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ABOUT JCTR

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ORIGINAL ARTICLE

Comparison of laminoplasty and posterior segment fusion with laminoplasty and anterior segment fusion for treating acute extensional cervical spinal cord injury in pre-existing canal stenosis: a multicenter retrospective study

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ABSTRACT

Background: Patients with pre-existing cervical spinal canal stenosis (CSCS) are more likely to suffer from an extensional spinal cord injury (SCI). However, the appropriate surgical alternatives for extended cervical SCIs in individuals with pre-existing CSCS remain unknown. The purpose of this study was to evaluate the clinical efficacies of laminoplasty and posterior short-segment fusion (PSF) with laminoplasty and anterior short-segment fusion (ASF) in the treatment of these patients.

Methods: The clinical data of 258 patients from six spine centers were included in this retrospective study. Patients were divided into two different groups based on the surgical approach: laminoplasty and PSF (PSF group) and laminoplasty and ASF (ASF group). ASIA grades and JOA scores were obtained before and after surgery to assess neurological function.

Results: There were 116 patients in the PSF group and 142 patients in the ASF group. The average operation time, intraoperative blood loss, and hospital stay were 188 min, 298 ml, and 7.6 days, respectively, in PSF group, compared to 245 min, 366 ml, and 10.4 days in PSF group, respectively. Complete decompression was achieved in all patients, and fusion was achieved 6 months after surgery. A post-operative computed tomography scan revealed that 39/464 (8.4%) screws had perforated, but no neurovascular complications occurred. Both surgical strategies improved the ASIA grade and there was no significant difference between the two groups ($P = 0.926$). The JOA score improved from 6.21 ± 1.85 to 10.90 ± 3.56 in the PSF group and from 6.45 ± 2.17 to 11.48 ± 3.62 in the ASF group, but at the final follow-up, there was no significant difference between the two groups ($P = 0.134$). The incidence of post-operative complications in the ASF group (24/142, 16.9%) ($P = 0.043$) was higher than that in the PSF group (6/116, 5.17%).

Conclusions: Cervical laminoplasty combined with short-segment transpedicular screw fixation is a reliable option to treat extensional cervical SCIs in patients with CSCS. This surgical strategy is beneficial for achieving sufficient cervical spinal cord decompression, preserving cervical spine stability, and avoiding extra anterior cervical decompression and fusion, thereby reducing surgery time, intraoperative blood loss volume, post-operative complication rate, and length of hospital stay.

Relevance for Patients: Cervical laminoplasty combined with posterior segmental fusion (PSF) reduces operative time, bleeding, and complications and achieves adequate spinal cord decompression in the treatment of extension cervical SCI in patients with CSCS.

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1. Introduction

Cervical spinal canal stenosis (CSCS) is a disorder in which the spinal cord or nerve roots are compressed, resulting in symptoms such as pain, paraesthesia, and dyskinesia. Pre-existing cervical spondylotic changes, cervical ossification of the posterior longitudinal ligament, or developmental cervical stenosis are the most common pathologic mechanisms leading to CSCS [1,2]. A hyperextension injury, which is accompanied by anterior longitudinal ligament rupture, intervertebral disc destruction, or cervical fracture-dislocation, induces cervical instability and raises the risk of cervical spinal cord injury (SCI) due to pre-existing CSCS [3]. Anterior longitudinal ligament and intervertebral disc rupture are common findings on magnetic resonance imaging (MRI) in patients with a hyperextension cervical injury without fracture or dislocation [1,4,5]. The risk of cervical instability demands surgical stabilization to prevent additional harm.

Cervical laminoplasty is a preferred technique to achieve complete decompression in patients with an extensional cervical spinal injury coupled with multilevel cervical stenosis. Moreover, an extra-anterior approach fusion at the disruption level is required to stabilize the cervical spine after laminoplasty. Despite the ability to achieve both complete decompression and satisfactory reconstruction, the posterior-anterior combined approach is criticized for necessitating a longer surgery time and triggering complications. Therefore, we advocate only the posterior approach, including laminoplasty and segment transpedicular screw fixation, to achieve both decompression and stabilization. In this study, we compared the clinical outcomes of different surgery regimens, such as laminoplasty combined with anterior fusion and laminoplasty associated with transpedicular screw instrumentation, to treat extensional cervical spinal injury in patients with CSCS.

2. Materials and Methods

2.1. Study participants

The clinical data of 258 patients with acute extensional cervical spinal injuries and pre-existing CSCS who were admitted to six spine centers between April 2010 and January 2022 were recruited for this retrospective study. Patients with the following characteristics were enrolled: aged 18 – 70 years, sustained an extensional cervical spinal injury within 24 h, suffered from pre-existing degenerative cervical stenosis, and developed cervical stenosis or stenosis involving ossification of the cervical posterior longitudinal ligament (OPLL). Patients with cervical dislocation, cervical infection, tumor, tuberculous disease, and brain injury were excluded from this study. The disrupted anterior longitudinal ligament or intervertebral disc was confirmed by gradient-echo T2 (T2-weighted GRE) and STIR-weighted MRI pulse sequences. The present study was approved by the institutional review board of each participating hospital.

2.2. Surgical procedures

In the laminoplasty and posterior short-segment fusion group, each patient was positioned in a Concorde position after being administered general anesthesia. Using the posterior middle approach, the extensional muscles were detached from the spinous process and lamina to expose the mass from C3 to C6. If C7 was involved, the C7 articular mass also needed to be exposed. The lateral margin of the articular masses of the injured level needs to be extra-exposed. The notch-referred technique was used to place cervical pedicle screws (CPS) [6]. Being virtually unaffected by bony encumbrances or erosive articular surface variants, the lateral vertebral notch is a reliable and consistent anatomical landmark for lower-axis CPS placement, providing an accurate and safe reference point for subaxial CPS placement. When short-segmental transpedicular screw instrumentation was completed in the involved cervical spine, laminoplasty was conducted from C3 to C6 (C7 may be necessary if involved). By sparing the nerve roots and spinal cord and enlarging the spinal canal, surgery reduces the pressure on the spinal cord and nerve roots. At the end of the procedure, the surgeon closed the incision layer by layer to promote healing [7]. In the treatment of a cervical SCI without fracture or dislocation, single-opening laminoplasty has satisfactory efficacy in the recovery of post-operative neurological function, reduction of pain, and improvement of daily life behaviors compared to total laminectomy with lateral mass screw fixation. Moreover, single-opening laminoplasty achieves lesser trauma and is associated with a lower complication rate. Therefore, given its advantages, posterior single-opening laminoplasty coupled with pedicle screw fixation was our preferred choice of surgical plan. The facets and masses were decorticated and bone grafted for fusion (Figure 1). In the laminoplasty and anterior short segment fusion group, the patient was initially placed in a prone position to receive laminoplasty and then placed in a supine position to be treated with anterior discectomy and fusion at the involved intervertebral disc.

2.3. Clinical assessment

Routine post-operative X-ray, computed tomography (CT), and MRI examinations were performed to confirm the instrument's position and the adequacy of decompression (Figure 1). In addition, all patients performed neurofunctional rehabilitation exercises in the rehabilitation department soon after surgery. Post-operative and follow-up assessments were performed to assess and determine the neurological function (ASIA scale and JOA score), bone-graft fusion, instrument's location, surgery time, intraoperative blood loss volume, and length of hospital stay of the patients in the two groups. The improvement rate of neurological function was calculated using the following formula:

$$\text{Improvement rate of neurological function}(\%) = \frac{\text{Postoperative JOA score} - \text{Preoperative JOA score}}{17 - \text{preoperative JOA score}} \times 100$$

2.4. Statistical analyses

Continuous variables between the two groups were compared using *t*-test. Chi-squared tests were used to compare categorical variables between the two groups. The software package IBM SPSS Statistics version 22 (IBM, USA) was used to perform

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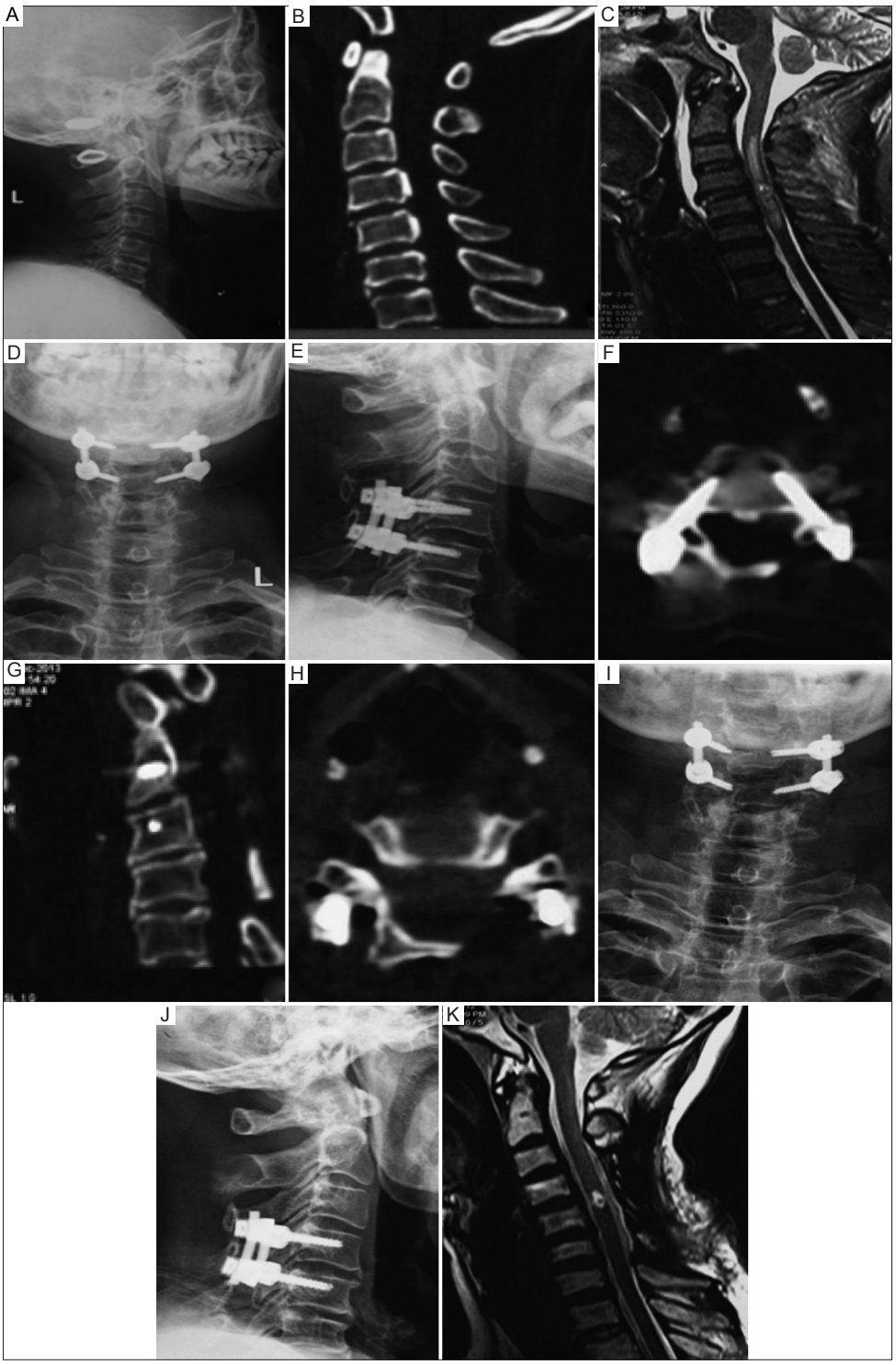

Figure 1. Laminoplasty with short-segmental transpedicular screw fixation in treating extensional cervical injuries in patients with cervical spinal canal stenosis (CSCS). Pre-operative lateral X-ray and sagittal computed tomography (CT) scans showed CSCS and segmental ossification of the posterior longitudinal ligament at C4-C5 (A and B). Pre-operative sagittal T2-weighted images showed an inhomogeneous high signal intensity of the cervical cord extending from C3 to C6. A swollen cervical cord, discontinuity of the anterior longitudinal ligament, and disc rupture at C4/5 were observed (C). X-ray immediately after the surgery showed that the screws were inserted in the appropriate position and that the osseous cervical canal was significantly expanded after laminoplasty from C3 to C6 (D and E). Horizontal CT scan showed that the screw trajectories were appropriate (F). A sagittal CT scan further confirmed that the osseous cervical canal was significantly expanded after laminoplasty (G). The horizontal CT scan 6 months after surgery showed that the hinged side of the cervical lamina was fused (H). The X-ray one year after surgery showed a good position of internal fixation. (I-J) Magnetic resonance imaging scan conducted 24 months after the surgery indicated that the cervical canal remained expanded and that post-traumatic syringomyelia had developed (K).

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the statistical analyses. Results with a $P < 0.05$ were considered statistically significant.

3. Results

Figure 1. Laminoplasty with short-segmental transpedicular screw fixation in treating extensional cervical injuries in patients with cervical spinal canal stenosis (CSCS). Pre-operative lateral X-ray and sagittal computed tomography (CT) scans showed CSCS and segmental ossification of the posterior longitudinal ligament at C4-C5 (A and B). Pre-operative sagittal T2-weighted images showed an inhomogeneous high signal intensity of the cervical cord extending from C3 to C6. A swollen cervical cord, discontinuity of the anterior longitudinal ligament, and disc rupture at C4/5 were observed (C). X-ray immediately after the surgery showed that the screws were inserted in the appropriate position and that the osseous cervical canal was significantly expanded after laminoplasty from C3 to C6 (D and E). Horizontal CT scan showed that the screw trajectories were appropriate (F). A sagittal CT scan further confirmed that the osseous cervical canal was significantly expanded after laminoplasty (G). The horizontal CT scan 6 months after surgery showed that the hinged side of the cervical lamina was fused (H). The X-ray one year after surgery showed a good position of internal fixation. (I-J) Magnetic resonance imaging scan conducted 24 months after the surgery indicated that the cervical canal remained expanded and that post-traumatic syringomyelia had developed (K).

Two hundred and fifty-eight patients grappling with acute extensional cervical spinal injuries, as confirmed by MRI, and pre-existing CSCS were followed up. One hundred and sixteen patients who underwent posterior laminoplasty and received transpedicular screw implantation were included in the PSF group. One hundred forty-two patients who underwent laminoplasty combined with anterior fusion were included in the ASF group. The mean follow-up time was 23.5 ± 2.8 months. The demographic characteristics of the patients are shown in Table 1, which shows that the patient profiles were not significantly different between the two groups.

3.1. Surgery time, blood loss, and length of hospital stay

In the PSF group, the average surgery time was 188 ± 23 minutes, intraoperative blood loss was 298 ± 42 ml, and length of hospital stay was 7.6 ± 2.9 days. However, in the ASF group, the average surgery time was 245 ± 25 min, intraoperative blood loss was 366 ± 51 ml, and length of hospital stay was 10.4 ± 3.3 days, which significantly outstripped those in the PSF group. The results are shown in Table 2.

3.2. Complications

All patients showed complete decompression on radiography, and bone fusion was achieved 6 months postoperatively. A post-operative CT scan revealed that 39 screws (8.4%) of 464 screws perforated the cortex of the pedicles. However, no neurovascular complications were involved. One patient in the ASF group died of respiratory failure. A total of 30 complications occurred, including lung infection, cranial spinal fluid leakage, surficial wound infection, dysphagia, and instrument failure in both groups, but the occurrence rate significantly differed between the two groups. Four instrument failures occurred in the ASF group, including dislodged plates, although good positioning of anterior fixation was verified intra-operatively. An additional revisional surgery was conducted for instrument failure. The results are shown in Table 2.

3.3. Neurological function improvement and analysis

Preoperatively, 258 patients had neurological deficits with ASIA grades C (50%) and B (31.4%), followed by grades D (11.6%) and A (7.0%). After surgery, the neurological function of patients in both groups improved to ASIA grades D (43.8%) and C (26.0%), followed by grades E (13.5%), B (12.8%), and A (3.9%). The assessments of the ASIA grades are shown in Table 3.

The mean pre-operative JOA score was 6.21 ± 1.85 in the PSF group and 6.45 ± 2.17 in the ASF group. The mean final follow-up JOA score was 10.90 ± 3.56 in the PSF group and 11.48 ± 3.62 in the ASF group, without a significant difference. The recovery rate was 43.5% in the PSF group and 47.7% in the ASF group, and no significant difference was found between the groups (P >0.05). The results are shown in Table 4.

Table 1. Demographic data of the patients

General information	PSF group (*n*=116)	ASF group (*n*=142)	*P*-value
Age (years)	47.8±8.7	48.6±8.2	0.446
Sex			
Male	87	101	0.486
Female	29	41	
Injury time (h)	4.9±2.4	5.1±2.9	0.548
Injured level			
C2/3	2 (1.7%)	5 (3.5%)	0.850
C3/4	14 (12.1%)	19 (13.4%)	
C4/5	55 (47.4%)	70 (49.3%)	
C5/6	39 (33.6%)	41 (28.9%)	
C6/7	6 (5.2%)	7 (4.9%)	

Abbreviations: PSF: Posterior short-segment fusion, ASF: Anterior short segment fusion

Table 2. Surgery time, blood loss, length of hospital stay, and complications between the two groups

Surgery-related index	PSF group (*n*=116)	ASF group (*n*=142)	*P*-value
Surgery time (min)	188±23	245±25	<0.001
Blood loss (ml)	298±42	366±51	<0.001
Length of hospital stay (days)	7.6±2.9	10.4±3.3	<0.001
Complications	6	24	
CSF leaking	3	1	0.043
Lung infection	1	7	
Wound infection	2	8	
Instrument failure	0	4	
Dysphagia	0	4	

Abbreviations: CSF: Cranial spinal fluid, PSF: Posterior short-segment fusion, ASF: Anterior short segment fusion

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Table 3. Comparison of pre-operative and final follow-up ASIA grades between the two groups

ASIA grades	PSF group (*n*=116) (%)	ASF group (*n*=142) (%)	Total (*n*=258)	*P*-value
Pre-operative ASIA grades				
A	7 (6.0)	11 (7.7)	18 (7.0)	0.690
B	39 (33.6)	42 (29.6)	81 (31.4)	
C	59 (50.9)	70 (49.3)	129 (50.0)	
D	11 (9.5)	19 (13.4)	30 (11.6)	
Final follow-up ASIA grades				
A	4 (3.4)	6 (4.2)	10 (3.9)	0.926
B	15 (12.9)	18 (12.7)	33 (12.8)	
C	29 (25.0)	38 (26.8)	67 (26.0)	
D	54 (46.6)	59 (41.5)	113 (43.8)	
E	14 (12.1)	21 (14.8)	35 (13.5)	

Abbreviations: PSF: Posterior short-segment fusion, ASF: Anterior short-segment fusion

Table 4. Comparison of pre-operative and final follow-up JOA scores between the two groups

JOA	PSF group (*n*=116)	ASF group (*n*=142)	*P*-value
Pre-operative JOA	6.21±1.85	6.45±2.17	0.345
Final follow-up JOA	10.90±3.56	11.48±3.62	0.134

Abbreviations: PSF: Posterior short-segment fusion, ASF: Anterior short-segment fusion

4. Discussion

Owing to the risk for paralysis, sensory impairment, bowel, bladder, and sexual dysfunction, acute cervical SCI is a potentially devastating condition. Individuals with cervical canal stenosis are known to be at high risk for developing cervical SCI when injured. Among cervical SCI patients with cervical stenosis, an extensional injury is the most common injury mechanism and occurs in many patients without any radiological evidence of fracture or dislocation. This injury should be classified as a distractive extension type according to Allen's report or as a B3 type injury according to the AO classification [8], which requires surgical intervention. Extensional injuries are characterized by progressive failure of the motion segment in an anterior-to-posterior direction, which consists of failure of the anterior longitudinal ligament and annulus fibrosus. Widening of the disc space could be seen on the X-ray under extension. There may be a small avulsion fracture at the anterior margin of the disc space in some cases. If extensional force continued, posterior subluxation could occur. It is also common that the magnitude of posterior displacement could often vanish following flexion of the head. Extensional injuries were often the result of a fall on the face. Therefore, for patients with a facial injury who are diagnosed with an SCI even without fracture or dislocation, attention should be given to an extensive cervical spinal injury. In this study, we advocated that cervical laminoplasty combined with transpedicular screw fixation is a preferred approach to treating an extensional cervical spinal injury in patients with stenosis. This study retrospectively analyzed the clinical outcomes of patients who underwent anterior decompression and fusion surgery and those who underwent posterior hybrid surgery [9]. PHT was as effective as ADF in the treatment of cervical SCI, based on the fact that PHT was superior to ADF in improving the patient's health-related quality of life and preserving cervical spine mobility in the long-term follow-up period.

Early decompression surgery for extensional cervical spinal injury had a beneficial outcome [10]. The optimal timing for surgical intervention remains unclear. La Rosa *et al*. [11] reported that early decompression surgery within 24 h of trauma exerted a significantly better effect [12,13] than late surgical management. Guest *et al*. also reported that early surgery (within 24 h of injury) improves overall motor recovery in patients whose traumatic central cord syndrome was related to acute disc herniation or fracture [14]. The patients in this cohort received early decompression, and an obvious improvement in post-surgical neurological scores was observed. In contrast, other studies reported that surgical treatment was not superior to conservative treatment for traumatic CSCI without major fracture or dislocation with spinal cord compression in the acute phase [15-17]. No relationships between pre-existing CSCS and neurological outcomes were evident after traumatic CSCI. These results suggest that decompression surgery might not be recommended for traumatic CSCI without major fracture or dislocation despite pre-existing CSCS. Some results suggest that prophylactic surgical treatment for CSCS may not have a significant impact on the incidence of traumatic CSCI. However, patients with pre-existing CSCS do have an increased incidence of CSCI, which is noteworthy [18]. We considered that the injured cord would be more severely squeezed in patients with a pre-conditioned stenotic canal under the rapid development of edema in the early stage after spinal cord trauma. Therefore, in this scenario, laminoplasty provides a feasible approach for the complete decompression of cervical spinal cord, with multisegmental decompression in particular delivering more effective outcomes. A study has shown that patients with minimal cord changes on MRI have the best outcome, followed by those with cord edema, and patients with parenchymatous hemorrhage and contusion on MRI fare poorly [19]. MRI of the latter cases frequently reveals hematomas and intramedullary edema [20].

According to relevant studies, dynamic changes in the cervical spine and spinal cord in cervical spinal cord injury patients without fractures or dislocations were assessed by kinematic MRI. Kinematic MRI showed dynamic patho-anatomical changes in patients with a cervical SCI, such as spinal stenosis in different locations without fractures or dislocations. The injured segments had small spinal canal diameters, high Muhle grades, little space available for the spinal cord, and a high spinal cord diameter to vertebral canal diameter ratio. MRI techniques can be utilized to examine SCI in patients [21].

Moreover, posterior laminoplasty is a relatively simple procedure that can preserve cervical mobility without engendering substantial post-operative complications. Laminoplasty achieves a decompressive effect for patients with SCI, and the combined anterior spinal fusion in the ASF group as well as the posterior decompression fusion in the PFS group enabled long-term post-operative stabilization, increasing the patient's range of motion and their ability to perform daily activities and ultimately enhancing their post-operative quality of life, which is

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not dissimilar to the idea of a study investigating the impact of spinal fusion on quality of life score improvement during the post-operative period [22]. This line of evidence proves that laminoplasty not only can lead to complete decompression but also hinder the development of anterior approach-associated complications in this cohort. Extra anterior approach-related complications, such as dysphagia, were observed in the ASF group, but no analogical complications were observed in the PSF group [23,24].

For patients with OPLL, we also compared the post-operative neurological recovery rates, which were similar in the ASF and PSF groups, as seen in related studies. In patients with giant OPLL with posterior convex malalignment, the neurological recovery rate was better after laminoplasty and ASF than after laminoplasty and PSF. In addition, post-operative neck pain was less severe in the ASF group. However, perioperative complications were more common in the ASF group. Although there was no statistically significant difference in the post-operative nerve recovery rate between the two groups, the recovery rate was higher in the ASF group in patients with kyphosis alignment (C2-C7 angle <0°). Post-operative cervical pain was greater and intraoperative blood loss was much more in the PSF group. The improvement in C2-C7 alignment was greater and the operative time was longer in the ASF group. Approach-related complications were more frequently observed in the ASF group than in the PSF group. Drawing upon the comparison between ASF group and PSF group, we concluded that the PSF group benefited substantially from the treatment, the patients in this group were assigned in terms of surgery time, length of hospital stay, blood loss, and complications compared to the ASF group. In the treatment of spinal cervical spondylosis due to OPLL, overall post-operative neurologic function (irrespective of the canal-occupying ratio) was better with the anterior approach than with the posterior approach. We believe that the anterior approach is particularly desirable for patients with a canal-occupancy ratio of >50%, although it leads to higher incidences of surgical trauma and surgery-related complications. The posterior approach is relatively safe, with lower rates of surgical trauma and complications. For patients with a canal-occupancy ratio of <50%, a posterior approach was recommended, bringing the post-operative neurologic function recovery to a level similar to patients treated with an anterior approach. Therefore, in patients with less severe OPLL, we also recommend the posterior approach for safety reasons and to minimize the chances of developing post-operative complications and patient discomfort. When it comes to treating patients with OPLL, there is no fixed set of criteria to determine the type of treatment because the optimal surgical plan should be decided by the attending surgeon after performing a comprehensive evaluation. Patients in both groups had sufficient decompression verified by post-operative MRI. Laminoplasty decompression conducted in all patients may explain why neurological function recovery was not significantly different between the two groups, as reflected in the post-operative ASIA grades and JOA scores.

Performing immediate post-operative cervical stabilization is another key factor for forestalling secondary injury in patients with an extensional cervical spinal injury. Laminoplasty can sufficiently decompress the stenotic canal, but it could aggravate the stability of the cervical spine with a pre-existing anterior longitudinal ligament and intervertebral disc disruption. Masaki *et al*. [25] reported that hypermobility of vertebrae at the cord compression level is a risk factor for poor surgical outcomes after laminoplasty. Therefore, stabilization intervention is needed for this cohort with options of extra anterior fusion or posterior instrumentation. Studies have indicated that posterior transpedicular screw fixation is biomechanically stronger than anterior fixation in the cervical spine [26,27]. The strategy of short-segmental fixation was to better preserve the mobility of cervical segments, thereby decreasing the stiffness of the cervical spine, retarding cervical degeneration, and maintaining post-operative range of motion. We found that there were no instrument failures in the PSF group, whereas four instruments became dislodged in the ASF group. Two post-operative fixation displacements occurred due to the excessive intervertebral cage height, which resulted in focal hyperlordosis of the involved segment. Two other instruments became dislodged postoperatively in a patient with hyperextension of the cervical spine.

In this study, compared to laminoplasty combined with anterior fusion procedure, laminoplasty associated with transpedicular screw instrumentation decreased intraoperative blood loss, surgery time, and length hospital stay. This was not difficult to understand because there was only one surgery approach conducted in PSF group associated with less surgical trauma, and patients in this group benefitted more and recovered much faster. Xu and Lun [28] reported that laminoplasty in combination with posterior fixation contributed to several clinical advantages, including less surgical trauma, less intraoperative blood loss, and satisfactory stability in treating multilevel CSCS and SCI in the trauma population. Our results from this comparative study supported Xu and Lun's findings to some extent.

There are some limitations in the study. First, in this retrospective multicenter study, the surgical proficiency for decompression and fixation was not controlled in different institutions. Different expertise in surgery would result in technical bias in the treatment. However, the number of surgeries performed in this study was not small, potentially reducing the risk of bias in the results. Second, although all cases were labeled acute cervical spine injuries within 24 h, it should be noted that controlling the time from injury to surgery in different institutions was a challenging endeavor, which might influence the treatment results in some aspects.

5. Conclusion

Cervical laminoplasty combined with short-segment transpedicular screw instrumentation is a reliable and preferred option to treat extensional cervical injuries in patients with pre-existing CSCS. This treatment regimen holds the promise of achieving sufficient cervical spinal cord decompression, instant three-column fixation, and immediate cervical spine restabilization, as well as preventing extra anterior cervical fusion, and reducing post-operative complications.

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Conflict of Interest

The authors declare they have no competing interests.

Ethics Approval and Consent to Participate

As ours was a retrospective study, no ethical application was required according to the hospital's rules. Written consent was obtained from the patients included in the study.

Consent for Publication

Written consent was obtained from all patients enrolled in this study.

Availability of Data

Data are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Subtotal cholecystectomy as an approach to preventing injury in the left-sided gallbladder in the emergency surgery setting: A case study

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ABSTRACT

A left-sided gallbladder is an unusual anatomic variation that makes gallbladder surgery challenging. Two systematic reviews on surgery for left-sided gallbladder highlighted high iatrogenic bile duct injury rates of 4.4% and 7.3%. This paper reports a female in her 40s with symptoms of acute calculous cholecystitis admitted to a secondary health-care center. After inserting four ports through standard sites for conventional gallbladder surgery, laparoscopic inspection revealed a phlegmonous left-sided gallbladder. No discordant situs of abdominal viscera was noted. Laparoscopic surgery was converted to open subtotal closed-tract cholecystectomy. No post-operative complications related to the surgical site were observed. A left-sided gallbladder affected by severe inflammation and infection is an extraordinary condition that should be considered as a risk factor. If an inflamed left-sided gallbladder is encountered, emergency subtotal cholecystectomy is an alternative to total cholecystectomy when the circumstances to adopt the strategies of a culture of safety in cholecystectomy for complete removal of the gallbladder are unfavorable.

Relevance for Patients: Subtotal cholecystectomy in patients with left-sided gallbladder reduces the risk for bile duct injuries, outweighing the potential side effects stemming from this surgical approach.

1. Introduction

Anatomical variants within the biliary ductal system are common [1,2]; contrarily, aberrations are rare. The true left-sided gallbladder is one of them, with an estimated incidence rate of <0.3% [3,4].

A recent systematic review summarized 53 case reports and case series on managing the left-sided gallbladder [5]. Briefly, cholecystectomy for symptomatic gallstones was performed in 90 (80.4%) of 112 total patients. A major iatrogenic injury to the common duct requiring hepaticojejunostomy occurred in four patients (4.4%). Another review paper revealed a higher bile duct injury rate (7.3%) in 55 patients during left-sided cholecystectomy [6].

Despite the high rates of bile duct injury in this subpopulation of patients, a left-sided gallbladder has not yet been elucidated as a surgical factor requiring pre-operative and (or) intraoperative consideration in applying the gallbladder bailout surgery principle, which is always directed at iatrogenic injury prevention [7-9]. This is especially important in an emergency general surgery setting. In this paper, we aim to describe an incidental surgical finding from a clinical case of an urgent subtotal cholecystectomy for a left-sided gallbladder. Insufficient awareness of the potential dangers associated with this anatomical variation of the gallbladder can expose a patient to a substantially higher risk of having life-changing consequences related to biliary or vascular injuries.

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2. Materials and Methods

2.1. Case presentation

A right-handed female patient in her 40s with severe central epigastric and right-sided hypochondrium pain was admitted to the emergency general surgery ward of the acute care hospital. The pain was associated with vomiting and diarrhea. Her only concomitant diseases were essential hypertension, which was controlled using 5 mg ramipril daily, and constipation. Her body mass index was 35.8 kg/m^2 on admission.

The patient was afebrile (37.1°C), with a satisfactory oxygen saturation level at 98%, sinus heart rhythm at 81 beats/min, and high arterial blood pressure at 179/98 mmHg. Further objective examination revealed severely tender right hypochondrium, central epigastrium, and positive Murphy's sign.

Her white blood cell count was 16.1 × 10^9/L. Her neutrophils comprised 89.4% leukocytes (14.4 × 10^9/L). The total serum bilirubin concentration was within the standard range (9 μmol/L; 0.53 mg/dL). However, her serum γ-glutamyl transferase concentration was 3.8 times above the standard level of <40 U/L. Her serum C-reactive protein concentration was within the standard range (4 mg/L). Hyperlactatemia of 3.8 mmol/L was also detected in the patient. The radiographs did not reveal pneumoperitoneum or chest infection.

The radiologist performed a transabdominal ultrasound scan within 24 h of admission. Signs of fatty liver disease with hepatomegaly and cholecystolithiasis were reported. Two annotated ultrasonograms are illustrated in Figure 1.

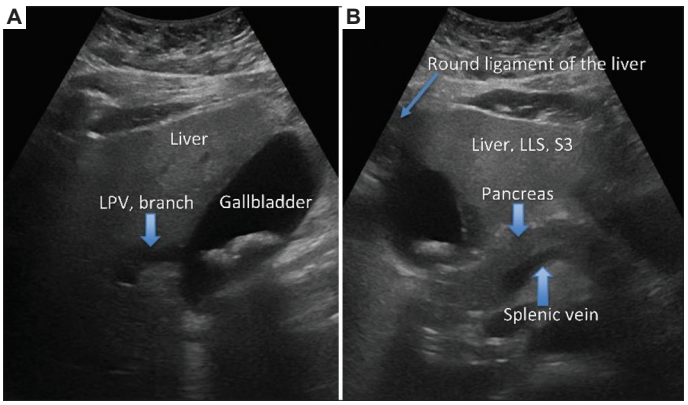

Figure 1. Transabdominal ultrasonography of the gallbladder and surrounding anatomical structures: (A) longitudinal view of the gallbladder reveals a distended organ and large calculi in its neck; the block arrow is directed at the tubular structure which, by our interpretation, is a branch of the left portal vein; (B) transverse view of the gallbladder shows a calculus within it; most importantly, the head and the body (the upper block arrow) of the pancreas, and splenic vein behind this organ (the lower block arrow) are visible. A line arrow is directed at a hypoechogenic area, a site of the round ligament of the liver.
Abbreviations: LLS: Left lateral section; LPT: Left portal vein; S3: The third segment of the liver.

2.2. Differential diagnosis

A working diagnosis of acute calculous cholecystitis was apparent; however, four diagnostic detail points should be briefly overviewed. First, according to Tokyo Guidelines 2007 (TG07), 2013 (TG13), and 2018 (TG18), grading the acute cholecystitis severity should be emphasized during admission and pre-operative diagnosis. Our case should have been classified as acute moderate cholecystitis – grade 2 – as it was associated with a duration of acute symptoms of >72 h [10].

Second, precise radiological characterization of the gallbladder and its site is crucial in managing acute cholecystitis. However, detecting an atypical gallbladder anatomical location is difficult (although possible) when performing an urgent transabdominal ultrasound scan (further details are provided in the discussion). Therefore, the left-sided gallbladder is identified during surgery in over 80% of cases [5].

Third, intraoperative characterization of the anatomy of the gallbladder, liver, and its ligaments facilitates decision-making during laparoscopic or open surgery. Also, it is essential in education and academic surgery. In the absence of *situs viscerum inversus*, the sinistroposition, a true left-sided gallbladder (our patient), usually with hypoplastic segment 4 of the liver, should be differentiated from the medioposition of the gallbladder [11], when it is medially displaced to lie on the undersurface of the quadrate lobe (i.e., inferior subsegment of segment 4) of the left hemiliver.

Fourth, a right-sided round liver ligament is another rare anatomical variant, which can be associated (but not always; our patient is an example) with the left-sided gallbladder and frequent intrahepatic vascular and biliary anomalies [12].

2.3. Therapeutic interventions

A standard conservative treatment scheme, including antibiotics, was established for this patient. We infused 100 mg of tigecycline and 240 mg of gentamycin through the peripheric vein, and a regular tigecycline dose of 50 mg every 12 h for 5 days was prescribed. Pyrexia during the hospital stay, local signs of peritoneal irritation, and serum C-reactive protein raised to 88 mg/L were key indicators to consider an urgent index admission laparoscopic cholecystectomy on the 4th day of hospitalization. Informed consent was obtained as a part of the routine pre-operative actions.

After inserting the first 11-mm diameter port below the umbilicus, a capnoperitoneum of up to 12 mmHg was achieved. Standard sites of the right upper quadrant of the abdominal wall were used to insert the other three ports for conventional cholecystectomy. Laparoscopic inspection revealed a distended thick-walled phlegmonous gallbladder on the left side of the round and falciform ligaments of the enlarged liver on the anterior wall of the distal portion of the stomach (Figure 2). A proximal portion of the gallbladder anterior to the hepatic hilum, inflamed tissues of the hepatoduodenal ligament, unclear segmental anatomy (such as the presence or absence of segment 4) despite the apparent sulcus on the visceral surface of the liver, and a sizeable umbilical fissure

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of the liver were other features of the case's surgical anatomy. Also, it was the first time an experienced consultant surgeon operated on a patient with a true left-sided gallbladder. An additional 5-mm diameter port was inserted into the peritoneal cavity through the left upper quadrant of the abdominal wall. An attempt was made to detach the gallbladder's fundus from the visceral surface of segment 3 of the liver. However, this procedure was aborted. A decision was made to convert a laparoscopic to open surgery through an upper midline laparotomy.

The fundus-first technique was further employed to detach 80% of the hepatic wall of the gallbladder from the cystic plate, which was edematous and hemorrhagic. Thereafter, the gallbladder's fundus was opened, infected bile was suctioned out, and moderate-sized gallstones were removed from the cavity of the gallbladder. When a good backflow of fresh bile was noticed from the internal orifice of the cystic duct, situated quite superiorly, a final decision was made to perform a subtotal cholecystectomy.

No attempt was made to dissect the cystic pedicle. The gallbladder was transected circumferentially at the level of the Hartmann's pouch. The remnant was closed using two continuous polyglactin 910 (Vicryl® 2/0) and polydioxanone (PDS II 2/0) sutures to obliterate the cavity of the remnant gallbladder. Floseal®, a human gelatine-thrombin matric sealant, was used to ensure hemostasis from the liver. The Portex®Robinson drainage system 20 Ch was used for the subhepatic space of the peritoneal cavity.

2.4. Outcome and follow-up

No surgical complications were observed. However, on post-operative day 2, a fever episode (38.1°C), supraventricular tachycardia (>200 beats/min), and hypotension were documented and managed according to hospital guidelines. Furthermore, on post-operative day 3, the patient was tested positive for influenza B. The patient was isolated in a side room with droplet precautions. The drain was removed from the peritoneal cavity on post-operative day 6, the day she was discharged from the hospital. Histopathological investigation of the excised part of the gallbladder of 60 × 33 × 24 mm dimensions revealed a 6-mm wall thickness, necrotic mucosa, and signs of diffuse chronic inflammation.

No other side effects and readmissions to the hospital occurred within 90 post-operative days. The patient underwent three-dimensional magnetic resonance cholangiopancreatography as an outpatient (Figure 3).

A follow-up visit to the surgical assessment unit on post-operative day 111 revealed that the patient had made an excellent post-operative recovery. We used the Gastrointestinal Quality of Life Index-10 (GIQLI-10, English; point range 0–40; a maximal score indicates perfect health) to assess the quality of life related to health [13]. The summative score was 28. However, only diarrhea (score 2 out of 4) had increased since the surgery, which was due to intake of high-fat or high-sugar foods. The other two low-score (1 out of 4) symptoms – strong burping/belching and tiredness/

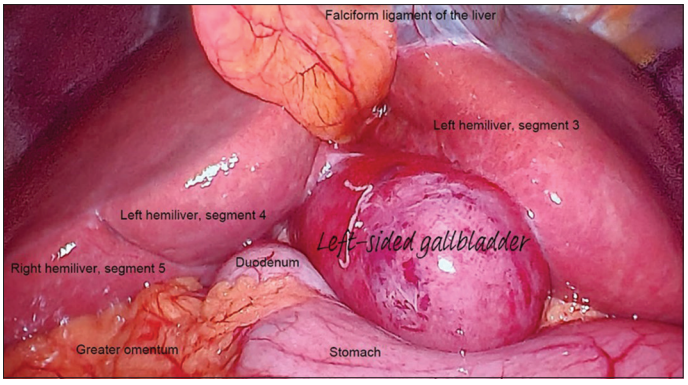

Figure 2. Laparoscopic inspection reveals a left-sided gallbladder and acute cholecystitis. The fissure on the visceral surface of the liver between segment 4 of the left hemiliver and segment 5 of the right hemiliver can be interpreted as an external hallmark of the Cantlie-Serege-Rex plane separating the right hemiliver from the left hemiliver.

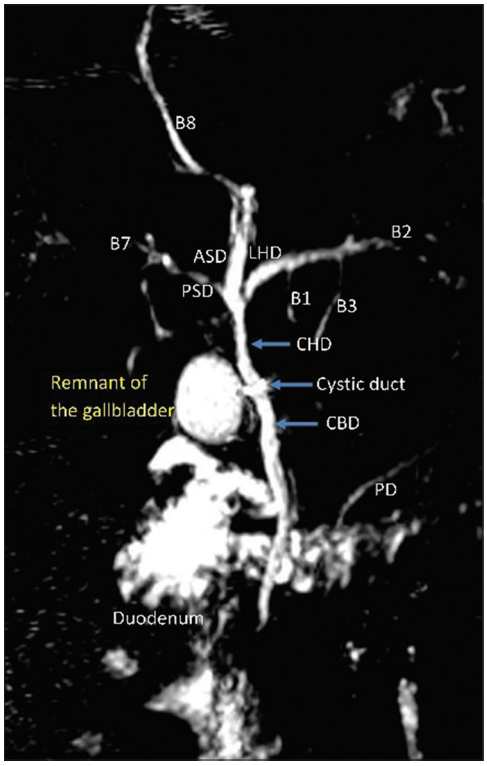

Figure 3. Magnetic resonance cholangiopancreatography (MRCP) on the 47th post-operative day. The gallbladder remnant is on the right side of the common hepatic and bile ducts, situating adjacent to them. This image suggests that the fusion of the cystic duct with the common hepatic duct is on the left of the main bile duct after a U-shaped turn from right to left anteriorly to the main bile duct. Other anomalies of the biliary ductal system are highly probable as the right hepatic duct (RHD) is not identifiable in MRCP images.
Abbreviations: ASD: Anterior sectional duct; CBD: Common bile duct; CHD: Common hepatic duct; LHD: Left hepatic duct; PD: Pancreatic duct; PSD: Posterior sectional duct; S3: The third segment of the liver; B1: Left-sided duct for caudate lobe; B2, B3, B7, and B8 are segmental bile ducts; B4, B5, and B6 are not highlighted.

