetron PILs, especially for the statements about pregnancy, seems relatively positive. While there is no major difference in pregnancy-related expressions when comparing original and generic preparations in SmPCs, the fact that there is a major difference in approximately 10% of lactation-related expressions

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can be associated with the inability to produce generally accepted information due to literature limitations in this field. As a matter of fact, it has been reported that there are no studies on the use of trimethobenzamide, ondansetron, granisetron and palonosetron in lactation, which are the active substances with a major difference in lactation information between the original and generic preparations (23).

According to the current guidelines of the The American College of Obstetricians and Gynecologists (ACOG), metoclopramide is at the advanced stages of the nausea and vomiting treatment algorithm during pregnancy (24). When symptoms persisted following the first pharmacological treatment, it was reported that metoclopramide was one of the drugs that could be considered for auxiliary treatment of nausea and vomiting in pregnant women and did not cause an increase in the risk of teratogenicity (24,25). Widespread use of the drug among pregnant women has been reported up to 86% (26). In our study, stating that the drug is contraindicated during pregnancy in the SmPCs of two of every five metoclopramide preparations is remarkable in terms of showing that the SmPCs can fall behind the current literature in this regard. On the other hand, the fact that pregnancy is among the contraindications in the SmPCs of nearly half of metoclopramide preparations while the pregnancy category of the drug is "B", and all of the tropisetron preparations in category "C" suggests that there are inconsistencies among the mentioned reference sources. In addition, lactation, near half of the metoclodraid preparations, was under the heading "contraindications". Although there have been studies that report that metoclopramidine may cause mild gastrointestinal adverse effects in infants and postpartum depression in mothers, the drug is classified as "compatible with lactation" (23,27,28). Accordingly, it can be said that the preparations that accept the lactation period as contraindications diverge with both other preparations and existing literature information. On the other hand, metoclopramide can be used by some mothers to enhance milk off-label (29). Conflicting results on milk-enhancing effect were reported in studies examining the use of the drug for this purpose (29,30). Moreover, it is reported that the recommended maximum doses and durations can be exceeded in order to achieve this desired effect (31). Therefore, taking into account the risks of abuse, it can be stated that the information about the use of the drug for limited indications such as nausea and vomiting, for the shortest possible time and at the lowest dose, and the information about the risks that may arise if the restrictions are not followed during use should be added to the content of the metoclopramide SmPCs.

In two of the three preparations of dimenhydrinate active substance, lactation was among the contraindications of the drug. Despite the fact that dimenhydrinate can reduce milk production in high doses and prolonged use, and can lead to symptoms such as irritability and drowsiness in infant, it has been reported that low doses and short-term use are not expected to cause such adverse effects and that it is appropriate to use the drug during lactation in the light of long-term clinical experience (32-34). Although the use of the drug for limited doses and durations seems safe in the light of this data, the classification of lactation period as contraindicated in most preparations indicates that these SmPCs/PILs are incompatible with the updated literature and should be reviewed.

The SmPCs/PILs of two preparations, including the original, of the active substance of domperidone, of which a total of seven preparations were evaluated in the study, contained statements such as "discontinuation of one of the drugs or lactation due to adverse effects that may develop in the baby", and the documents of the other two preparations "the drug is not recommended during the lactation period". Inconsistencies between preparations and inconsistencies with current literature information in other active substances were also in question for domperidone. Domperidone can also be used off-label to increase breast milk during lactation (30). Considering the abundance of the studies related to its use in this field in the literature, the importance of including more standard and compatible expressions about the use of the drug in lactation in the SmPCs/PILs can be better understood (28,35-38).