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fatigue – were regarded as regular occurrences suffered similarly before subtotal cholecystectomy.

3. Discussion

The primarily aims of surgical care are to save the patient's life, prevent the patient from further disease complications or reduce the risk of sustaining them, improve the patient's quality of life, and eliminate the possibility of iatrogenic injury associated with surgery. Gallbladder surgery for benign biliary disease is an excellent example of this concept because injury to any classified bile duct is considered avoidable [14-16]. This paper highlights the decision-making during and the technical details of gallbladder surgery related to double conversion in an acute surgery setting with atypical gallbladder anatomy. Conversions from laparoscopic to open surgery and pre-planned total to subtotal cholecystectomy with the closure of the gallbladder remnant guaranteed no intraoperative risks, satisfactory surgical outcomes, and effective physical rehabilitation following the arduous gallbladder surgery. Seven other themes related to the left-sided gallbladder –precision in radiological diagnostics, the importance of laparoscopic inspection, detailed informed consenting, extraordinarily high bile duct injury rates, variations of ductal anatomy, intraoperative fluorescent cholangiography, and decision-making to perform a less-than-total gallbladder removal – emerged from the details of this case report.

First, pre-operative identification of the left-sided gallbladder is difficult, especially in emergency admission patients whose radiological investigations are restricted to a real-time ultrasound scan of the gallbladder [17]. Table 1 describes why it is difficult to reveal a left-sided gallbladder through standard examination and imaging techniques before surgery [3,18-22]. On the contrary, a left-sided gallbladder and the variations of the biliary tract – a frequent combination of biliary anomalies – can be diagnosed preoperatively using intravenous contrast-enhanced reconstructive three-dimensional computed tomography (CT)-cholangiography [23-25]. However, a three-dimensional CT-cholangiography is not a routine investigation in an acute care surgery environment. It can be considered when a congenital anomaly of the gallbladder is suspected during an ultrasound scan examination. The same logic is relevant for applying an urgent magnetic resonance cholangiopancreatography.

Second, a targeted laparoscopic inspection of the liver and gallbladder through a first port and the rationale for correctly using other laparoscopic ports and instruments are fundamental principles of safe laparoscopic surgery for all, as an element of uncertainty is a satellite of every surgery. Unfortunately, the gallbladder anatomy-related intraoperative problem was not identified and acknowledged during the primary inspection of the hepatobiliary area. This determined the standard insertion of the other three laparoscopic ports through the right upper quadrant of the abdominal wall. If the problem had been identified during the primary inspection, the second port would have been inserted into the peritoneal cavity through the left lateral quadrant laterally to create an adequate workspace between the round and falciform

Table 1. Comparison of selected characteristics of right-sided and left-sided gallbladders and their clinical implications

Characteristics	Right-sided gallbladder	Left-sided gallbladder	Explanation	Implications
Embryogenesis	The same primary structure for the gallbladder and one extrahepatic bile duct	The same primary structure for the gallbladder and one extrahepatic bile duct	It is a cholecystic axis; hepatic ducts appear much later as lateral buds	Locational variations of the gallbladder are rare: migration to the left side or primary formation on the left side of the liver
Incidence	≥99.7%	<0.3%	See embryogenesis	Increased risk of injuries during left-sided gallbladder surgery
Innervation	Sympathetic and sensory: coeliac plexus, T7–9 Parasympathetic: the right vagus nerve through its hepatic branch	Standard and identical to right-sided gallbladder	No evidence of different innervation of the left-sided gallbladder is available	The same dermatomes may be affected Boas' sign for both anatomical variations: a change detected by lightly drawing a pin down the back of the patient's chest
Pain	Right hypochondrium and epigastrium, with or without radiation to the back close to the tip of the right scapula	Identical afferent pain pathway	See innervation	Murphy's sign for both anatomical variations
US scanning	Conventional description includes the measurements of the gallbladder size, wall thickness, gallstones, and polyps	Not the main investigation to clarify the anatomical relationship with the liver (see CT scanning)	Left-sided gallbladder is an occasional event; other anatomical variations, such as floating gallbladder, are possible.	The aim: gallbladder disease diagnosis; US scan is the first and, in most cases, the last choice of testing approach to diagnosing cholecystolithiasis and acute cholecystitis
Standard IV contrast-enhanced CT scanning	Assessment of the gallbladder and surrounding anatomical structures in general surgical practice	Specific target when planning elective liver resection and transplantations	A positive predictive value of 60% for left-sided gallbladder using standard CT scan technique	Collective discussion with hepatobiliary radiologists is warranted regarding the application of specific CT scan protocols

Abbreviations: CT: Computed tomography; IV: Intravenous; US: Ultrasound

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ligaments, left lateral section of the liver, and the gallbladder [25]. The location of the third, fourth, and (if the need arises) fifth ports for traction of the gallbladder should be adapted according to the anatomical situation and surgeons' preferences. This point should be regarded as a reminder to the surgeons to inspect the liver and gallbladder after the insertion of the laparoscope through the periumbilical port and early recognize the abnormal position of the gallbladder to allow the standard port placements to be modified [26].

Third, the theoretical reasons for performing alternative gallbladder surgeries should be discussed with the patient comprehensively for informed consent [27]. The options for managing a left-sided gallbladder were not discussed with our patient preoperatively. Interestingly, the incidences of a left-sided gallbladder (not routinely discussed while providing information for informed consent) and major bile duct injury (discussed routinely) are similar. It is approximately 0.3%.

Fourth, comparisons of bile duct injury rates from both reviews on cholecystectomy for a left-sided gallbladder [5,6] with the CholeS Study Group [28] data for conventional cholecystectomy, are concerning. For example, four patients in the cholecystectomy for a left-sided gallbladder cohort had an injury to the bile duct with a rate of 7.3% [6], which is 4.3 times higher than the bile duct injury rate (1.7%) for the most difficult grade 4 and 5 cholecystectomies. Furthermore, it is almost 43 times higher than the bile duct injury rate (0.17%) for grade-3 difficulty-specific cholecystectomies and 29 times higher than the overall bile duct injury rate of 0.25% in the CholeS Study [28]. Such comparisons have methodological drawbacks; nonetheless, they indicate that a left-sided gallbladder and associated variations in biliary ductal anatomy present challenges in intraoperative decision-making and the technical execution of the surgical procedure [29].

Fifth, the atypical position of the gallbladder predetermines the cystic duct's atypical anatomical relationship with the main bile ducts, first- and second-order bile ducts, and the entire hepatic pedicle. Specifically, the left-sided gallbladder, anterior to the hepatic pedicle, changes Calot's triangle planes from horizontal and lateral to vertical and anterior, bringing the gallbladder closer to the extrahepatic biliary tract (Figure 3) [3]. Five topographical patterns of the fusion of the cystic duct with the extrahepatic bile duct, including common hepatic, lobar, and sectional, were described in 41 patients with a left-sided gallbladder [5]. In descending order by incidence, they were on the right side of the common hepatic duct after a U-shaped turn anterior to this duct (65.6%), on the left of the common hepatic duct (9.5%), with the left hepatic duct (9.5%), with the right hepatic duct (7.6%), and with the smaller order bile duct (sectional, most probable) to the right hepatic duct (2.4%). Furthermore, six patients (14.6%) had other minor biliary anomalies, and one had a duplicate common bile duct. The selected magnetic resonance cholangiopancreatography image (Figure 3) strongly suggests the fusion of the cystic duct on the left with the common hepatic duct after its U-shaped turn anterior to this duct. Also, the congenital absence of the right hepatic duct is highly probable. Therefore, dissection of the left-sided gallbladder close to its wall is key to preventing the patient from injuries to the highly probable anomalous extrahepatic bile ducts.

Variations of biliary anatomy at the hepatic hilum are more frequent in patients with left-sided gallbladder, especially in those with abnormal intrahepatic portal vein branching [23]. The understanding of infraportal bile duct anatomy, classified as joining the hepatic duct caudally to the transverse portion of the left portal vein [30], is of paramount importance for safe cholecystectomy planning. A few variations in infraportal courses of segmental and sectional bile ducts were reported. They should be considered before, as it is possible to identify them using contrast-enhanced computed tomography and magnetic resonance-based imaging, and during gallbladder surgery. The examples include infraportal B11 (it is one of the bile ducts of segment 1 which drains Spiegel's lobe) joining the left or common hepatic duct [30], right posterior sectional bile duct joining the right anterior sectional bile duct with an infraportal course [31], right posterior sectional duct joining the common bile duct [32], and infraportal B3 [33]. Encountering another infraportal bile duct of the left hemiliver is always possible, as a true left-sided gallbladder is more associated with the left-sided biliary tract variations. Thus, infraportal variations of biliary anatomy at the hepatic hilum are the second reason a surgeon should initiate the dissection of the left-sided gallbladder as close to its wall as possible to prevent infraportal bile duct injury [24]. It is a prerequisite for safe total cholecystectomy.

Sixth, an intraoperative fluorescent cholangiography method using indocyanine green and a near-infrared light source is a new imaging method in laparoscopic cholecystectomy to improve the visualization of the extrahepatic biliary anatomy (despite a long history of indocyanine green utilization in liver surgery) [34]. However, it should be noted that surgical care providers can use intraoperative imaging methods approved by the individual health-care organization.

Seventh, when in doubt, an anatomic dissection of the proximal portion of the gallbladder and cystic pedicle cannot be performed safely, or the hepatic wall of the gallbladder cannot be safely detached from surrounding tissues [25], a less-than-total gallbladder removal should be performed [3-6,25]. At present, two medical terms are used to name a less-than-total cholecystectomy – a subtotal cholecystectomy [9] and a partial cholecystectomy [35]. The question regarding the probability of symptomatic gallbladder remnant events in the future and the necessity of elective completion cholecystectomy remains open, as this depends on the number of specific factors associated with subtotal cholecystectomy. Examples of these factors include the type of completion of subtotal cholecystectomy (controversial conclusions) [7,35-38], the presence or absence of bile leak after subtotal cholecystectomy [39], and retained gallstones within the gallbladder remnant [40]. According to a systematic review, the overall incidence of retained gallstones, recurrent biliary events, and completion cholecystectomy ranges between 0.8% and 3% [8]. During the follow-up visit, our patient was instructed to contact the consulting surgeons if the symptoms resurfaced.

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Two limitations of this case study should be acknowledged. First, we were unable to obtain systematic data on the occurrence of the left-sided gallbladder in our institution, which is a large hepatobiliary and general emergency surgery center for the region. The absence of figures indicating clinical and histopathological correlations is another limitation of this paper. However, the key messages arising from this paper are fully supported by the clinical information provided and the discussion points.

4. Conclusions

Iatrogenic bile duct injury rates are the highest in the subpopulation of patients with left-sided gallbladder. A left-sided gallbladder, therefore, should be considered a risk factor. Subtotal cholecystectomy, especially in an emergency surgery setting, is an alternative to total cholecystectomy to avoid gallbladder surgery-related risks and prevent a patient from iatrogenic injuries to the bile ducts when a safe cholecystectomy cannot be performed due to a lack of clarity or knowledge on the extrahepatic biliary anatomy, which might deviate from the typical anatomy in patients with left-sided gallbladder. It is essential to discuss with patients about managing the left-sided gallbladder during the consent-taking step.

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Conflict of Interest

The authors declare no competing interests in relation to the publication of this work.

Ethics Approval and Consent to Participate

This case report was not classified as an object for approval by the local ethics, research, or clinical audit committee according to local policies. Informed consent in written form was obtained from the patient before drafting the paper.

Consent for Publication

Informed consent was obtained from the patient.

Availability of Data

Additional data are available from the corresponding author upon reasonable request.

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Appendix

Patient's Perspective

I have had what I believed to be severe trapped wind for at least 12 years. I was in extreme discomfort and pain whenever I overindulged in rich food. I never visited the general practitioner to investigate; I just accepted this as part of life. The usual pain began at midday, and by 6 pm, I was in unbearable agony. I visited the accident and emergency center, and after initial triage and assessment by the surgical team, I was informed that I had an inflamed gallbladder and may need surgery. I was in the surgical assessment unit department for another 4 days receiving antibiotics to manage the inflammation. As the pain was not subsiding and my temperature remained elevated, it was decided that I would need surgery to remove the gallbladder. I was informed of the risks and was told that the surgeon would try for a keyhole surgery but that it could also lead to open surgery. After the surgery, I was informed of the situation and that I needed open surgery because my gallbladder was on the left side of my liver. I was in pain, but it was managed well by the doctors and nurses. I had an episode where my blood pressure dropped, and my heart went tachycardic, which was very scary. It was dealt with promptly and efficiently, and I have since been referred to a cardiologist for further investigation. My recovery has been smooth, with no complications. My life postoperatively is much better, and I have not had the pain I previously experienced. Despite not knowing that I had gallstones, I feel very lucky that it was diagnosed and the surgery was successful. I want to thank the surgeons for looking after me so well and bringing me back to health.

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ORIGINAL ARTICLE

Association between hematological parameters, serum retinol, and glycemic indices in diabetes mellitus: a preliminary case–control study

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ABSTRACT

Background: The global prevalence of type 2 diabetes mellitus (T2DM) is on the rise. Hyperglycemia, free radical damage, and inflammation are commonly implicated as the etiopathological factors of diabetes mellitus. This preliminary study aims to investigate the association of the disease with serum retinol and hematological parameters and compare these parameters with non-diabetic controls.
Methods: The biophysical profiles of 85 subjects with diabetes and the same number of healthy controls were recorded using standard techniques. Biochemical and hematological investigations were carried out. The data are expressed as median with interquartile range (IQR) values. Mann–Whitney *U*-test was conducted to assess the difference between the two groups.
Results: There were a significant increase in median values of glycated hemoglobin (HbA1c), fasting blood glucose (FPG), and white blood cells (WBC) and a significant decrease in median values of monocytes in subjects with T2DM as compared to controls. There was a significant negative correlation between eosinophils and FPG in subjects with T2DM. In healthy controls, there was a significant positive correlation between serum retinol, certain hematological parameters, and HbA1c; and there was a significant negative correlation between WBC and FPG. The T2DM group had a significant negative correlation between eosinophil count and FBG.
Conclusion: Our study shows that serum retinol levels are not reflective of oxidative stress, but a routine WBC and differential count can shed light on the chronic inflammatory status. These results help with the formulation of targeted treatment to delay progression of the disease and prevent its complications.
Relevance for Patients: Vitamin A plays a pivotal role safeguarding the immunity and eye health for diabetic patients, but serum retinol estimation is not reflective of inflammatory or glycemic control status in diabetic patients. They would benefit from a hematocrit test.

1. Introduction

The current global prevalence of type 2 diabetes mellitus (T2DM) is 8.5%, almost double from 4.7% in 1980 [1]. In India, there has been a rapid increase in the prevalence of T2DM, from 26 million (5.5%) in 1990 to 65 million (7.7%) in 2016 [2].

Insulin resistance, impaired insulin secretion, abnormal fat metabolism, and excessive hepatic glucose production are the major contributors of hyperglycemia in subjects with T2DM [3]. Hyperglycemia leads to increased production of superoxide radicals, resulting in increased generation of free radicals and impairment in antioxidant defence mechanisms [4]. Oxidative stress due to hyperglycemia also further worsens insulin resistance [5]. The increase in reactive oxygen species (ROS) plays a significant role in the onset, progression, and pathogenesis of diabetic complications [6]. Further, the interaction between advanced

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glycation end-products (AGE) and their receptors (RAGE) results in the transduction of various signaling pathways, leading to the generation of ROS, pro-inflammatory cytokines, and chemokines that would trigger cellular dysfunction [7].

In the development of diabetic complications, unfavorable hyperglycemia induces biochemical as well as hematological changes. Due to altered biochemical and blood tissue products, their interactions lead to alteration in erythrocyte functional properties, leukocyte indices, and platelet indices in diabetes mellitus [8]. A large cohort study in Israel found that a rise in white blood cell (WBC) count serves as an independent risk factor for T2DM development in normoglycemic subjects not being affected by other risk factors such as obesity, family history, or dyslipidemia [9]. Total count, differential number of WBC, and neutrophil/lymphocyte ratio (NLR) are known markers of inflammation. High NLR is associated with insulin resistance and acts as a prognostic marker in T2DM along with glycated hemoglobin (HbA1c) [10].

Vitamin A, an antioxidant [8], can be obtained as provitamin A carotenoids like β-carotene from edible plants or as retinyl esters from animal sources [11]. The data regarding serum vitamin A levels in T2DM patients are ambiguous. Despite the controversy surrounding the vitamin A status changes in T2DM patients, new evidence lends credibility to decreased vitamin A levels in individuals suffering from T2DM [4,12].

As there is a paucity of reports on the association of serum retinol and hematological parameters with T2DM, the preliminary study was designed to determine the association of the above parameters in subjects with T2DM and healthy controls. The objectives of this study were to estimate the serum retinol, hematological parameters, and indicators of glycemic control in T2DM and healthy controls; compare them between the two groups; and investigate the correlation of fasting blood glucose and HbA1c with serum retinol and hematological parameters in the two groups.

2. Methods

The patients who attended our non-communicable disease (NCD) prevention clinic in AIIMS, Bhubaneswar, India, from August 2018 to August 2019, were recruited to this cross-sectional, observational study after giving consent to participate. Ethics approval was granted to this study (IEC approval number: IEC/AIIMS BBSR/PG Thesis/2018-19/10 dated – 13th July 2018). Convenient sampling was conducted to recruit cases and age-matched healthy controls. The procedures used in this study adhered to the tenets of the Declaration of Helsinki. Study participants were given clear explanations regarding this study in languages they understand, and informed written consent was obtained from each of them. Participants were coded with numbers to ensure anonymity.

The inclusion criteria of cases and controls are as follows:

a. Adults who consented to participate in the study
b. T2DM adults with HbA1c levels ≥6.5% (as cases)
c. Non-diabetic adults with HbA1c <5.7% (as control)
d. Adults aged 18–65 years
e. Treatment-naïve patients

Individuals with the following features were excluded from the study:

a. Pre-diabetic adults with HbA1c between 5.7% and 6.4%
b. Individuals using oral hypoglycemic agents or insulin for the management of diabetes
c. Individuals using vitamin A or multivitamin supplemented with vitamin A for any non-related conditions
d. Subjects addicted to smoking/alcohol/drug abuse
e. Pregnant or lactating women
f. Individuals with acute or chronic liver disease
g. Subjects who had received blood transfusions in the previous 3 months
h. Individuals with symptomatic thyroid dysfunction
i. Patients on lipid-lowering drugs
j. Patients on hormone replacement therapy, including oral contraceptive drugs
k. Patients with acute infections
l. Patients who were too ill to participate or had emergency health conditions
m. Patients on antiepileptic drugs

The demographic data and relevant history were obtained using a questionnaire. The biophysical profiles of subjects, encompassing height, weight, waist circumference (WC), and hip circumference (HC) were recorded using standard techniques. Single-trained personnel recorded weight using the same digital weighing scale with a minimum graduation of 10 grams. The height of each individual (standing straight with arms at the side and knees kept together) was measured using the same stadiometer with a minimum graduation of 1 mm. The WC and HC were measured using a measuring tape; the WC was measured at the narrowest portion of the waist above the umbilicus when the individual was standing upright and the HC was measured at the broadest part of the hips. The body mass index (BMI) and waist–hip ratio (WHR) were calculated.

Fasting venous blood was collected for all biochemical and hematological investigations. HbA1c and fasting plasma glucose (FPG) were estimated using Beckman Coulter AU 5800 fully automated chemistry analyzer coupled with reagent kits from Beckman Coulter (Ireland), after quality checks using QC material from Bio-Rad. Serum retinol was estimated by reverse-phase high-pressure liquid chromatography (HPLC) in HPLC Agilent LC Infinity 1200. Measurements of WBC count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count, red cell distribution width–coefficient of variation (RDW-CV), platelet distribution width (PDW), mean platelet volume (MPV), platelet larger cell ratio (P-LCR), plateletcrit (PCT), neutrophil, lymphocyte, monocyte, and eosinophil parameters of complete hemogram were performed in fully automated analyzer SYSMEX XN 1000 (Sysmex America, Inc.,

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IL, USA). Neutrophil–lymphocyte ratio (NLR) was calculated by dividing the number of neutrophils by lymphocytes.

Considering a 15% difference in serum retinol levels between cases and controls, the sample size calculated was 85 in each arm with an alpha error of 0.05 and 90% power of the study [13].

The data were analyzed using SPSS version 25.0. All data were tested for normality using the Kolmogorov–Smirnov test. Most of the data were not normally distributed, so the data are expressed as median with interquartile range (IQR) values. The difference between the two groups was assessed with Mann–Whitney *U*-test (2-tailed). A *P*-value of less than 0.05 was considered statistically significant.

3. Results

The participants were selected after screening 335 patients. They were selected on the basis of their glycated hemoglobin levels; 85 treatment-naive T2DM and 85 normal healthy individuals were recruited in the study (Figure 1). A total of 165 subjects were excluded due to one or more exclusion criteria. The demographic and general clinical characteristics of T2DM and control are expressed as median with IQR values in Table 1. There was no significant difference in median values of BMI, WC, WHR, systolic and diastolic blood pressure (SBP and DBP), serum retinol, RBC, Hb, HCT, MCV, MCH, MCHC, PLT, RDW-CV, PDW, MPV, P-LCR, PCT, neutrophils, lymphocytes, eosinophils, and NLR between T2DM and control subjects. However, there were a significant increase in median values of HbA1c, FPG, and WBC and a significant decrease in median values of monocytes in subjects with T2DM as compared to controls (Table 1).

In T2DM patients, the eosinophil count was significantly negatively correlated with FPG (Table 2). In controls (non-diabetic subjects), there was a significant positive correlation between serum retinol, RBC, Hb, HCT, MCV, MCH, MCHC, and HbA1c (Table 2). A significant negative correlation between RDW-CV and HbA1c was also observed in controls (Table 2). Further, in subjects with non-diabetes, WBC count was negatively correlated with FPG (Table 2). The T2DM group had a significant negative correlation between eosinophil count and FBG. In both groups, there was a significant positive correlation between FPG and HbA1c (not shown in the Table 2).

Table 1. Demographic, anthropometry, biochemical and hematological parameters in diabetic and non-diabetic subjects

Parameters (unit)	Type 2 diabetic subjects	Non-diabetic subjects	*P*
Age (years)	43 (37, 59)	43 (36, 59)	0.959
T2DM duration (years)	3 (2, 5)	-	-
BMI (kg/m²)	25.4 (22.8, 27.9)	25.3 (22.2, 26.7)	0.285
Waist circumference (cm)			
Male (N=48,49)	90.5 (87.0, 95.0)	86.0 (83.0, 94.0)	0.088
Female (N=37,36)	82.0 (78.0, 88.5)	83.0 (73.0, 92.0)	0.808
Waist-hip ratio			
Male	0.96 (0.92, 0.99)	0.94 (0.91, 0.98)	0.358
Female	0.89 (0.85, 0.90)	0.90 (0.83, 0.93)	0.526
Systolic BP (mm/Hg)	120 (114, 130)	120 (110, 130)	0.111
Diastolic BP (mm/Hg)	80 (70, 83)	80 (70, 81)	0.252
HbA1c (%)	8.1 (7.15,9.65)	5.1 (4.8, 5.4)	<0.001
FPG (mg/dL)	165.0 (138.5, 221.0)	96.0 (90.0, 101.5)	<0.001
Serum retinol (µg/dL)	31.4 (27.8, 41.5)	32.53 (26.0, 40.5)	0.863
WBC (10^3/µL)	8.3 (7.0, 9.7)	7.4 (6.8, 8.7)	0.014
RBC (10^6/µL)			
Male	5.3 (4.8, 5.6)	5.2 (4.8, 5.5)	0.613
Female	4.7 (4.4, 5.1)	4.5 (4.2, 4.8)	0.066
Hb (g/dL)			
Male	14.1 (12.7, 15.1)	13.5 (13.1, 15.1)	0.920
Female	12.4 (10.9, 13.2)	11.9 (10.9, 12.7)	0.357
HCT (%)			
Male	44.8 (41.0, 47.3)	44.2 (41.6, 46.8)	0.843
Female	40.3 (36.1, 41.8)	38.7 (35.8, 41.7)	0.508
MCV (fL)	85.1 (80, 89.4)	87.2 (81.5, 90.2)	0.315
MCH (pg)	27.0 (24.4, 28.4)	27.2 (25.1, 28.5)	0.448
MCHC (g/dL)	31.1 (30.3, 32)	31.1 (30.2, 32.0)	0.745
Platelet count (10≥/µL)	277.0 (229.5, 326.5)	280.0 (235.0, 327.5)	0.959
RDW-CV (%)	14.0 (13.2, 15.1)	14.3 (13.5, 15.1)	0.277
PDW (fL)	13.35 (11.3, 16.5)	13.85 (10.93, 17.45)	0.735
MPV (fL)	11.0 (9.8, 12.3)	11.25 (10.15, 12.50)	0.267
P-LCR (%)	33.9 (23.8, 43.1)	35.4 (25.4, 45.2)	0.256
PCT (%)	0.31 (0.25, 0.37)	0.32 (0.27, 0.36)	0.548
Neutrophils (%)	62.6 (56.5, 69.5)	62.5 (56.8, 66.4)	0.252
Lymphocytes (%)	28.9 (23.0, 36.1)	29.9 (26.6, 35.2)	0.255
Monocytes (%)	2.2 (1.3, 3.0)	2.6 (1.6, 3.5)	0.034
Eosinophils (%)	3.5 (1.6, 6.3)	2.9 (2.1, 5.2)	0.888
Basophils (%)	0.3 (0.2, 0.5)	0.4 (0.3, 0.5)	0.079
NLR ratio	2.2 (1.6, 2.9)	2.1 (1.6, 2.5)	0.193

Data are given as median and IQR (Interquartile range).
Abbreviations: BMI: Body mass index; FPG: Fasting blood glucose; Hb: Hemoglobin; HbA1c: Glycated hemoglobin; HCT: Hematocrit; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MPV: Mean platelet volume; NLR, Neutrophil/lymphocyte ratio; PCT: Plateletcrit; PDW: Platelet distribution width; P-LCR: Platelet larger cell ratio; RBC: Red blood cell; RDW-CV: Red cell distribution width–coefficient of variation; T2DM: Type 2 diabetes mellitus; WBC: White blood cell

4. Discussion

In this case–control study, we found no statistically significant difference in serum retinol levels between T2DM and non-diabetic individuals, and the serum retinol levels of both groups remained within normal limits. This finding was consistent with other studies where normal serum retinol level was observed in T2DM patients [13-15]. Despite the report of lower serum retinol levels in diabetic individuals than in healthy controls [16], the liver retinol levels were found to be higher in diabetic animal model, suggesting that the liver inhibits retinol mobilization in people with diabetes. There is no difference in serum retinol levels between subjects with T2DM and non-diabetes, probably because serum retinol levels are typically maintained within a narrow range in individuals with adequate liver vitamin A stores [17]. In this study, however, there was a significant positive correlation between serum retinol level and HbA1c in healthy controls,

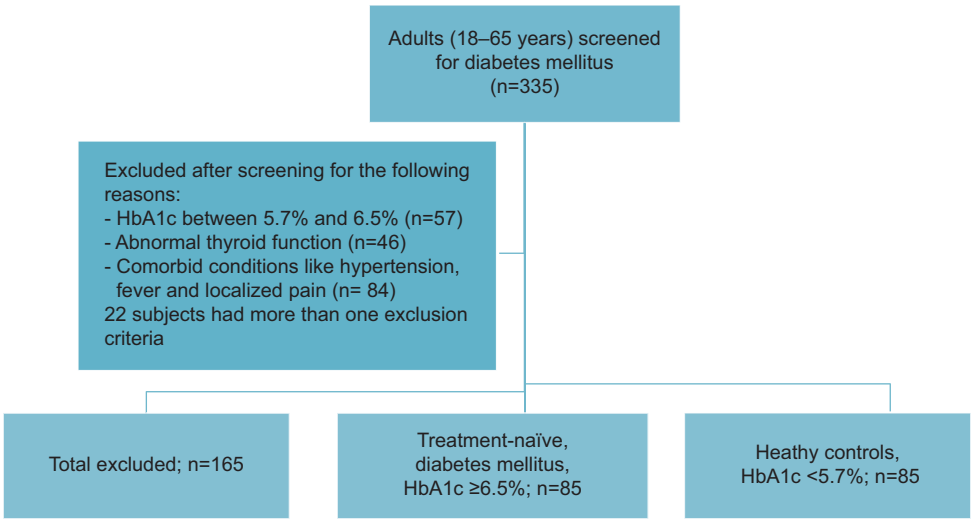

Figure 1. CONSORT flow diagram for patient enrolment.

Table 2. Correlation of glycemic indices with retinol and hematological parameters in diabetic and non-diabetic subjects

Parameters	Type 2 diabetic subjects (cases)				Non-diabetic subjects (controls)			
	HbA1C		FPG		HbA1C		FPG	
	R[a]	*P*[b]	*R*[a]	*P*[b]	*R*[a]	*P*[b]	*R*[a]	*P*[b]
Serum retinol	0.078	0.479	0.123	0.260	0.234	0.031	0.164	0.134
WBC	0.034	0.759	0.056	0.613	0.05	0.651	−0.3	0.005
RBC	0.038	0.731	−0.004	0.971	0.064	0.563	−0.002	0.989
Hb	−0.035	0.750	−0.06	0.588	0.251	0.021	−0.01	0.926
HCT	−0.008	0.939	−0.096	0.384	0.222	0.041	−0.056	0.610
MCV	−0.026	0.812	0.013	0.906	0.227	0.037	0.025	0.821
MCH	−0.009	0.937	0.042	0.700	0.256	0.018	0.101	0.356
MCHC	−0.073	0.504	0.007	0.952	0.256	0.018	0.173	0.113
Platelet count	−0.034	0.758	0.106	0.334	−0.095	0.387	0.016	0.884
RDW-CV	−0.087	0.427	−0.111	0.311	−0.342	0.001	−0.048	0.666
PDW	0.205	0.089	0.008	0.946	−0.105	0.395	−0.02	0.871
MPV	0.138	0.257	−0.021	0.867	−0.103	0.404	−0.09	0.464
P-LCR	0.145	0.231	0.002	0.989	−0.107	0.385	−0.088	0.475
PCT	−0.011	0.931	0.082	0.502	−0.233	0.056	−0.059	0.634
Neutrophils	0.004	0.968	0.135	0.218	0.017	0.876	−0.063	0.568
Lymphocytes	−0.08	0.464	−0.186	0.088	0.044	0.692	0.137	0.212
Monocytes	0.038	0.728	−0.018	0.871	0.106	0.333	0.032	0.770
Eosinophils	−0.164	0.135	−0.262	0.015	−0.028	0.801	−0.118	0.281
Basophils	0.145	0.186	0.033	0.766	0.102	0.353	−0.057	0.603
NLR ratio	0.059	0.589	0.17	0.119	−0.016	0.884	−0.108	0.326

[a]Spearman's correlation. [b]$P<0.05$.
Abbreviations: FPG: Fasting blood glucose; Hb: Hemoglobin; HbA1c: Glycated hemoglobin; HCT: Hematocrit; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MPV: Mean platelet volume; NLR: Neutrophil/lymphocyte ratio; PCT: Plateletcrit; PDW: Platelet distribution width; P-LCR: Platelet larger cell ratio; RBC: Red blood cell; RDW-CV: Red cell distribution width–coefficient of variation; WBC: White blood cell

possibly due to the mobilization of liver retinol stores to meet the demands of increased oxidative stress as a result of increased blood glucose levels [18]. The retinol-binding protein-4 (RBP4) is an inflammatory adipokine that is associated with insulin resistance and implicated in the pathogenesis of diabetes [19]. In this preliminary study, despite a decrease in retinol levels in the diabetic patients and a positive correlation with inflammation as seen in the NLR of the same group, our results were not statistically significant. This is in agreement with other studies showing that free RBP4 plays a role in the pathogenesis of atherosclerosis and diabetes mellitus [20]. Hence, either the low serum retinol levels or an increased synthesis of RBP4 from adipocytes needs to be investigated for the purpose of guiding treatment planning in future.

In the present study, there was a significant difference in WBC between subjects with T2DM and controls. Significant negative correlation between WBC and FPG was only observed in non-diabetic subjects. The increased WBC count is caused by chronic inflammation in T2DM resulting from insulin resistance and glucotoxicity [21,22]. Several other studies have also shown that WBC count might be associated with T2DM and its complications [23-27]. The chemical substances produced in leukocytes affect various tissues, such as vascular endothelial cells and pancreatic β cells, suppressing insulin secretion and its action and accelerating progression of T2DM [28]. It was also observed that there is an increase in baseline white cell counts in individuals who developed diabetes compared to those who had not developed diabetes at follow-up [29]. An increased circulating WBC count independently associated with worsening glucose metabolism even when the WBC level was within the normal range has been reported in a large Chinese population consisting of middle-aged and elderly subjects [30]. It was also reported that total leukocyte count is significantly increased in diabetic patients [30,31].

This study also showed that the difference in monocyte count between T2DM subjects and non-diabetic controls was significant. Decreased monocyte count may be attributed to monocyte adhesion to the endothelium, a critical factor in initiating early atherosclerotic lesions. The cause for increased monocyte

adhesion to the endothelium in diabetes may be secondary to advanced protein glycosylation of the endothelium [32]. There was no difference in the NLR between the two groups in this study, probably because of the convenient sampling used and that it was a hospital-based, instead of a community-based, study.

Among the non-diabetic, healthy controls in this study, there was a significant positive correlation between serum retinol, RBC, Hb, HCT, MCV, MCH, and MCHC with HbA1c, which was not seen in the T2DM cases. Such findings were congruent with a report by Bhutto *et al.* with a focus on T2DM [33]. A significant negative correlation between RDW-CV and HbA1c in controls indicates the role of these parameters in modulating cardiovascular risk [33,34].

5. Conclusion

Our study showed that serum retinol levels do not reflect oxidative stress, but a routine WBC and differential count can illuminate the chronic inflammatory status. These results may assist with the formulation of targeted treatment for delaying disease progression and preventing complications. A holistic approach to improving patient's well-being encompasses raising awareness for lifestyle changes, consuming healthy antioxidant-enriched diet and doing exercises (yoga, aerobics, meditation, *etc.*). Future studies should focus on investigating the efficacy of monitoring chronic inflammation in the normoglycemic population by means of hematological parameter measurements in disease prevention.

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None.

Conflict of interest

None of the authors have any competing interests to report in this study. This was a non-funded study.

Ethics approval and consent to participate

Ethics approval was granted to this study (IEC approval number: IEC/AIIMS BBSR/PG Thesis/2018-19/10 dated – 13th July 2018). Written consent was obtained from the study participants before their participation.

Consent for publication

Written consent was obtained from study participants for using their data without disclosing their identity.

Availability of data

Data are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Steroid-responsive intractable pruritus in drug-induced liver injury: a case series

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ABSTRACT

Background: Drug-induced liver injury (DILI) is commonly caused by modern medications, complementary and alternative medicines (CAMs), and other toxins. DILI is an umbrella term encompassing herb-induced liver injury (HILI) caused by herbs and CAMs, in addition to other medications. Apart from the cessation of the culprit drug and the supportive management, there are no definite treatment options for DILI. Although being used in DILI, steroids are not the standard medications for DILI, except that they are indicated for a few specific conditions.
Materials and Methods: We report five cases of DILI with pruritus who responded well to steroids used as rescue therapy. DILI in these five cases was caused by CAMs (1), anabolic steroids (2), dapsone (1), and antifungal drug itraconazole (1). All patients presented with jaundice and pruritus, and their conditions did not improve following the discontinuation of offending agents and the implementation of supportive care. We used the Roussel UCLAF Causality Assessment Method 2016 for causality assessment. R-value was used to describe the pattern of liver injury. All patients underwent comprehensive work-up including liver biopsy as part of the procedure to rule out other potential etiologies. Steroids were used as a last resort, and both clinical and biochemical measurements were conducted.
Results: The mean age of patients was 28.8 years, and the majority of them were males (80%). The median duration from symptom onset to presentation at our hospital was 4 weeks. The mean durations for pruritus improvement and complete biochemical improvement after steroid treatment were 3.2 weeks and 11.2 weeks, respectively. Extended follow-up was done for a mean period of 29.6 weeks from symptom presentation, and none of the patients had recurrence of liver injury after discontinuation of steroids.
Conclusions: Ssteroids can be used to treat as rescue therapy for severe DILI with intractable pruritus in patients with worsening liver function.
Relevance for Patients: DILI in selected cases can be therapeutically managed using steroids, which, however, should not be indicated as a first-line treatment.

1. Introduction

Drug-induced liver injury (DILI) is commonly caused by modern medications, complementary and alternative medicines (CAMs), and other toxins. DILI is an umbrella term encompassing herb-induced liver injury (HILI) resulted from herbs, CAMs, and other medications. DILI is divided into intrinsic (dose-dependent) and idiosyncratic (dose-independent) injuries [1]. Liver injury may be characterized as cholestatic, hepatocellular, or mixed injury, according to the results of liver function tests (LFT) and the calculation of the R-value. DILI may resemble liver diseases, particularly auto-immune hepatitis (AIH),

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making the diagnostic procedure challenging and necessitating differential diagnosis to rule out other liver diseases. In India, the combination of anti-tuberculosis (TB) drugs (46.4%), CAMs (13.9%), anti-epileptic drugs (8.1%), non-anti-TB antimicrobials (6.5%), anti-metabolites (3.8%), anti-retroviral drugs (3.5%), non-steroidal anti-inflammatory drugs (2.6%), hormones (2.5%), and statins (1.4%) represents the most common cause of DILI [2]. Management of DILI includes discontinuation of the culprit drug and administration of supportive care. However, in many patients, long after discontinuation of the culprit drug and implementation of supportive care, the injury fails to improve and progresses instead. Unfortunately, definitive management for such patients has not been developed. The role of steroids in the management of patients with DILI, except for those with immune checkpoint inhibitors [3] and drug-induced auto-immune hepatitis [4], remains doubtful. In other forms of DILI, the therapeutic effect of steroids has not been proven, especially when the injury is accompanied by pruritus. In the present study, we assessed the role of corticosteroids in five patients with DILI induced by different medications who had intractable pruritus and did not respond to conventional management.

2. Materials and Methods

Five patients were recruited in the Department of Gastroenterology, Banaras Hindu University, Varanasi, Uttar Pradesh, India, from January 2022 to December 2022. Patients were diagnosed with DILI secondary to CAMs (1), anabolic steroids (2), dapsone (1), and itraconazole (1). All these patients failed not respond adequately to the discontinuation of the offending agents and the supportive care and their condition even exacerbated. This case series depicts the etiology, clinical profile, management, and outcomes of patients with DILI and HILI. The R-value was calculated to define the patterns of liver injury. R-value was calculated by dividing alanine aminotransferase (ALT) by alkaline phosphatase (ALP), using multiples of the upper limit of normal (ULN) for both. R-value of >5 defines hepatocellular; <2, cholestatic; and between 2 and 5, a mixed pattern of liver injury. Patients were thoroughly evaluated to identify the alternative causes of liver injury, such as hepatotropic viruses, autoimmune liver diseases, Wilson's disease, and biliary obstruction by imaging. A liver biopsy was performed in all cases for histopathological examinations. We used the updated (2016) version of the Roussel UCLAF Causality Assessment Method (RUCAM) for causality assessment [5] (Table 1). For cases of non-response or worsening of liver injury and pruritus despite discontinuation of the offending agents, both corticosteroids and supportive care were administered to the patients. Prednisolone was started either at a dose of 40 mg/day (cases 1, 2a, and 3) or 1 mg/kg/day (cases 2b and 4) depending on the choice of treating hepatologist. Patients were followed to observe the outcomes in terms of improvement in pruritus, normalization of liver enzymes, intolerance or adverse effects of corticosteroids, and recurrence of liver injury. Informed consent was obtained from all patients or their nearest kin. This work is reported as per the CARE guidelines.

3. Results and Case Descriptions

3.1. Results

The mean age of the enrolled patients was 28.8 years, and the majority were males (80%). The median duration from the onset of symptoms to the presentation at our hospital was approximately 4 weeks. Case 1 had polycystic ovarian disease for which she took CAMs, and Case 3 took dapsone for leprosy. Cases 2a, 2b, and 4 had no underlying comorbidities. RUCAM scores were 5 for Case 1, 7 for case 2b, and 6 for each of the other three patients. R-values were 4.0, 3.6, 7.0, 2.4, and 2.5 for Cases 1, 2a, 2b, 3, and 4, respectively. Although these patients had severe DILI, none had acute liver failure. Mean durations for pruritus improvement and complete biochemical improvement after steroid treatment were 3.2 weeks and 11.2 weeks, respectively. All patients had good tolerance with corticosteroids without presenting any conspicuous side effects. Extended follow-up was done for a mean duration of 29.6 weeks from the presentation, and none of the patients had recurrence of liver injury after discontinuation of steroids (Table 2). Figure 1 depicts values of bilirubin, ALP, and ALT at different time points in all cases.

3.2. Case descriptions

3.2.1. Case 1 - CAM-induced liver injury with intractable pruritus in the background of doubtful choledocholithiasis

A 26-year-old female patient complained of abdominal pain, jaundice, and itching all over the body for 6 months before seeking medical consultation in our hospital. Her symptoms worsened at night, severely diminishing quality of sleep and life. On general examination, she had excoriating maculopapular skin lesions all over the body, with a few lesions showing oozing of blood (Figure 2A). Two weeks before presentation to our hospital, she underwent endoscopic retrograde cholangiopancreatography (ERCP) for biliary stone extraction and biliary stenting at another hospital. Her symptoms worsened, and she was admitted to our hospital and thoroughly investigated (Table 3). The biliary system was not dilated on imaging post-ERCP. Because there was no definitive diagnosis, a liver biopsy was performed. The results unveiled portal tract neutrophilic and eosinophilic infiltrates with hepatocellular and canalicular cholestasis with cholestatic rosettes predominantly in zone 3, suggesting mixed hepatocellular and cholestatic pathology (Figure 3, Case 1); DILI was considered a probable diagnosis. On re-inquiring, she admitted to having consumed CAMs for polycystic ovarian disease, which she started a few weeks before the onset of jaundice and stopped 15 days before presentation at our hospital. Her RUCAM score was five points, which suggests a possible DILI/HILI. The patient was given ursodeoxycholic acid (UDCA) 10 mg/kg/day. For treating pruritus, she received topical emollients, anti-histaminic, cholestyramine, and naltrexone; however, these medications were not therapeutically effective as her symptoms persisted during her hospital stay. She was started on prednisolone 40 mg/day which was slowly tapered later as her pruritus, jaundice, and skin lesions improved drastically at 3 weeks of follow-up (Figure 2B). Over

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Table 1. Detailed causality assessment in all patients using updated RUCAM# 2016 [5]

Updated RUCAM parameters	Case 1	Case 2a	Case 2b*	Case 3	Case 4
Time to onset from the beginning of the drug/herb consumption	+2	+2	+2	+2	+2
Course of ALP/ALT* after cessation of the drug/herb (percentage difference between ALP/ALT* peak and normal)	+1	+1	+2	+1	+1
Risk factors	0	0	0	0	0
Concomitant use of drugs/herbs	0	0	0	0	0
Search for alternative cause	+1	+1	+1	+1	+1
Previous hepatotoxicity of the drug/herb	+1	+2	+2	+2	+2
Response to unintentional re-exposure	0	0	0	0	0
Total score	5	6	7	6	6

#RUCAM score and causality grading: ≤0, excluded; 1–2, unlikely; 3–5, possible; 6–8, probable; ≥9, highly probable.
*Hepatocellular pattern of liver injury

Table 2. Duration of important events in all patients

Events	Case 1	Case 2a	Case 2b	Case 3	Case 4
Time to onset from the beginning of the drug/herb consumption (weeks)	3	10	8	6	Few weeks
Duration of from symptoms onset to presentation at our hospital (weeks)	24	4	3	6	3
Clinical (pruritus) improvement (weeks)	3	3	4	2	4
Biochemical improvement (normalization of LFT) (weeks)	11	11	14	8	12
Total follow-up duration without recurrence (data collected through phone call) (weeks)	36	32	24	28	28

Abbreviation: LFT: Liver function test.

the next 11 weeks, prednisolone was tapered and stopped with normalization of LFT parameters and resolution of pruritus. The patient was further followed for approximately 36 weeks, during which she did not manifest any symptoms and her liver enzyme levels were normal.

3.2.2. Case 2 - Anabolic steroid-induced liver injury

(A) Patient 2a

A 26-year-old male patient, who is a bodybuilder, presented with worsening jaundice, severe itching, malaise, and abdominal discomfort for 1 month. He had no comorbidities. His itching was more pronounced at night, disrupting his sleep. He had been taking stanozolol 50 mg intramuscularly on alternate days for 3 months to improve his physique. He discontinued the drug after the onset of symptoms. On physical examination, he had a body mass index of 27.6 kg/m^2, icterus, and hepatomegaly of 4 cm below the right costal margin. At admission, the patient's total bilirubin (TB) was 31.6 mg/dL. Other biochemical and serological parameters are illustrated in Table 3. Result from magnetic resonance cholangiography was normal. Despite the positive Kayser-Fleischer (KF) ring, his 24-h urinary copper and serum ceruloplasmin levels were normal. His RUCAM score was seven points. Examination of percutaneous liver biopsy showed that his liver had preserved architecture with portal tracts showing mild mixed inflammation, characterized by lymphomononuclear cells with a fair number of neutrophils and a mild ductular reaction. Hepatocytes showed intracellular and canalicular cholestasis predominantly in zone 3. Canalicular bile plugs, cholestatic rosettes, and prominent zone 3 perivenulitis were also noted. These findings were suggestive of mixed hepatocellular-cholestatic pathology compatible with DILI (Figure 3, Case 2a). The patient received UDCA, silymarin, anti-histaminic, and cholestyramine for several days, but his pruritus worsened and bilirubin rose to 42.2 mg/dL. The international normalized ratio (INR) increased to 1.6 from a baseline of 1. The patient was started on oral prednisolone 40 mg/day and naltrexone for severe pruritus. Over 3 weeks of follow-up, his pruritus and jaundice improved. The results of LFT are as follows: TB – 10.6 mg/dL, direct bilirubin (DB) – 6.8 mL/dL, aspartate aminotransferase (AST) – 45 IU/L, ALT – 68 IU/L, ALP – 90 IU/L, gamma-glutamyl transpeptidase (GGTP) – 40 IU/L, and INR – 1.1. The steroid was slowly tapered and discontinued over the next 8 weeks of follow-up when his liver function parameters became normal. He was followed for the next 21 weeks after stopping steroid therapy and was doing well.

(B) Patient 2b

A 24-year-old male, a gym enthusiast without any comorbidities approached us with worsening jaundice and severe itching, which had persisted for 20 days. He had been taking creatine and some steroid tablets for performance enhancement for 2 months and stopped after the onset of symptoms. The patient could not provide the exact details of the pills he was taking. TB and DB were 22.3 and 16.8 mg/dL, respectively, during presentation at our hospital. Other biochemical and serological parameters are summarized in Table 3. His RUCAM score was 7 points, suggesting a probable DILI. His R-value was seven, suggesting a hepatocellular pattern of liver injury. Histopathologically, his liver demonstrated a normal architecture, accompanied by few enlarged hepatocytes with mild intrahepatic and canalicular cholestasis, and lobular lymphocytic infiltrates with few eosinophils. Mild interface hepatitis was seen. Eosinophilic cholangitis with moderate chronic inflammatory cell infiltrate of the portal tract was also noted. Overall, these features were suggestive of cholestatic

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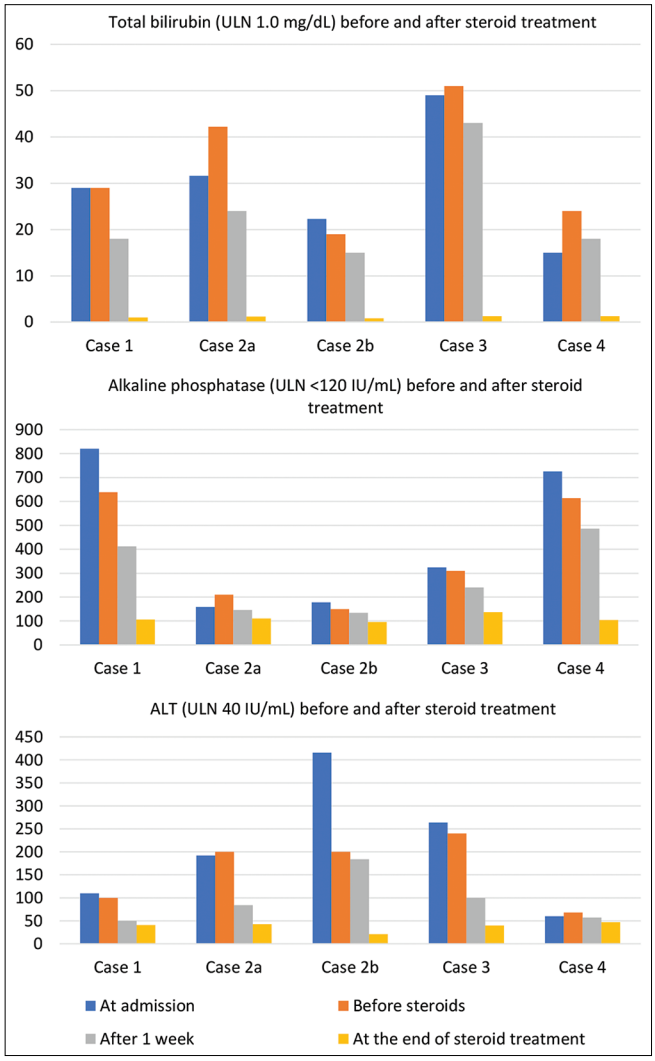

Figure 1. Total bilirubin, alkaline phosphatase, and alanine aminotransferase levels at different time points in all five patients.

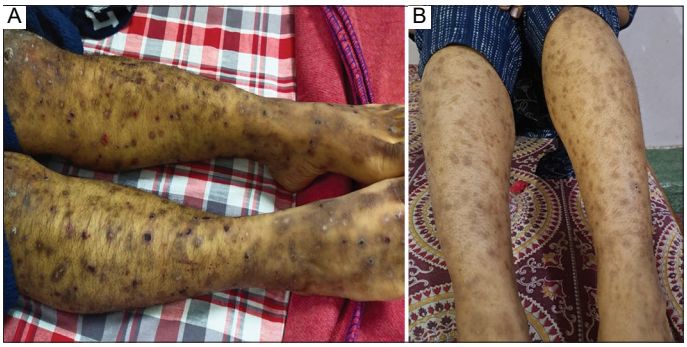

Figure 2. Skin lesions in Case 1 before (A) and after steroid therapy (B).

hepatitis with mild portal fibrosis and eosinophilic infiltrates with the possibility of DILI (Figure 3, Case 2b). He was given UDCA and anti-histaminic. However, since these medications were not effective, oral prednisolone (1 mg/kg/day) was administered. Over the next 4 weeks, his pruritus improved, and TB decreased to 7.3 mg/dL (direct – 5.8 mL/dL), ALT – 88 IU/L, AST – 65 IU/L, ALP – 104 IU/L, and GGTP – 92 IU/L. Prednisolone was tapered over the next 10 weeks and stopped after complete normalization of liver function. He was further followed for 10 weeks after steroid discontinuation, during which recurrence did not occur.

3.2.3. Case 3 - Dapsone-induced liver injury

A 52-year-old male patient presented with worsening jaundice and pruritus for the 1.5 months before seeking medical consultation at our hospital. He had been taking dapsone 100 mg daily as anti-leprosy treatment in the past 3 months. He was not taking any other medications, was a non-addict, and had no comorbidities, except leprosy. On physical examination, madarosis, contracture of upper limb fingers, and large hypopigmented hypoesthetic patches at the trunk and back were present. Features of hypersensitivity were absent. He had deep icterus but there were no clinical signs of liver failure. TB and DB were 49 and 40 mg/dL, respectively. KF rings were present in both eyes, and 24-h urinary copper was slightly elevated (Table 3). Serum ceruloplasmin was normal. His RUCAM score was 6 points, and his R-value was 2.4, suggesting a mixed pattern of DILI. Based on these results, dapsone was discontinued, and a liver biopsy was performed, demonstrating prominent acinar disarray, mild-to-moderate inflammatory infiltrates in the portal tract, and ductular reactions with focal neutrophilic cholangitis. Giant hepatocytes, zone 3 canalicular and intrahepatic cholestasis, and prominent zone 3 perivenulitis were also noted. Copper staining was negative. The overall picture suggested mixed hepatitis and cholestatic pattern, which was possibly drug-induced (Figure 3, Case 3). Emollients, anti-histaminic, and UDCA were given but liver functions continued to worsen, and oral prednisolone (40 mg/day) was started as rescue therapy. At 2 weeks of follow-up, his TB was 8.3 mg/dL (direct – 6.6 mL/dL). Other liver function parameters were ALT – 65 IU/L, AST – 98 IU/L, ALP – 154 IU/L, and GGTP – 171 IU/L. The patient's liver function parameters became normal after 8 weeks of treatment. Afterward, the patient was referred for further management of leprosy with special advice to avoid dapsone. Up to 18 weeks after steroid discontinuation, the patient did not report any signs of recurrence.

3.2.4. Case 4 - Antifungal-induced liver injury

Case 4 is a 16-year-old adolescent who had been taking itraconazole for Tinea corporis infection prescribed by a local physician, which he inadvertently continued for a prolonged period (several weeks). Following this, he developed jaundice, pruritus, and night blindness over approximately 3 weeks. Symptoms were worse at night, markedly hampering his quality of life. For these symptoms, he took some CAMs for the past 10 days, which were not clinically beneficial. Physical findings included exfoliated skin with intense scratch marks all over the body, deep icterus, Bitot's spots, and ecchymotic patches. TB and DB were 15 and 10 mg/dL, respectively, at presentation (Table 3). Although KF ring was bilaterally positive, 24-h urinary copper and serum ceruloplasmin were normal. His RUCAM score was 6 points, and his R-value was 2.4, indicating a mixed pattern of DILI. He had severe cholestasis

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Table 3. Summary of laboratory parameters of all patients during presentation at our hospital

Parameters	Case 1	Case 2a	Case 2b	Case 3	Case 4
Implicated drug	CAM	Anabolic steroid	Anabolic steroid	Dapsone/Rifampicin	Itraconazole
Age (years)	26	26	24	52	16
Gender	Female	Male	Male	Male	Male
BMI (18.5–23.5 kg/m^2)	24.5	27.6	25	20	16
Hemoglobin (12.5–15.5 g/dL)	12.7	15	14.6	13.9	11
TLC (4500–10,000/mm^3)	11000	8100	7200	6100	4500
Platelet count (1.5–4.5 L/mm^3)	2.9 L	3.5 L	2.8 L	2.6 L	1.8 L
ALT/AST/ALP (<40/<40/<120 IU/L)	110/96/820	192/107/159	416/187/178	264/272/324	60/112/725
GGT (<40 IU/L)	280	92	259	168	320
TB/DB (0.3–1.0/0–0.3 mg/dL)	29/21	31.6/22.3	22.3/16.8	49/40	15/10
Protein/albumin (6.0–8.3/3.5–5.0 g/dL)	6.9/3.9	6.5/4.3	7.1/4.6	6.5/4.1	6.0/4
PT/INR (<1.5)	16/1.2	14/1.0	14.3/1.1	13.9/0.9	74/7.7
Creatinine (0.2–1.0 mg/dL)	0.8	1.0	0.9	0.7	0.5
HBsAg/Anti-HCV	NR/NR	NR/NR	NR/NR	NR/NR	NR/NR
IgM Anti-HAV/HEV/HBc	NR/NR/NR	NR/NR/NR	NR/NR/NR	NR/NR/NR	NR/NR/NR
Autoimmune profile (ANA, ASMA, anti-LKM 1, anti-SLA, AMA-M2)	Negative	Negative	Negative	Negative	Negative
Total IgG (1200 –1600 mg/dL)	1180	1400	1250	1360	1150
Ceruloplasmin (20–60 mg/dL)	29	30	21	28	23
24-h urinary copper (<60 µg/day)	45	38	25	60	55
KF ring	Negative	Positive	Negative	Positive	Positive
R-value	4.0	3.6	7.0	2.4	2.5

Abbreviations: BMI: Body mass index; HCV: Hepatitis C virus; HAV: Hepatitis A virus; HEV: Hepatitis E virus; NR: Non-reactive; SLA: Soluble liver antigen; ANA: Anti-nuclear antibody; AMA: Anti-mitochondrial antibody; LKM: Liver kidney microsome; ASMA: Anti-smooth muscle antibody; IgG: Immunoglobulin G; KF: Kayser-Fleischer

as indicated by severe pruritus and fat-soluble vitamin deficiencies (Vitamin A and Vitamin K). As the patient had severe coagulopathy at presentation (prothrombin time – 74; and INR – 7.7), a trans-jugular liver biopsy was performed. Histopathological examination unveiled marked acinar disarray with areas of lobular inflammatory cell infiltrates and zone 3 cholestasis. Portal tracts showed mild lymphomononuclear inflammation with few admixed neutrophils and eosinophils. Hepatocytes showed focal ballooning degeneration, and canalicular cholestasis with cholestatic rosettes in zone 3. These findings suggested mixed hepatitis and cholestatic pathology compatible with DILI (Figure 3, Case 4). He was given UDCA, an anti-histaminic, and fat-soluble vitamin supplements. Despite all these measures, his pruritus and liver biochemical parameters did not improved and instead became worse. Hence, oral prednisolone (1 mg/kg/day) was prescribed. At 4 weeks of steroids administration, his pruritus and jaundice improved, and the stigmata of fat-soluble vitamin deficiencies disappeared. LFT results of this patient are as follows: TB – 2.3 mg/dL (direct – 1.5 mL/dL), ALT – 70 IU/L, AST – 54 IU/L, ALP – 150 IU/L, GGTP – 86 IU/L, and INR 1.2. Prednisolone was slowly tapered over 8 weeks, and his clinical and liver parameters became normalized. He was followed for the next 16 weeks after discontinuation of steroid therapy, during which recurrence of symptoms did not occur.

4. Discussion

Drugs, herbs, toxins, and CAMs are common causes of liver injury and are commonly seen in hepatology practice. In India, where medications can easily be obtained over the counter, it is not surprising to encounter cases afflicted with classical forms of DILIs, such as the case presenting itraconazole-induced hepatotoxicity described in this paper. Pruritus is a common symptom of cholestatic liver injury resulting from medications, and its treatment is often complex and difficult to achieve curative effect. Several agents are available for treating pruritus in different patterns of liver injury; however, the efficacy of these agents varies without any firm recommendations.

CAMs account for 14% of total DILI cases in Indian Network for DILI (INDILI) [2]. These drugs usually contain unknown constituents—possibly heavy metals—making it difficult to identify the culprit agent [6]. One Indian study reported that 6.5% of liver disease patients who presented to the outpatient and emergency departments had ayurvedic and herbal medicine-related severe DILI and one-third of these patients ingested them for gastrointestinal symptoms [7]. According to the U.S. Drug-induced Liver Injury Network (DILIN), dietary supplements were the causative agents in 16% of cases [8]. Multiple studies confirmed that anabolic steroid use can cause hepatotoxicity, such as cholestasis, steatohepatitis, peliosis hepatis, and hepatic tumors [9]. Dapsone is known to cause drug hypersensitivity syndromes (DHS), such as drug reaction with eosinophilia and systemic symptoms (DRESS) and DILI. DILI in these cases may be hepatocellular, cholestatic, or mixed, and is usually associated with hypersensitivity. A mixed pattern is the most common type of hepatotoxicity resulting from dapsone [10]. DILI induced by antifungal drugs such as azoles and echinocandin is one of the

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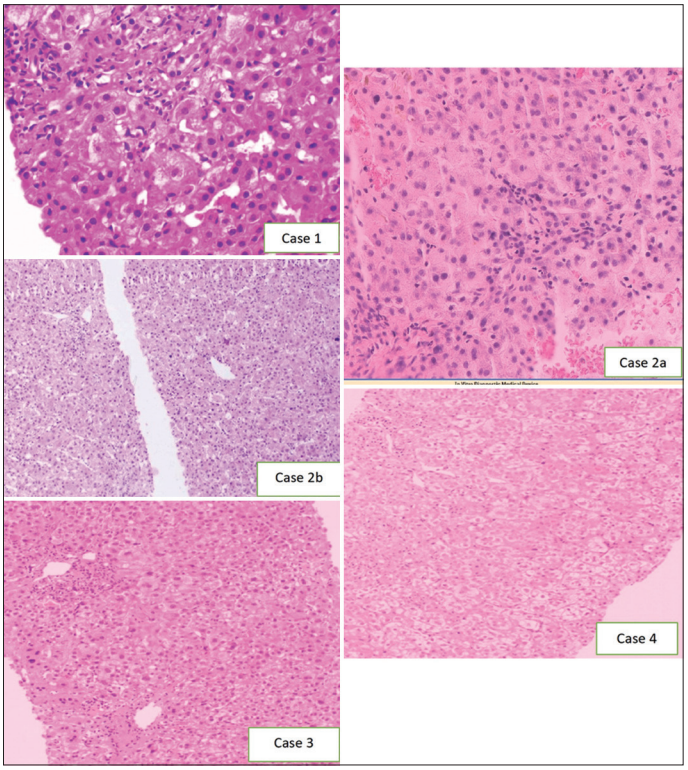

Figure 3. Histopathological micrographs of the liver (hematoxylin and eosin staining) of all patients.

most common adverse events [11] and contributes to 2.9% of all cases of DILI [12].

Identification and discontinuation of the culprit agents and avoidance of re-exposure are the mainstays of DILI management. In most patients, spontaneous recovery occurs after the culprit drug is discontinued [13]. DILI, in its natural course, may develop acute or sub-acute liver failure and may prove fatal if liver transplantation is not implemented [14]. Pruritus hampers the quality of life to a great extent, and in severe cases, patients may develop suicidal tendencies. For severe pruritus, drugs like antihistaminics, namely, hydroxyzine or diphenhydramine, topical emollients, and soothing agents like lactocalamine can be attempted. Cholestyramine provides symptomatic relief in patients with pruritus.

At present, there is no specific therapy for changing the natural course of DILI [15,16]. Steroids have been recommended in patients with drug-induced autoimmune hepatitis, DILI secondary to immune checkpoint inhibitors and biologicals, and DILI with features of hypersensitivity [17,18]. The role of steroids in other causes of DILI has been studied with mixed results presented in uncontrolled studies. A retrospective study showed that 15 patients treated with a combination of prednisolone and ursodeoxycholic acid exhibited a rapid reduction in bilirubin, liver enzymes, and INR [19]. Another retrospective study with a larger number of patients described a beneficial effect of corticosteroid therapy in terms of mortality benefit and rapid recovery in severe DILI [20]. In addition, budesonide has been reported to be beneficial in two patients without autoimmune features [21]. Contrary to these findings, two studies reported that corticosteroid administration was not found to be beneficial, but instead, was harmful to patients with severe DILI [22] and DILI-related acute liver failure [23]. A recent report from prospective DILI registries concluded that corticosteroid therapy did not worsen outcomes in DILI patients, and its administration led to a greater rate of normalization of liver enzymes in patients with severe DILI [24]. Amidst these mixed results on the role of corticosteroids in DILI/HILI, a recent open-label randomized controlled study concluded that steroids may accelerate the recovery of patients with severe DILI [25].

Despite a very limited number of patients, the present study demonstrates the beneficial role of corticosteroids in the treatment of DILI. In the present study, four patients had mixed patterns of liver injury with predominant cholestasis, leading to severe pruritus that significantly hampered quality of life in three patients (cases 1, 2a, and 4). The severity of pruritus in Case 1 was characterized by intense itching, which led to skin excoriations and bleeding. Case 2b had a hepatocellular pattern of liver injury (R-value = 7) with cholestatic symptoms, which can be explained by cholestatic pattern of liver injury by histopathologic means. Most patients with DILI recovered following the cessation of culprit agents, without showing signs of progression. Typically, patients with progressive liver injury may have severe DILI or end up with liver failure, in which secondary infections play a major role, and may face a very high mortality risk if liver transplantation is not implemented. Treatment with steroids in the setting of liver failure may increase the risk of secondary infections, posing a negative impact on the overall outcome. None of our patients had features of liver failure. Severe coagulopathy in Case 4 due to itraconazole-induced DILI can be explained by cholestasis leading to Vitamin K malabsorption. This patient had features of fat-soluble Vitamin A deficiency; therefore, he responded well to Vitamin A supplements in addition to corticosteroids.

Discontinuation of culprit medications along with supportive management was not sufficient to keep the patient's liver parameters and symptoms under control. Thus, steroids were prescribed as a rescue therapy for the patients, and all of them responded well to steroids without exhibiting any adverse effects. The beneficial effects of steroids in DILI may be attributed to its anti-inflammatory action. However, therapeutic effect of steroids in pruritus is conditional on the improvement in liver function and cholestasis. In the present study, improvement in pruritus was observed in a mean duration of 3.2 weeks and complete normalization of LFTs was achieved in a mean period of 11.2 weeks.

Several limitations of this study should be acknowledged. A small number of patients and the absence of a control group are significant limitations of this study, which prevent us from drawing a firm conclusion about the role of steroids in such scenarios. Furthermore, we did not perform tests for atypical viruses like herpes simplex virus, Epstein bar virus, varicella-zoster virus, and cytomegalovirus that can cause liver injury. Three patients had KF ring, as confirmed during slit lamp examination, but a genetic analysis for the *ATP7B* gene was not performed to completely rule out Wilson's disease in these cases.

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5. Conclusions

A short course (a few weeks) of steroids can be used to effectively treat DILI and intractable pruritus in patients without liver failure. However, large prospective controlled studies are needed to confirm the role of corticosteroids in these situations.

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None.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Approval and Consent to Participate

As this study was retrospective in nature, research ethics approval was not obtained. However, written informed consent was obtained from all patients for inclusion in the study.

Consent for Publication

Written informed consent was obtained from all patients for using their data in a publication.

Availability of data

Data are available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

Effect of combination of molnupiravir with clarithromycin on blood biomarkers in patients with mild-to-moderate COVID-19

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ABSTRACT

Background: Molnupiravir is a type of medication used to treat coronavirus disease 2019 (COVID-19). However, no evidence regarding the therapeutic effect of molnupiravir combined with clarithromycin (CAM) on blood biomarkers is available.

Methods: Of the 156 rehabilitation patients, 124 patients with mild-to-moderate COVID-19 were treated with molnupiravir. Among these 124 patients, 54 were treated with CAM. The remaining 28 rehabilitation patients were negative for COVID-19. Blood biomarkers were assessed after administration of molnupiravir in patients receiving molnupiravir plus CAM or molnupiravir alone.

Results: Among the measured blood biomarkers, lactate dehydrogenase, potassium, white blood cells, C-reacted protein, neutron–lymphocyte ratio, fibrin degradation product, and prothrombin time–international normalized ratio values were significantly higher ($P < 0.05$) in the molnupiravir alone group than in the molnupiravir plus CAM group, and lymphocytes were significantly lower ($P < 0.05$) on day 5 after admission. In the molnupiravir plus CAM group, immunoglobulin (Ig) A levels increased and soluble interleukin 2-receptor levels (sIL2R) decreased ($P < 0.05$) on day 14 after admission. In addition, COVID-19-negative patients had higher IgA levels and lower sIL2R levels compared to infected patients ($P < 0.05$). The concomitant administration of molnupiravir plus CAM resulted in fewer sequelae after 12 months, and the incidence of venous thromboembolism was significantly reduced ($P < 0.05$).

Conclusion: In patients with mild-to-moderate COVID-19, concomitant administration of molnupiravir plus CAM showed several non-worsening blood biomarkers, elevated immune activity, and reduced post-infection sequelae.

Relevance for Patients: After administration of molnupiravir to patients with mild-to-moderate COVID-19, administration of CAM to patients suffering from secondary macrolide-sensitive bacterial infection was compared with administration of molnupiravir alone. D-dimer, IgA, and sIL2R are potential predictive factors of disease severity in critically ill patients with COVID-19.

1. Introduction

Sudden deterioration and death of patients suffering from mild-to-moderate coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is common on the clinical scene. In some cases, patients become severely ill without being aware of it and suffer from oxygen deficiency. It is necessary to prevent deterioration of COVID-19, tested positive by means of SARS-CoV-2 antigen test or

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SARS-CoV-2 PCR test, among patients with mild-to-moderate symptoms before respiration failure takes place. At the height of the COVID-19 pandemic, the hospitalization rates for patients with severe infections were high; naturally, the mild-to-moderate cases were asked to self-quarantine at home. Therefore, an approach to treating COVID-19 patients with mild-to-moderate symptoms is essential.

Molnupiravir has been proven to be a well-tolerated, direct-acting oral antiviral agent that prevents symptom progression in patients with mild-to-moderate COVID-19 [1,2]. Given the context of rapid spread of infection among hospitalized patients in isolation and closed wards, oral molnupiravir serves as an ideal medication due to the ease of administration [3,4]. However, even if molnupiravir is administered in mild-to-moderate cases, the possibility of deterioration cannot be avoided if a secondary infection occurs. In this study, we selected patients with macrolide-susceptible bacteria among the patients with secondary infections.

Macrolide (clarithromycin or azithromycin) antibiotics were incorporated in the treatment regimen in the early stages of the COVID-19 pandemic, especially considering that they may have anti-inflammatory effects. Macrolides have been extensively researched as broad adjunctive therapy for COVID-19 due to its immunostimulant abilities [5]. Adding clarithromycin (CAM) or azithromycin to the therapeutic protocols for COVID-19 could be beneficial for early control of fever and early PCR-negative conversion in mild COVID-19 cases [6]. It has been reported the first COVID-19-positive patient who recovered from the symptoms after the use of chloroquine and CAM was reported in Colombia [7]. CAM has immunomodulatory properties superior to those of azithromycin [8] and enhances antiviral secretory-IgA production and neutralizing activities through the induction of IgA class switching recombination [9]. Interleukin (IL)-6 and IL-2 trigger cytokine release syndrome observed in severe cases of COVID19. CAM significantly inhibited the production of IL-6 by dendritic cells and significantly decreased IL-2 productions [10]. The results of The ACHIEVE Open-Label Single-Arm Trial demonstrated that early CAM treatment leads to clinical improvement in patients with moderate COVID-19 [11].

At present, there is no evidence regarding therapeutic effect of molnupiravir combined with CAM on blood biomarkers in COVID-19 patients. Thus, the aim of this study is to investigate blood biomarkers in patients with mild-to-moderate COVID-19, compounded by secondary infection with macrolide-sensitive bacteria, who were treated with molnupiravir followed by administration of CAM. At the same time, blood biomarkers were also evaluated for COVID-19-negative patients, who were used as controls in this study despite being quarantined together with other COVID-19 patients. In addition, we compared the degree of sequelae 12 months after the COVID-19 treatment with molnupiravir plus CAM or molnupiravir alone.

2. Methods

2.1. Study design

A total of 156 patients who were hospitalized in a rehabilitation ward from May 2022 to July 2022 were recruited in this study. Of the 156 patients, 4 severe COVID-19 patients were transferred to other hospitals where endotracheal intubation and ventilator treatment were available. We administered molnupiravir (800 mg twice daily for 5 days) to 124 mild-to-moderate COVID-19 patients (Supplementary Data 1). Chest X-ray, computed tomography, and bacteriological examination were performed to detect the occurrence of secondary infection. A respiratory physician diagnosed 87 secondary infections and administered antibiotics based on the results of bacterial sensitivity test. Fifty four of the 124 patients had mycoplasma infection, Gram-positive coccal infection, pneumococcal infection, or Haemophilus influenza infection and received CAM (400 mg twice daily for 3 days). Of the 124 patients, 33 received antibiotics other than CAM. Twenty eight of the 156 patients were negative for COVID-19 despite being quarantined with other COVID-19-positive patients and were not treated with any drugs for COVID-19 (Figure 1).

2.2. Laboratory examinations

Nucleic acid detection tests and antigen tests were performed in accordance with the "Guidelines for Pathogen Testing of Novel Coronavirus Infectious Disease (COVID-19) March 17, 2022, Version 5.1" published by the Ministry of Health, Labor and Welfare of Japan [12]. Real-time RT-PCR was performed to detect SARS-CoV-2 nucleic acids using GeneFinder™ COVID-19 PLUS RealAmp Kit and ELITe InGenius® instrument. Antigen qualitative tests were conducted using the SARS CoV-2 Rapid Antigen Test Nasal kit (SD BIOSENSOR, Roche; REF: 9901-NCOV-03G; LOT: QCO 3811951). During hospitalization, blood samples were collected on the 5th day and 14th day from the start of oral molnupiravir administration at the time of infection according to the doctor's instruction. Blood biomarkers were assessed using an automated hematology analyzer. Uninfected patients were assumed to be infected when their roommates started taking molnupiravir, and their blood was collected for medical assessment, considering the possibility of infection from infected patients in the same room. To investigate predictive factors of molnupiravir plus CAM combination therapy in COVID-19 patients, the following biomarkers were measured: lactate dehydrogenase (LDH) [13], total cholesterol, triglyceride [14], uric acid [15], creatinine [16], potassium [17], white blood cells (WBC), hemoglobin (Hb) [18], platelet (PLT) [13], C-reactive protein (CRP) [19], neutrophils, lymphocytes [20], neutrophil–lymphocyte ratio (NLR) [21], fibrinogen [22], fibrin degradation product (FDP) [23], D-dimer [24], prothrombin time-international normalized ratio (PT-INR) [25], creatinine phosphokinase (CPK) [26], brain natriuretic peptide (BNP) [27], IgG [28], IgA [29], IgM [30], and soluble IL-2 receptor (sIL2R) [31]. Cutoff values are listed in Supplementary Data 2 and 3.

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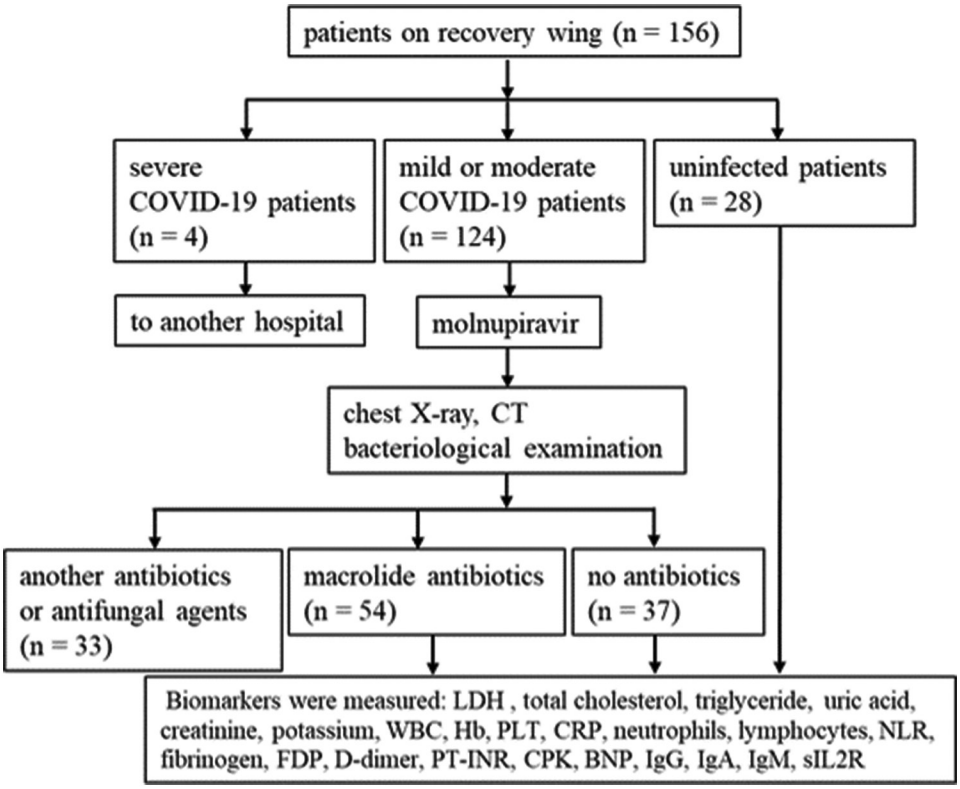

Figure 1. CONSORT flow chart detailing patient enrollment allocation, follow-up, and analysis. Out of the 156 rehabilitation patients, 124 patients with mild-to-moderate COVID-19 received molnupiravir (800 mg twice daily for 5 days), and 54 of this subset of patients received clarithromycin (400 mg twice daily for 3 days). The remaining 28 patients were negative for COVID-19 despite being isolated from other COVID-19-positive patients and were not administered drugs.
Abbreviations: CT: Computed tomography, LDH: Lactate dehydrogenase, WBC: White blood cells, Hb: Hemoglobin, PLT: Platelet, CRP: C-reacted protein, NLR: Neutron–lymphocyte ratio, FDP: Fibrin degradation product, PT-INR: Prothrombin time–International Normalized Ratio, CPK: Creatinine phosphor kinase, BNP: Brain natriuretic peptide, Ig; Immunoglobulin, sIL2R: Soluble interleukin 2 receptor.

2.3. Statistics analysis

Student's *t*-test was used for statistical data analysis. A $P < 0.05$ was considered significant. Data are presented as SD±. Cox proportional hazard models were utilized to assess the impact of risk factors. SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

3. Results

3.1. Baseline characteristics of COVID-19 patients treated with molnupiravir plus clarithromycin and molnupiravir alone and uninfected patients

Between the molnupiravir plus CAM group and molnupiravir alone group, significant differences were observed in baseline symptoms of fever, cough and sputa, shortness of breath, chest tightness, and dyspnea, and in baseline comorbidity of respiratory diseases ($P < 0.05$). Some baseline symptoms were significantly different between uninfected rehabilitation patients and those receiving molnupiravir plus CAM or molnupiravir alone. However, there was no significant difference in baseline comorbidity (Supplementary Data 4).

On Okinawa Island, the rollout of Pfizer-BioNTech COVID-19 mRNA vaccines began in December 2021. More than 90% of the patients had received at least one vaccine shot within 6 months before admission, and none of them received vaccine during hospitalization. However, only 89.3% of the uninfected rehabilitation patients had been vaccinated (Supplementary Data 4).

3.2. Dynamics of blood biomarkers during disease progression in molnupiravir alone group, molnupiravir plus clarithromycin group, and uninfected group

LDH, potassium, WBC, CRP, NLR, FDP, and PT-INR values were significantly higher ($P < 0.05$) in the molnupiravir alone group than in the molnupiravir plus CAM combination group on day 5, and lymphocyte count was significantly lower ($P < 0.05$). On the 14th day, WBC, CRP, neutrophils, and NLR were all significantly high ($P < 0.05$), and lymphocyte count was significantly low ($P < 0.05$).

In the molnupiravir alone group, LDH, potassium, WBC, CRP, neutrophils, NLR, fibrinogen, FDP, PT-INR, and CPK significantly increased on day 5 after admission ($P < 0.05$) and total cholesterol, triglyceride, uric acid, PLT, and lymphocytes

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significantly decreased ($P < 0.05$). D-dimer decreased significantly on day 14 after admission ($P < 0.05$), and CRP was significantly elevated ($P < 0.05$).

In the molnupiravir plus CAM group, there were no significant differences in any of the biomarkers between admission and day 5. In addition, the values of D-dimer decreased significantly on day 14 after admission ($P < 0.05$).

In the uninfected group, no significant differences in biomarkers were observed before infection, on day 5, and on day 14. The values of biomarkers, except for BNP, were within normal limits (Figure 2 and Supplementary Data 2).

3.3. Dynamics of IgG, IgA, IgM, and sIl2R in advanced stages of disease in molnupiravir alone group, molnupiravir plus clarithromycin group, and uninfected group

We measured baseline levels of immunological biomarkers on days 5 and 14 from the start of oral administration at the time of infection in the molnupiravir alone group and the molnupiravir plus CAM group and on the day of admission in the uninfected group (Figure 3 and Supplementary Data 3).

The IgA level at admission in the uninfected group was significantly higher than the IgA level on day 5 in both the molnupiravir alone group and the molnupiravir plus CAM group ($P < 0.05$).

The sIL2R level at admission in the uninfected group was significantly lower than the sIL2R level on day 5 in both the molnupiravir alone group and the molnupiravir plus CAM group ($P < 0.05$).

In the molnupiravir plus CAM group, the IgA level on day 14 was significantly higher than that on day 5, and the sIL2R level on day 14 was significantly lower than that on day 5 ($P < 0.05$).

In the molnupiravir plus CAM group, the IgA level on day 14 was significantly higher ($P < 0.05$) and the sIL2R level was significantly lower ($P < 0.05$) than in the molnupiravir alone group. The values of IgG and IgM were within the normal limit.