It is understood that the statement in the original PILs of ondansetron that "the drug is not recommended for use in pregnancy" is not found in more than one-third of the generic PILs. Contrary to the statement in the original PIL, ondansetron is shown among the alternative agents that can be used in resistant nausea and vomiting during pregnancy in the ACOG guideline (24). The use of ondansetron, which has the highest number of preparations among the active substances included in the study, during pregnancy has become widespread in recent years. For example, it was reported in the USA in 2014 that women who were prescribed ondansetron during pregnancy accounted for about a quarter of all pregnant women (39). However, despite its frequent use, important results that may contradict each other have been reported from recent studies on fetal safety data of the drug. (40-42). Accordingly, in 2019, EMA stated that the drug should not be used in the period in question due to the conflicting results regarding the cardiac malformations that may develop in the fetus due to ondansetron and the possible increased risk of orofacial malformation in the first trimester (43). However, the EMA's sharp approach was not accepted by the European Network of Teratology Information Services (ENTIS) (44). Unlike the EMA, the British Medicines and Healthcare products Regulatory Agency recommended that the benefit/risk calculation be made before the use of ondansetron by women in the first trimester of pregnancy (45). The contradictory results reported in the literature may have paved the way for the incompatibility between the preparations of ondansetron compared to the other drugs with original-generic preparation differences in our study. Moreover, the fact that there is still no consensus among international health authorities on the fetal safety status of the drug may make it difficult to include clear recommendations on the use of the drug in SmPCs/PILs. However, it is clear that attempts to standardize these documents are needed to ensure more rational use of the drug by physicians and patients.

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Study Limitations

In our study, the current market availability of the preparations whose SmPCs/PILs were examined were not taken into consideration, and the most up-to-date documents in TMMDA were evaluated within the data collection process of these products within the scope of the research. SmPCs/PILs information on preparations that are not available in the market may contain some differences compared to preparations available in the market. The fact that this situation could not be evaluated in detail within the scope of the study can be accepted as a limitation. However, the fact that all preparations registered in TMMDA have been examined, has enabled a broader data to be presented regarding the differences detected in SmPCs/PILs. Other limitations of the study are that the differences between the generic preparations of the same drug were not elaborated and the combined preparations were not examined. In addition, since it is not known whether the differences determined in PILs make a difference in the patient's perception of the use of the drug, the relevant inferences should be interpreted in this context and verified with studies including reading tests.

Conclusion

It is noteworthy that none of the nausea and vomiting drugs used in special populations such as pregnant women and lactation mothers have indications of these periods in their SmPCs or PILs. The fact that different expressions are encountered in the information that should be included in these documents may be associated with the absence of these indications. Although the differences between the original and generic preparations of drugs are mostly evaluated as minor, the detection of a major difference in at least one of the titles examined in the SmPCs or PILs of all preparations except aprepitant points out the necessity of standardization by reflecting the changes in the current literature to these documents at frequent intervals. However, the differences determined in PILs may cause confusion in terms of interlocutors, especially patients. The impression of this situation on the patients needs to be evaluated with more comprehensive reading tests. It is thought that the determinations made in this study will contribute to the elimination of obstacles on the way that SmPCs/PILs contain up-to-date information, are more guiding and user friendly, from the perspective of pregnancy and lactation.

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Relapse of T-Cell Lymphoma as Isolated Brachial Plexus Neurolymphomatosis: A Case Report

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ABSTRACT

Neurolymphomatosis (NL) is the neoplastic endoneurial invasion of the peripheral nervous system. It is a rare and challenging diagnosis, but it should be taken into account in the differential diagnosis of peripheral neuropathy, particularly in patients with a documented history of haematologic malignancy. Magnetic resonance neurography is very useful in diagnosis and especially when correlated with positron emission tomography/computed tomography (PET/CT). In equivocal cases, nerve biopsy can be considered when the benefit outweighs the risk. We aimed to report a case of a 30-year-old male patient who was in complete remission from T-lymphoblastic lymphoma, presenting with clinical findings indicating initially ulnar entrapment. However, with the demonstration of brachial plexopathy with axonal loss in electrodiagnostic studies, magnetic resonance imaging neurography dedicated to brachial plexus was carried out and revealed pathological enhancement associated with mild fluorodeoxyglucose (FDG) uptake on PET/CT. Moreover, NL, due to the relapse of T-cell lymphoma, was diagnosed through incisional biopsy, showing diffuse infiltration of blast cells positive for terminal deoxynucleotidyl transferase, CD3 and CD10. Further, radiotherapy and systemic chemotherapy were initiated, and symptoms recovered with regression of pathological FDG uptake.