3.4. Predictive factors as blood biomarker in molnupiravir alone group and molnupiravir plus clarithromycin group

Multivariate logistic regression analysis based on the collection and analysis of clinical and laboratory data for the

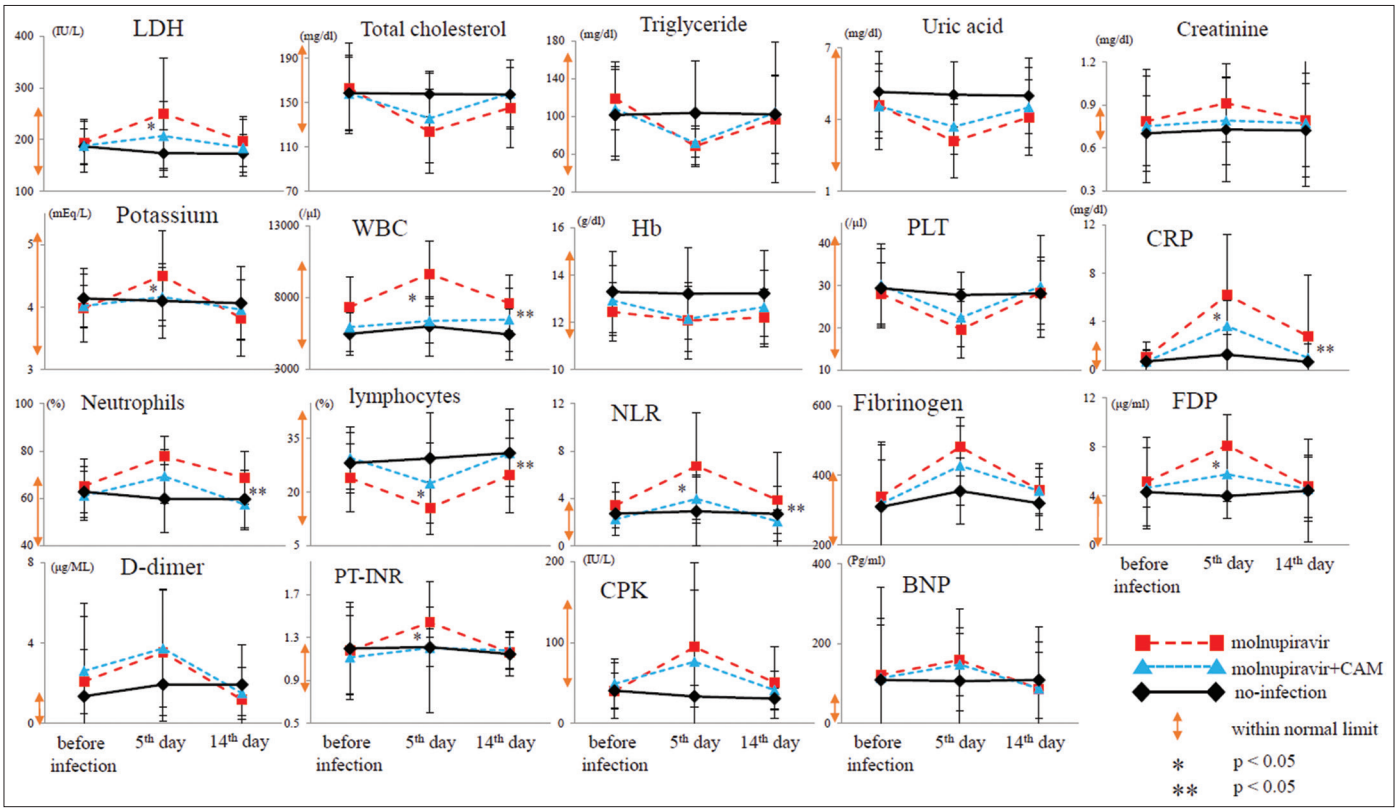

Figure 2. Time-series dynamics of blood biomarkers in the molnupiravir alone group, the molnupiravir plus clarithromycin (CAM) group, and the uninfected group. The molnupiravir plus CAM group had lower or higher values than the molnupiravir alone group and were close to the values of the uninfected group. The vertical axis indicates the value of each biomarker, while the horizontal axis indicates before infection, 5th days and 14th days after administration of molnupiravir. Red squares and dashed lines indicate molnupiravir; blue triangles and dotted lines indicate molnupiravir + CAM; black diamonds and bar-shaped line indicate no infection; double-headed arrow indicates within normal limits. *$P < 0.05$, **$P < 0.05$.
Abbreviations: LDH: Lactate dehydrogenase, WBC: White blood cells, Hb: Hemoglobin, PLT: Platelet, CRP: C-reacted protein, NLR: Neutron–lymphocyte ratio, FDP: Fibrin degradation product, PT-INR: Prothrombin time–International Normalized Ratio, CPK: Creatinine phosphor kinase, BNP: Brain natriuretic peptide.

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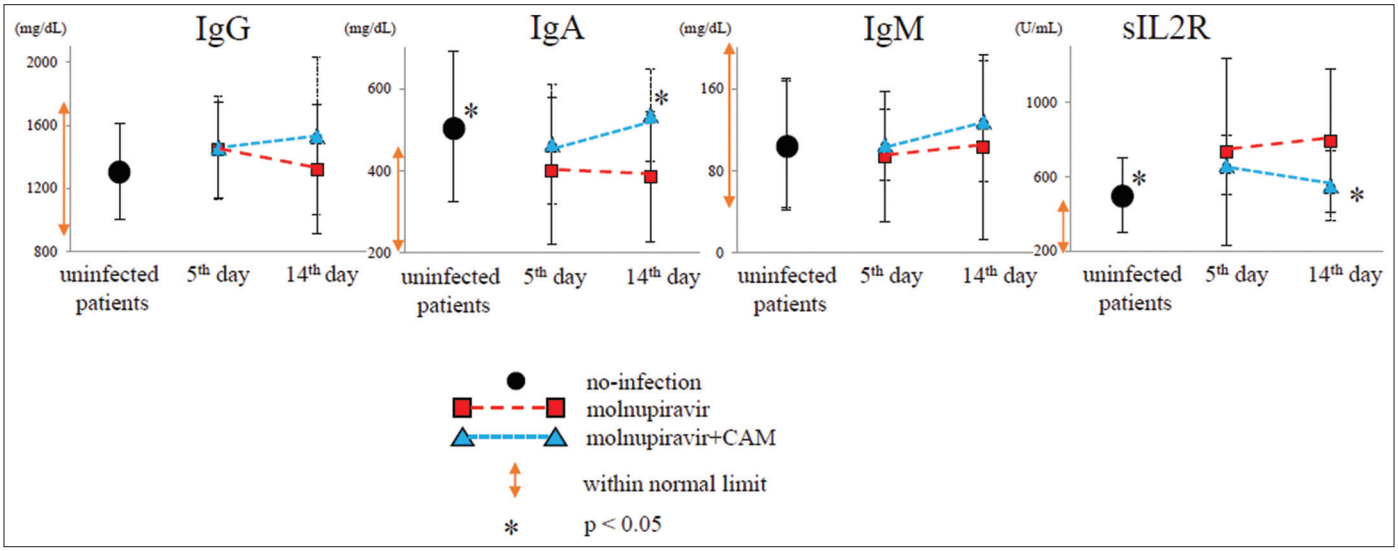

Figure 3. Time-series dynamics of immune biomarkers in the molnupiravir alone group, the molnupiravir plus clarithromycin (CAM) group, and the uninfected group. Uninfected individuals had higher IgA levels and lower sIL2R levels than infected individuals. IgA levels increased and sIL2R levels decreased in the molnupiravir plus CAM group. On day 14 after administration, IgA levels were higher and sIL2R levels were lower in the molnupiravir plus CAM group than in the molnupiravir alone group. The vertical axis indicates the value of each biomarker, while the horizontal axis indicates the duration of hospitalization for an uninfected patient during drug administration, and the 5th and 14th days for an infected patient after administration. Red squares and dashed lines indicate molnupiravir; blue triangles and dotted line indicate molnupiravir + CAM; double-headed arrow indicates within normal limit. *$P < 0.05$.

molnupiravir alone group and the molnupiravir plus CAM group showed that predictive factors were D-dimer (OR = 1.08, 95% CI = 1.05 – 1.11, $P < 0.05$), IgA (OR = 1.06, 95% CI = 1.02 – 1.10, $P < 0.05$), and sIL2R (OR = 1.13, 95% CI = 1.09 – 1.17, $P < 0.05$) (Supplementary Data 5).

3.5. Comparison of severity of sequelae 12 months after COVID-19 treatment with molnupiravir plus clarithromycin or molnupiravir alone

Administration of molnupiravir plus CAM significantly reduced the incidence of venous thromboembolism compared to administration of molnupiravir alone ($P < 0.05$). There were no significant differences in other sequelae, but the incidence of sequelae was generally lower in the administration of molnupiravir plus CAM (Supplementary Data 6).

4. Discussion

Okinawa consists of more than 160 archipelagos, situated between Taiwan and the main island of Japan in the East China Sea. Remote islanders do not always have adequate medical care if they contract COVID-19, even if they become severely ill. Many of the islanders are over 90 years old. However, elderly patients with pre-existing conditions are more likely to become seriously ill if infected. Therefore, it is imperative to prevent the deterioration from mild or moderate COVID-19 to severe form, necessitating ventilator management among elderly patients, and it is also our responsibility as medical professionals to engage in the treatment of COVID-19-infected patients [32]. Preventing mild or moderate COVID-19 from worsening in medical settings stands as a model treatment approach in the face of the rapid surge in COVID-19 infections.

Almost all biomarkers were reduced surprisingly by the combined administration of molnupiravir and CAM as compared to the molnupiravir alone administration (Figure 2). However, even in the molnupiravir plus CAM group, CRP, neutrophils, fibrinogen, and FDP exceeded the standard values. Thus, it is necessary to consider medications other than CAM considering drug–drug interactions (DDI), anti-inflammatory effect, and thrombosis prevention. In the molnupiravir plus CAM group, the values were almost equivalent to those of uninfected patients, and within at least 2 weeks, the biomarkers fell within the reference range, suggesting the abrogation of COVID-19 deterioration regardless of symptoms at the time of infection. The presence of cerebrovascular and cardiovascular disorders is the reason that D-dimer and BNP values exceeded the standard values regardless of the duration of drug administration in patients, whether uninfected or infected. Interestingly, the D-dimer and BNP levels on the 14th day after administration of molnupiravir were lower than those before infection and were also lower than those in uninfected subjects. Molnupiravir may be effective in preventing thrombosis and improving heart failure.

Furthermore, after 12 months, the incidence of venous thromboembolism was significantly reduced in the molnupiravir plus CAM group (Supplementary Data 6), suggesting that D-dimer is a potential predictive factor. On the same note, identifying D-dimer as a predictive factor for venous thromboembolism is tantamount to recognizing the thrombotic tendency among COVD-19 patients. Both the target patients and rehabilitation patients at our hospital suffered from cerebrovascular and cardiovascular disorders, and

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their D-dimer and BNP levels surpassed the standard values at the time of admission, requiring anticoagulants for thrombosis prevention. It has been reported that thrombosis can be induced in patients with COVD-19. However, the current findings show that fibrinogen and FDP levels remain high after infection, but they became lower than before infection, although not within the standard values, after 14 days of molnupiravir administration. Furthermore, concomitant administration of CAM improved coagulation factor elevation and intravascular coagulation, and venous thromboembolism and pulmonary thromboembolism were not observed in the target patients. This might be the reason why no patients from the current cohort died of COVID-19 and were successfully discharged from the hospital.

Based on our results, IgA and sIL2R are potential predictive factors. Uninfected individuals had significantly higher IgA levels and lower sIL2R levels than infected individuals. This can be one of the reasons why there were no new infection cases even after 14 days although the rehabilitation patients had had close contact with COVID-19-positive roommates in a closed ward (Figure 3). In addition, molnupiravir administration sustained the effect of IgM even on day 14, but molnupiravir plus CAM administration resulted in higher IgG levels on day 14. Although there was no significant difference, CAM might maintain humoral immunity even after 10 days of administration. SARS-CoV-2, the virus that causes COVID-19, infects cells on mucosal surfaces. Serum-neutralizing antibody responses are variable, and neutralizing antibodies appear to be generally low in patients with COVID-19. Potent IgG antibodies neutralize the virus, but secretory antibody responses such as IgA, which can affect initial viral spread and transmissibility across mucosa, may be of particular value for defense against SARS-CoV-2 [33]. This result is consistent with the effect of molnupiravir plus CAM administration, *i.e.*, increase in IgA and decrease in sIL2R (Figure 3).

Molnupiravir (MK-4482, EIDD-2801) is a promising broad-spectrum experimental antiviral agent developed by Merck & Co. It was originally developed to treat influenza infections because it exerts antiviral activity through RNA-dependent RNA polymerase to induce huge numbers of copy errors. Molnupiravir has demonstrated potent *in vitro* antiviral activity with low cytotoxicity and high resistance barrier against positive-sense RNA viruses, including SARS-CoV-2 [34]. When the pandemic began, molnupiravir was in pre-clinical development for the treatment of seasonal influenza but has evolved into a potential agent for the prevention and treatment of COVID-19 [35]. Influenza infections occur year-round on the main island, and we have reported that the non-infected individuals had higher IgA levels than the influenza-infected individuals [36]. IgA levels in nasal discharge and alveolar lavage fluid, which were markedly decreased when anti-influenza drugs were administered alone, significantly recovered to high levels when the drug was administered in combination with CAM, a macrolide with immuno-modulating effects. In addition, IgA production was significantly enhanced by nearly 10-fold in patients who were co-administered with CAM, although it was not observed after the administration of anti-influenza drugs alone [9]. CAM combined administration may increase IgA, which is low in the human body, by encouraging its production, as observed in this study.

Indeed, vaccines can induce secretory IgA antibodies and are effective. However, there is still an urgent need for antiviral drugs with potent activity to defend against the emerging SARS-CoV-2 variants for which existing vaccines may be less effective. Certainly, early treatment with molnupiravir reduced the risk of hospitalization or death in unvaccinated adults at risk for COVID-19 (Funded by Merck Sharp and Dohme; MOVe-OUT ClinicalTrials.gov number, NCT04575597) [37]. As an early treatment for patients with COVID-19, molnupiravir administration is therapeutically effective, but there is the potential for co-infection or potentially life-threatening recurrence in patients after treatment. The need for highly synergistic agent integrating molnupiravir is also becoming increasingly important. To control for confounding factors, we investigated the number of vaccinations in rehabilitation patients who were uninfected despite being quarantined with other COVID-19 patients. As a result, uninfected patients received fewer vaccinations than those with COVID-19. Therefore, in this study, vaccination was excluded as a confounding factor, and blood biomarkers were analyzed for their capacity, without the influence of vaccination, to prevent COVID-19 deterioration.

Our results show that IgA levels increased when molnupiravir was co-administered with CAM. CAM effects are not limited to only anti-inflammatory [38]. Molnupiravir plus CAM may alter and improve the clinical course of patients with COVID-19 infection, at least through an indirect mechanism that relies on several variable anti-inflammatory and/or immunomodulatory effects in addition to its well-known antibacterial activity. Compared to uninfected patients, IgA may be a predictive factor for the improvement of COVID-19 infection, and sIL2R may be a predictive factor for the prevention of secondary infection.

There are several limitations in this study. First, the present work is essentially an exploratory retrospective study. There is no previous clinical evidence evaluating the therapeutic efficacy of molnupiravir and CAM against COVID-19, and the sample size was based on the feasible number of consented patients who were hospitalized. Second, without knowing DDI, many antivirals and anti-inflammatory drugs have been approved to treat COVID-19 patients, but potential DDIs that can enhance the safety and efficacy of molnupiravir and CAM remain elusive [39]. Third, the target sample size of this study is small. Fourth, uninfected patients who could not be transferred were managed in a closed ward and inspection was performed on both the infected and uninfected individuals, but there might be a bias in terms of examination date. Furthermore, bias from patients and attending physicians cannot be completely avoided. Fifth, this study was conducted only at medical institutions on the main island of Okinawa and included only Japanese patients undergoing rehabilitation treatment. Sixth, macrolides increase the risk of some cardiac side effects, especially in the elderly. These constraints have limited generalization of the current set of findings.

5. Conclusion

D-dimer, IgA, and sIL2R are potential predictive factors of COVID-19 severity in patients with mild-to-moderate symptoms receiving molnupiravir plus CAM.

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Conflict of Interest

The authors declare no competing interest in this study or its publication.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and it was registered and approved by the Ethics Committee and Review Board of Daido Central Hospital (approval ID: daido0036) (May 2022). Written informed consent was obtained from all the subjects before their participation in the study.

Consent for Publication

Written informed consent for releasing their data and/or images in this paper was obtained from all the subjects in the study.

Availability of Data

Data are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Effect of combination of molnupiravir with clarithromycin on blood biomarkers in patients with mild-to-moderate COVID-19

Supplementary File

Supplementary Data 1

Main inclusion/exclusion criteria for the global phase III study [MOVe-OUT (002) study]

1. SARS-CoV-2-positive patients (confirmed by PCR test, etc. using samples collected within 5 days before randomization).
2. SARS-CoV-2 infection symptom onset within 5 days and 1 or more symptoms associated with SARS-CoV-2 infection can be recognized (cough, sore throat, nasal congestion, runny nose, shortness of breath or difficulty breathing during exertion, muscle or body pain, fatigue, fever [above 38.0°C], chills, headache, nausea, vomiting, diarrhea, loss of sense of smell, and loss of taste).
3. Mild or moderate patient as defined below.

[Mild] Satisfy A and B

A. All of the following are recognized.
Breathing rate is <20 breaths/min, heart rate is less than 90 times/min, SpO_2 is more than 93% (value in indoor air or in a state where oxygen is administered for reasons other than infection with SARS-CoV-2 and the amount of oxygen has not been increased since the onset of symptoms of infection with SARS-CoV-2)

B. Neither of the following are recognized.
Shortness of breath at rest or during exertion or incomplete breathing (when one or more of the following (1) to (4) is required: (1) endotracheal intubation and ventilator, (2) high-flow oxygen therapy using nasal cannula [flow rate over 20 L/min, oxygen ratio of 0.5 or more], (3) non-invasive positive pressure ventilation, (4) ECMO, shock state, multi-organ dysfunction)

[Moderate disease] Satisfy all of A to B

A. One or more of the following is recognized
Shortness of breath during exertion, respiratory rate is more than 20 times/min but less than 30 times/min, heart rate of 90 beats/min or more and less than 125 beats/min

B. Any of the following is recognized
   a. SpO_2 is over 93% (value in indoor air or in a state where oxygen is administered for reasons other than infection with SARS-CoV-2, and the amount of oxygen has not been increased since the onset of symptoms of infection with SARS-CoV-2)
   b. Requiring oxygen administration of 4 L/min or less due to infection by SARS-CoV-2 regardless of SpO_2.

C. None of the following are recognized
Shortness of breath at rest or incomplete breathing (when one or more of the following (1) to (4) is required: (1) endotracheal intubation and ventilator, (2) high-flow oxygen therapy using nasal cannula [flow rate over 20 L/min, oxygen ratio of 0.5 or more], (3) non-invasive positive pressure ventilation, (4) ECMO, shock state, multi-organ dysfunction)

4. Have one or more of the following risk factors for severe SARS-CoV-2 infection:
   (1) 61 years old or older active cancers (excludes cancers that do not involve immunosuppression or high mortality)
   (2) Chronic kidney disease
   (3) Chronic obstructive pulmonary disease
   (4) Obesity (BMI 30 kg/m^2 more)
   (5) Serious heart disease (incomplete heart, coronary artery disease, or cardiomyopathy)
   (6) Diabetes

Molnupiravir has been evaluated in phase I, phase II, and phase III trials that have demonstrated favorable efficacy, dose-dependent pharmacokinetics, and a robust safety profile. In an interim analysis of a phase III trial, treatment with molnupiravir reduced the risk of hospitalization or death in COVID-19 patients by 50% [1]. On December 24, 2021, the Japanese Ministry of Health, Labor and Welfare granted special approval for domestic marketing of molnupiravir, an oral antiviral drug for COVID-19, based on the evaluation in the phase III trial. Molnupiravir can be administered to non-hospitalized patients aged 18 years and older with mild-to-moderate disease who have risk factors for severe disease [2]. For the use of molnupiravir, we referred to the guidelines from the World Health Organization (WHO) [3], Food and Drug Administration (FDA) [4], and National Institutes of Health (NIH) [5].

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Supplementary Data 2

Table 1. Baseline levels of blood biomarkers on admission, 5 days, and 14 days after starting treatment for infection in molnupiravir alone, molnupiravir plus clarithromycin (CAM), and unaffected groups

Blood biomarkers	Admission	Day 5	*P*-value[1]		Day 14	*P*-value[2]
Biochemistry biomarkers						
LDH (IU/L)						
Molnupiravir	193.1±41.2	250.2±107.3	<0.05		196.7±48.4	n.s.
Molnupiravir + CAM	187.7±50.7	206.7±67.3	n.s.		183.7±55.3	n.s.
Uninfected	186.5±33.5	173.1±45.2	n.s.		172.5±36.4	n.s.
P-value*	n.s.	<0.05		*P*-value**	n.s.	
Total cholesterol (mg/dl)						
Molnupiravir	163±41.1	123.7±37.9	<0.05		145.2±35.9	n.s.
Molnupiravir + CAM	158.0±32.5	135.7±40.4	n.s.		158.2±30.2	n.s.
Uninfected	158.6±34.0	157.7±20.0	n.s.		157.2±31.0	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
Triglyceride (mg/dl)						
Molnupiravir	118.8±33.5	68.3±21.5	<0.05		96.1±46.2	n.s.
Molnupiravir + CAM	107.5±49.8	71.6±14.9	n.s.		103.9±74.4	n.s.
Uninfected	101.3±47.8	103.4±55.0	n.s.		101.8±41.5	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
Uric acid (mg/dl)						
Molnupiravir	4.6±1.4	3.0±1.5	<0.05		4.0±1.5	n.s.
Molnupiravir + CAM	4.5±1.8	3.7±1.1	n.s.		4.5±1.6	n.s.
Uninfected	5.1±1.6	5.0±1.3	n.s.		5±1.5	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
Creatinine (mg/dl)						
Molnupiravir	0.78±0.31	0.91±0.27	n.s.		0.79±0.32	n.s.
Molnupiravir + CAM	0.75±0.39	0.79±0.30	n.s.		0.77±0.44	n.s.
Uninfected	0.70±0.26	0.72±0.36	n.s.		0.72±0.32	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
Potassium (mEq/l)						
Molnupiravir	3.9±0.5	4.5±0.7	<0.05		3.8±0.6	n.s.
Molnupiravir + CAM	4.0±0.3	4.1±0.4	n.s.		3.9±0.4	n.s.
Uninfected	4.1±0.4	4.1±0.6	n.s.		4.0±0.5	n.s.
P-value*	n.s.	<0.05		*P*-value**	n.s.	
Blood routine biomarkers						
WBC ($\times 10^2/\mu l$)						
Molnupiravir	73.2±20.7	96.4±22.5	<0.05		76.0±19.3	n.s.

(*Contd...*)

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Table 1. (*Continued*)

Blood biomarkers	Admission	Day 5	*P*-value[1]		Day 14	*P*-value[2]
Molnupiravir + CAM	59.2±16.8	63.5±15.3	n.s.		64.4±22.0	n.s.
Uninfected	54.5±14.6	59.8±20.7	n.s.		54.1±17.9	n.s.
P-value*	n.s.	<0.05		*P*-value**	<0.05	
Hb (g/dl)						
Molnupiravir	12.4±1.2	12.0±1.6	n.s.		12.2±1.2	n.s.
Molnupiravir + CAM	12.9±1.4	12.1±1.3	n.s.		12.6±1.5	n.s.
Uninfected	13.2±1.7	13.2±1.9	n.s.		13.2±1.8	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
PLT (/µl)						
Molnupiravir	28.1±7.2	19.6±6.7	<0.05		28.4±7.5	n.s.
Molnupiravir + CAM	30.1±9.6	22.4±6.8	n.s.		29.8±12.0	n.s.
Uninfected	29.4±9.4	27.8±5.4	n.s.		28.1±8.6	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
Inflammatory biomarkers						
CRP (mg/dl)						
Molnupiravir	1.0±1.2	6.1±4.9	<0.05		2.7±5.0	<0.05
Molnupiravir + CAM	0.6±0.8	3.6±2.1	n.s.		0.9±1.4	n.s.
Uninfected	0.6±0.9	1.2±1.6	n.s.		0.6±1.4	n.s.
P-value*	n.s.	<0.05		*P*-value**	<0.05	
Neutrophils (%)						
Molnupiravir	65.2±11.3	77.5±8.5	<0.05		68.5±11.2	n.s.
Molnupiravir + CAM	61.0±10.1	69.2±11.4	n.s.		57.2±10.4	n.s.
Uninfected	62.7±10.6	59.7±14.4	n.s.		59.5±12.0	n.s.
P-value*	n.s.	n.s.		*P*-value**	<0.05	
Lymphocytes (%)						
Molnupiravir	23.8±9.5	15.5±7.4	<0.05		24.6±10.3	n.s.
Molnupiravir + CAM	29.5±8.6	22.3±11.2	n.s.		30.9±9.3	n.s.
Uninfected	28.1±8.4	29.4±12.8	n.s.		30.9±12.2	n.s.
P-value*	n.s.	<0.05		*P*-value**	<0.05	
NLR						
Molnupiravir	3.4±1.9	6.7±4.5	<0.05		3.8±4.0	n.s.
Molnupiravir + CAM	2.2±1.4	3.9±2.0	n.s.		2.0±0.9	n.s.
Uninfected	2.7±1.8	2.9±2.9	n.s.		2.6±2.3	n.s.
P-value*	n.s.	<0.05		*P*-value**	<0.05	
Coagulation biomarkers						
Fibrinogen (mg/dl)						
Molnupiravir	340±156.9	482±83.8	<0.05		358.4±74.8	n.s.
Molnupiravir + CAM	319.8±169.1	428.8±113.1	n.s.		356.7±65.3	n.s.
Uninfected	310.3±134.0	355.0±94.4	n.s.		320.4±76.0	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
FDP (µg/ml)						
Molnupiravir	5.1±3.6	8.1±2.5	<0.05		4.7±2.5	n.s.
Molnupiravir + CAM	4.6±3.3	5.7±2.2	n.s.		4.5±2.6	n.s.
Uninfected	4.3±1.2	4±1.7	n.s.		4.4±4.1	n.s.
P-value*	n.s.	<0.05		*P*-value**	n.s.	
D-dimer (µg/ML)						
Molnupiravir	2.0±1.5	3.5±3.1	n.s.		1.1±0.7	<0.05
Molnupiravir + CAM	2.6±3.3	3.7±2.9	n.s.		1.4±1.2	<0.05
Uninfected	1.3±3.9	1.9±1.7	n.s.		1.9±1.9	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	

(*Contd...*)

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Table 1. (*Continued*)

Blood biomarkers	Admission	Day 5	*P*-value[1]		Day 14	*P*-value[2]
PT-INR						
Molnupiravir	1.1±0.4	1.4±0.1	<0.05		1.1±0.1	n.s.
Molnupiravir + CAM	1.1±0.3	1.2±0.1	n.s.		1.1±0.1	n.s.
Uninfected	1.1±0.4	1.2±0.6	n.s.		1.1±0.2	n.s.
P-value*	n.s.	<0.05		*P*-value**	n.s.	
Cardiac biomarkers						
CPK (IU/L)						
Molnupiravir	40±22.2	94.3±103.5	<0.05		50.6±44.4	n.s.
Molnupiravir + CAM	49.0±30.5	76.25±88.2	n.s.		41.1±22.7	n.s.
Uninfected	40.3±34.0	33.3±13.2	n.s.		30.5±13.5	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	
BNP (pg/ml)						
Molnupiravir	121.3±142.3	158.4±128.2	n.s.		86.3±92.6	n.s.
Molnupiravir + CAM	113.4±228.6	147.0±78.3	n.s.		86.8±153.9	n.s.
Uninfected	108.3±139.6	105.7±132.8	n.s.		108.4±96.1	n.s.
P-value*	n.s.	n.s.		*P*-value**	n.s.	

Abbreviations: CAM: Clarithromycin, n.s.: No significant difference, LDH: Lactate dehydrogenase, WBC: White blood cells, Hb: Hemoglobin, PLT: Platelet, CRP: C-reacted protein, NLR: Neutron–lymphocyte ratio, FDP: Fibrin degradation product, PT-INR: Prothrombin time-International Normalized Ratio, CPK: Creatinine phosphor kinase, BNP: Brain natriuretic peptide.
P-value*: Molnupiravir alone group and molnupiravir + CAM group on the 5th day.
P-value**: Molnupiravir alone group and molnupiravir + CAM group on the 14th day.
P-value^{1}: The time of admission and on the 5th day from the start of admission.
P-value^{2}: The time of admission and on the 14th day from the start of admission.
Notes: The cutoff values were as follows: LDH (115 – 245 IU/L), total cholesterol (130 – 219 mg/dl), triglyceride (30 – 149 mg/dl), uric acid (2.5 – 7.0 mg/dl), creatinine (0.47 – 0.79 mg/dl), potassium (3.4 – 5.0 mEq/L), WBC (35 – 90×10^{2}/μl), Hb (11.5 – 15.2 g/dl), PLT (12 – 40×10^{4}/μl), CRP (<0.3 mg/dl), neutrophils (28 – 68%), lymphocytes (18 – 51%), NLR (0.55 – 3.78), fibrinogen (150 – 400 mg/dl), FDP (<4 μg/ml), D-dimer (<1.0 μg/ML), PT-INR (0.8 – 1.2), CPK (45 – 163 IU/L), BNP (<18.4 pg/ml)

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Supplementary Data 3

Table 2. Baseline levels of immunological biomarkers at admission, 5 days, and 14 days after infection in the molnupiravir alone, molnupiravir plus clarithromycin (CAM), and unaffected groups

Immunological biomarkers	Admission	Day 5	*p*-value[1]	Day 14	*p*-value[2]
IgG (mg/dl)					
Uninfected	1310.1±304.7	-		-	
Molnupiravir + CAM	-	1460±326.3	n.s.	1532.75±500.3	n.s.
Molnupiravir	-	1445.5±303.1	n.s.	1321.3±409.8	n.s.
P-value*		n.s.		n.s.	
IgA (mg/dl)					
Uninfected	509.7±183.6	-		-	
Molnupiravir + CAM	-	465.3±146.6	<0.05	536±112.7	<0.05
Molnupiravir	-	400.2±178.4	<0.05	385.2±159.7	n.s.
P-value*		n.s.		<0.05	
IgM (mg/dl)					
Uninfected	106.0±63.8	-		-	
Molnupiravir + CAM	-	105.4±34.7	n.s.	128.4±58.6	<0.05
Molnupiravir	-	94.2±63.7	n.s.	103.4±90.4	n.s.
P-value*		n.s.		n.s.	
sIL2R (U/dl)					
Uninfected	502.3±199.9	-		-	
Molnupiravir + CAM	-	661.2±158.6	<0.05	552.5±188.0	<0.05
Molnupiravir	-	732.3±500.3	<0.05	792.8±383.3	n.s.
P-value*		n.s.		<0.05	

Abbreviations: CAM: Clarithromycin, n.s.: No significant difference, Ig: Immunoglobulin, sIL2R: Soluble interleukin 2-receptor.

P-value[1]: Admission in unaffected patients and on the 5th day from the start of admission; administration at the time of infection (molnupiravir [5 days] + CAM [3 days], molnupiravir [5 days]).

P-value[2]: 5th day and 14th day from the start of admission; administration after infection (molnupiravir [5 days] + CAM [3 days], molnupiravir [5 days]).

P-value*: Molnupiravir + CAM group and molnupiravir alone group.

Notes: IgA, IgG, and IgM levels were measured using the immunoturbidimetric method, in which the antibody reacts with an antigen to form an immune complex precipitate; the aggregate is irradiated with light, and the attenuation (absorbance) of the irradiated light due to scattering is automatically detected (SRL Inc., Tokyo, Japan). The serum concentrations of sIL2R were determined using a chemiluminescent enzyme immunoassay (SRL Inc.). The cutoff values were as follows: IgG (870 – 1700 mg/dl), IgA (110 – 410 mg/dl), IgM (46 – 260 mg/dl), and sIL2R (157 – 474 U/ml). Data are presented as means±SD. The *P*-values were determined using Student's t-test. A *p*-value <0.05 was considered significant. SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

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Supplementary Data 4

Table 3a. Baseline characteristics of COVID-19 patients receiving molnupiravir plus clarithromycin or molnupiravir alone

Characteristics	Molnupiravir+ CAM (*n*=54)	Molnupiravir (*n*=37)	*P*-value
Female gender	26 (48.1%)	23 (62.2%)	n.s.
Age (years)	78 (49 – 96)	76 (67 – 100)	n.s.
Male gender	28 (51.9%)	14 (37.8%)	n.s.
Age (years)	74 (49 – 91)	73 (64 – 96)	n.s.
Baseline symptoms			
Fever	54 (100%)	0 (0%)	<0.05
Cough and sputa	53 (98.1%)	0 (0%)	<0.05
Fatigue	42 (77.8%)	21 (56.7%)	n.s.
Shortness of breath	36 (66.7%)	0 (0%)	<0.05
Chest tightness	32 (59.2%)	0 (0%)	<0.05
Dyspnea (93% < SpO_2< 96%)	30 (55.6%)	0 (0%)	<0.05
Nausea	21 (38.9%)	19 (51.3%)	n.s.
Vomit	17 (31.5%)	11 (29.7%)	n.s.
Headache	6 (11.1%)	4 (10.8%)	n.s.
Chills	5 (9.2%)	0 (0%)	n.s.
Diarrhea	3 (5.5%)	2 (5.4%)	n.s.
Hair removal	3 (5.5%)	1 (2.7%)	n.s.
Cingulum	2 (3.7%)	1 (2.7%)	n.s.
Skin rash	2 (3.7%)	2 (5.4%)	n.s.
Conjunctiva inflammation	1 (1.8%)	1 (2.7%)	n.s.
Myalgia	1 (1.8%)	0 (0%)	n.s.
Abdominal pain	1 (1.8%)	0 (0%)	n.s.
Blood cough	1 (1.8%)	0 (0%)	n.s.
Charlson index	25 (46.2%)	16 (43.2%)	n.s.
Baseline comorbidity			
Cardiovascular disease[1]	51 (94.4%)	34 (91.9%)	n.s.
Cerebrovascular disease[2]	42 (77.8%)	32 (86.5%)	n.s.
Respiratory diseases[3]	38 (70.3%)	0 (0%)	<0.05
Orthopedic disease[4]	36 (66.7%)	28 (75.7%)	n.s.
Hypertension	32 (59.2%)	26 (70.3%)	n.s.
Diabetes mellitus	28 (51.8%)	15 (40.5%)	n.s.
Cancer[5]	17 (31.5%)	13 (35.1%)	n.s.
Chronic renal failure	9 (16.6%)	8 (21.6%)	n.s.
Vaccination	51/54 (94.4%)	35/37 (94.6%)	n.s.
Within 6 months before admission	49/51 (96.1%)	33/35 (94.2%)	n.s.
1 vaccination	47/49 (95.9%)	31/33 (93.9%)	n.s.
2 vaccinations	2/49 (4.0%)	2/33 (6.1%)	n.s.
Within 3 months before admission	2/51 (3.9%)	2/35 (5.7%)	n.s.
1 vaccination	1/2 (50%)	1/2 (50%)	n.s.
2 vaccinations	1/2 (50%)	1/2 (50%)	n.s.

Abbreviations: CAM: Clarithromycin, n.s.: No significant difference.

[1]Heart failure, myocardial infarction, angina pectoris, arrhythmia, dilatation of the myocarditis, dissecting aneurysm of aorta

[2]Cerebral infarction, brain hemorrhage, subarachnoid hemorrhage, cerebral aneurysm

[3]Chronic obstructive pulmonary disease, chronic bronchitis, chronic interstitial pneumonia, bronchial asthma

[4]Fracture, prosthesis replacement

[5]Esophageal cancer, gastric cancer, pancreatic cancer, liver cancer, cholangiocarcinoma, colorectal cancer, lung cancer, breast cancer, ovarian cancer, laryngeal cancer, tongue cancer, meningioma

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Table 3b. Baseline characteristics of COVID-19 patients receiving molnupiravir plus clarithromycin (CAM) or uninfected patients

Characteristics	Molnupiravir + CAM (n=54)	Uninfected (n=28)	*P*-value
Female gender	26 (48.1%)	12 (42.8%)	n.s.
Age (years)	78 (49 – 96)	76 (69 – 100)	n.s.
Male gender	28 (51.9%)	16 (57.1%)	n.s.
Age (years)	74 (49 – 91)	73 (74 – 100)	n.s.
Baseline symptoms			
Fever	54 (100%)	0 (0%)	<0.05
Cough and sputa	53 (98.1%)	0 (0%)	<0.05
Fatigue	42 (77.8%)	0 (0%)	<0.05
Shortness of breath	36 (66.7%)	0 (0%)	<0.05
Chest tightness	32 (59.2%)	0 (0%)	<0.05
Dyspnea (93% < SpO_2< 96%)	30 (55.6%)	0 (0%)	<0.05
Nausea	21 (38.9%)	0 (0%)	<0.05
Vomit	17 (31.5%)	0 (0%)	<0.05
Headache	6 (11.1%)	0 (0%)	<0.05
Chills	5 (9.2%)	0 (0%)	<0.05
Diarrhea	3 (5.5%)	0 (0%)	<0.05
Hair removal	3 (5.5%)	0 (0%)	<0.05
Cingulum	2 (3.7%)	0 (0%)	<0.05
Skin rash	2 (3.7%)	0 (0%)	<0.05
Conjunctiva inflammation	1 (1.8%)	0 (0%)	<0.05
Myalgia	1 (1.8%)	0 (0%)	<0.05
Abdominal pain	1 (1.8%)	0 (0%)	<0.05
Blood cough	1 (1.8%)	0 (0%)	<0.05
Charlson index	25 (46.2%)	0 (0%)	<0.05
Baseline comorbidity			
Cardiovascular disease[1]	51 (94.4%)	24 (85.7%)	n.s.
Cerebrovascular disease[2]	42 (77.8%)	23 (82.1%)	n.s.
Respiratory diseases[3]	38 (70.3%)	19 (67.9%)	n.s.
Orthopedic disease[4]	36 (66.7%)	22 (78.6%)	n.s.
Hypertension	32 (59.2%)	21 (65.6%)	n.s.
Diabetes mellitus	28 (51.8%)	18 (64.3%)	n.s.
Cancer[5]	17 (31.5%)	15 (53.6%)	n.s.
Chronic renal failure	9 (16.6%)	11 (39.3%)	<0.05
Vaccination	51/54 (94.4%)	25/28 (89.3%)	n.s.
Within 6 months before admission	49/51 (96.1%)	21/25 (84%)	n.s.
1 vaccination	47/49 (95.9%)	19/21 (90.4%)	n.s.
2 vaccinations	2/49 (4.0%)	1/21 (4.7%)	n.s.
Within 3 months before admission	2/51 (3.9%)	1/25 (4.0%)	n.s.
1 vaccination	1/2 (50%)	0/1 (0%)	<0.05
2 vaccinations	1/2 (50%)	0/1 (0%)	<0.05

Abbreviations: CAM: Clarithromycin, n.s.: No significant difference.

[1]Heart failure, myocardial infarction, angina pectoris, arrhythmia, dilatation of the myocarditis, dissecting aneurysm of aorta

[2]Cerebral infarction, brain hemorrhage, subarachnoid hemorrhage, cerebral aneurysm

[3]Chronic obstructive pulmonary disease, chronic bronchitis, chronic interstitial pneumonia, bronchial asthma

[4]Fracture, prosthesis replacement

[5]Esophageal cancer, gastric cancer, pancreatic cancer, liver cancer, cholangiocarcinoma, colorectal cancer, lung cancer, breast cancer, ovarian cancer, laryngeal cancer, tongue cancer, meningioma.

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Table 3c. Baseline characteristics of COVID-19 patients receiving molnupiravir alone or uninfected patients

Characteristics	Molnupiravir (*n*=37)	Uninfected (*n*=28)	*P*-value
Female gender	23 (62.2%)	12 (42.8%)	n.s.
Age (years)	76 (67 – 100)	76 (69 – 100)	n.s.
Male gender	14 (37.8%)	16 (57.1%)	n.s.
Age (years)	73 (64 – 96)	73 (74 – 100)	n.s.
Baseline symptoms			
Fever	0 (0%)	0 (0%)	n.s.
Cough and sputa	0 (0%)	0 (0%)	n.s.
Fatigue	21 (56.7%)	0 (0%)	<0.05
Shortness of breath	0 (0%)	0 (0%)	n.s.
Chest tightness	0 (0%)	0 (0%)	n.s.
Dyspnea (93% < SpO_2 < 96%)	0 (0%)	0 (0%)	n.s.
Nausea	19 (51.3%)	0 (0%)	<0.05
Vomit	11 (29.7%)	0 (0%)	<0.05
Headache	4 (10.8%)	0 (0%)	<0.05
Chills	0 (0%)	0 (0%)	n.s.
Diarrhea	2 (5.4%)	0 (0%)	<0.05
Hair removal	1 (2.7%)	0 (0%)	<0.05
Cingulum	1 (2.7%)	0 (0%)	<0.05
Skin rash	2 (5.4%)	0 (0%)	<0.05
Conjunctiva inflammation	1 (2.7%)	0 (0%)	<0.05
Myalgia	0 (0%)	0 (0%)	n.s.
Abdominal pain	0 (0%)	0 (0%)	n.s.
Blood cough	0 (0%)	0 (0%)	n.s.
Charlson index	16 (43.2%)	0 (0%)	<0.05
Baseline comorbidity			
Cardiovascular disease[1]	34 (91.9%)	24 (85.7%)	n.s.
Cerebrovascular disease[2]	32 (86.5%)	23 (82.1%)	n.s.
Respiratory diseases[3]	0 (0%)	19 (67.9%)	n.s.
Orthopedic disease[4]	28 (75.7%)	22 (78.6%)	n.s.
Hypertension	26 (70.3%)	21 (65.6%)	n.s.
Diabetes mellitus	15 (40.5%)	18 (64.3%)	n.s.
Cancer[5]	13 (35.1%)	15 (53.6%)	n.s.
Chronic renal failure	8 (21.6%)	11 (39.3%)	n.s.
Vaccination	35/37 (94.6%)	25/28 (89.3%)	n.s.
Within 6 months before admission	33/35 (94.2%)	21/25 (84%)	n.s.
1 vaccination	31/33 (93.9%)	19/21 (90.4%)	n.s.
2 vaccinations	2/33 (6.1%)	1/21 (4.7%)	n.s.
Within 3 months before admission	2/35 (5.7%)	1/25 (4.0%)	n.s.
1 vaccination	1/2 (50%)	0/1 (0%)	<0.05
2 vaccinations	1/2 (50%)	0/1 (0%)	<0.05

Abbreviation: n.s.: No significant difference

[1]Heart failure, myocardial infarction, angina pectoris, arrhythmia, dilatation of the myocarditis, dissecting aneurysm of aorta

[2]Cerebral infarction, brain hemorrhage, subarachnoid hemorrhage, cerebral aneurysm

[3]Chronic obstructive pulmonary disease, chronic bronchitis, chronic interstitial pneumonia, bronchial asthma

[4]Fracture, prosthesis replacement

[5]Esophageal cancer, gastric cancer, pancreatic cancer, liver cancer, cholangiocarcinoma, colorectal cancer, lung cancer, breast cancer, ovarian cancer, laryngeal cancer, tongue cancer, meningioma

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Supplementary Data 5

Table 4. Risk predictive factors associated with clinical outcome in COVID-19 patients treated with molnupiravir alone and molnupiravir plus clarithromycin analyzed using a Cox proportional hazards model

Risk predictive factors	Univariate analysis*		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Biochemistry biomarkers				
Total cholesterol	0.46 (0.31 – 0.61)	<0.05		
Triglyceride	0.51 (0.46 – 0.56)	<0.05		
Uric acid	0.56 (0.45 – 0.67)	<0.05		
Blood routine biomarkers				
Hemoglobin	0.37 (0.25 – 0.49)	<0.01		
Platelet	0.41 (0.38 – 0.44)	<0.05		
Inflammatory biomarkers				
C-reactive protein	1.37 (1.29 – 1.45)	<0.01		
Neutrophils	1.25 (1.16 – 1.34)	<0.05		
Lymphocytes	0.33 (0.28 – 0.38)	<0.05		
Coagulation biomarkers				
Fibrinogen	1.37 (1.23 – 0.51)	<0.05		
FDP	1.14 (1.02 – 1.26)	<0.05		
D-dimer	1.32 (1.24 – 1.40)	<0.05	1.08 (1.05 – 1.11)	<0.05
Cardiac biomarkers				
CPK	1.36 (1.23 – 1.49)	<0.05		
BNP	1.03 (1.01 – 1.05)	<0.05		
Immunological markers				
IgA	1.32 (1.25 – 1.39)	<0.05	1.06 (1.02 – 1.10)	<0.05
sIL2R	0.46 (0.37 – 0.55)	<0.05	1.13 (1.09 – 1.17)	<0.05

Abbreviations: FDP: Fibrin degradation product; CPK: Creatinine phosphor kinase; BNP: Brain natriuretic peptide; Ig: Immunoglobulin; sIL2R: Soluble interleukin 2-receptor. *A total of 23 biomarkers were tested in the survival analysis and only variables with a significant hazard ratio (HR) at the significance level of $P<0.05$ are listed in table.