Keywords: Neurolymphomatosis, T-cell lymphoblastic lymphoma, brachial plexopathy

INTRODUCTION

Neurolymphomatosis (NL) is the neoplastic endoneurial invasion of the peripheral nervous system (PNS). It differs from meningeal lymphomatosis, which is the infiltration of the PNS due to subarachnoid seeding (1). Furthermore, NL occurs more frequently with the spread of a known malignancy into the PNS from systemic sites or from the primary central nervous system (CNS) lymphoma (PCNSL) as a progression in the course of the disease or as a relapse following complete remission. More rarely, it may occur as an initial manifestation of the disease. We report a case of a 30-year-old male patient with right brachial plexopathy caused by the relapse of T-lymphoblastic lymphoma.

CASE PRESENTATION

A 30-year-old male patient presented with right medial forearm and hand pain and numbing in the fourth and fifth fingers, initially indicating ulnar nerve entrapment. A year ago, he was treated for T-lymphoblastic leukaemia/lymphoma and achieved complete remission with no pathological fluorodeoxyglucose

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(FDG) uptake in positron emission tomography/computed tomography (PET/CT). In neurological examination, muscular strengths of the forearm flexors and intrinsic hand muscles were poor, and there was pain-related paraesthesia. Moreover, no disc impingement was demonstrated by cervical magnetic resonance imaging (MRI). Ulnar nerve entrapment was considered; however, electrodiagnostic studies revealed right lower truncus brachial plexopathy associated with axonal loss and active denervation. Gadolinium-enhanced MRI neurography showed a 25x32x41 mm-sized enhancing extrapulmonary lesion located at the apical part of the right hemithorax, affecting the middle and lower part of the brachial plexus (BP) (Figure 1). No pathological signal was detected in the adjacent pulmonary parenchyma. Also, there was no extension into the spinal canal. PET/CT showed a mild hypermetabolic FDG uptake (maximum standardized uptake value: 3.04) in the lesion. Moreover, NL was considered and incisional biopsy was performed to make an exact diagnosis. During the antigenic assessment, diffuse infiltration of blast cells positive for terminal deoxynucleotidyl transferase, CD3 and CD10 was demonstrated (Figure 2). Once again, radiotherapy and systemic chemotherapy was started. With the regression of pathological FDG uptake in PET/CT, his symptoms recovered with improvement of muscle strengths from 3/5 to 5/5.

Written informed consent has been taken from the patient.

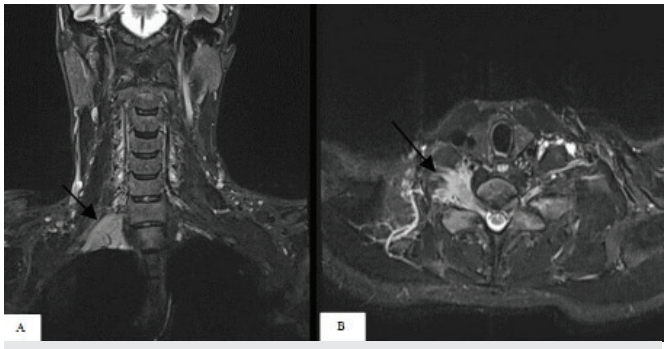

Figure 1. Brachial plexus magnetic resonance imaging neurography with gadolinium, short-tau inversion recovery sequence sagittal (A) and axial (B) images showing pathologic enhancement in the right brachial plexus (black arrows)

DISCUSSION

NL is a very rare neurological manifestation of haematologic malignancies that should be distinguished from the more prevalent causes of PNS involvement, such as radiation-induced peripheral neuropathy or paraneoplastic syndromes. Most cases of NL are associated with diffuse large B-cell non-Hodgkin's lymphoma (DLBCL) but can also occur in T-cell and NK-cell lymphomas or acute leukaemias. According to the report of the International Primary CNS Lymphoma Collaborative Group, NL developed in 50 patients over a 16-year period, of which 90% were associated with non-Hodgkin's lymphoma (NHL) and 10%

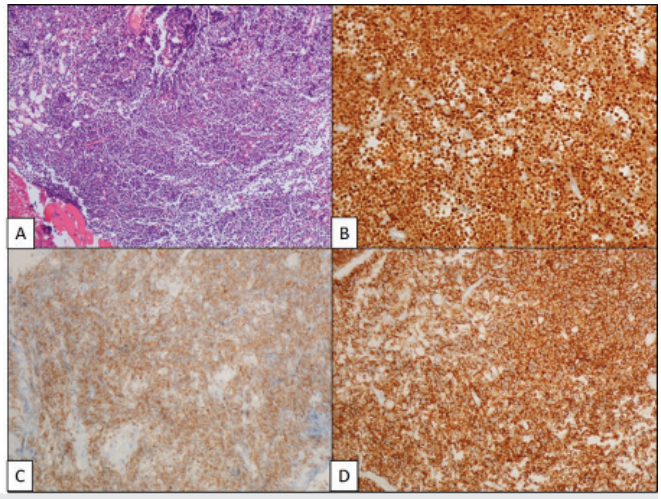

Figure 2. Incisional biopsy of the brachial plexus lesion showing diffuse infiltration of blast cells (H&E) (A), which were positive for terminal deoxynucleotidyl transferase (B), CD3 (C) and CD10 (D) in the antigenic assessment

with acute leukaemia (2). In a 12-year retrospective analysis of 1,181 patients with NHL, 22 cases of NL were identified, of which 20 patients were associated with DLBCL, 1 patient with mantle cell lymphoma, and 1 patient with peripheral T-cell lymphoma (3). In another report of 23 cases with NL of lumbosacral plexus, B-cell histology was seen in 21 cases and T-cell histology in only 2 cases (4). In our patient, NL was related to a relapse of T-cell lymphoma. Since the clinical findings are not clear, it is frequently misdiagnosed, especially when the neuropathy is the initial presentation or manifests after a period of complete remission, as in our case. When the BP is affected, the presenting symptoms are weakness, paraesthesia and pain radiating down to the upper limb or localised in the forearm and hand in cases of middle and lower trunk involvement, as in our case. In contrast to the radiation-induced brachial plexopathy that commonly affects the upper trunk or metastatic brachial plexopathy that has a predilection for the lower trunk, all parts of BP may be invaded by lymphomatous cells from the roots to the distal branches with no distinctive distribution (5). As suggestive of NL, severe pain, rapid evolution and asymmetric distribution have been reported (2). However, relatively painless or symmetrical neuropathy has also been defined (6,7). Although axonal sensory motor neuropathy is more frequent in nerve conduction studies, pure demyelinating and mixed neuropathy have also been reported (7). Moreover, due to its low sensitivity, cerebrospinal fluid (CSF) cytology is used in the diagnosis but is not always helpful (6). Neuroimaging studies with MRI neurography often provide a correct diagnosis, especially when performed together with PET/CT. On MRI, normal nerves are usually smaller than the accompanying arteries, showing gradual tapering in a well-organised fascicular distribution with isointensity to skeletal muscles on both T1w and T2w images without enhancement, except the dorsal root ganglia which should not be confused with a tumoral process. In NL, focal or diffuse nerve enlargement, fascicular disorganisation,

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hyperintensity on T2w or short-tau inversion recovery sequences and significant focal or diffuse gadolinium enhancement are seen. Although these are not specific for NL, they are highly suggestive when associated with PET/CT, which is the most accurate modality in the evaluation of nodal and extranodal spread of lymphoma (8,9). In nerve pathology, the gold standard method of diagnosis is nerve biopsy, but it is not performed in all patients because of a significant risk of permanent nerve damage. Moreover, it is concerned when the benefit outweighs the risk and the imaging studies along with CSF examinations are inconclusive. In general, treatment of NL is similar to that of PCNSL, which includes systemic and/or intrathecal chemotherapy with or without radiotherapy (8). In rare instances, surgical intervention, such as emergent decompression, is needed. Our patient was treated with radiotherapy and systemic chemotherapy with achievement of complete remission. Since the relapse of the lymphoma in our patient was localised to the right BP, radiotherapy was performed with limited-field radiation. Despite the treatment, the prognosis is worse in patients with NL than in those without (10). Although the lack of optimal treatment one of the causes, it may also be due to misdiagnosis that leads to delayed targeted treatment.

CONCLUSION

NL remains a rare and challenging diagnosis. It should be considered when a patient with a known history of haematologic malignancy presents with peripheral neuropathy, especially when the symptoms are asymmetric and rapidly evolving. MRI neurography and PET/CT are very helpful and should be used together to improve sensitivity. In equivocal cases, nerve biopsy can be used for early diagnosis when the benefit outweighs the risk, especially when, as in our case, there is an already existing axonal pattern of damage.

Informed Consent: Written informed consent has been taken from the patient.

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