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Supplementary Data 6

Table 5. Comparison of severity of sequelae 12 months after COVID-19 treatment with molnupiravir+clarithromycin (CAM) or molnupiravir alone

Sequelae	Molnupiravir + CAM (*n*=54)	Molnupiravir (*n*=37)	*P*-value
Baseline symptoms			
Fever	0 (0%)	0 (0%)	n.s.
Fatigue	17 (31.5%)	13 (35.1%)	n.s.
Chest pain	6 (11.1%)	5 (13.5%)	n.s.
Dyspnea (93% < SpO_2 < 96%)	10 (18.5%)	8 (21.6%)	n.s.
Nausea	2 (3.7%)	2 (5.4%)	n.s.
Headache	8 (14.8%)	6 (16.2%)	n.s.
Sleep disorder	15 (27.8%)	11 (29.7%)	n.s.
Depression	12 (22.2%)	9 (24.3%)	n.s.
Anxiety	14 (25.9%)	10 (27.0%)	n.s.
Palpitations	4 (7.4%)	4 (10.8%)	n.s.
Effort intolerance	23 (42.6%)	16 (43.2%)	n.s.
Diarrhea	2 (3.7%)	2 (5.4%)	n.s.
Hair loss	4 (7.4%)	3 (8.1%)	n.s.
Loss of smell	9 (16.7%)	7 (18.9%)	n.s.
Loss of taste	6 (11.1%)	5 (13.5%)	n.s.
Cingulum	1 (1.9%)	2 (5.4%)	n.s.
Skin rash	0 (0%)	0 (0%)	n.s.
Conjunctiva inflammation	0 (0%)	0 (0%)	n.s.
Myalgia	10 (18.5%)	8 (21.6%)	n.s.
Joint pain	12 (22.2%)	9 (24.3%)	n.s.
Baseline comorbidity			
Cardiovascular disease[1]	53 (98.1%)	35 (94.6%)	n.s.
Cerebrovascular disease[2]	43 (80.0%)	33 (89.2%)	n.s.
Respiratory diseases[3]	39 (72.2%)	2 (5.4%)	n.s.
Orthopedic disease[4]	37 (68.5%)	29 (78.4%)	n.s.
Hypertension	34 (62.9.2%)	28 (75.7%)	n.s.
Diabetes mellitus	29 (53.7%)	18 (48.6%)	n.s.
Renal failure	10 (18.5%)	9 (24.3%)	n.s.
Skeletal muscle damages[5]	27 (50.0%)	19 (51.4%)	n.s.
Venous thromboembolism	13 (24.1%)	21 (56.8%)	<0.05
Lower urinary tract symptoms[6]	29 (53.7%)	20 (54.1%)	n.s.

Abbreviations: CAM: Clarithromycin; n.s.: No significant difference.
[1]Heart failure, myocardial infarction, angina pectoris, arrhythmia, dilatation of the myocarditis, dissecting aneurysm of aorta.
[2]Cerebral infarction, brain hemorrhage, subarachnoid hemorrhage, cerebral aneurysm.
[3]Chronic obstructive pulmonary disease, chronic bronchitis, chronic interstitial pneumonia, bronchial asthma.
[4]Fracture, prosthesis replacement.
[5]Reduction in the maximal voluntary contraction for quadriceps and biceps in recovering patients (54% and 69% of the predicted normal value, respectively).
[6]Postvoiding residual urine and voiding volume

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ORIGINAL ARTICLE

Effects of motor imagery and action observation on respiratory function in mild smokers: a randomized single-blind controlled pilot trial

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ABSTRACT

Background: Motor imagery (MI) and action observation (AO) training can activate brain areas involved in planning, adjusting, and automating voluntary movement in a manner similar to that when these activities are being performed.
Aim: The main objective of this study was to assess the effects of MI and AO training on respiratory function in mild smokers.
Methods: A single-blind placebo-controlled pilot trial was designed. A total of 27 mild smokers were randomized into three groups: MI (n = 9), AO (n = 9), and sham observation (SO; n = 9) groups. The MI and AO groups performed mental training of breathing exercises while the SO group observed a landscape without a human agent. The primary outcomes were pulmonary function parameters (forced expiratory volume during the 1st s [FEV_1], forced vital capacity [FVC], FEV_1/FVC ratio, maximum voluntary ventilation [MVV], and peak expiratory flow [PEF]), and the secondary outcomes were maximal inspiratory/expiratory pressures (MIP/MEP) and perceived fatigue. All outcome measures were assessed at baseline and post-intervention.
Results: Regarding the pulmonary function parameters, only the AO group showed significant within-group differences in FEV_1 (mean differences [MD] = 0.37 L (0.17 – 0.56), P = 0.001), FVC (MD = 0.1 L (0.02 – 0.16), P = 0.008), and PEF (MD = 0.74 L/s (0.29 – 1.18), P = 0.002) with a small-to-moderate effect size. No differences were found in FEV_1/FVC ratio and MVV. With regard to the maximal static pressures, only the AO group showed significant within-group differences in MEP with a small effect size (MD = 11.22 cm H_2O (0.19 – 22.2), P = 0.046). Finally, both AO and MI groups showed significantly greater perceived fatigue with regard to SO group with a large effect size (P < 0.05).
Conclusion: AO training has a slight impact on some pulmonary function parameters, such as FEV_1, FVC, or PEF, as well as on MEP when applied in isolation and in a single session.
Relevance for Patients: Although it is still early to draw some solid conclusions, AO training could be used in combination with respiratory exercises to see if the effect is greater than exercises in isolation. The study of movement representation strategies on pulmonary function is a field that has been sparingly explored so far. This paper offers some interesting data to be considered for further research.

1. Introduction

Motor imagery (MI) is defined as the creation and maintenance of a movement image without actually executing it [1]. In addition, action observation (AO) training is defined as the real-time visualization of a motion image without actually performing it [2]. Both neurosensory-motor training tools cause an activation of the cortical areas related to the planning, adjustment, and automation of voluntary movement that is qualitatively equal to,

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but quantitatively less than, the action actually being performed [3]. Regarding the neurophysiology behind these neurosensory-motor training tools, there appears to be an overlap in the activity of some brain areas during MI, AO, and actual performance [4]. Hardwick *et al.* [4] found that MI and AO training recruited similar premotor-parietal cortical networks but, while MI recruited a subcortical network similar to that found during actual movement execution, AO training showed no activity in any subcortical area.

In addition, AO training and MI generation processes can be carried out in different modalities. Both methods of movement representation can be implemented in two perspectives. First, there is the first-person perspective, where the person observes or imagines him/herself showing his/her own point of view. On the other hand, based on the third-person perspective, the person observes or imagines him/herself from the outside, as an external observer. Both forms have been described and studied in the scientific literature [5-11]. In addition to the first-person or third-person perspective, also called internal or external perspective, respectively, MI is specifically subclassified into two other modalities, namely visual MI and kinesthetic MI [12,13]. Theoretically, the differences between these two modalities of construction and generation of MI lie in their execution. On the one hand, in kinesthetic MI, the ability to feel is incorporated at the same time as the MI task is performed, causing, at the neurophysiological level, some differences with respect to visual MI [14]. For example, during kinesthetic MI, there is a greater increase in electromyographic activity than in the visual modality [15]. These findings were also found in the stimulation of the corticospinal system evaluated by neuroimaging [16]. Even at the level of neurovegetative system activity, the kinesthetic modality has also been found to elicit higher levels of heart rate, respiratory rate, skin conductance, *etc.* [17,18]. Visual MI refers to creating a motor image being, therefore, a representation devoid of any stimulation of the somatosensory system [12,14].

Interest in the study of the effects of MI and AO training on some sensorimotor variables has grown in recent years. For example, Cuenca-Martínez *et al.* [19] found that adding MI to an usual treatment improved active range of motion in patients subjected to immobilization. Furthermore, they also showed that MI maintained significantly greater strength and speed in patients undergoing surgery [19]. In addition to this, it has been found that adding MI to an usual treatment improved pain intensity and strength to a greater degree than usual treatment alone in patients undergoing a total knee arthroplasty [20]. Both techniques have been shown to improve the motor learning process both in isolation [21,22] and in combination with physical exercise [23]. Losana-Ferrer *et al.* [24] found that both AO and MI, in combination with actual practice, elicited higher levels of strength as well as electromyographic activity than physical practice in isolation. This increase in strength has also been found when AO and MI training were combined in isolation, without the presence of actual practice [25]. It seems therefore that MI and AO training, both in isolation and in combination with physical practice, leads to improvements in some clinical variables of interest.

Physical training has been widely used in respiratory rehabilitation. In fact, some systematic reviews have shown that respiratory muscle training improves several pulmonary function parameters and maximal static pressures in some clinical populations such as patients with chronic obstructive pulmonary disease [26], lung cancer survivors [27], asthma [28], obstructive sleep apnea [29], or tobacco smokers [30,31]. Therefore, we believe that the addition of mental practice along with the performance of respiratory muscle training could have an impact on these clinical populations. However, it is too early to be certain of this statement. To date, we believe that there is no study that has evaluated the effect of MI and AO on pulmonary function parameters and maximal static pressures. There are a few studies that have evaluated the effect of MI on breath-holding performance [32,33]. Therefore, we set out the following pilot study with the aim to evaluate the effect of MI and AO training in isolation to see if it had any impact on maximal static respiratory pressures and several pulmonary function parameters. The authors hypothesize that mental practice in isolation may have a significant impact on these variables and in future studies, it could be combined with respiratory muscle training to see if it increases its clinical effect.

Because there are currently no studies that aim to assess the impact of movement representation techniques in isolation on pulmonary function, the main aim of this pilot study was to assess the effects of MI and AO in isolation on respiratory function in mild smokers.

2. Methods

2.1. Study design

This study was a randomized, single-blind, placebo-controlled pilot trial, which was planned and conducted in accordance with Consolidated Standards of Reporting Trials (CONSORT) requirements and was approved by The Ethics Committee of Research in Humans of the Ethics Commission in Experimental Research of University of Valencia (number: 2301127). This study was registered in the United States Randomized Trials Registry on clinicaltrial.gov (trial registry number: NCT05662072). All the participants were briefed on the study procedures, which were planned according to the ethical standards of the Helsinki Declaration.

2.2. Participants

All data were collected at the University of Valencia (between November 2022 and February 2023) by email and social networks. All participants were currently smokers aged >18 years and had a pack per year index of <5 (mild smoking index). This population was chosen because we were looking for a population as close as possible to healthy subjects but with room for improvement in the assessment tests. This study excluded those who presented a respiratory pathology, cardiac, systematic (hypertension, diabetes, viral infections, *etc.*), or metabolic disease, history of recent surgery (in the last year), vertebral fracture, or osteoarticular disorders of the spine area.

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2.3. Randomization

Randomization was performed using a computer-generated random sequence table with a balanced three-block design (GraphPad Software, Inc., CA, USA). An independent researcher generated the randomization list, and a member of the research team who was not involved in the assessment of the participants or the intervention was in charge of the randomization and maintained the list. The patients included were randomly assigned to one of the three groups using the random sequence list, ensuring concealed allocation.

2.4. Blinding

The assessments and interventions were performed by different physical therapists. The evaluator was blinded to the participants' group assignment. All the intervention procedures were performed by the same physical therapist who had experience in the field and was blinded to the purpose of the study. All participants were blinded to their group allocation.

2.5. Interventions

2.5.1. MI

The participants who carried out the MI training performed 10 sets of 1 min per set. In each minute of imagining, participants had to imagine themselves, as the first person, kinesthetically (*i.e.*, trying to feel at all times what they were imagining), forcibly taking in air and pulling it out by inflating a balloon as hard as they could. The imagination process lasted for an uninterrupted duration of 50 s. For the remaining 10 s, participants had to imagine taking in as much air as possible by expanding their chest box as much as they could to perform a forced expiration technique (high expiratory flow technique known as FET). During the intervention, the physical therapist gave small neutral guidelines such as "*keep imagining*," or "*try to feel what you are imagining*" (Figure 1).

2.5.2. AO

The participants in the AO group performed the same exercise intervention as the MI group, but instead of imagining the motor gestures, they had to observe a person performing the respiratory exercises. The duration and distribution of the intervention were the same as in the MI group (10 sets of 1 min per set) (Figure 1).

2.5.3. Sham observation (SO)

Participants in this group underwent a SO protocol. A video only composed of nature images was visualized for 10 min, without visualizing any motor gesture. This kind of SO protocol has been used in previous research [34,35] (Figure 1).

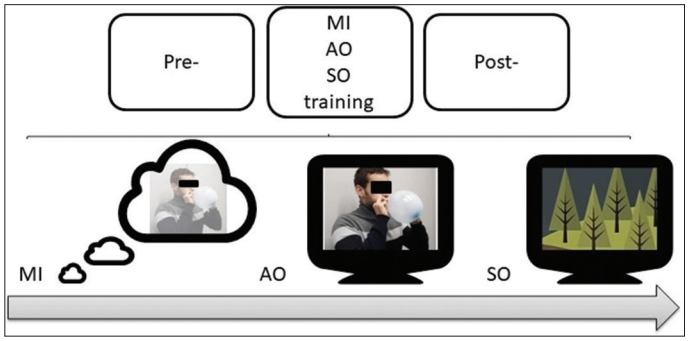

Figure 1. An illustration of the intervention.
Abbreviations: AO: Action observation; MI: Motor imagery; SO: Sham observation.

2.6. Smoking index

The pack per year index is a smoking load tool for lifetime tobacco exposure. A pack per year index is defined as 20 cigarettes smoked every day for 1 year. It was calculated using the formula (number of smoked cigarettes per day × number of years smoking)/20 [36]. The levels of the smoking index were mild (<5 packs), moderate (5 – 15 packs), or strong (>16 packs).

2.7. Outcome measures

2.7.1. Baseline variables

(A) Physical activity levels

The level of physical activity was objectified through the International Physical Activity Questionnaire (IPAQ), which allows the participants to be divided into three groups according to their level of activity: high, moderate, and low or inactive [37]. This questionnaire has shown acceptable validity and psychometric properties to measure total physical activity. Therefore, the psychometric properties of the questionnaire were accepted for use in studies that required the measurement of physical activity; reliability was approximately 0.65 (r = 0.76; 95% CI 0.73 – 0.77) [38].

(B) Imagery ability

The movement imagery questionnaire-revised (MIQ-R) is an 8-item self-report inventory used to assess visual and kinesthetic MI ability. Four different movements are included in the MIQ-R, which comprises four visual and four kinesthetic items. Each participant rated the ease or difficulty of generating the mental image on a 7-point scale in which 7 indicated "very easy to see/feel" and 1 "very difficult to see/feel." The internal consistencies of the MIQ-R have been adequate, with Cronbach's α coefficients ranging above 0.84 for the total scale, 0.80 for the visual subscale, and 0.84 for the kinesthetic subscale [39].

2.7.2. Primary outcomes

(A) Pulmonary function

Pulmonary function was assessed by performing forced spirometry (Spirodoc, Medical International Research, Roma, Italy) following the American Thoracic Society's (ATS) criteria [40] to obtain the following parameters: forced expiratory volume during the 1st s (FEV_1), forced vital capacity (FVC), forced expiratory ratio (FEV_1/FVC), maximum voluntary ventilation (MVV), and peak expiratory flow (PEF). The patient was seated in a chair with the backrest supporting his back, and during the

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respiratory maneuvers required to perform spirometry, nasal clips were placed to prevent air leakage through the nose. The participant was then instructed to undertake an initial maximal inspiration to reach total lung capacity, followed by a forced maximal expiration for at least 6 seconds until its expiratory limit was reached. To ensure proper test execution, the maneuver was repeated at least three times (up to a maximum of eight times), with a 1-min break in between repetitions. As advised by the ATS, spirometry maneuvers with performance artifacts or variations of more than 0.150 L between the highest FEV_1 and/or FVC values were discarded. The three repeats' greatest value was recorded (Figure 2).

2.7.3. Secondary outcomes

(A) Maximal inspiratory (MIP)/expiratory pressure (MEP)

The MIP and MEP pressures were measured using a digital respiratory dynamometer (MicroRPM, CareFusion, Basingstoke, UK) [41]. To minimize air leakage through the nose during testing, nasal clips were placed on the subjects who were seated. Patients were instructed to exert their hardest possible inhalation and exhalation efforts and hold them for at least 1.5 seconds. To obtain the maximum value of three maneuvers with <10% variation, MIP was evaluated at residual volume and MEP at total lung capacity according to the ATS statement [41] (Figure 2).

(B) Perceived fatigue

We employed the Visual Analog Scale of fatigue (VAS-f) to quantify the participants' perceived fatigue after performing the training session. The VAS-f uses an analog scale of 0 – 100 mm, with 0 representing minimum fatigue (no fatigue) and 100 representing maximum fatigue. The VAS-f scale is useful, sensitive, and easy to apply [42].

2.8. Procedures

Each participant completed an informed consent document to participate in the study, in addition to a set of questionnaires to complete before starting the intervention. These questionnaires included the IPAQ form and a questionnaire about age, gender, weight, height, and smoking index. Then, MIQ-R was assessed. Each participant was then seated and underwent an assessment of pre-intervention outcome measures (pulmonary function tests through forced spirometry and maximal static respiratory pressure). At this time and in a sitting position, patients performed the MI training, AO protocol, or SO according to the randomized allocation. Immediately after the intervention, a blinded evaluator measured all outcome measures (post-intervention). In addition, just at the end of the intervention, perceived mental training fatigue was also assessed.

Figure 2. An image of a participant performing the pulmonary function tests. On the left, the maximal static pressure strength is assessed. On the right, forced spirometry is performed.

2.9. Data analysis

The statistical data analysis was performed using statistical SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA). The normality of the variables was evaluated by the Shapiro – Wilk test. Descriptive statistics were used to summarize the data for continuous variables and are presented as mean ± standard deviation, with 95% confidence interval. The categorical variables are presented as absolute (number) and relative frequencies (percentage). A two-way repeated measures analysis of variance (ANOVA) was conducted to study the effect of the between-participant "intervention group" factor on each of the three categories (MI, AO, and SO) and the within-participant "time" factor, as well as on each of two categories (pre- and post-intervention) of all the dependent variables. A *post hoc* analysis with Bonferroni correction was performed in the case of significant ANOVA findings for multiple comparisons between variables. Effect sizes (*d*) were calculated according to Cohen's method, in which the magnitude of the effect was classified as small (0.20 – 0.49), moderate (0.50 – 0.79), or large (0.8) [43]. The α level was set at 0.05 for all tests. In addition, we compared the baseline variables between groups with a one-factor ANOVA to explore whether the groups were homogeneous at the start of the study. The perceived fatigue outcome measure was also explored with a one-factor ANOVA.

3. Results

A total of 27 mild smokers participants were included and were randomly allocated into three groups of 9 participants per group. All the variables presented a normal distribution. No statistically significant differences were found between groups for any of the primary variables, demographic data or self-report variables were present at baseline between the groups (Table 1). There were no adverse events reported in either group.

3.1. Pulmonary function

3.1.1. FEV_1

The ANOVA revealed significant changes in the FEV_1 (L) parameter during time ($F = 10.52$, $P = 0.003$, $\eta_p^2 = 0.305$) and also during group * time interaction ($F = 3.39$, $P = 0.049$, $\eta_p^2 = 0.221$). The *post hoc* analysis revealed significant within-group differences in the AO group with a moderate effect size

(mean differences [MD] = 0.37 L (0.17 – 0.56), P = 0.001, d = 0.51). In addition, the *post hoc* analysis revealed significant inter-group differences between the AO and MI group with a large effect size (MD = 0.724 L (0.07 – 1.37), P = 0.026, d = 1.41) (Figure 3).

Moreover, the ANOVA revealed significant changes in the FEV_1 (%) parameter during time (F = 6.74, P = 0.016, η_p^2 = 0.22) but not, during group * time interaction (F = 1.93, P = 0.16, η_p^2 = 0.11). The *post hoc* analysis revealed significant within-group differences in the AO group with a large effect size (MD = 8.55% (2.69 – 14.4), P = 0.006, d = 0.99). This implies that participants who underwent AO training significantly increased their expiratory air volume in the 1st s after the end of the intervention.

3.1.2. FVC

The ANOVA revealed significant changes in the FVC (L) parameter during time (F = 6.35, P = 0.019, η_p^2 = 0.20) but not, during group * time interaction (F = 1.68, P = 0.20, η_p^2 = 0.10). The *post hoc* analysis revealed significant within-group differences in the AO group with a trivial effect size (MD = 0.1 L (0.02 – 0.16), P = 0.008, d = 0.13).

Moreover, the ANOVA revealed significant changes in the FVC (%) parameter during time (F = 5.08, P = 0.033, η_p^2 = 0.17) but not, during group * time interaction (F = 1.19, P = 0.32, η_p^2 = 0.08). The *post hoc* analysis revealed significant within-group differences in the AO group also with a trivial effect size (MD = 1.89% (0.30 – 3.47), P = 0.021, d = 0.17). The results seem to show that the forcibly assessed vital capacity increased slightly in the participants who undertook AO training.

3.1.3. FEV1/FVC ratio

The ANOVA revealed no significant changes in the FEV_1/FVC ratio parameter during time (F = 3.2, P = 0.08, η_p^2 = 0.12) and during group * time interaction (F = 0.57, P = 0.56, η_p^2 = 0.04).

3.1.4. MVV

The ANOVA revealed no significant changes in the MVV parameter during time (F = 1.73, P = 0.20, η_p^2 = 0.06) and during group * time interaction (F = 0.51, P = 0.60, η_p^2 = 0.041).

3.1.5. PEF

The ANOVA revealed significant changes in the PEF parameter during time (F = 13.77, P = 0.001, η_p^2 = 0.36), but not during group * time interaction (F = 1.61, P = 0.21, η_p^2 = 0.11). The *post hoc* analysis revealed significant within-group differences in the AO group with a small effect size (MD = 0.74 L/s (0.29 – 1.18), P = 0.002, d = 0.42) (Figure 4). The results showed that peak exhaled airflow increased slightly after AO training.

3.2. Maximal respiratory pressure

3.2.1. MIP

The ANOVA revealed no significant changes in the MIP measurement during time (F = 0.35, P = 0.55, η_p^2 = 0.01) and during group * time interaction (F = 1.79, P = 0.18, η_p^2 = 0.13).

Table 1. Descriptive statistics of sociodemographic and baseline data

Measures	MI (n=9)	AO (n=9)	SO (n=9)	P-value
Age	21.6±3.6	24.6±4.2	20.9±1.1	0.057
BMI (kg/m^2)	24.5±3.7	21.6±3.8	23.9±2.5	0.173
Smoking index	2.3±1.5	2.2±1.7	1.4±1.0	0.314
IPAQ	2517.1±407.0	1861.6±397.7	2326.5±836.8	0.069
MIQR-T	46±4.6	47.0±4.0	46.5±4.7	0.895
MIQR-K	22.7±2.4	23.0±2.5	23.3±2.6	0.88
MIQR-V	23.2±2.4	24.0±1.8	23.2±2.5	0.71
Gender				0.09
Male	1 (11.1)	2 (22.2)	5 (55.6)	
Female	8 (88.9)	7 (77.8)	4 (44.4)	

Abbreviations: AO: Action observation; MI: Motor imagery; SO: Sham observation; m: Meter; kg: Kilogram; BMI: Body mass index; MIQR: Movement Imagery Questionnaire-Revised; T: Total; K: Kinesthetic subscale; V: Visual subscale; IPAQ: International physical activity questionnaire.

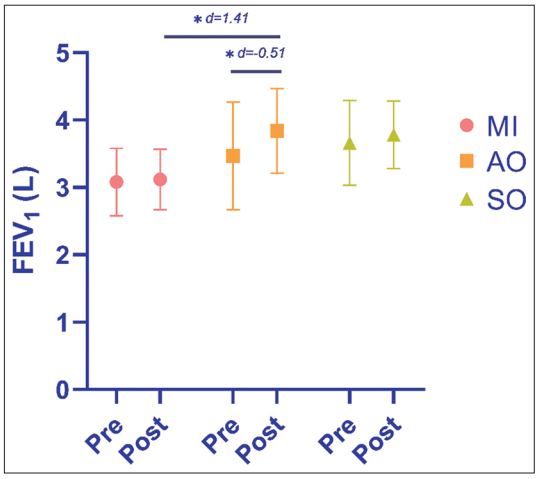

Figure 3. Results of FEV_1.
Abbreviations: FEV_1: Forced expiratory volume during the 1st s; L: liters; AO: Action observation; MI: Motor imagery; SO: Sham observation.

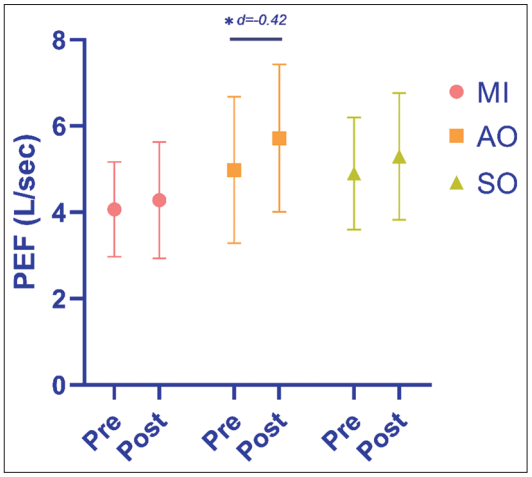

Figure 4. Results of PEF.
Abbreviations: PEF: Peak expiratory flow; L/s: Liters per second; AO: Action observation; MI: Motor imagery; SO: Sham observation.

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3.2.2. MEP

The ANOVA revealed significant changes in the MEP measurement during time (F = 3.95, $P = 0.048$, $\eta_p^2 = 0.144$) but not, during group * time interaction (F = 1.26, $P = 0.30$, $\eta_p^2 = 0.09$). The *post hoc* analysis revealed significant within-group differences in the AO group with a small effect size (MD = 11.22 cmH_20 (0.19 – 22.2), $P = 0.046$, $d = 0.33$). The MI group showed an increase in MEP variable, but it was not statistically significant (MD = 5.33 cmH_20 (−5.7 – 16.3), $P = 0.33$) (Figure 5). The results showed that peak expiratory pressure increased slightly after AO training.

3.3. Perceived fatigue

With regard the perceived fatigue, the one-way ANOVA showed statistically significant differences (F = 10.6, $P < 0.001$). The *post hoc* analysis showed statistically significant between-group differences in AO group in comparison with SO group and also in MI group in comparison with SO group both with a large effect size (MD = 17.5 (1.8 – 33.3), $P = 0.026$, $d = 1.45$, and MD = 28.0 (12.2 – 43.7), $P < 0.001$, $d = 2.58$, respectively), showing greater levels of perceived fatigue in mental practice groups (Figure 6).

3.4. Sample size calculation

The sample size was estimated with the program G * Power 3.1.7 for Windows (G * Power© from University of Dusseldorf, Germany) [44]. The sample size calculation was considered as a power calculation to detect between-group differences in a primary outcome measure (FEV_1). We considered 3 groups and 2 measurements for primary outcomes to obtain 95% statistical power (1-β error probability) with an α error level probability of 0.05 using ANOVA of repeated measures, between factors, and an effect size of $\eta_p^2 = 0.221$ obtained from our results. This generated a sample size of a total of 45 participants (15 per group).

4. Discussion

The main objective of this pilot study was to assess the effects of MI and AO in isolation on respiratory function in mild smokers. Regarding pulmonary function parameters, the results showed that AO training caused a significant increase in the FEV_1 pre-post-intervention as an absolute value with a moderate effect size. This result was not observed for either the MI group or the SO group. Furthermore, if we look at the FEV_1 value as a percentage of the theoretical values, the AO group showed a statistically significant pre-post intervention increase with a large effect size. This result was also not found in the MI and SO groups. Moreover, this increase in FEV_1 in absolute value was significantly greater than that found by the MI group at the post-intervention time. With respect to FVC, significant pre-post-intervention differences were found only in the AO group, although with an almost negligible effect size. Concerning the PEF parameter, only the AO group showed a significant pre-post-intervention increase with a small effect size. Neither the MI group nor the SO group showed significant intra-group differences in these variables. However, no significant differences were found in either group for FEV_1/FVC ratio parameter nor for MVV. Regarding the maximal static respiratory pressure, only the AO group showed statistically significant differences with respect to MEP with a small effect size. However, these differences were not statistically superior to the MI and SO groups. In relation to MIP, no significant differences were found in either intervention group. Finally, both mental training groups (AO and MI) showed greater perceived fatigue than the SO group, featuring differences with a large effect size.

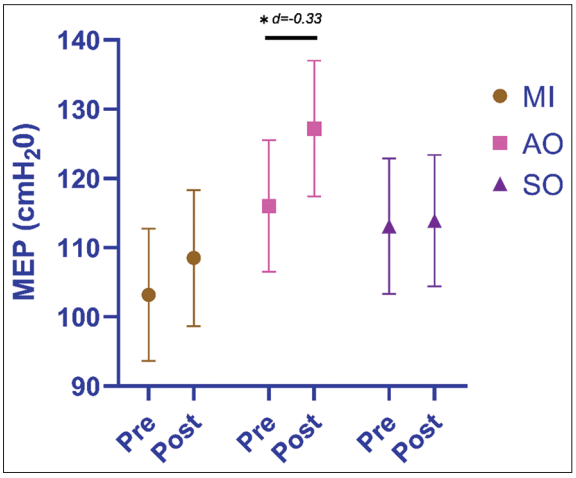

Figure 5. Results of maximal expiratory pressure variable. Abbreviations: MEP: Maximal expiratory pressure; AO: Action observation; MI: Motor imagery; SO: Sham observation; cmH_20: Centimeters of water pressure.

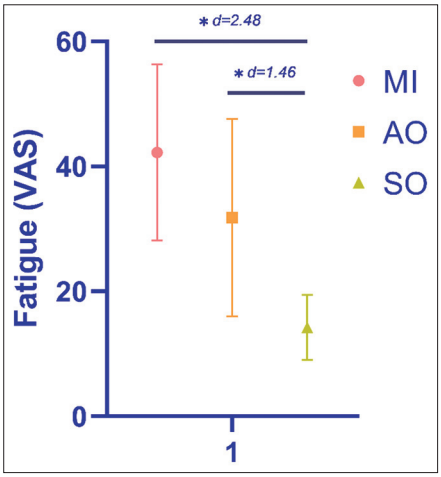

Figure 6. Results of post-intervention perceived fatigue. Abbreviations: VAS: Visual Analog Scale; AO: Action observation; MI: Motor imagery; SO: Sham observation.

These results seem to indicate that AO training has a slight impact on some pulmonary function parameters, as well as on MEP. It is likely that the improvement in MEP will translate into an improvement in some parameters of forced spirometry such as PEF or FEV_1. As the improvement in strength seems to be slight, the improvement in some pulmonary parameters also seems to be minimal. At this point, it is important to answer the question why mental training, such as AO training in isolation, could have an

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impact on maximal strength variables and pulmonary volumes and flows. Several research studies indicate that both mental practice techniques (MI and AO) provoke a neurophysiological activation of the areas related to the planning and adjustment of voluntary movement in a way very similar to when the execution is carried out [3,4,45]. This is due to the activity of mirror neurons, discovered by Rizzolatti *et al.* in the 1990s [46]. This mirror neuron system seems to function more efficiently through AO training than through MI, as it is less demanding, in terms of cognitive load, to maintain an image than to create and also maintain it [45]. This could be a justification for why AO training elicits greater changes than MI when both are applied in isolation. In previous research, we found that AO elicits greater and longer-lasting motor learning than MI [21], as well as a better sense of short-term cervical joint repositioning [22]. With respect to the other variables, AO training appears to lead to greater pain modulation, as well as greater heart rate response in patients with cervical pain, as compared with MI [47]. In addition, Cuenca- Martínez *et al.* [45] commented that some variables could influence the process of building a movement image, such as motor experience. The musculature involved in breathing seems difficult to train, and therefore, visual input could be more effective than direct imagination when a motor gesture is complex to perform, as could be the training of the respiratory musculature, both at tidal volume and in a forced manner. This could also partly justify why the MI group did not show intra-group differences. Movement is a cortical expression because it is planned before it is executed. The voluntary initiation of both imagined/observed and actual action is linked to breathing. It is suggested that the respiratory system is involved in these processes of voluntary movement planning regardless of whether it culminates in overt movements [48].

Perceived fatigue was also assessed, with the aim of confirming that the participants undergoing mental practice training, specifically the MI group, were actually performing the MI protocol. It has been widely reported that mental fatigue could be the main determinant of MI [45,49], because the person would stop imagining in conditions of high mental fatigue, especially in motor gestures with great difficulty, or if the time of the imagining task is maintained in a sustained manner. This was also argued earlier by Buccino [2], who advocates that MI has some intrinsic limits that AO training does not exhibit because MI is a more demanding tool, in terms of attention and concentration, compared with AO training. The loss of attention, as well as the difficulty of the breathing training exercises, could explain the poor effect of MI in this study.

At the clinical level, it appears that AO training has an impact on the activity of the expiratory musculature that results in a slight improvement in maximal strength that also appears to translate into small improvements in some pulmonary function parameters. Although it is still early to draw solid conclusions, AO training could be used in combination with respiratory exercise to see if the effect is greater than exercise alone. For example, in other populations such as patients with acute cerebral infarction, mental practice in combination with a conventional rehabilitation program has been shown to elicit a greater clinical effect, including improved blood oxygen to brain tissue, than the conventional rehabilitation program alone as assessed with functional near-infrared spectroscopy (fNIRs) technology [50]. In addition, in patients where actual therapeutic exercise is not possible (e.g., bedridden, or after surgery), mental practice training could be performed with the aim of minimizing the impact of immobilization. However, research studies should be carried out to determine these effects in different clinical populations with ventilatory disorders, such as chronic obstructive pulmonary disease, asthma, and lung cancer, and also to evaluate the medium- and long-term impact.

The present study has some limitations that should be taken into consideration. First, the main limitation is the small sample size. Probably, a larger sample would give slightly different results although this is only an assumption. This pilot study was used to make an estimate of the sample size and we found that the final study should contain at least 15 participants for each group. Second, this study has a theoretical perspective with the aim of looking at the impact of mental practice in isolation. To have a more clinical perspective, future studies should evaluate whether the combination of movement representation techniques with actual respiratory training would lead to an improvement of exercise capacity or assess the impact of airway disease on health status and perceived wellbeing, as compared to actual exercise in isolation. Finally, the results were derived from the analysis of the very short-term data. Future studies should include a follow-up to see if the changes generated by the intervention are sustained over time. For all these reasons, the results should be interpreted with caution as this is a preliminary study.

5. Conclusions

AO training has a slight impact on some pulmonary function parameters, such as FEV_1, FVC, or PEF, as well as on MEP when applied in isolation and in a single session. The impact of MI seems almost non-existent, at least in isolation and in a single session. At the clinical level, it seems that AO training has an effect on the activity of the expiratory musculature, resulting in a slight improvement in maximal strength that also appears to translate into small improvements in some pulmonary function parameters. Future studies should combine AO with breathing exercises to assess whether the effects are more pronounced than those stemming from breathing exercises in isolation.

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Not applicable.

Conflict of Interest

None declared.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Research in Humans of the Ethics Commission in Experimental Research of

University of Valencia (number: 2301127). Signed written consent was obtained from the participants before the start of this study.

Consent for Publication

Signed consent was obtained from the participants to use their images and their data for this study.

Availability of Data

Data are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Prognostic factors and nomogram construction for primary retroperitoneal myxoid/round cell liposarcoma: an analysis of population-based data

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ABSTRACT

Background: It has been reported that the prognosis for myxoid/round cell liposarcoma (MLPS/RCLPS) is inconsistent across different sites. However, there are neither prognostic studies nor predictive models that focused on MLPS/RCLPS of retroperitoneal origin.

Methods: Utilizing the Surveillance, Epidemiology, and End Results database, we selected 171 primary retroperitoneal MLPS/RCLPS cases from the period between 2000 and 2019. Prognostic factors influencing disease-specific survival were identified through Cox regression analysis. These independent prognostic factors were then used to construct a DSS nomogram prediction model. The accuracy and reliability of this nomogram were evaluated using the concordance index (C-index) and calibration plots. Furthermore, we categorized patient prognosis using an X-tile based on the nomogram score.

Results: The observed 5-year and 10-year DSS rates for all patients were 64.0% (95% CI: 56.2% – 71.8%) and 47.1% (95% CI: 38.1% – 56.1%), respectively. The patient cohort had a median age of 64 years, ranging from 24 to 92 years, with a slight male predominance (n = 92, 53.8%) over females (n = 79, 46.2%). Distant metastases were diagnosed in 24 patients (14%). The distribution of MLPS and RCLPS was 89.5% and 10.5%, respectively. In terms of treatment, adjuvant radiotherapy was administered to 33 patients (19.3%), neoadjuvant radiotherapy to 9 patients (5.3%), and chemotherapy to 20 patients (11.7%), while a significant majority (83.6%) underwent surgical procedures. Independent prognostic factors for DSS included age (HR = 1.039, P < 0.001), marital status (P = 0.029), history of previous tumors (HR = 0.257, P = 0.007), presence of metastatic disease (HR = 2.206, P = 0.027), and surgical treatment (HR = 0.490, P = 0.036). A nomogram prediction model was constructed to forecast 1-, 5-, and 10-year DSS rates, with a C-index of 0.739. Calibration plots demonstrated a strong correlation between the nomogram's predictions and actual observations. Based on the prediction model, patients were stratified into three groups, and significant differences in prognosis were observed between these groups.

Conclusion: A poorer prognosis is associated with retroperitoneal-derived MLPS/RCLPS than with other sites. The nomogram prediction model we built can be used to assist patients in consulting with their doctors and selecting patients for clinical trials.

Relevance for Patients: Our study highlights the unique challenges and prognosis variations in retroperitoneal myxoid/RCLPS. The developed nomogram serves as a valuable tool for patients, aiding informed discussions with doctors and guiding decisions on treatment and clinical trial participation.

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1. Introduction

Retroperitoneal soft-tissue sarcoma (RPS) accounts for approximately 15% of all soft-tissue malignancies, whereas myxoid/round cell liposarcoma (MLPS/RCLPS) represents less than 5% of all RPS [1-4]. MLPS/RCLPS is the most prevalent lipomatous malignancy in children and adolescents [5,6]. The onset of MLPS/RCLPS occurs earlier than that of other subtypes of liposarcoma and reaches its incidence peak in middle age.

The diagnosis of MLPS/RCLPS is definitive due to its distinctive morphology, which is rarely mistaken for other monomorphic soft tissue tumors with myxoid stromal and lipomatous differentiation [7]. In addition, specific chromosomal translocations were identified, including *FUS* and *CHOP* gene fusions [(t12;16)(q13;p11)] and *EWS* and *CHOP* gene fusions [(t12;22)(q13;q12)] in 90% of tumors in >5% of tumors. The detection of these translocations with polymerase chain reaction (PCR) techniques enables pathologists to make precise diagnoses in difficult cases [8,9]. RCLPS refers to MLPS with round cells, accounting for more than 5% of all cases [7]. In terms of aggressiveness, RCLPS has a worse prognosis than MLPS [10].

MLPS/RCLPS is distinguished from other soft-tissue sarcomas by a number of characteristics. First, it is more susceptible to extrapulmonary metastases than other sarcomas [11,12]; second, it is more sensitive to radiotherapy and chemotherapy than other liposarcomas [13]; third, the prognosis is favorable, with the 5-year disease-specific survival (DSS) rate for local diseases exceeding 90% [13].

Several crucial prognostic factors impact patient survival, including both distant and local recurrence. Key factors include the completeness and negative margins of surgical resection, histological grade reflecting differentiation in myxoid/round cell liposarcoma patients [14], patient age, and the role of tumor biomarkers for treatment monitoring, prognosis assessment, early diagnosis, and treatment prediction.

Furthermore, analysis suggests that high FGF-21 expression improves prognosis [15]. Multivariate analysis considers clinicopathological factors, such as tumor site, round cell (RC) components, high MIB-1 labeling index, and p53 missense mutation as unfavorable indicators. In cases of MLS/RCLS, reduced p14 protein expression and p53 mutations associate with poor prognosis. In addition, the RC component is identified as a negative prognostic factor, potentially involving the p14ARF/p53 pathway in its development [16].

Commonly mutated genes such as *TP53*, *NF1*, and *PIK3CA* are identified in STS through genome studies. *PIK3CA* mutations, more frequent in myxoid/round cell and pleomorphic tumors compared to well-differentiated/dedifferentiated tumors, suggest *PIK3CA* as a potential driver gene and therapeutic target. Survival analysis reveals that patients with increased *PIK3CA* copy numbers have worse prognosis, highlighting its significance [17]. NY-ESO-1's association with higher tumor grade and shorter survival establishes it as a valuable prognostic marker for myxoid liposarcoma. In addition, PRAME's high expression is correlated with unfavorable prognosis and elevated levels in myxoid liposarcoma, indicating its role as a prognostic factor [18]. Elevated levels of SIRT1 and VEGF are linked to unfavorable clinical characteristics and prognosis, suggesting SIRT1 as a potential therapeutic target [19]. Finally, modulation of FGFR signaling and its inhibitors show promise in high-grade liposarcoma treatment, which highlights the potential of developing targeted therapies [20]. Moreover, CXCR4 and AXL are emerging as promising therapeutic targets in the management of aggressive MLPS behavior.

Although it has been known for some time that the prognosis of MLPS/RCLPS of different primary sites varies [21,22], there is currently no prognostic study on MLPS/RCLPS of retroperitoneal origin and no prognostic tool. Therefore, we analyzed the surveillance, epidemiology, and end results (SEER) database, which provides data from 17 geographically variable cancer registries representing approximately 26% of the U.S. population [21,22], to investigate the DSS-related prognostic factors in MLPS and to attempt to develop a prognosis nomogram prediction model.

2. Materials and Methods

Using SEER*Stat 8.4.0.1, patients diagnosed with MLPS/RCLPS between 2000 and 2019 were identified from the SEER database, in which all cases were reported from the United States. The following were the criteria for inclusion: (1) the International Classification of Diseases (ICD) code O-3 morphology 8852 or 8853; (2) the primary site recodes of ICD-O-3 was the retroperitoneum; and (3) active patient monitoring to ensure a reliable patient status. The following were the criteria for exclusion: (1) patients with non-primary tumor and (2) patients younger than 18 years old. Myxoid/Round cell liposarcoma is diagnosed through a combination of histological, immunohistochemical, and genetic examinations. Pathologically, it is characterized by abundant myxoid stroma and a round cell component, with varying degrees of lipogenic differentiation. Immunohistochemically, these tumors typically express S-100 protein, CDK4, and MDM2. A critical aspect of the diagnosis is the identification of hallmark genetic alterations, particularly the *FUS-CHOP* or *EWS-CHOP* fusion genes, often detected through molecular tests like reverse-transcription PCR or fluorescence *in situ* hybridization.

The primary endpoint of this study was DSS. We collected and analyzed data on gender, age, marital status, race, history of previous tumors, the interval between diagnosis and treatment, presence of metastatic disease, histologic subtypes, tumor differentiation, tumor size, and treatment methods including radiotherapy, chemotherapy, and surgery. Information regarding the interval between diagnosis and treatment and tumor size was missing for 20 (11.7%) and 21 (12.3%) patients, respectively. Given the rarity of retroperitoneal MLPS/RCLPS, we chose not to exclude these patients, instead substituting the missing values with their respective medians (1 month, 20 cm). All the aforementioned variables were included in the univariate Cox model analysis. Variables with $P < 0.1$ were further included in the multivariate analysis. Variables with $P < 0.05$ in the Cox multivariate regression model were selected for the nomogram prediction model. The accuracy of the nomogram was subsequently validated using the

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C-index and calibration curve. Based on the nomogram score, patients were stratified into low-, intermediate-, and high-risk groups. Survival differences between these groups were compared using the Kaplan–Meier curve and the log-rank test. The risk stratification cutoff point was determined using X-tile, a novel bioinformatics tool for biomarker assessment and outcome-based cut-point optimization.

All tests were conducted with two-tailed statistics, and $P < 0.05$ was considered statistically significant. Data were analyzed using R statistical software (version 4.1.2, http://www.r-project.org).

3. Results

3.1. Patient and tumor characteristics

A total of 171 patients fulfilled the inclusion criteria, with 77 succumbing to their disease by the time of the last follow-up. The median follow-up duration for all surviving patients was 87 months (IQR: 25 – 156 months). Patient characteristics are detailed in Table 1. The patient cohort had a median age of 64 years, ranging from 24 to 92 years, with a slight male predominance (n = 92, 53.8%) over females (n = 79, 46.2%). Marital status was distributed as follows: married (53.8%, n = 92), single (18.1%, n= 31), widowed (14.0%, n = 24), and divorced or separated (8.8%, n = 15). The majority of patients were white (n = 138, 80.7%), and 83.0% had no history of other tumors. Distant metastases were diagnosed in 24 patients (14%). The distribution of MLPS and RCLPS was 89.5% and 10.5%, respectively. In terms of treatment, adjuvant radiotherapy was administered to 33 patients (19.3%), neoadjuvant radiotherapy to 9 patients (5.3%), and chemotherapy to 20 patients (11.7%), while a significant majority (83.6%) underwent surgical procedures.

Table 1. Patient and tumor characteristics in 171 patients with primary retroperitoneal myxoid/round cell liposarcoma

Characteristics	N=171	% of total
Gender		
Male	92	53.8
Female	79	46.2
Age, years median (range)	64 (24 – 92)	
Marital status		
Married	92	53.8
Single	31	18.1
Widowed	24	14.0
Divorced	15	8.8
Separated	2	1.2
Unknown	7	4.1
Race		
White	138	80.7
Asian or Pacific Islander	17	9.9
Black	15	8.8
Unknown	1	0.6
Past tumor history		
No	142	83.0
Yes	29	17.0
Months from diagnosis to treatment	1 (0 – 6)	
Metastasis disease		
Yes	24	14
No	147	86
Histologic subtypes		
Myxoid liposarcoma	153	89.5
Round cell liposarcoma	18	10.5
Tumor size, cm median (range)	200 (15 – 750)	
Tumor differentiation		
Well-differentiated	60	35.1
Moderate-differentiated	30	17.5
Poor-differentiated	16	9.4
Undifferentiated	14	8.2
Unknown	51	29.8
Radiation		
Adjuvant	33	19.3
Neoadjuvant	9	5.3
No/Unknown	129	75.4
Chemotherapy		
Yes	20	11.7
No/Unknown	151	88.3
Surgery		
Performed	143	83.6
Not performed	28	16.4
Dead because of disease		
Yes	77	45.0
No	94	55.0

3.2. Survival analysis

The 1-year, 5-year, and 10-year DSS rates (Figure 1) and overall survival (OS) rates (Figure 2) for all patients were 86.7% (95% CI, 81.6 – 81.8), 64.0% (95% CI, 56.2 – 71.8), 47.1% (95% CI, 38.1 – 56.1) and 83.1% (95% CI, 77.4 – 88.8), 55.2% (95% CI, 47.4 – 63.0), 35.5% (95% CI, 27.5 – 43.5), respectively.

In the univariate analysis, factors such as patient age ($P < 0.001$), marital status ($P = 0.002$), history of previous tumors ($P = 0.044$), presence of metastatic disease (P = 0.001), tumor differentiation (P = 0.009), radiotherapy (P = 0.030), and surgery (P = 0.003) were found to be associated with DSS (Table 2). Variables with $P < 0.1$ in the univariate analysis were subsequently included in the multivariate analysis of the Cox model. The multivariate analysis revealed that patient age (HR = 1.039, $P < 0.001$), marital status ($P = 0.029$), history of previous tumors (HR = 0.257, $P = 0.007$), presence of metastatic disease (HR = 2.206, P = 0.027), and surgical treatment (HR = 0.456, P = 0.036) were independent prognostic factors for DSS (Table 2).

3.3. Development and validation of nomograms

Subsequently, a DSS nomogram prediction model was developed using a Cox regression model, based on the results of the aforementioned multivariate analysis (Figure 3). This model accurately predicts 1-year, 5-year, and 10-year DSS. Calibration plots (Figure 4) demonstrate a strong correlation between the nomogram's predictions and the actual outcomes. The concordance

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Table 2. Univariable and multivariable analyses to determine independent predictors of disease-specific survival in primary retroperitoneal myxoid/round cell liposarcoma

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Gender female versus male	0.950 (0.607 – 1.487)	0.823		
Age (continuous)	1.033 (1.015 – 1.051)	<0.001	1.039 (1.017 – 1.061)	<0.001
Marital status		0.002		0.029
Single vs. married	0.448 (0.209 – 0.960)		0.408 (0.181 – 0.921)	
Widowed vs. married	1.288 (0.701 – 2.369)		0.722 (0.363 – 1.436)	
Divorced vs. married	2.053 (1.018 – 4.143)		1.916 (0.931 – 3.940)	
Separated vs. married	4.972 (1.184 – 20.887)		4.701 (0.818 – 27.029)	
Unknown vs. married	3.792 (1.132 – 12.699)		0.734 (0.155 – 3.483)	
Race		1.000		
Asian Pacific Islander vs. White	0.971 (0.465 – 2.030)			
Black vs. White	0.982 (0.449 – 2.148)			
Unknown vs. White	NA			
Past tumor history yes vs. no	0.628 (0.399 – 0.988)	0.044	0.257 (0.096 – 0.688)	0.007
Months from diagnosis to treatment (continuous)	0.907 (0.707 – 1.163)	0.907		
Metastatic disease yes vs. no	2.666 (1.510 – 4.707)	0.001	2.206 (1.096 – 4.438)	0.027
Histologic subtypes round cell vs. myxoid	1.759 (0.902 – 3.433)	0.098	1.936 (0.776 – 4.825)	0.156
Tumor size (continuous)	1.001 (1.000 – 1.003)	0.120		
Tumor differentiation		0.009		0.471
Moderate vs. well	0.961 (0.513 – 1.801)		0.961 (0.478 – 1.929)	
Poor vs. well	3.123 (1.545 – 6.315)		1.993 (0.754 – 5.273)	
Undifferentiated vs. well	0.585 (0.205 – 1.671)		0631 (0.178 – 2.233)	
Unknown vs. well	1.220 (0.680 – 2.189)		0.939 (0.498 – 1.771)	
Radiotherapy yes vs. no		0.030		0.170
Adjuvant vs. no/unknown	0.432 (0.227 – 0.823)		0.567 (0.262 – 1.228)	
Neoadjuvant vs. no/unknown	0.539 (.0132 – 2.204)		0.385 (0.086 – 1.722)	
Chemotherapy yes vs. no/unknown	1.695 (0.867 – 3.314)	0.123		
Surgery performed vs. not performed	0.423 (0.240 – 0.747)	0.003	0.490 (0.251 – 0.954)	0.036

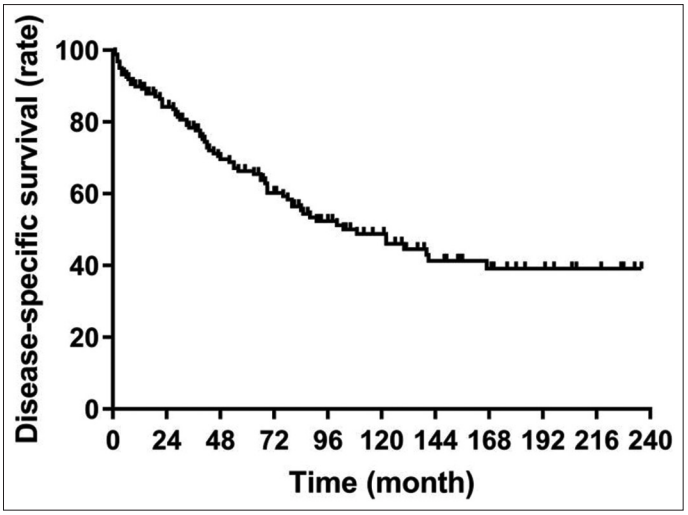

Figure 1. Disease-specific survival in patients with primary retroperitoneal myxoid/round cell liposarcoma.

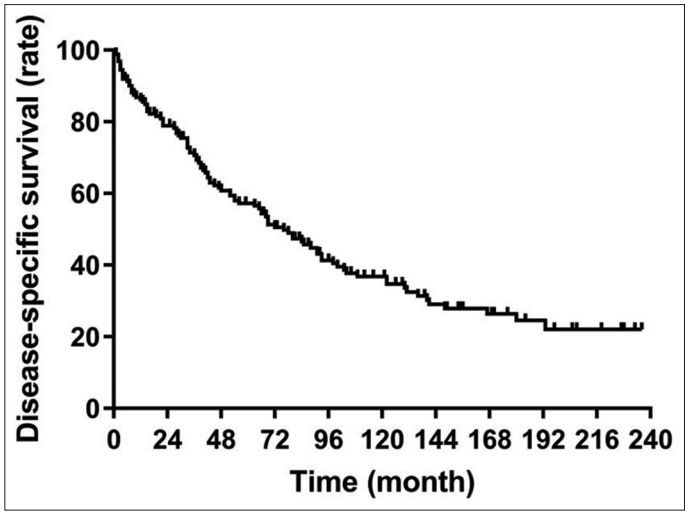

Figure 2. Overall survival in patients with primary retroperitoneal myxoid/round cell liposarcoma.

indices and bootstrapped 95% confidence intervals (95% CI) for the nomogram were 0.739 (0.616 – 0.862).

Utilizing X-tile software, patients were stratified into high-risk (>165), intermediate-risk (141 – 165), and low-risk (<141)

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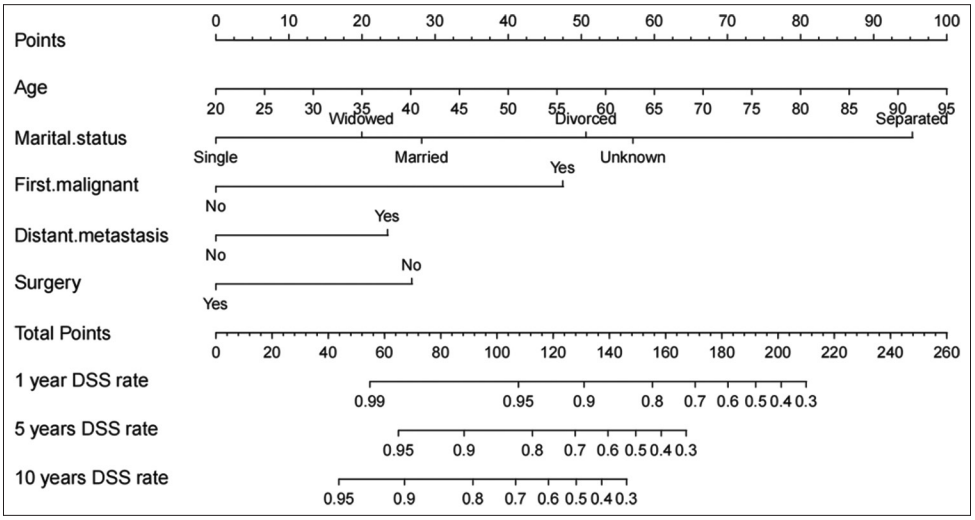

Figure 3. Nomogram for 1-year, 5-year, and 10-year disease-specific survival (DSS) in patients with primary retroperitoneal myxoid/round cell liposarcoma.

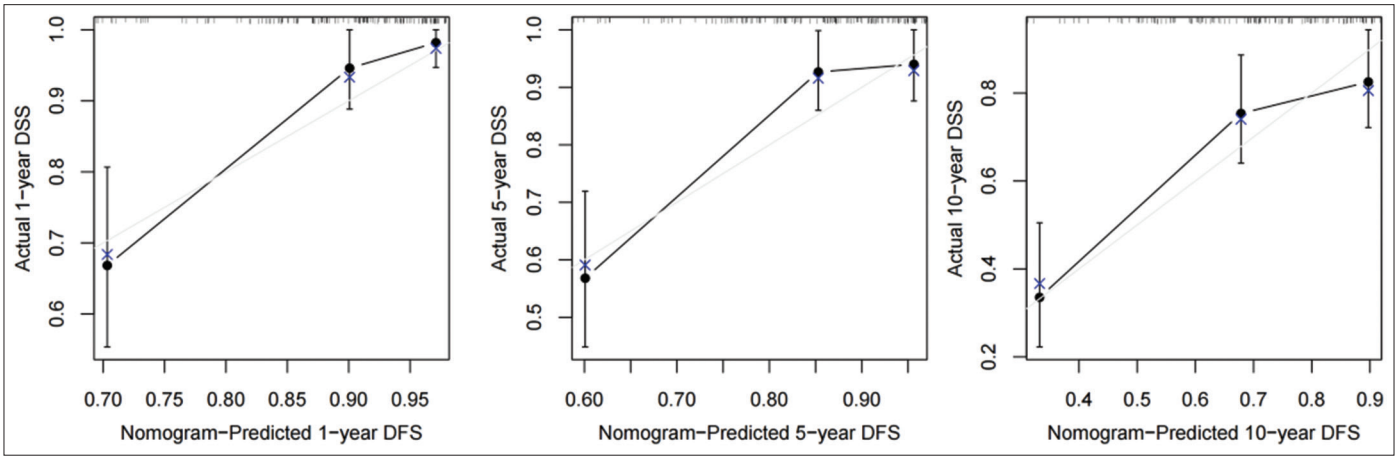

Figure 4. Calibration plots for internal validation of 1-year, 5-year, and 10-year disease-specific survival nomogram.

groups according to their nomogram scores. Figure 5 illustrates the DSS of the three groups; the median DSS was 7.0 months (95% CI: 0.0 – 26.4), 25.4 months (95% CI: 2.2 – 101.8), and 167 months (95% CI: NA), respectively (p <0.001). The usage of the nomogram prediction model is as follows. Assume an MLPS patient comes for a consultation in the outpatient department, with the following basic information: a 65-year-old (60 points) divorced (50 points) woman with no history of malignant tumors (48 points). She is diagnosed without distant metastasis (0 points), but the lesion is inoperable (28 points). Therefore, the total score for this patient is 176 points, placing her in the high-risk group (>165 points), with a corresponding prediction of less than 30% for 5-year DSS. If existing medications are ineffective in controlling the condition, we recommend considering clinical trial enrollment for this patient.

4. Discussion

Retroperitoneal MLPS/RCLPS represents a rare subset of an already rare group of tumors. According to the only reported cohort focused on retroperitoneal MLPS/RCLPS to date, based on five cases from the National Cancer Center Hospital in Tokyo, MLPS/RCLPS accounted for a mere 2.3% of RPS and 3.2% of all sites [2]. In a large cohort study on retroperitoneal liposarcoma, the proportion of MLPS/RCLPS was less than 10% [3,23]. In this study, we retrospectively examined prognostic factors and reported long-term survival status based on the SEER database. For the first time, we identified age (HR = 1.039, P < 0.001), marital status (P = 0.029), previous tumor history (HR = 0.257, P = 0.007), and presence of distant metastasis (HR = 2.206, P = 0.04) as risk factors for DSS. Furthermore, we developed a DSS prediction model for retroperitoneal MLPS/RCLPS that accurately forecasts patients' prognoses. Patients were stratified into three groups based on the nomogram scores. Twenty patients (11.6%) in the high-risk group and thirty-four patients (19.8%) in the intermediate-risk group had a median DSS of only 7.0 (95% CI, 0.0 – 26.4) months and 25.4 months (95% CI, 2.2 – 101.8 months), respectively. In contrast, the median DSS for patients in the low-risk group was 167 (95% CI, NA) months. Based on these

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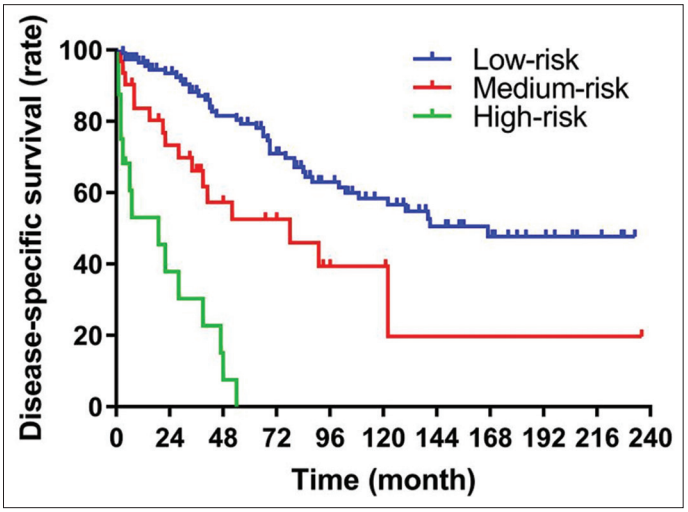

Figure 5. Disease-specific survival in patients with low-, medium-, and high-risk groups.

findings, we recommend more active follow-up for patients in the middle- and high-risk groups and consideration for clinical trials when permissible.

In a retrospective cohort study, we found that retroperitoneal MLPS/RCLPS differed from MLPS/RCLPS at other sites in several ways. Firstly, the prognosis was poorer. In a study conducted by Hans Roland Dürr in 2018 involving 43 cases of MLPS/RCLPS, the 5-year and 10-year OS rates were 81% and 72%, respectively [24]. In a study involving 174 cases of primary MLPS/RCLPS reported by Fiore *et al.*, the 5-year and 10-year DSS rates for the MLPS and RCLPS group were 93% and 92%, and 87% and 77%, respectively. However, only 7% of patients in the cohort were of retroperitoneal origin [10]. Based on 85 patients with MLPS, Chowdhry *et al.* found that tumor size was the only factor affecting OS, and the 5-year OS in this study was 87.5% [25]. In 2020, 89 patients with MLPS/RCLPS participated in a multicenter prospective cohort study, and their 3-year DSS was as high as 96%. Similarly, no retroperitoneal patients were included in this study [26]. The patients in this study cohort had a significantly worse prognosis than those in the preceding cohorts (5-year and 10-year DSS were only 64.0% and 47.1%, respectively). Second, the median age and tumor diameter of patients with retroperitoneal MLPS/RCLPS were also significantly different. As an example, the median age of patients in this study was 64 years, whereas in previous studies, it was less than 50 years; the median tumor size was 20 cm, as opposed to approximately 10 cm in previous studies. The Trans-Atlantic RPS Working Group reported that the 10-year OS of RPS was 46%, the median age of patients in this cohort of 1007 patients was 58 years, and the median tumor size was 20 cm [27]. Retroperitoneal MLPS/RCLPS appears to have a prognosis more comparable to that of an RPS than systemic MLPS/RCLPS. In other words, even in MLPS/RCLPS, the primary site may be as crucial to the patient's prognosis as the pathological subtype.

As early as 2003, Memorial Sloan-Kettering Cancer Center (MSKCC) conducted a nomogram study on retroperitoneal liposarcoma. There were 177 patients in the study, and the 5-year DSS for all patients was 60%. There were only 13 (7%) patients with MLPS and RCLPS in the cohort, so the accuracy of prediction for these patients was limited even though pathological type was an independent prognostic factor for RLPS in multivariate analysis [23]. Subsequently, MSKCC developed a DSS nomogram prediction model using data from 801 liposarcoma patients (including 144 MLPS and 81 RCLPS). Despite the 12-year DSS of 72% for the entire cohort, the 12-year DSS for liposarcomas of retroperitoneal origin in subgroups by site was only 32%. This is consistent with our previous findings that the prognosis for retroperitoneal MLPS/RCLPS is worse than other sites. The researchers also developed a 5-year and 12-year DSS prediction model with good verification based on age, presentation status, primary site, histologic variant, tumor burden, and gross margin status (C-index = 0.776) [28]. Gronchi *et al.* developed a nomogram prediction model for RPS using data from 523 patients in 2013. The cohort's 5-year OS rate was 56.8%. Although this study did not differentiate the pathological subtype of myxoliposarcoma, it has been externally validated and can accurately predict the DFS and OS of patients, presenting significant implications for the diagnosis and treatment of RPS [29]. On the basis of pathological classification, MSKCC subsequently developed a DSS nomogram prediction model for RPS. This nomogram can predict DSS at 3, 5, and 10 years after surgery with high accuracy (C-index = 0.71). In addition, it is also a fly in the ointment that, due to the rarity of MLPS and RCLPS, MLPS is classified as WDLPS and RCLPS as DDLPS [30]. Compared to the aforementioned studies, the nomogram established in this research focuses on retroperitoneal MLPS/RCLPS, providing more precise diagnosis and treatment for this relatively rare disease.

In recent years, numerous studies have explored the impact of psychosocial factors on cancer outcomes, highlighting marital status as an independent predictor of survival across different cancer types. Research shows that unmarried individuals with cancer tend to experience more advanced disease stages than their married counterparts. Married patients typically enjoy higher socioeconomic status and better access to quality healthcare. They also benefit from emotional and financial support from their spouses, enhancing their focus on the healing process. Notably, partner-provided emotional support can alleviate the stress associated with cancer treatment. Social support within a marriage may influence cancer survival by affecting neuroendocrine, neurological, and immune interactions. For instance, higher social support levels are associated with increased activity of natural killer (NK) cells, which play a crucial role in recognizing and eliminating cancer cells. In addition, oxytocin hormone release during social interactions may indirectly inhibit cancer cell growth by suppressing stress responses [31-34].

Marital status was found to be a risk factor for DSS. In univariate analysis, the tumor-specific survival of married patients was greater than that of widowed, divorced, and separated patients [35-37]. However, contrary to previous research, we found that married patients had twice the risk of dying from cancer compared to single (never-married) patients, even after

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adjusting for other confounding variables (Table 2). To determine the reason, we compared baseline characteristics of single and married patients, but there was no difference between the two groups (data not shown). Intriguingly, when patients were divided into four groups consisting of married men, single men, married women, and single women, the DSS of single women was significantly higher than that of the other groups. In particular, the 10-year DSS for single women, married women, single men, and married women was 87.5 (95% CI, 75.6% – 100.0)%, 51.3 (95% CI, 32.5% – 70.1)%, 60.5 (95% CI, 35.8% – 85.2)%, and 44.8 (95% CI, 28.1% – 61.5)%. Most analyses indicate that women have better outcomes than men, but few studies indicate that single women have better outcomes than other demographic groups. Based on the current data, we cannot conclude the reasons for the above differences for the time being, and further in-depth research is needed.

The current study considered that an anthracycline-based combination chemotherapy regimen is the first-line treatment option and trabectedin may be considered in first-line therapy when anthracyclines cannot be used. Although doxorubicin ± ifosfamide remains the first-line treatment for most STS subtypes, some STSs (alveolar soft part sarcoma, clear cell sarcoma, epithelioid sarcoma, and extraosseous myxoid chondrosarcoma) have been reported to show little response to these cytotoxic chemotherapies [38]. In addition to chemotherapy, new treatments are also being investigated, some of which have already shown considerable results. Trabectedin is a marine-derived antitumor drug that achieves anti-tumor cell activity by inhibiting transcription, anti-angiogenesis, and immune regulation. Related tests show that MRCL and other translocation-related sarcomas (liposarcoma and leiomyosarcoma) are the most sensitive types of sarcoma related to trabectedin [39]. The French Sarcoma Group conducted a randomized phase III study evaluating the efficacy of trabectedin versus best supportive care (BSC) in patients with advanced STS. Patients were randomized (1:1) to receive trabectedin (1.5 mg/m^2 24 h intravenous infusion every 3 weeks) or BSC. The median PFS was 3.1 months in the trabectedin group, and the median PFS was 1.5 months in the BSC group. It can be seen that trabectedin is better for disease control than BSC [40]. Eribulin is a non-taxane microtubule inhibitor, which is more sensitive to leiomyosarcomas and liposarcomas. Eribulin has now become an effective treatment for MRCL. Several recent trials of eribulin combined with other drugs for advanced liposarcoma have prolonged the median PFS in patients with considerable results [39]. In a phase II trial of eribulin-gemcitabine combination in patients with advanced liposarcoma, a 12-week PFS rate was 70.6% (n = 12/17) in the liposarcoma cohort, with a median PFS of 5.7 months [41].

Radiotherapy is often used in combination with surgery and allows for preoperative, intraoperative, or postoperative radiotherapy. Preoperative radiotherapy may enable surgery for unresectable tumors. Different liposarcoma subtypes differ in their sensitivity to radiotherapy, and MLPS is highly radiosensitive [42]. Neoadjuvant radiotherapy is mostly used in patients with mucoid LPS because of its great radiosensitivity. The effects of radiotherapy may be initiated by reducing the myxoid stroma produced by tumor cells as well as promoting adipocyte maturation [43]. Radiotherapy can also cause a change in tumor size. Studies have shown that pre-operative radiotherapy to patients can reduce tumor seeding during surgery, but the disadvantage of pre-operative radiotherapy is that it can affect wound healing [44]. Pre-operative radiotherapy may improve the prognosis of low-grade retroperitoneal sarcoma without much benefit for high-grade retroperitoneal sarcoma [45]. Post-operative radiotherapy can be effective in improving local control in patients with positive surgical margins, but the associated side effects of post-operative radiotherapy will also increase [44]. For soft-tissue sarcomas, tumor size is an important prognostic factor and is associated with both the local recurrence rate and overall survival. Magnetic resonance imaging (MRI) examination of tumor size before and after radiotherapy revealed a median maximum tumor size of 12.4 cm and a median tumor volume of 298.9 cm^3. After radiotherapy, the median maximum tumor size on MRI was 8.7 cm, and the median tumor volume was 106.9 cm^3 [46]. However, the role of radiotherapy in liposarcoma should be explored in a prospective trial.

Recently, significant advances have been made in an increasing number of targeted therapies, with some targeted agents showing promising results in patients with advanced or metastatic STS. Unlike other liposarcomas, the tumor microenvironment of MRCL is relatively "cold" immunologically, rendering MRCL less sensitive to immunotherapy. However, the cancer testicular antigens in these tumors are highly expressed, such as NY-ESO-1 and MAGEA4. Therefore, these two antigens have become ideal targets for the treatment of MRCL patients [39]. Others such as PPARγ agonists, PI3KCA inhibitors, and tyrosine kinase inhibitors have been shown to play a key role in the treatment of MRCL patients.

This research has the following limitations: First, because it is a retrospective study, there were unavoidable selection bias; second, data used in this study were derived from the SEER database, and some information was missing; third, although 171 cases of MLPS/RCLPS represent the largest cohort to date, the nomogram prediction model established in this study had only been internally validated, and additional external validation is required to increase the confidence of the prediction.

5. Conclusion

One hundred and seventy-one patients with primary retroperitoneal MLPS/RCLPS were retrospectively analyzed for prognostic factors using the SEER database, and the findings indicate that age, marital status, previous tumor history, metastatic disease, and whether surgery was performed are associated with DSS. In addition, we developed the first retroperitoneal MLPS/RCLPS prognostic prediction model. By dividing patients into three risk categories, it may useful for outpatient consultations and patient selection for clinical trials.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

Author Contributions

Aobo Zhuang, Yingxue Cheng, and Yue Wang contributed to the design of the study. Zhe Xi, Guangting Yan, and Gen Zhang contributed to recruiting patients. Jialiang Zheng, Lingwei Gu, and Peng Li contributed to collecting data. Wengang Li, Lanlan Lian, Xi Li, Fuan Xie, and Ting Wu approved the final version of this manuscript and were responsible for the decision to submit the manuscript.

Ethics Approval and Consent to Participate

This article does not contain any studies with human or animal subjects performed by any of the authors.

Consent for Publication

Not applicable.

Availability of Data

The data that support the findings of this study are available from SEER database. Data are available from the authors on reasonable request and with permission of SEER database.

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ORIGINAL ARTICLE

Self-medication practice and associated factor among adult household members in Gurage Zone, Southern Ethiopia, Ethiopia, 2022: a cross-sectional study

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ABSTRACT

Background: Self-medication refers to the use of medicinal products by the consumers to treat self-recognized disorders or symptoms, or the intermittent or continued use of medication is not prescribed by a physician for chronic or recurring diseases or symptoms. Globally, the prevalence of self-medication is estimated to be around 27 – 99.4% according to different studies.
Methods: A cross-sectional study was conducted to assess self-medication practice and associated factors among adult household members of Gubre town from April 28 to June 27, 2022. Data collection from 399 households was conducted by means of a systematic random sampling technique using a pre-tested questionnaire.
Result: Among 398 respondents, 113 (28.4%) of them were found to practice self-medication. Two hundred and forty-three (61.05%) participants reported to have fallen sick in the 2 weeks before the survey. Being married (adjusted odds ratio [AOR] = 1.599, 95% confidence interval [CI]: 1.09 – 2.621), being literate (AOR = 1.672, 95% CI: 1.032 – 2.01), perceiving self-medication as an acceptable practice (AOR = 1.652, 95% CI: 1.32 – 1.887), peer influence (AOR = 1.54, 95% CI: 1.304 – 2.321), and access to medical information (AOR = 1.452, 95% CI: 1.263 – 1.570) were significantly associated with self-medication practice.
Conclusion: Nearly a quarter of the study participants practice self-medication. Being married, being literate, perceiving self-medication as an acceptable practice, peer influence, and access to medical information were significantly associated with self-medication practice.
Relevance for Patients: Tailored interventions designed by policymakers, program designer, and implementers should aim to reduce or eliminate non-prescribed drug use among the community through the education on the impact of self-medication on individual health.

1. Introduction

Self-medication refers to the use of medicinal products by the consumers to treat self-recognized disorders or symptoms, or the intermittent or continued use of medication that is not prescribed by a physician for chronic or recurring diseases or symptoms [1]. Alternatively, such practice is also defined by many authors as the utilization of medicines by a patient on his initiative or on the recommendation of a non-professional or a layperson instead of seeking advice from a health-care provider [2,3].

Self-medication is a commonly employed practice in an attempt to treat a perceived illness [4]. The type or extent of self-medication and the reasons for it may vary from

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country to country. In developing countries, both modern drugs and traditional medicines are commonly used for self-medication [5].

It was also noted that prescription-only medications could easily be obtained without prescriptions for self-medication in developing countries like Ethiopia [6]. Utilizing drugs without prescription from physicians may not produce the maximum beneficial effects or may even jeopardize the consumer's health. The efficacy and safety of most traditional medicines used in Ethiopia are not scientifically proven, and the dosage prescribed by traditional healers is always imprecise [7].

Inappropriate and ineffective self-medication can also delay timely and appropriate treatment, culminating in tragic consequences [8]. Unused medications are often kept well beyond their use-by dates or stored without appropriate identification (such as being mixed in a container without original packaging), leading to potential misuse. Some consumers may attempt to mix drugs that are contraindicated to each other, exposing themselves to the heightened risk of adverse drug interactions and reactions [9]. Furthermore, 21% of drugs stored in family medical kits are often expired, and drug packaging inserts are missing (18%) [9].

Only a small proportion of the many symptoms facing an individual, accounting for 10 – 30% of the symptoms, is reportedly brought to the attention of physicians. A prevailing presumption is that the majority of the symptoms are either tolerated or self-medicated [5]. According to different studies, the prevalence of self-medication is estimated to be 27% in Spain and 99.4% in Nigeria [10,11]. The extent of self-medication practice varies across countries; for instance, in the United States of America, about 71% of men claimed to have self-medicated at least once within 6 months before survey [12]. About 41.5% of respondents in the United Kingdom [13], 27% in Spain, [10], and 50% in Ethiopia [14] reported to have practiced self-medication. Several factors are associated with a likelihood of practicing self-medication. These factors include age, gender, expenditure, self-care orientation, socioeconomic status, satisfaction of efficacy, and seriousness of illnesses [15,16].

Inappropriate self-medication results in drug dependencies, wastage of resources, and serious health hazards. Self-medication in southern Ethiopia is quite common but there is little information regarding the extent of practice and associated factors. Therefore, this study aimed to determine the magnitude and factors associated with self-medication practices among the various segments of the community, with the ultimate purpose of formulating appropriate health education programs to halt self-medication-related public health problems. It is crucial to synthesize available evidence for better decision-making and help Gurage zone residents wean off the habit of self-medication.

2. Methods

2.1. Study design and setting

A community-based cross-sectional study was conducted, from April 28 to June 27, 2022, at Gubre town in the Southern Nations Nationalities and Peoples Region, Gurage Zone, located 178 km southwest of Addis Ababa and 20 km east of Wolkite town.

2.2. Inclusion and exclusion criteria

Individuals that match these inclusion criteria were recruited: aged 18 years or above, available during data collection, able to communicate by either speaking or writing, and had lived in the town for at least 6 months.

Exclusion criteria include individuals who are unable to communicate (a problem with hearing) and non-volunteer.

2.3. Sample size calculation and sampling procedure

The sample size was determined using the single population proportion formula in consideration of the following assumptions: 95% CI and 5% margin of error. The maximum sample size was determined from an assumption of the total households of the town by the single population formula:

$$n= ([Z^{\alpha}/2]^2P\ [1\text{-}P])/d^2$$

Where n = Minimum sample size; p = Estimate of the prevalence of self-medication (to estimate, p = 0.45 is used [17]); $Z^{\alpha}_{/2}$ = Standard normal variable at 1.96; α (confidence level) is mostly 5% (*i.e.*, with 95% confidence level); d = Tolerated error 0.05 (5%).

$$n = ([1.96]^2\ 0.45[1\text{-}0.45])/(0.05)^2$$

n = 380

Topping up with 10% for the non-response rate, the final sample size = 380 + (380 × 0.1) = 418.

Systematic random sampling was applied to select households, which were chosen from 1996 households, that is every 5th (every 1996/418th) household. An individual aged 18 and above was randomly selected from a selected household for interview. After clearly explaining the aim of the study, informed consent was obtained from all study participants before data collection. The standardized data collection tool was developed by reviewing related literature.

2.4. Operational definition

(i). Drug retailed outlet: Community pharmacist who sells drugs to prescribers [18].

(ii). Over-the-counter drugs: Drugs that are purchased by users without prescription [18].

(iii). Self-oral medication practice: Use of drug(s) by the study participants without consulting a qualified health practitioner [19].

2.5. Data quality assurance

Before the actual data collection began, a pre-test targeting 5% of the total sample (20 samples) was carried out at Agena town. Training was given to data collectors and supervisors on how to manage the data collection process. The data were collected in face-to-face interviews. First, data cleaning was done in the three steps during template formation to search for ensure consistency of values, by adhering to a good skipping pattern and controlling data entry. Second, cleaning during data entry was conducted

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by two data clerks using two computers in a blinded fashion; principal investigator was responsible for counter checking entered data, assessing 5 – 10% of daily-entered data. Third, after data cleaning, we calculated simple frequency, tabulated variables for consistency, and addressed layers and missing values.

2.6. Data processing and analysis procedure

The data was compiled, analyzed, and presented with tables, and analysis was performed using SPSS version 24 software. Logistic regression was performed and all variables with $P < 0.25$ in bivariable logistic regression were fitted into the backward stepwise multivariable logistic regression model. Adjusted odds ratio (AOR) along with a 95% confidence interval (CI) and $P < 0.05$ was used to interpret the findings of research from final models of multivariable regression tables.

3. Results

A very high response rate, measuring 95.2% of the total sample, was noted, while the remaining 4.8% were non-response rates. Among the 398 selected household individuals, 281 (70.6%) were females. Regarding the age of the respondents, 118 (29.6%) were 35 – 44 years. Two hundred and seventy (67.8%) were married. Around half of the respondents' were orthodox believers (198; 49.7%). In terms of educational status, 122 (30.7%) of the respondents had an education background of grade 9 – 12. Most of the respondents were employed (264; 66.3%), and the average monthly income was between 1500 and 5000 Ethiopian birr (Table 1).

Of the 398 respondents, 113 (28.4%) of them practice self-medication, and 243 (61.1%) reported to have fallen sick within the 2 weeks before the study period. Among the reported symptoms, headache (62, 25.5%) was the most common, followed by cough (39, 16.0%) (Table 2).

Among 113 respondents who practice self-medication, 43 (38%) used analgesic/antipyretics, 27 (23.9%) used anti-helminths, 26 (23%) used antimicrobials, 9 (8%) used antacids, and 8 (7%) used others as self-medication agents. Among households that practice self-medication, 57 (50.4%) reported that the practice worsened their health condition, 41 (36.3%) reported improved health condition, and 15 (13.3%) reported no change after self-medication. Besides, 265 (66.6%) of them reported that the health service they received was costly, and about 217 (54.5%) of them complained of sluggish service.

Among those who practice self-medication, 37 (32.7%) of them were male, 35 (31%) were aged 35 – 44 years, 70 (62%) were married, and 30 (26.5%) had only received elementary education (Table 3).

Based on bivariate analysis, marital status, educational status, occupation, thinking about self-medication, peer influence, and income were found to be significant factors influencing the adoption of self-medication. These factors were entered into multivariable logistic regression for further analysis to control for confounding factors. However, a significant association was observed between self-medication and study variables, such as marital status, educational status, thinking about self-medication, and peer influence (Table 4).

Table 1. Social and demographic factors of self-medication practice and associated factors among adult household members of Gubre town, Gurage Zone, Ethiopia

Variables	Category	Frequency	Percent
Sex	Male	117	29.4
	Female	281	70.6
Age	18 – 24	96	24.1
	25 – 34	106	26.6
	35 – 44	118	29.6
	45 – 54	41	10.3
	≥55	37	9.3
Marital status	Single	91	22.9
	Married	270	67.8
	Widowed	31	7.8
	Divorce	6	1.5
Religion	Orthodox	198	49.7
	Muslim	154	38.7
	Protestant	36	9.0
	Catholic	10	2.5
Educational status	Illiterate	77	19.3
	Read and write	74	18.6
	Elementary (1 – 8)	74	18.6
	Secondary (9 – 12)	122	30.7
	Higher (12+)	51	12.8
Occupation	Employed	264	66.3
	Non-employed	134	33.7
Ethnicity	Gurage	343	86.2
	Oromo	16	4.0
	Amhara	15	3.8
	Others	24	6.0
Income per month (Ethiopian birr)	<1500	162	40.7
	1500 – 5000	226	56.8
	>5000	10	2.2

Table 2. Frequency of symptoms reported by household members of Gubre town, Gurage Zone, Ethiopia

Illness/symptom	Frequency	Percent
Headache	62	25.5
Cough	39	16.0
Fever	35	14.4
Abdominal pain	34	14.0
Diarrhea and vomiting	24	9.9
Heartburn	23	9.5
Difficulty of swallowing	14	5.8
Others	12	4.9

4. Discussion

This study aimed to estimate the prevalence and factors concerning self-medication in Gubre town.

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Table 3. Self-medication practice among adult household members of Gubre town, Gurage Zone, Ethiopia

Variables	Self-medication		Total frequency
	Yes	No	
	Number (%)	Number (%)	Number (%)
Sex			
Male	37 (31.6%)	80 (68.4%)	117 (29.4%)
Female	76 (27.0%)	205 (73.0%)	281 (70.6%)
Age			
18 – 24	20 (20.8%)	76 (79.2%)	96 (24.1%)
25 – 34	28 (25.9%)	80 (74.1%)	108 (27.1%)
35 – 44	35 (31.0%)	78 (69.0%)	113 (28.4%)
45 – 54	14 (34.1%)	27 (65.9%)	41 (10.3%)
>55	16 (45.7%)	24 (54.3%)	40 (10.1%)
Religion			
Orthodox	63 (31.8%)	135 (68.2%)	198 (49.7%)
Muslim	35 (22.7%)	119 (77.3%)	154 (38.7%)
Protestants	14 (38.9%)	22 (61.1%)	36 (9.0%)
Catholic	1 (10.0%)	9 (90.0%)	10 (2.5%)
Marital status			
Single	27 (29.7%)	64 (70.3%)	91 (22.9%)
Married	70 (26.0%)	199 (74.0%)	269 (67.6%)
Divorced	2 (28.6%)	5 (71.4%)	7 (1.8%)
Widowed	14 (45.2%)	17 (54.8%)	31 (7.8%)
Ethnicity			
Gurage	98 (28.6%)	245 (71.4%)	343 (86.2%)
Oromo	3 (18.8%)	13 (81.3%)	16 (4.0%)
Amhara	6 (40.0%)	9 (60.0%)	15 (3.8%)
Other	6 (25.0%)	18 (75.0%)	24 (5.0%)
Education status			
Illiterate	20 (25.6%)	58 (74.4%)	78 (19.6%)
Read and write	26 (35.1%)	48 (64.9%)	74 (18.6%)
Elementary (1 – 8)	30 (39.5%)	46 (60.5%)	76 (19.1%)
Secondary (9 – 12)	26 (21.8%)	93 (78.2%)	119 (29.9%)
Higher (12+)	11 (21.6%)	40 (78.4%)	51 (12.8%)
Occupation			
Employed	59 (22.4%)	205 (77.6%)	264 (65.4%)
Non-employed	54 (40.3%)	80 (59.7%)	134 (33.6%)
Monthly income			
<1500	46 (28.4%)	116 (71.6%)	162 (40.7%)
1500 – 5000	66 (29.2%)	160 (70.8%)	226 (56.8%)
>5000	1 (10.0%)	9 (90.0%)	10 (2.5%)
Thinking about self-medication			
A good practice	93 (41.7%)	227 (58.3%)	320 (80.4%)
Not an acceptable practice	20 (3.9%)	58 (96.1%)	78 (19.6%)
Peer influence on self-medication			
Yes	70 (26.9%)	190 (73.1%)	260 (65.3%)
No	43 (31.2%)	95 (68.8%)	138 (34.7%)

Our study showed that the prevalence of self-medication was 28.4%. However, this rate was lower than those reported in previous studies: 48% in Khartoum state, Sudan and 43.24% in Ayder campus of Mekele University. The prevalence of self-medication practice in our study was greater than that of a study in Jimma town (27.6%); this discrepancy was probably attributed to the higher accessibility of medical information in towns like Jimma than in rural areas [20-22].

The most common illnesses that led to self-medication in this study, such as headache, common cold, and fever, were also reported in northwestern Ethiopia, Ayder campus of Mekele University. Half of the ill people who had headaches and abdominal pain sought medical help, suggesting that headaches and abdominal pain are important signals that make patients visit health facilities. Thus, the type of illness is a contributing factor to the patient's response toward their illness [5,21,22].

In this study, some of the most common reasons for the practice of self-medication were the previous experience with similar ailments, emergency care, and milder illnesses. The rationale is almost similar to a previous study conducted in the Ayder campus of Mekele University, but another study conducted in Jima regarded the low cost of practicing self-medication as the main reason [17,21,22].

Analgesics (paracetamol), anti-helminths, and anti-microbial were the most commonly used class of drug, because common cold and headache are the most common symptoms reported by respondents, and they obtained over-the-counter paracetamol for self-medication purposes. This rate was almost similar to a previous study in six Latin America countries and the Mekele University study [21,23]. Most of the drugs mentioned in this study were over-the-counter drugs and leftover drugs in the house, a finding almost similar to that reported in northwest Ethiopia, Amhara, and Jimma [5,21,22,24].

Marital status was found to be significantly associated with self-medication practice. Married respondents were about 1.6 times more likely to adopt self-medication as compared to single respondents (AOR = 1.099, 95% CI: 1.09 – 2.621). Respondents who were widowed were about 1.06 times more likely to adopt self-medication as compared to respondents who were single (AOR = 1.055, 95% CI: 1.001 – 1.922). In addition, educational status was found in significant association with self-medication practice. Literate respondents were 1.67 times more likely to self-medicate than those who were illiterate (AOR = 1.672, 95% CI: 1.032 – 2.01), a result similar to a study conducted in Somaliland in Borama district, which also showed that self-medication is significantly associated with educational status. This phenomenon can be explained by the ability of literate people, who are also of good socioeconomic standing, to access information on drug use and to purchase drugs from pharmacies. Similarly, a study conducted in Nigeria also showed that there was an association between self-medication practices and educational status [20].

Thinking about self-medication was found to significantly contribute to the practicing of self-medication. Respondents who thought that self-medication is an acceptable practice were about 1.65 times more likely to self-medicate as compared to respondents who thought that self-medication was not acceptable (AOR = 1.65, 95% CI: 1.32 – 1.887). A plausible reason is that they think self-medication does not negatively impact health.

Peer influence was also found to be significantly associated with self-medication. Respondents who had peer influence were

Table 4. Bivariate model and final multivariable model of risk factors for self-medication in Gubre town

Factor	Self-medication		Crude OR (95% CI)	Adjusted OR (95% CI)
	Yes	No		
Marital status				
Single	27	64	1	1
Married	70	199	1.199 (2.097 – 2.996)**	1.59 (1.09 – 2.62)*
Divorced	2	5	1.05 (0.84 – 4.51)	1.01 (0.22 – 1.19)
Widowed	14	17	0.51 (2.35 – 2.82)	1.06 (1.01 – 1.92)
Educational status				
Illiterate	20	58	1	1
Read and write	26	48	0.636 (0.54 – 2.90)	1.10 (0.76 – 1.97)
Elementary (1 – 8)	30	46	0.528 (0.27 – 4.47)	1.33 (0.75 – 3.21)
Secondary (9 – 12)	26	93	1.02 (0.458 – 2.25)	1.87 (0.34 – 1.67)
Higher (12+)	11	40	1.25 (1.55 – 2.47)**	1.672 (1.032 – 2.01)**
Occupation				
Employed	59	205	0.39 (0.253 – 2.59)	0.93 (0.12 – 2.02)
Non-Employed	54	80	1	1
Income (Ethiopian birr)				
<1500	46	116	0.280 (0.04 – 0.97)	0.56 (0.33 – 1.76)
1500 – 5000	66	160	0.27 (0.16 – 0.89)	0.431 (0.32 – 0.90)
>5000	1	9	1	1
Thinking about self-medication				
A good practice	93	227	1.494 (1.12 – 1.78)**	1.65 (1.32 – 1.89)**
Not an acceptable practice	20		1	1
Peer influence for self-medication				
Yes	70	190	1.23 (1.02 – 1.73)**	1.54 (1.30 – 2.32)**
No	43	95	1	1

NB: *0.05> P>0.01; **P≤0.01. OR: Odds ratio; CI: Confidence interval

about 1.54 times more likely to self-medicate as compared to respondents who had no peer influence (AOR = 1.54, 95% CI: 1.304 – 2.321). This result was supported by a study carried out in the Meket district in northeast Ethiopia. Peer influence on self-medication might stem from the belief in oneself and compliance with what is considered normal by one's friends.

Access to medical information was also implicated in a significant association with self-medication. Respondents who had no access to medical information were about 1.452 times more likely to self-medicate as compared to respondents who had access to medical information (AOR = 1.452, 95% CI: 1.263 – 1.570). This finding was supported by another study [16]. Possibly, the increased prevalence of self-medication was precipitated by a lack of adequate knowledge about adverse reactions and side effects.

A notable limitation of this cross-sectional study is that a cause-effect relationship cannot be delineated.

5. Conclusion

According to this study, nearly a quarter of the study participants practice self-medication. Headache, cough, fever, and abdominal pain are identified as the most common symptoms for respondents to practice self-medication. Being married, being literate, perceiving self-medication as an acceptable practice, peer influence, and access to medical information were significantly associated with self-medication practice. Health education should be given to the Gubre town residents to minimize inappropriate self-medication practices. It is highly essential to disseminate health information to create awareness among people regarding the disadvantages of self-medication practice through leaflets, mass media, and health education.

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None.

Conflict of Interest

The authors declare that they have no competing interest.

Ethical Approval and Consent to Participate

Ethical clearance was obtained from the Wolkite University, College of Medicine and Health Science Institutional Ethical Review Board. All procedures were performed in adherence with the guidelines and regulations relevant to human research. Both verbal and written informed consent was obtained from all subjects for the study. Legally authorized representatives of illiterate participants provided informed consent for the study.

Before data collection, the participants were instructed to sign on free and informed consent form. The personal identification of study participants was not recorded to ensure anonymity.

Consent for Publication

Not applicable.

Availability of Data

Data are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Indiscriminate use of psychotropic drugs by health discipline students at a private university in Colatina

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ABSTRACT

Background: Psychotropic drugs are used to treat disorders involving central nervous system. However, several drugs are indiscriminately used by individuals seeking better academic or professional performance or for esthetic or recreational purposes. Indiscriminate utilization of psychotropic drugs is deleterious and can cause insomnia, anxiety, and emotional ability in short term, as well as physical or psychological dependence, cardiovascular, cognitive, and motor alterations in long term, in addition to the risk of overdose.
Aim: This study was designed to characterize the prevalence of indiscriminate use of psychotropic drugs among students in the health disciplines in Colatina, Espírito Santo, to decipher the factors driving this practice. The findings from this study can lend themselves for designing preventive measures, in addition to providing a source of information about the harmful impacts of this habit.
Methods: A descriptive study on a sample of 122 college students from courses in the health disciplines was carried out using online questionnaires. The data were tabulated in GraphPad Prism 9 software, with 95% reliability ($P < 0.05$), and analyzed using Fisher's test.
Results: Half of the college students interviewed claimed to have used psychotropic drugs at some points in their lives, and 21.3% had attempted self-medication. Utilization of psychotropic drugs became a common habit among students after entering academic life (62.3%).
Conclusions: Self-medication is a common practice among the interviewed students and academic life, which has an significant impact on mental health, galvanizes this practice. These findings point to the need for better attention to the mental health of the university students and more education regarding the practice of self-medication in the population as a whole.
Relevance for Patients: The study highlights the need for greater attention by health professionals to this practice of self-medication, and more primary preventive measures focused on educating these users to prevent jeopardizing the health of individuals indiscriminately used psychopharmaceuticals and to consequently reduce health system costs.

1. Introduction

According to the National Health Surveillance Agency (ANVISA), psychotropic drugs are classified as "substances subject to special control" as they can cause physical or psychological dependence, in addition to significant side effects. Therefore, its commercialization requires retention of a controlled prescription (under inspection by ANVISA, issued by an authorized prescriber) [1].

Self-medication, defined as the selection and use of drugs to treat self-recognized symptoms including mental problems without consulting a physician, is recognized as a

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global issue [2]. In several international studies, the prevalence of self-medication stands at an average of 12% – 90% [3-6]. During COVID-19 pandemic, the self-medication rate increased exponentially and numerous adverse effects from self-medication have been documented [7,8].

Some individuals self-medicate themselves with psychotropic drugs for better academic and professional performance or esthetic or recreational purposes. The prevalence of psychotropic drug misuse is found to be high among university students, professionals who live under a high level of stress, and businessmen. This practice is deleterious and can cause insomnia, anxiety, and emotional ability in the short term. Moreover, it is associated with long-term physical or psychological dependence, cardiovascular, cognitive, motor alterations, and risk of overdose [9]. Therefore, this study aimed to characterize the prevalence of indiscriminate use of psychotropic drugs among students in the health discipline in Colatina, Espírito Santo, to decipher the factors that spur on this practice. A better understanding of these factors can help with the formulation of preventive measures.

2. Materials and Methods

We evaluated the use of psychotropic drugs and associated factors among students enrolled in health courses at a university center in Colatina, Espírito Santo. For this purpose, we carried out a descriptive study, since the objective was to determine the distribution of health-related conditions (the use of psychotropic drugs), according to time, place, and/or characteristics of individuals, in a single analysis. We chose students of health courses because previous studies have shown a higher prevalence of self-medication among students, which is attributable to higher workload, social pressure, greater access to information, and easy way of obtaining medication due to the lack of more stringent regulations. The institution had approximately 4000 students enrolled during the period in which the questionnaire was applied (between July and August 2020), of which 1743 were from courses in the health discipline. At first, we expected to obtain a sample of 149 students, considering the 12% prevalence of psychotropic use in Brazil [10]; however, due to the COVID-19 pandemic, we obtained a sample smaller than expected. Finally, we included 122 students for the research (confidence level was estimated at 95% and the sampling error with a margin of 5%), excluding students younger than 18 years old. The percentage of students interviewed was proportional to the number of students enrolled in each course. Only students enrolled in any heath discipline courses in the institution and aged 18 years old or older were included in this study; students younger than 18 years old were excluded.

For data collection, an online questionnaire adapted from the "IV survey on drug use among the first- and second-grade students in 10 Brazilian capitals" was utilized [11]. The survey took an average of 5 min to complete. The questionnaire was applied only once and included questions about the following variables: gender, age, course, course period, health data, self-assessment of physical and mental health, and pattern of use of psychotropic drugs and observed effects. Only individuals who agreed to the terms set forth in the Informed Consent Form (TCLE) form agreed to complete the survey. The data were tabulated in GraphPad Prism 9 software (Boston, MA, USA), with 95% reliability ($P < 0.05$). Fisher's test was used for the analysis of the variables.

The research was approved by the Human Research Ethics Committee of the UNESC, following the ethical principles defined by Resolution 466/12 of the National Health Council for carrying out research involving human beings (Certificate of Presentation for Ethical Appreciation [CAAE] in 33291620.1.0000.5062, opinion no. 4,091,372).

Colatina has 111,788 inhabitants and is a regional indicator of health status in the entire Northwest macro-region of Espírito Santo in Brazil. Colatina is an important medical and hospital care center that provides a series of treatments, ranging from basic care to highly complex examinations. This hub comprises seven hospitals, 54 municipal health units, 15 clinical analysis laboratories, six radiology clinics, two hemodialysis centers, a Regional Specialty Center, a blood center, and a Municipal Health Surveillance Center, among others. The university under study is responsible for training professionals for these institutions.

3. Results

The sample obtained consisted of 122 students who agreed to answer the questionnaire, equivalent to 7% of the total population of 1743 students regularly enrolled in health courses. In Table 1, we present the characteristics of the students evaluated, the

Table 1. Evaluation of characteristics and health of the students (n=122)

Variable	N (%)
Gender	
Female	96 (78.7)
Male	26 (21.3)
Age (years)	
18–24	91 (74.6)
25–34	23 (18.8)
35 or above	8 (6.6)
Course	
Nursing	24 (19.7)
Pharmacy	9 (7.4)
Physiotherapy	20 (16.4)
Medicine	45 (36.9)
Nutrition	17 (13.9)
Dentistry	7 (5.7)
Course period	
1–3 semester	17 (13.9)
4–6 semester	77 (63.2)
7–12 semester	28 (22.9)
Had used psychotropic drugs at some points in life	
Yes	61 (50.0)
No	61 (50.0)
Consider mental health issues is a priority	
As a priority and requires medical attention	116 (95.1)
As a priority but does not require medical attention	3 (2.5)
As a phase in life and does not require medical attention	3 (2.5)

Colatina, Espírito Santo, Brazil, 2020

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statement of use of psychotropic drugs during some period of their lives, and the priority they give to mental health. It was found that the largest population of this sample was female (78.7%; *n* = 96) and that the most prevalent age group was between 18 and 24 years old with (74.6%; *n* = 91). The data showed that 95.1% (*n* = 116) of the interviewees considered that medical follow-up is required for assessing a good mental health state. Regarding the use of psychoactive substances, half of the population (50%; *n* = 61), stated that they use or have used psychotropic drugs at some point in their lives.

Table 2 shows the mental health characteristics of students who admitted to using psychotropic drugs at some point in their lives (*n* = 61). On entering academic life, the use of psychotropic drugs become a common practice in 62.3% (*n* = 38) of the surveyed students. On the other hand, regarding the classification of the assessment of their own health in general terms, 18% (*n* = 11) considered it excellent, 37.7% (*n* = 23) very good, and 37.7% (*n* = 23) good. As for the evaluation of the impact on mental health after entering college, 52.4% (*n* = 32) and 9.9% (*n* = 6) stated that there was a slight and major worsening, respectively, after starting academic life.

Many students, amounting to 82.0% (*n* = 50), reported that the time spent on other activities has reduced due to the high demand of academic activities. With regard to their own emotional self-assessment, about 49.2% (*n* = 30) considered that they felt nervous most of the time. Among these students who use or have used psychotropic drugs, 49.2% (*n* = 30) and 19.7% (*n* = 12) feel exhausted most of the time and all of the time, respectively. As for depression, about 64.4% (*n* = 40) felt depressed some time. Regarding the most used class of psychotropic drugs, antidepressants stand as the most commonly used class of drugs, measuring at 68.8% (*n* = 42) of the surveyed students, followed by stimulants among 47.5% (*n* = 29) and benzodiazepines among 37.7% (*n* = 23) students. A complete profile of the drugs used is available in Figure 1.

Of the surveyed college students, 21.3% (*n* = 26) reported self-medication with psychotropic drugs. Evaluating this portion, 69.2% (*n* = 18) declared that they have health insurance; 30.8% (*n* = 8) have regular medical appointments; 53.8% (*n* = 14) have regular medical appointments only when necessary; 15.4% (*n* = 4) do not have regular medical appointments. As for mental health, 92.4% (*n* = 24) consider it a priority problem, which requires medical follow-up; 3.8% (*n* = 1) consider it a priority problem, which does not require medical follow-up; 3.8% (*n* = 1) consider it a temporary problem that does not require medical attention. Regarding the perception of the risk of psychotropic drug use: 61.6% (*n* = 16) of the students rated it as high risk, 34.6% (*n* = 9) as moderate risk, and 3.8% (*n* = 1) as low risk.

Table 3 depicts the relationship between self-medication, admission into college, and students' mental health. However, there is no statistically significant association between self-medication, student's admission into college, and their mental health changes. Interestingly, there is discrepancy between the ones that think mental health needs treatment prescribed by a specialist and the ones that regularly go to a doctor.

4. Discussion

The results of this study revealed that self-medication was practiced by 50% of the surveyed students, who admitted to using psychotropic drugs during some points of their lives. Current findings are in concordance with other studies on students in the health courses [12-14]. Half of the drugs named by the students in this survey can only be dispensed with a prescription, and

Table 2. Self-assessment of mental health of students who used psychotropic drugs according to the characteristics of students (*n*=61)

Variable	n (%)	*p*	Odds ratio
The beginning of the use of psychotropic drugs was…			
Before graduation	23 (37.7)	0.4	0.5 (0.2–1.6)
After graduation	38 (62.3)		
What do you think about your health after entering college?			
The same or better	23 (37.7)	0.2	0.49 (0.17–1.37)
Worse	38 (62.3)		
Did you reduce the time for other activities after college?			
Yes	50 (82.0)	0.3	2.27 (0.5–8.5)
No	11 (18.0)		
Have you ever felt nervous during your academic life?			
All the time or most of the time	41 (67.2)	>0.9	1.1 (0.4–3.3)
Some time or never	20 (32.8)		
Have you ever felt depressed during your academic life?			
All the time or most of the time	11 (19.4)	0.7	1.4 (0.4–5.0)
Some time or never	50 (80.6)		
Have you ever felt drained out during your academic life?			
All the time or most of the time	42 (68.9)	0.2	1.9 (0.6–6.6)
Some time or never	19 (31.1)		

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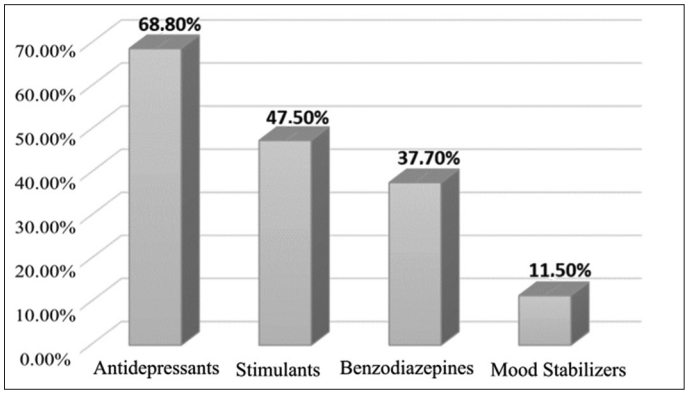

Figure 1. Profile of drugs used without medical prescription (n = 122). Colatina, Espírito Santo, Brazil, 2020.

Table 3. Relationship between self-medication and characteristics of academics among students who had used psychotropic drugs (n = 61)

Variable	*n* (%)
Do you have a health plan?	
Yes	18 (69.2)
No	8 (30.8)
Do you have regular medical appointments?	
Yes	8 (30.8)
No	4 (15.4)
Only when needed	14 (53.8)
Do you think mental health issue is a priority?	
As a priority and requires medical follow-up	24 (92.4)
As a priority, but does not require medical supervision	1 (3.8)
As a phase in life and does not require medical supervision	1 (3.8)
How do you consider the risk of using psychotropic drugs?	
High risk	16 (61.6)
Moderate risk	9 (34.6)
Low risk	1 (3.8)

Colatina, Espírito Santo, Brazil, 2020

drugs such as benzodiazepines, stimulants, and antidepressants were among the most cited drugs in this study. Indiscriminate use of these drugs can cause mild symptoms such as intestinal dysfunction, medium symptoms such as tachycardia, nervousness, and excessive excitement, and even, under rare circumstances, hepatitis, leukopenia, Parkinsonian syndrome, mental confusion, hypomania, restlessness, myoclonus, hyperreflexia, chills, tremors, diarrhea, and incoordination [12]. Some symptoms require immediate medical attention because negligence in delivering appropriate treatments can result in sequelae, such as anorexia, disturbed sleep, sexual dysfunction, increased appetite and weight, and muscle twitching [15].

Among the interviewees, those who report issues in carrying out their daily activities are more inclined to practicing self-medication. Among the reasons that may be related to this finding is the attempt to overcome symptoms that hinder adaptation to the demands of university life [16]. The difficulty in carrying out activities may also be related to changes in mental health among students after entering college: 49.2% felt nervous most of the time, 64.4% felt depressed some time, and 49.2% and 19.7% felt exhausted most of the time or all the time, respectively. In this scenario, psychotherapy can be a solution, which, in addition to being safe, is effective as an alternative form of pharmacological treatment depending on the condition, bringing a range of benefits. However, they also have limitations which are worthy of discussion. The administration of pharmacological treatment leads to more rapid symptom remission but at the risk of adverse side effects, whereas psychotherapy requiring weekly follow-up with the patients can be conducted in a completely safe manner, with therapeutic progress becoming more noticeable over time [17].

Another factor related to self-medication is that students who did not have a private health plan were the dominant group of individuals self-medicating themselves with psychotropic drugs (69.2%). However, students who could afford specialized care, such as psychotherapeutic assistance, also reported the practice of self-medication and substance abuse. This number is within normal limits, since many treatments such as psychotherapy are not offered free of charge, and they are beyond the economic reach of many university students [15]. Thus, the irregular use of medicines is more common among those who seek a quick and low-cost solution, especially the group without a good medical insurance. Self-medication can be defined as a practice of treating health problems with approved and available medications, without prescription and monitoring by a qualified professional, thus reducing the effectiveness and safety of the drug in question [18]. At present, a standardization instrument, such as clinical follow-up protocols established and prescribed by qualified professionals is not available for the identification of treatments and their diagnoses [15]. In addition, many cases of anxiety and major depressive disorder would have a better prognosis if non-drug therapeutic measures were associated with pharmacological treatment, which in certain cases are sufficient for remission of symptoms in milder cases [19].

Of those who stated that they had self-medicated at some point in their lives, 66.6% had some type of problem related to this practice. Although other studies did not portray the prevalence of this phenomenon, some outlined the risks of practicing self-medication, such as serious adverse reactions or poisoning that can lead to death. One of the hypotheses raised by the researchers carrying out this study was that having training in the health discipline could influence the prevalence of self-medication practice, but this remains to be validated.

Despite some limitations inherent to the design of the present study, as the analysis is based on the self-reported responses, our findings corroborate the literature, evidencing the need for professional support for students due to their extensive academic curriculum and pressures, which serve as a trigger for mental disorders such as anxiety, depression, and psychoactive substance abuse. It should be noted that some students have attempted inappropriate self-medication that put their health in hazard; a plausible explanation for this phenomenon is that they have incomplete knowledge about medical practice and limited experience, but the same justification cannot be applied to the already trained and qualified professionals, who have attempted self-medication and face the same problems [20-22].

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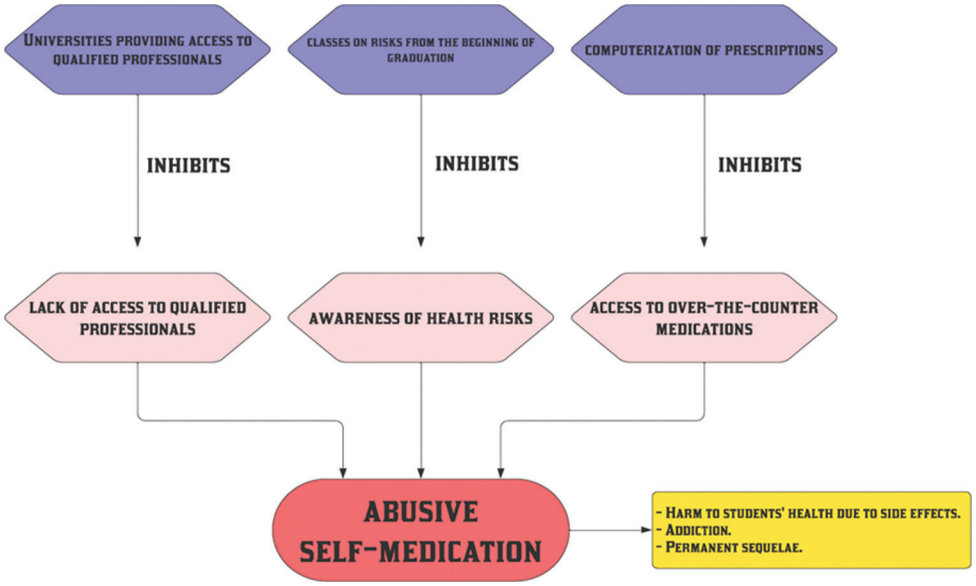

Figure 2. Abusive self-medication: Causes, consequences and proposed solution. Colatina, Espírito Santo, Brazil, 2020.

This survey also highlighted the lack of information regarding the adverse effects caused by self-medication. In Brazil, the population's low perception of risk also has its roots in the lack of social debate on the issue in the media, which only focus on illicit drugs as the significant problem in the country. In addition, drugs such as antidepressants appear to have a reasonable margin of safety and undeniable efficacy, justifying their popularity among doctors and the lay population. In the researched group, 18% and 37.7% consider their own mental health excellent and very good, respectively, while 37.7% consider it just good; 52.4% observed a slight deterioration in mental health after entering academic life, and 9.9% observed a major deterioration. Among the academics who assumed they had self-medicated with psychotropic drugs, 92.4% consider mental health a priority problem that requires medical follow-up. For the group that self-medicated with psychotropic drugs, only 61.6% considered using psychotropic drugs a highly risky practice. These findings point to the need for better attention to the mental health of the university students and more education regarding the practice of self-medication for the population as a whole.

The survey period, especially during which data collection was ongoing, coincided with the COVID-19 pandemic, which significantly stymied the data collection process because of the discontinuation of face-to-face academic activities and the enforcement of preventive measures against infection. To surmount the communication challenges, we used a digital communication platform, *Whatsapp*, to get in touch with the respondents during the study. Therefore, the lack of face-to-face survey is a limitation to data collection. The fact that this survey focuses on students of only one university significantly limits the generalizability of the current findings to other populations. In addition, given the anonymity requirements, respondents might omit their actual personal experiences during the survey, providing inaccurate responses that contribute to the reduced veracity of the current findings.

5. Conclusion

Students in the health discipline, despite their training, use psychotropic drugs for self-medication, a practice that can cause serious intestinal, cardiac, neurological, and psychiatric problems, and may cause permanent sequelae. The declining mental health after entering college and the difficulty in seeking medical care is among the possible causes justifying the misuse of psychotropic drugs. Therefore, the universities, once aware of the problem, should expand their support to students by offering professional help from psychologists and psychiatrists, if deemed necessary, so that they do not resort to self-medication.

In the same vein, to better prevent attempted self-medication, a brief orientation or lecture on the consequences and side effects of self-medication, as well as ethical problems in dealing with health professionals should be included in the first lesson of every course. Thus, having a better understanding of the risks of indiscriminate use of medications could serve as a disincentive to the practice, from the moment they enter university.

It is also necessary to intensify supervision over the dispensing of prescription drugs, with an emphasis on combating illegal supply, which is responsible for drug access without a prescription and consequent indiscriminate use of the drugs in question. A pivotal step in intensifying supervision entails the integration of the controlled prescriptions within a computerized system, which enables the trace mapping of a particular medication, facilitating inspection by the National Health Surveillance Agency. The supervision begins from the drug prescriptions by a medical professional to the user only, reducing incidence of possible fraud, transfer, and misappropriations. All the conclusions are summarized in Figure 2.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Approval and Consent to Participate

The research was approved by the Human Research Ethics Committee of the UNESC, following the ethical principles defined by Resolution 466/12 of the National Health Council for carrying out research involving human beings (CAAE in 33291620.1.0000.5062, opinion no. 4,091,372). Only individuals who agreed to the TCLE form agreed to complete the survey.

Consent for Publication

All participants agreed to the terms set forth in the Informed Consent Form.

Availability of Data

Data are available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

Prognostic nutritional index instead of serum Vitamin D levels as a determinant of the presence of osteoporosis in adult male patients with neurological impairment

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Abstract

Background: Complications of non-traumatic fractures and osteoporosis, which reduce mobility and quality of life, should not be ignored in patients with neurological impairment (NI).
Aim: To diagnose osteoporosis in adult patients with NI, a readily available and easily obtained index, instead of serum Vitamin D level or bone mineral density (BMD), was explored.
Methods: This was a single-center retrospective study. The participants were inpatients with NI admitted between August 2020 and June 2022. Patient data regarding (1) patient information, (2) blood data, including the prognostic nutrition index (PNI), which predicts outcomes of various diseases, (3) body composition, (4) T-score by BMD, (5) nutritional measures, and (6) outcome measures were collected. Enrolled patients were divided into two groups, with or without osteoporosis, according to their T-score. The data were analyzed by three methods: (1) comparison of all collected data between the two groups to analyze the factors influencing osteoporosis; (2) multiple logistic regression analysis; and (3) receiving operating characteristic curve analysis.
Results: Patients with osteoporosis had a significantly lower PNI (45 vs. 49, $P = 0.045$), and higher Vitamin D insufficiency (71% vs. 31%, $P = 0.031$). PNI was the strongest influencing factor, and its cutoff value for osteoporosis was 50.
Conclusion: The PNI is the strongest determinant of osteoporosis in patients with NI. Therefore, PNI can potentially be used as a surrogate for BMD instead of serum Vitamin D levels in institutionalized and homebound patients who do not have BMD measurement devices.
Relevance for Patients: Prognostic nutrition index, which is a simple blood test, outperforms serum vitamin D concentration as a good indicator for early detection of osteoporosis.

1. Introduction

Patients with neurological impairments (NI), including cerebral palsy (CP), are often affected by osteoporosis, associated fractures, and bone pain, which often result in reduced mobility. In this context, these complications, which reduce quality of life (QoL), cannot be ignored. Bone pain associated with osteoporosis occurs in 56.4% of patients, followed by deformity and fatigue in 44.2% and 36.9% of patients, respectively [1]. Osteoporosis is a debilitating bone disease characterized by low bone mass and poor bone quality. A major consequence of osteoporosis is the increased risk of fragility and non-traumatic fractures (NTFx). These fractures are a major cause of functional disability, morbidity, impaired QoL, and early mortality. Recent studies reported that the prevalence of osteoporosis in patients with NI showed an odds ratio ranging from 5.76 to 30.5 compared to those without NI [2,3], suggesting that NI is an osteoporosis

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risk factor. Diagnosing osteoporosis with comorbidities is difficult, especially in patients with NI who have spent long periods in nursing homes or other facilities without bone mineral density (BMD) measurement equipment. Overlooking the complications of osteoporosis in patients with NI can lead to the development of fractures and non-traumatic brain injury and reduce their QoL. Therefore, in this study, we developed an easy-to-measure index that could serve as a substitute for BMD measurement and an alternative index that would aid in diagnosing osteoporosis in patients with NI. The study showed that an alternative index to BMD can help detect osteoporosis and its related complications at early stages in patients with NI, allowing early treatment and improving their QoL. Institutionalized patients with NI often present with fragile bones as facilities often do not have the necessary equipment for measuring BMD, for example, in the case of care-related fractures. Therefore, addressing this issue can improve the patient's condition as well as their family, caregivers, and facility staff. However, not all facilities have the equipment to measure BMD to diagnose osteoporosis. A surrogate index can help diagnose osteoporosis in patients living in such institutions without adequate BMD measurements. Consequently, the objective was to develop an easy and reliable index for patients with NI to diagnose osteoporosis when BMD measuring equipment is unavailable.

2. Methods

2.1. Participants

In this single-center and retrospective study, all patients with NI hospitalized at a single medical center between August 2020 and June 2022 were included in the study. Patients diagnosed with NI who stayed at the hospital for more than 3 months during the study period were included. Patients were excluded from the study if they were as follows: (1) female, (2) younger than 18 years, (3) had missing BMD data, (4) had hepatic and renal dysfunction (serum total bilirubin level 1.5 mg/dL or serum creatinine level 1.5 mg/dL, and (5) died during hospitalization.

2.2. Data collection

Data for catabolic measurements were collected in the same month as the body composition measurements. The following information was collected:

(1) Patient information including age, sex, height, weight, antiepileptic drug (AED) use, and gross motor function classification scale (GMFCS) score (Appendix) evaluated by a single physician.
(2) Blood work data, including serum albumin level (Alb), serum creatinine level, serum total bilirubin level, hemoglobin level, platelet count, total lymphocyte count (TLC), neutrophil count, eosinophil count, basophil count, monocyte count, 25-hydroxyvitamin D (25-(OH) VD), and Onodera's prognostic nutritional index (PNI) calculated by 10 × Alb + 0.005 × TLC [4].
(3) Body composition indices measured using a bioelectrical impedance analysis (BIA) Inbody S10 (Inbody, Tokyo, Japan) device and the following components were measured: body mass index, lean mass, body water content, muscle mass, body fat mass, body fat percentage, extracellular water/total body water ratio, skeletal muscle mass, protein content, bone mineral content, somatic cell mass, basal metabolic rate, appendicular skeletal muscle mass index, and phase angle.
(4) BMD measurements, including the T-score of lumbar vertebrae L1-4 that was measured by dual-energy X-ray absorptiometry (DEX) using PRODIGY (Lunar iDXA; GE Healthcare Japan Co., Tokyo, Japan). T-score was calculated using the following equation: ([measured BMD – young adult average BMD]/[BMD-SD of young adult aged 20 – 44 years of the same sex and ethnicity]) [5]. Patients with a T-score of <−2.5 SD were diagnosed with osteoporosis.
(5) Nutritional measures, including average energy intake (kcal/kg/day) and average protein intake (g/kg/day) for a total of 3 days, including the days before and after the day of body composition measurement. The average Vitamin D intake (μg/day) and average calcium intake (mg/day) were based on the 42-day cycle menu of the research center's diet, and the intake of both Vitamin D and calcium varied daily, and the average salary for a 42-day cycle was used
(6) Outcome measures.

The primary outcome was the presence of osteoporosis (diagnosed using the T-score), and the factors that most influenced this outcome were compared. These data did not suffer from any source bias since the blind collection methodology was adopted.

As hormones strongly influence bone mineral quantification in female participants, they were excluded from this study. All collected data were compared between those with and without osteoporosis to characterize the group with osteoporosis. In addition to BMD, we examined the presence or absence of indicators that can be used to diagnose osteoporosis, particularly using test data that can be more easily collected.

- Method 1: Participants were divided into two groups, and the data collected were compared between the two groups to analyze the factors influencing osteoporosis.
- Method 2: Multiple logistic regression analysis on the factors identified in method 1 was performed to clarify the factors influencing osteoporosis.
- Method 3: Receiving operating characteristic (ROC) curve analysis was used to determine the cutoff values for the most influential factors in osteoporosis.

All procedures conformed to the ethical standards of the institutional and national review boards and the tenets of the 1964 Declaration of Helsinki.

2.3. Statistical analysis

We used a thumb rule for at least 12 people in each group and listed the main cross-tabulations required to ensure that the total number of participants in each table cell would be adequate and decided on the number of subjects.

In method 1, data were presented as medians and 25%, 75%, or percentage points, and differences between the two groups

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were evaluated using the Mann–Whitney *U*-test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables. In method 2, multiple logistic regression analysis was performed, and in method 3, the area under the curve (AUC) and 95% confidence interval (CI) were determined using ROC curve analysis. The AUC with 95% CI was considered significant when the AUC was 1.0 but not when the AUC was 0.5. The point with the highest sensitivity of (1 - specificity) was defined as the more effective cutoff value. Statistical significance was defined as $P < 0.05$. SPSS version 29 (IBM, Armonk, NY, USA) was used for all the statistical analyses.

3. Results

The study had 68 inpatients, 34 of whom were excluded. Of the 34 males, four with missing BMD data were excluded, and the remaining 30 were included in the analysis (Figure 1). All subjects were bedridden, and their motor function was assessed as equally impaired (level V of complete dependence on mobility) using the gross motor function classification scale (GMFCS) [6], which classifies motor function into five levels, ranging from level I with no limitations (walking, running, and climbing stairs) to level V with complete dependence on mobility support [6]. A single physician performed this assessment. The assessment is highly correlated with mobility, as represented by the World Health Organization "handicap score" [7], and can also be considered an indication of the degree of mobility and dysphagia leading to malnutrition [6].

3.1. An association of osteoporosis with lower PNI and vitamin insufficiency

Table 1 shows the results of the comparison between patients with NI with and without osteoporosis (T-score $-2.5 \leq$ vs. < -2.5). As a result, Alb and PNI were significantly lower in the osteoporosis group (3.6 g/dL vs. 3.9 g/dL, $P = 0.002$; 45 vs. 49, $P = 0.045$). Furthermore, the number of patients with 25-OH VD levels of <30 ng/mL was significantly higher in the osteoporosis group (71% vs. 31%, $P = 0.031$). These results indicate that, compared with patients without osteoporosis, patients diagnosed with osteoporosis by their T-score had (1) significantly lower Alb and PNI and (2) serum 25-OH VD <30 ng/mL and the number of patients with Vitamin D insufficiency was significantly higher in the osteoporosis group.

3.2. Lower PNI and Vitamin D insufficiency influence osteoporosis in patients with NI

Multiple logistic regression analysis revealed that PNI and 25-(OH) VD levels <30 mg/dL remained significant factors that influence osteoporosis or low BMD, with odds ratios of 1.233 ($P = 0.037$) and 0.132 ($P = 0.033$), respectively (Table 2). From the analysis of the factors influencing osteoporosis using method 2, the PNI was considered the strongest influencing factor.

3.3. ROC curve analysis results of factors that influence osteoporosis the most

The cutoff value for osteoporosis was calculated using ROC curve analysis. The cutoff value for the PNI without osteoporosis was 50 for severely disabled male subjects. The sensitivity and specificity were 0.500 and 0.857, respectively, and AUC was 0.714 ($P = 0.046$) (Figure 2).

4. Discussion

4.1. PNI can help predict health outcomes of various diseases, including cancer and non-cancer diagnoses

Onodera *et al.* reported that the PNI can help predict postoperative complications in patients with colorectal cancer [4]. Since then,

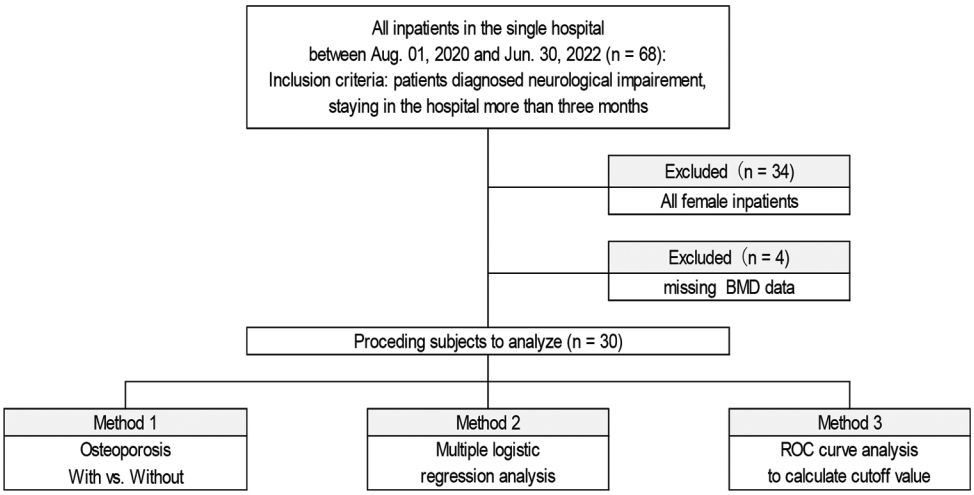

Figure 1. Flowchart of the study. A total of 68 adult patients with NI were enrolled in this study. Inclusion criteria were patients who had stayed at the study hospital for more than 3 months during the study period and were diagnosed with NI. To eliminate the confounding effect of gender, all female patients were excluded. In addition, four male patients were also excluded due to lack of BMD data. The remaining 30 male patients with NI were then further evaluated using the three methods shown in this flowchart.
Abbreviations, Aug: August; BMD: Bone mineral density; Jun: June; NI: Neurological impairment; ROC: Receiver operating characteristic.

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Table 1. Comparison of male CP patients with a T-score<2.5 SD (with osteoporosis) and a group above (without osteoporosis)

Parameters	Total	Osteoporosis (T-score≤−2.5)			BMD decrease (Z-score≤−2.0)		
		Present	Absent	*P*-value	With	Without	*P*-value
Characteristics							
Subject number	30	14	16		16	14	
Age, years	55 (48, 66)	60 (51, 67)	50 (34, 62)	0.092	56 (49, 67)	51 (44, 63)	0.519
BMI, kg/m^2	16.8 (15.0, 19.8)	17.5 (14.9, 20.1)	16.4 (15.0, 18.8)	0.506	17.1 (14.9, 19.9)	16.5 (15.1, 19.3)	0.901
Weight, kg	41.3 (36.0, 46.7)	41.5 (35.2, 45.8)	40.2 (35.5, 47.6)	0.868	41.5 (32.8, 44.5)	40.2 (36.0, 48.7)	0.934
Blood sampling parameters							
Alb, g/dL	3.8 (3.5, 4.0)	3.6 (3.2, 3.8)	3.9 (3.7, 4.2)	0.002	3.6 (3.2, 3.9)	3.9 (3.7, 4.2)	0.024
Hb, g/dL	13.7 (12.5, 14.6)	12.9 (11.6, 14.2)	14.5 (12.9, 14.9)	0.067	13.3 (11.9, 14.4)	14.1 (12.8, 14.8)	0.279
Plt, ×10^4/μL	20.8 (17.7, 25.8)	19.6 (17.1, 22.9)	22.4 (17.9, 27.5)	0.262	20.8 (17.4, 23.8)	20.5 (17.7, 27.8)	0.575
WBC, counts/μL	5315 (4770, 6540)	5240 (4698, 5933)	5485 (4805, 6580)	0.394	5240 (4725, 6498)	5485 (4780, 6540)	0.693
TLC, counts/μL	1748 (1290, 2254)	1915 (1399, 2254)	1694 (1177, 2427)	0.603	1915 (1355, 2325)	1694 (1242, 2308)	0.603
Onodera-PNI	46 (43, 51)	45 (41, 48)	49 (43, 53)	0.045	45 (41, 50)	48 (43, 54)	0.208
25(OH) vitamin D, ng/mL	31.4 (22.5, 37.4)	24.7 (20.5, 35.6)	34.3 (24.6, 38.1)	0.146	24.7 (20.7, 33.9)	35.1 (30.7, 38.3)]	0.081
25(OH) vitamin D<30 ng/mL, n (%)	15 (50)	10 (71)	5 (31)	0.031	12 (75)	3 (21)	0.004
Body composition measures							
Fat mass, %	29 (20, 43)	35 (20, 45)	25 (21, 36)	0.467	28 (19, 45)	29 (21, 38)	0.950
Fat-free mass, %	35 (29, 41)	31.5 (27.5, 40.2)	38 (32, 41)	0.244	34 (28, 42)	36 (30, 41)	0.708
ASMI, kg/m^2	4.40 (3.48, 5.43)	4.1 (3.3, 5.5)	4.6 (4.2, 5.2)	0.190	4.2 (3.3, 5.5)	4.6 (4.0, 5.1)	0.417
BMD measures							
T-score	−2.35 (−2.93, −1.43)	−2.95 (−3.53, −2.60)	−1.55 (−2.00, −0.83)	<0.001	−2.85 (−3.45, −2.53)	−1.35 (−1.93, −0.80)	<0.001
Z-score	−2.10 (−2.40, −1.18)	−2.50 (−3.43, −2.20)	−1.35 (−1.78, −0.55)	<0.001	−2.30 (−3.35, −2.20)	−1.15 (−1.63, −0.45)	<0.001
Medication							
AED, n (%)	18 (60)	9 (64)	9 (56)	0.659	10 (63)	8 (57)	0.769

Compared with male CP patients with a T-score<2.5 SD (with osteoporosis) and a group above (without osteoporosis), male CP patients with osteoporosis had a lower Onodera PNI score and a higher prevalence of serum Vitamin D concentration<30 ng/mL than those without osteoporosis.
Notes: (i) Continuous variables were tested using Mann–Whitney *U* test; (ii) categorical variables were tested using Chi-square test or Fisher's exact test.
Abbreviations: Alb: Serum albumin concentration; ALI: Advanced lung cancer inflammation index; BMD: Bone mineral density; BMI: Body mass index; ECW: Extra-cellular water; Hb: Hemoglobin; PNI: Prognostic nutritional index; TBW: Total body water; SII: Systemic immune-inflammation index; TLC: Total lymphocyte count; WBC: White blood cell; AED: Antiepileptic drugs.

Table 2. Osteoporosis variables and their odds ratios, 95% confidence intervals, and *P* values

Variable	OR	(95% CI)	*P*-value
PNI	1.233	(1.013, 1.501)	0.037
25(OH) Vit.D<30	0.132	(0.021, 0.849)	0.033

The multiple logistic regression analysis performed on the factors to clarify the factors influencing osteoporosis, both PNI and serum 25-(OH) Vitamin D concentration<30 ng/mL. According to these results, lower PNI and serum Vitamin D levels below 30 ng/mL indicate that patients with NI have a higher prevalence of osteoporosis.
Abbreviations: CI: Confidence interval; OR: Odds ratio; PNI: Prognostic nutritional index; Vit.D: Vitamin D.

more than 3,000 studies have been published for similar clinical purposes for various diseases, mainly in patients with cancer and less frequently in patients with other ailments [8-11]. As PNI is a comprehensive index of anti-inflammatory response and immune competence [8], it suggests that our subjects may be relatively acceptable in terms of the stabilization of inflammatory cytokines and oxidative stress markers, which play important roles in regulating albumin [11]. In this context, it can be interpreted that patients with NI with osteoporosis have a lower PNI, greater inflammation, and lower levels of Alb, which have an anti-inflammatory effect, than patients without osteoporosis [12-14].

4.2. PNI as a surrogate to identify osteoporosis in male patients with NI

It was recently reported to be 8.0%, 10.3%, 14.5%, and 25.9% in adults aged 18–30, 31–40, 41–50, and >50 years, respectively [15]. These observations suggest that the prevalence of osteoporosis in patients with CP increases at a rate of 1.5-fold per decade from 30 to >50 years. The present study suggested that PNI is the strongest factor influencing the coexistence of osteoporosis in patients with NI. Therefore, the PNI can potentially be a surrogate for BMD, especially in institutionalized and homebound patients who lack BMD measurement instruments. According to a report that examined the prevalence of osteoporosis in patients with CP by different age group with 10-year difference, female patients in their 30s to over 70 years of age accounted for up to 33.1% of the total prevalence, showing a linearly increasing trend with age. In contrast, male patients showed a peak of 12.4% in their 60s and a decrease to 10% in their 70s, indicating a clear sex difference in the prevalence [15]. However, detecting osteoporosis in patients with NI at home or in facilities without BMD measurement equipment is challenging. As factors such as sex and AED usage correlate with decreased BMD, we restricted the subjects in this study to

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males and included AED usage as a factor for our study. When the osteoporosis group was classified according to BMD measurements using a T-score of 2.5 SD or less as the criterion for osteoporosis, 14 patients (47%) in the osteoporosis group had significantly lower PNI values than those without osteoporosis. Therefore, a male patient with NI can be diagnosed with osteoporosis using the PNI. A previous study showed that NTFx was associated with an increased risk of 12-month mortality in adults with CP compared to those without CP, and NTFx is considered a major contributor to functional disability [13]. Therefore, special attention should be paid to adverse events occurring during the 12 months following NTFx. Based on these results, the PNI appears to be a surrogate for identifying the coexistence of osteoporosis in adult male patients with NI. Taken together, PNI is an indicator of adverse events such as aspiration, pneumonia, fractures, NTFx, and death.

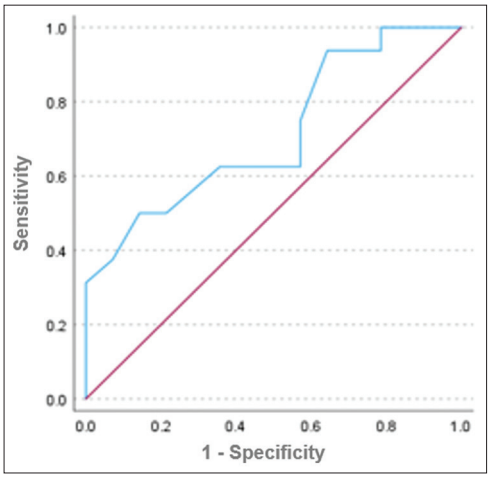

Figure 2. ROC curve analysis for determining cutoff value of PNI. Based on this curve, the cutoff value of PNI in adult NI patients to detect the association of osteoporosis was below the cutoff value of 50 of PNI. The sensitivity and specificity were 0.500 and 0.857, respectively, and the area under the curve AUC was 0.714 ($P = 0.046$).
Abbreviations: AUC: Area under curve; NI: Neurological impairment; PNI: Prognostic nutritional index; ROC: Receiver operating characteristic.

4.3. PNI cutoff values analyzed from the latest 1000 PNI-related articles

We reviewed 1000 articles on PNI studies published recently, except meta-analyses or reviews, to determine the cutoff value for PNI in patients with NI. Among these papers, 201 indicated clear cutoff values for both patients with cancer (125 papers) and patients with other ailments (76 papers) (Figure 3). This reflects that the first report of the PNI involved a study of patients with colorectal cancer. The latest PNI-related research, published in 2023, also used systematic reviews and meta-analyses to show that PNI is a helpful indicator for predicting outcomes in patients with cancer [16,17]. However, among patients with other ailments, there have been no studies on the PNI in patients with osteoporosis undergoing NI. To the best of our knowledge, this is the first report of PNI in a patient with NI and osteoporosis. From the comparison results of the two box plots showing the PNI cutoff values for patients with cancer and less frequently reported non-cancer diseases, at least two factors were found. First, the PNI of patients with cancer was closely scattered around 46 compared to those without cancer, and second, the PNI cutoff for patients without cancer was significantly lower than that for patients with cancer. Furthermore, in nearly 3000 PNI-related articles in addition to the 1000 papers, we analyzed, no study has examined NI and PNI in patients with osteoporosis. Therefore, we used PNI to assess patients with NI who also had osteoporosis. This study provides valuable results that can be used as indicators for osteoporosis risk assessment. Moreover, when 75 PNI articles in the non-cancer disease group

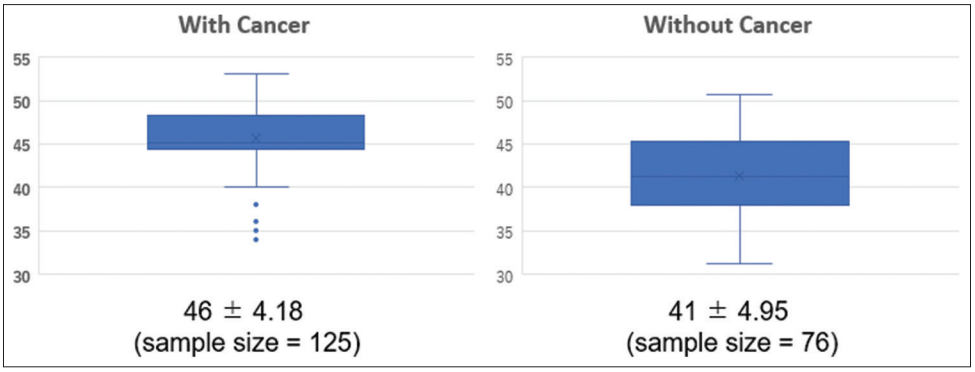

Figure 3. Comparison of the cutoff value of PNI in patients with and without cancer. Left: PNI cutoff value in patients with cancer was 46 ± 4 (sample size = 125). Right: PNI cutoff value in patients without cancer was 41 ± 5 (sample size = 76). The PNI cutoff for non-cancer patients was significantly lower than that for cancer patients. Furthermore, comparing this result with the PNI cutoff value of 50.0 in NI patients with osteoporosis in this study, PNI is a comprehensive index of nutritional status and immune competence. In addition, among the latest 1000 PNI articles, specifically, there were 201 arcs with cutoff values for PNI, of which 125 were PNI from patients with different types of cancer. This may reflect the fact that the first report of PNI was a study of patients with colorectal cancer. On the other hand, among non-cancer patients, there were no studies of PNI in patients with osteoporosis and NI, and this study appears to be the first report on PNI in patients with NI and osteoporosis.
Abbreviations: NI: Neurological impairment; PNI: Prognostic nutritional index.

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were arranged in descending order of PNI value, the PNI cutoff value of 50 for patients with NI shown in this study was next to the cutoff value for non-ST segment elevation myocardial infarction, which was 50.7 [18]. The PNI cutoff in this study was the second highest. The cutoff value to identify osteoporosis in patients with NI was 50, which is the second highest among non-cancer diseases. It has been suggested that this may be largely due to the loss of mobility. However, there is insufficient evidence to conclude that the magnitude of the PNI cutoff indicates the magnitude of inflammation underlying the target disease. Therefore, it is necessary to investigate whether diseases with the same PNI cutoff can be considered equivalent in terms of the incidence of inflammation and inflammation-related adverse events based on other aspects, such as cytokine storm parameters.

4.4. Strengths and limitations

To the best of our knowledge, this is the first study to report that PNI can be used to diagnose osteoporosis in patients with NI. We added the PNI cutoff value for patients with NI who are bedridden in hospitals. If these patients have osteoporosis with a PNI lower than the cutoff value, they may have a high probability of developing osteoporosis and experiencing adverse events. This information can notify caregivers to pay care-related attention to them to avoid adverse events such as aspiration pneumonia or choking during eating or drinking. In addition, for patients living at home or in facilities without BMD measurement equipment, PNI can be a feasible substitute if it can be measured through blood testing instead of a bone density scan. In subsequent care, this may help screen for osteoporosis and prevent complications, such as aspiration or pneumonia.

This study has several limitations. In the first study, the PNI was shown to be effective in predicting adverse events in patients with cancer [4,16,17]. Since the publication of the first article, there have been at least 76 reports on the efficacy of PNI to establish it as a predictor of noncancerous AEs (Figure 2). The present study demonstrated that PNI can help detect coexisting osteoporosis in bedridden male patients with NI. Therefore, the relationship between PNI and osteoporosis and between PNI and death was clarified. However, the relationship between PNI and death was not clarified. Second, during body composition analysis, we did not observe an association between Vitamin D deficiency and osteoporosis, skeletal sarcopenia, or sarcopenia. Recently, it has been proposed that sarcopenia and osteoporosis simultaneously affect muscles and bones, which are the target organs for Vitamin D hormones. The coexistence of these pathologies, termed "osteosarcopenia" [19], could not be demonstrated in the subjects of this study. In addition to the small number of subjects, it is unclear whether the essential existence of this pathology is problematic. Third, the number of studies included for assessment was too small to draw definitive conclusions. A larger number of participants must be included to gain more statistical power to detect all relevant associations and obtain a strong conclusion. In addition, a larger number of subjects may provide an additional and more accurate parameter for predicting osteoporosis comorbidity than the PNI.

5. Conclusion

The PNI was found to be the strongest determinant of osteoporosis in patients with NI. The PNI instead of serum Vitamin D levels can potentially be used as a surrogate for BMD in institutionalized and homebound patients who do not have BMD measurement devices.

Acknowledgments

We are grateful to Professor Eiji Yamato from the Department of Food Science and Nutrition, Mukogawa Women's University, Japan, for his collaboration on all parts of this study.

Funding

None to declare.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Approval and Consent to Participate

Informed consent was obtained using the opt-out method and the study was approved by the Research Center's Ethics Committee (R04-001).

Consent for Publication

The authors obtained the written informed consent from the subjects for publishing their data.

Availability of Data

Datasets generated during and/or analyzed during the present study, as well as the list of 1000 PNI-related articles and the extracted PNI cutoff values from 76 articles, are available from the corresponding author on reasonable request.

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Appendix

GMFCS scoring system

- Level I: No limitations (walks, runs, climbs stairs, etc.); impaired coordination.
- Level II: Limitations walking on uneven surfaces or long distances; needs support climbing stairs; difficulties running and jumping.
- Level III: Walks with a cane or crutches; needs a wheelchair to travel long distances.
- Level IV: Uses a walker at home; used in a wheelchair in other situations.
- Level V: Complete dependence for mobility.

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ORIGINAL ARTICLE

Three-dimensional analysis of pharyngeal airway volume in Class I, II, and III malocclusion

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ABSTRACT

Aim: This study aimed to evaluate pharyngeal airway dimensions using cone-beam computed tomography (CBCT) in patients with Class I, II, and III malocclusions and normal growth patterns.
Methods: All CBCT images were categorized into three groups: Class I (0° < ANB < 4°, −1 mm < Wits < 0 mm), Class II (ANB > 4°, Wits > 0 mm), and Class III (ANB < 0°, Wits < −1 mm). CBCT images were obtained from individuals with normal growth patterns (32 ± 5° = GN/GO – SN), where GN represents gonion, GN is gnathion, and SN equates to the sella-nasion distance. Measurements were taken for total pharyngeal volume, velopharyngeal volume, glossopharyngeal volume, and oropharyngeal volume, and the narrowest area of the airway was measured. ANOVA and Tukey's *post hoc* test were used to compare the airway dimensions among skeletal classes I, II, and III.
Results: The CBCT images were captured from 90 patients (45 males and 45 females) aged 17 to 39. The mean volume of the total pharyngeal airway, velopharyngeal, glossopharyngeal, and oropharyngeal and the most constricted area were significantly greater in patients with skeletal Class III malocclusion compared to patients with skeletal Class II malocclusion showing normal growth pattern. Total pharyngeal airway, velopharyngeal, and oropharyngeal volumes were lower in Class II patients compared to Class I and III patients with normal growth patterns. There was a significant difference in the pharyngeal space between males and females with Class II malocclusion. Pharyngeal space in female Class II malocclusion was higher than that in males. There was no difference regarding airway space between female and male patients with Class 1 malocclusion. Pharyngeal space between females and males with Class III malocclusion showed no difference.
Conclusion: Class III pharyngeal volumes were generally larger in Class I and II malocclusions. Sex differences in the volumes of various pharyngeal spaces were only present in the case of Class II malocclusions.
Relevance for Patients: Class II pharyngeal volumes were generally smaller in Class I and III malocclusions.

1. Introduction

Since the 19th century, there has been a significant focus on investigating the connection between craniofacial morphology and respiratory function [1]. Numerous studies reviewed in the literature have suggested that the transition from two-dimensional (2D) radiography to three-dimensional (3D) cone-beam computed tomography (CBCT) represents a dependable and consistent approach capable of substituting conventional radiography [2-5].

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Some studies supported the association between skeletal pattern and the airway, while others did not show such a relationship. A study by Jadhav *et al.* [6] demonstrated that there was no significant correlation between the total airway volume and three sagittal skeletal groups. Alhammadi *et al.* [7] reported that the volume of the palatopharyngeal and glossopharyngeal airways and the narrowest point of the palatopharyngeal airway were greater in Class II skeletal than in other skeletal groups. Alves *et al.* [8] found that the type of malocclusion did not influence the dimensions and volumes of the airway in most cases. On the other hand, Tseng *et al.* [9] showed that individuals with Class II skeletal malocclusion have smaller airway volumes than individuals with Class I and III malocclusion. In a study by Shokri *et al.* [10], it was shown that the volume and area of the airway were significantly greater in Class III patients than in Class I or II. Zeng *et al.* [11] demonstrated that the volume of the pharyngeal airway was significantly greater in Class III and Class I patients compared to Class II patients. Due to the significant discrepancies in the results among the studies mentioned above and the lack of research regarding different sagittal malocclusion with normal growth patterns in the Middle Eastern population, the necessity of conducting this research became evident. Therefore, this study aimed to measure the relationship between the airway volume and skeletal Class I, Class II, and Class III malocclusions with normal growth patterns in individuals aged 17 – 39 years using CBCT.

2. Patients and Methods

In this study, we conducted a comprehensive analysis of CBCT obtained from the Department of Oral and Maxillofacial Radiology archives at Tehran Medical Sciences, Islamic Azad University. The survey was conducted in accordance with the guidelines of the Declaration of Helsinki. All human research was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, revised in 2013. Ethical approval was obtained from the Islamic Azad University Local Research Ethics Committees (protocol identifier IR.IAU.DENTAL, REC; 1400.041).

In this cross-sectional analytical study, the 90 CBCT images were divided into three groups, with 30 patients in each class, namely Class I, II, and III malocclusions. These CBCT images were obtained using a Sirona Galileos Sirona Dentsply device in Germany; all images were prepared by the Scan-Fast protocol, with a scan time of 14 s, a field of view of 15 cm × 15 cm, 98 kV, and 3 mA. All CBCTs were performed when the patients assumed the standing position, and patients stood when looking at themselves in the mirror. All images were taken from CBCT scans where the teeth were in occlusion, and all cephalograms of CBCT scans are completely real because they were extracted from CBCT images captured using the Sirona Galileos device, Germany. The patients were divided into three groups: Class I, Class II, and Class III, based on the ANB angle and Wits appraisal. The SNA and SNB angles were measured using the following points: S (the center of the sella turcica), N (the intersection points of the nasion and the frontal bone in the sagittal view), A (the innermost point on the anterior contour of the maxilla below the maxillary plane), and B (the innermost point on the anterior mandibular shape above the pogonion). The Wits appraisal is the measured distance between A and B along the mid-sagittal reference line. GoGn-SN angle was measured between the line of the gonion (Go) and gnathion (Gn) and the sella-nasion (SN) line. All patients were middle easterners and had normal growth patterns.

All CBCT images were selected from patients with a mandibular plane angle of 32 ± 5 = GoGn-SN.

- Class I: 0° < ANB < 4°; −1 mm < Wits < 0 mm
- Class II: ANB > 4°; 0 mm < Wits
- Class III: ANB < 0°; Wits < −1 mm

Exclusion criteria of this study are as follows:

The patients who had no history of orthognathic surgery, nasal surgery, syndromes, trauma, or pathology in the airway and pharynx.

CBCT images that lacked diagnostic value.

CBCT images were converted to DICOM format and transferred to 3D Dolphin software (Management & Imaging Solutions, Chatsworth, CA, USA). The overall volume of the pharyngeal airway and the most constricted area (mm^2) were assessed and determined (Figures 1 and 2). The measurements were performed by two researchers, and the intraclass correlation coefficient (ICC) was calculated to determine the reliability of the two researchers. In this study, the ICC was above 80%, indicating the reliability of the two researchers.

The definitions used throughout this study are as follows:

2.1. Total pharyngeal airway volume (TP)

The upper bound of the pharyngeal airway passes through PNS and is parallel to the standard horizontal plane; the lower bound passes through C4 and is parallel to the standard horizontal plane.

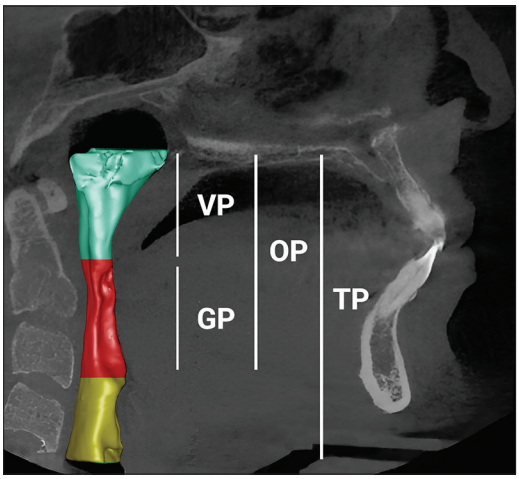

Figure 1. Three-dimensional upper airway model.
Abbreviations: VP: Velopharyngeal airway volume; GP: Glossopharyngeal airway volume; OP: Oropharyngeal volume; TP: Total pharyngeal airway volume.

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2.2. Velopharyngeal airway volume (VP)

The upper bound of the velopharyngeal airway passes through PNS and is parallel to the standard horizontal plane; the lower bound passes through the tip at the end of the soft palate and is parallel to the standard horizontal plane.

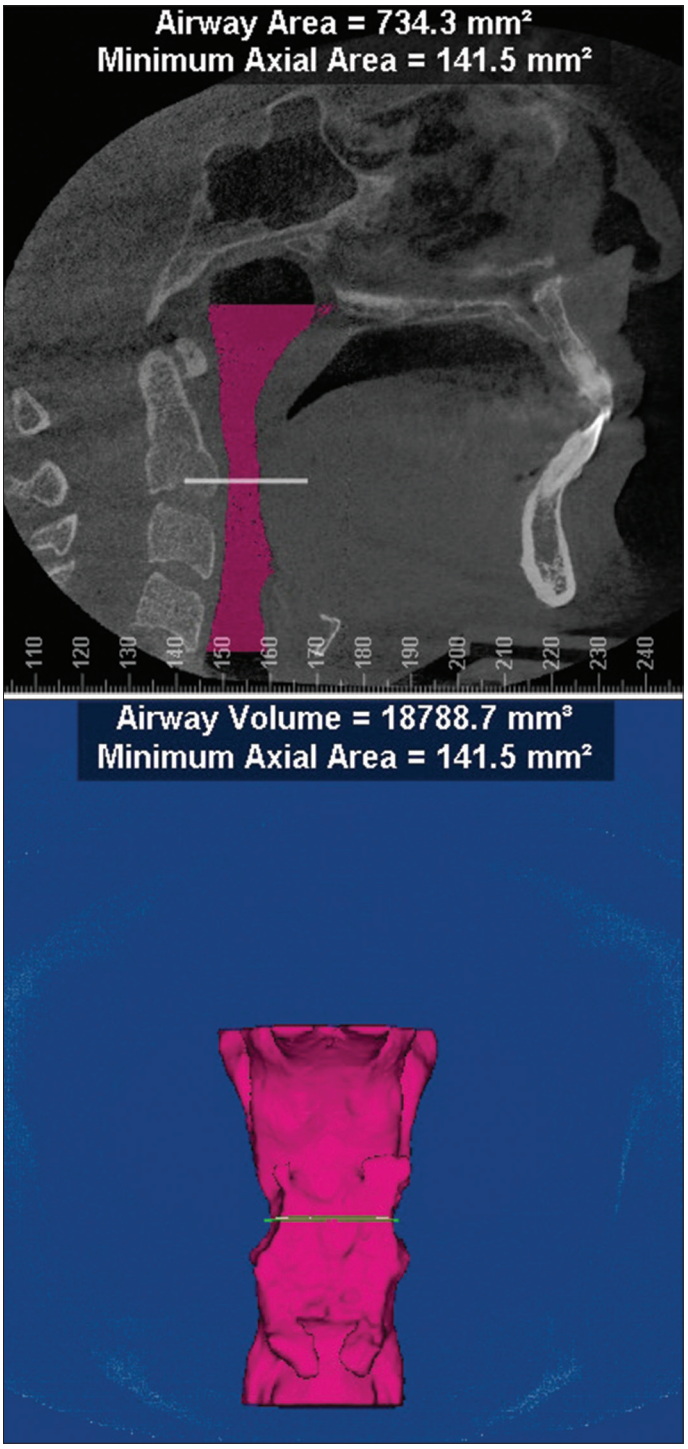

Figure 2. Three-dimensional upper airway model. Pink color refers to 3D pharyngeal volume.

2.3. Glossopharyngeal airway volume (GP)

The upper bound of the glossopharyngeal airway passes through the tip at the end of the soft palate and is parallel to the standard horizontal plane; the lower bound passes through the upper tip at the end of the epiglottis and is parallel to the standard horizontal plane.

2.4. Oropharyngeal airway volume (OP)

VP + GP, the velopharyngeal and glossopharyngeal airways are together known as the oropharyngeal airway.

2.5. Most constricted area

The smallest cross-sectional views of the upper respiratory tract of the image were measured.

The data were analyzed using SPSS software version 22.0 (IBM, Armonk, NY, USA). Analysis of variance (ANOVA) and Tukey's *post-hoc* correction were used to compare the dimensions of the airway among the skeletal malocclusion groups (Class I, II, and III). $P < 0.05$ was considered statistically significant.

3. Results

This study analyzed CBCT images of 90 patients (45 males and 45 females) aged 17 – 39 years. The three groups did not have any significant difference in terms of gender and age. All data were normally distributed. According to ANOVA results, there was a significant difference in the means of SNA, SNB, and ANB angles and Wits appraisal among the three malocclusion classes. However, the mean GoGn-SN angle did not show any significant difference among the three classes (Table 1).

The total pharyngeal airway volume was 19.483 ± 3.071, 16.091 ± 2.788, and 23.235 ± 5.684 mm^3 in Class I, II, and III malocclusions, respectively ($P < 0.001$). The volume of the total pharyngeal airway, velopharyngeal, glossopharyngeal, and oropharyngeal and the most constricted area in Class II malocclusion were less than Class I and III malocclusions ($P < 0.001$). The volume of velopharyngeal, glossopharyngeal, and oropharyngeal regions and the most constricted area were

Table 1. Comparison of cephalometric measurements according to the skeletal malocclusion

Variable	Type of malocclusion			*P*-value[1]
	Class I	Class II	Class III	
SNA[2]	81.8±1.9	81.5±2.0	79.8±2.2	0.005*
SNB[2]	79.3±2.1	76.1±2.8	81.5±2.2	<0.001*
ANB[2]	2.6±1.0	5.9±1.7	−1.8±1.4	<0.001 *
Wits	−3.0±0.3	4.0±2.3	−4.2±2.1	<0.001*
Sn-Go-Gn[2]	32.7±2.7	32.3±2.5	32.6±2.4	0.845

Data are expressed as mean±standard deviation.
[1]ANOVA; *statistically significant.
[2]S, the center of the sella turcica; N, the intersection points of the nasion and the frontal bone in the sagittal view; A, the innermost point on the anterior contour of the maxilla below the maxillary plane; and B, the innermost point on the anterior mandibular shape above the pogonion

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higher in Class III malocclusion compared to Class I and II malocclusions ($P < 0.001$; Table 2).

A significant difference in volume was observed in all pharyngeal space pairs between the two malocclusions, except glossopharyngeal volume in Class I versus II and Class I versus III. Furthermore, there were no significant differences in the most constricted area between classes I and II and between classes I and III (Table 3).

The independent *t*-test showed that there was no significant difference in pharyngeal space between males and females in Class I and III malocclusion. However, in Class II malocclusion, there was a significant difference between females and males (Table 4).

4. Discussion

The respiratory tract has a crucial role in swallowing, breathing, and articulation [12,13]. Airway space also affects the body posture and head inclination [14,15]. The mean volume of the pharyngeal, velopharynx, oropharynx, and glossopharynx and the mean area of the narrowest region airway in patients with skeletal Class III malocclusion were significantly larger than in patients with skeletal Class I and II malocclusions. Class III patients have a more protruding mandible and the tongue is positioned more anteriorly. These anatomical features consequently widen the distance between the posterior pharyngeal wall and the dorsum of the tongue, creating a larger airway space in skeletal Class III malocclusion than in classes I and II [9].

Several studies have evaluated the relationship between skeletal pattern, craniofacial morphology, and pharyngeal airway volume

Table 2. Comparison of the total pharyngeal airway, velopharyngeal, glossopharyngeal, oropharyngeal, and most constricted area per skeletal malocclusion

Variable	Type of malocclusion			*P*-value[1]
	Class I	Class II	Class III	
Total pharyngeal airway volume (mm^3)	19.483±3.071	16.091±2.788	23.235±5.684	0.001*
Velopharyngeal volume (mm^3)	9.995±2.230	8.258±1.922	11.670±3.514	<0.001*
Glossopharyngeal volume (mm^3)	3.050±1.225	2.298±1.207	3.735±1.771	0.001*
Oropharyngeal volume (mm^3)	13.112±2324	10.556±2596	15.405±4.612	<0.001*
Most constricted area (mm^2)	154±45	119±46	173±92	<0.001*

Data are expressed as mean±standard deviation.
[1]ANOVA; *statistically significant

Table 3. Comparison of airway measurements in pairs between two malocclusions

Variable	Pair comparison	Result of comparison	*P*-value[1]
Total pharyngeal airway volume (mm^3)	I versus II	I>II	0.005*
	I versus III	III>I	0.002*
	II versus III	III>II	< 0.001*
Velopharyngeal volume (mm^3)	I versus II	I>II	0.034*
	I versus III	III>I	0.043*
	II versus III	III>II	<0.001*
Glossopharyngeal volume (mm^3)	I versus II	I>II	0.108
	I versus III	III>I	0.156
	II versus III	III>II	<0.001*
Oropharyngeal volume (mm^3)	I versus II	I>II	0.011*
	I versus III	III>I	0.025*
	II versus III	III>II	<0.001*
Most constricted area (mm^2)	I versus II	I>II	0.095
	I versus III	III>I	0.460
	II versus III	III>II	0.004*

[†]Tukey's *post-hoc* correction; *statistically significant

Table 4. Sex-based comparative analysis of the total pharyngeal airway, velopharyngeal, glossopharyngeal, oropharyngeal, and most constricted area according to the class of skeletal malocclusion.

Malocclusion class	Gender	Mean and standard deviation	*P*-value[1]
I			
Total pharyngeal airway volume (mm^3)	Male	19.023±2.877	0.421
	Female	19.944±3.288	
Velopharyngeal volume (mm^3)	Male	9.807±2.445	0.653
	Female	10.182±2.061	
Glossopharyngeal volume (mm^3)	Male	3.029±1.415	0.928
	Female	3.071±1.051	
Oropharyngeal volume (mm^3)	Male	13.024±2.563	0.839
	Female	13.201±2.146	
Most constricted area (mm^2)	Male	148±54	0.533
	Female	159±34	
II			
Total pharyngeal airway volume (mm^3)	Male	14.873±2.479	0.014*
	Female	17.309±2.603	
Velopharyngeal volume (mm^3)	Male	7.379±1.790	0.01*
	Female	9.137±1.671	
Glossopharyngeal volume (mm^3)	Male	1.584±554	0.001*
	Female	30.12±1.273	
Oropharyngeal volume (mm^3)	Male	8.963±2.176	0.001*
	Female	12.149±1.947	
Most constricted area (mm^2)	Male	100±38	0.021*
	Female	137±46	
III			
Total pharyngeal airway volume (mm^3)	Male	23.462±5.695	0.831
	Female	23.008±5.863	
Velopharyngeal volume (mm^3)	Male	11.570±3.380	0.880
	Female	11.770±3.760	
Glossopharyngeal volume (mm^3)	Male	3.681±1.706	0.871
	Female	3.789±1.892	
Oropharyngeal volume (mm^3)	Male	15.251±4.673	0.859
	Female	15.559±4.708	
Most constricted area (mm^2)	Male	186±90	0.462
	Female	161±94	

[1]Independent *t*-test; *statistically significant

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using CBCT, and reported conflicting results. Elagib *et al.* [16] found that skeletal patterns affect airway volume. However, the authors reported that age and sex also significantly influence airway variables. The findings in the current study differ from the data by Elagib *et al.*, who reported a significant difference between Class I, II, and III malocclusions with respect to pharyngeal space. In the current study, the age of patients was from 17 to 39 years, while the patients' age in the study by Elagib *et al.* was from 12 to 19 years. Furthermore, the race of patients in both studies was different, and both sex and age significantly affect pharyngeal space.

Tseng *et al.* measured the differences in airway volume and the smallest cross-sectional area of the pharynx among 90 patients with three skeletal patterns using CBCT. The authors observed that the mean overall volume of the respiratory pharyngeal airway, velopharynx, glossopharynx, oropharynx, and hypopharynx and the smallest cross-sectional area of the respiratory airway in Class III patients and Class I patients were significantly larger than in Class II patients. The study further revealed that the airway volume of the patients in skeletal Class II was only two-thirds of the airway volume of those in skeletal Class III, which is comparable to our study.

Opdebeeck *et al.* [17] reported that patients with a vertical growth pattern have a smaller airway space than those with a horizontal growth patterns. The difference between the findings of Opdebeeck *et al.* and current study is due to the growth pattern, which was normal in our study. It appears that the growth pattern affects the dimensions of the pharyngeal space.

Di Carlo *et al.* [18] evaluated the airway space of 90 young adult patients with Class I, II, and III malocclusions. They found no statistical difference between airway dimension and sagittal malocclusion. In contrast to our study, the CBCTs were acquired in supine position, where the patients were lying in a bed with their head fitted in a molded pillow. Moreover, Di Carlo *et al.* selected the CBCT images according to the following criteria: Class I ($0.5° < ANB < 4.5°$), Class II ($ANB > 4.5°$), and Class III ($ANB < 0.5°$). Future studies should attempt to evaluate the area of nasal vestibules, which is a restricting factor in nasal airflow. It is also recommended that the position of the hyoid bone be assessed about its muscular attachment.

One of the limitations of this study was that CBCT scans of patients with horizontal or vertical growth patterns were not included due to the insufficient number of such cases. It is expected that in future studies, airway dimensions will be evaluated in patients with Class I, II, and III malocclusions, including vertical, normal, and horizontal growth patterns, and the volume of the nasopharynx will also be measured.

5. Conclusions

The mean volume of the total pharyngeal airway, velopharyngeal, glossopharyngeal, and oropharyngeal and the most constricted areas were significantly greater in patients with skeletal Class III malocclusion compared to patients with skeletal Class II malocclusion with normal growth pattern. Total pharyngeal airway, velopharyngeal, and oropharyngeal volumes were lower in Class II patients compared to Class I and III patients with normal growth patterns. There was a significant difference in pharyngeal space between males and females with Class II malocclusion. Pharyngeal space in females with Class II malocclusion was larger than that in their male counterparts. There was no difference in airway space between female and male patients with Class 1 malocclusion. Pharyngeal space between females and males showed no difference in Class III malocclusion.

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Conflict of interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate

Ethical approval was obtained from the Islamic Azad University Local Research Ethics Committees (protocol identifier IR.IAU. DENTAL, REC;1400.041). Informed consent was obtained from the patients.

Consent for publication

Informed consent for releasing the patients data and/or images in this paper was obtained.

Availability of data

Data are available from the corresponding author upon reasonable request.

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