Gülhane Medical Journal

ISSN: 1302-0471
e-ISSN: 2146-8052

Gulhane Med J June 2022 Volume 64 Issue 2

www.gulhanemedj.org

64/2

Gülhane
Medical Journal
Gülhane Tıp Dergisi

Gülhane Medical Journal is the official scientific publication of the Gülhane Faculty of Medicine, University of Health Sciences Türkiye.

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Publisher Certificate Number: 14521

Printing at: Son Sürat Daktilo Dijital Baskı Merkezi Sanayi ve Ticaret Ltd. Şti.
Gayrettepe Mahallesi, Yıldızposta Caddesi, Evren Sitesi A Blok No: 32 D: 1 - D: 3 34349 Beşiktaş, İstanbul, Türkiye
Phone: +90 (212) 288 45 75 **E-mail:** info@sonsuratdaktilo.com
Printing Date: June 2022
ISSN: 1302-0471 **E-ISSN:** 2146-8052
International scientific journal published quarterly.

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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

In the second issue of 2022, we have prepared interesting original articles, review articles, and case reports for our readers. As the journal's publishing team, we tried to cover a wide range of articles from different disciplines.

I would like to express my gratitude to all submitting authors, reviewers, and editors for their contributions.

Prof. Dr. M. Ali Gülçelik
Editor-in-Chief

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DOI: 10.4274/gulhane.galenos.2021.72692
Gulhane Med J 2022;64:128-35

Use of high flow nasal cannula as a modality of acute respiratory failure due to COVID-19

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Date submitted:
28.04.2021
Date accepted:
13.06.2021
Online publication date:
15.06.2022

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Keywords: High-flow nasal cannula, HFNC, COVID-19, acute respiratory distress

ABSTRACT

Oxygen (O_2) therapy is the first-line therapy for acute respiratory distress with hypoxemia due to Coronavirus disease-2019 (COVID-19). High-flow nasal cannula (HFNC) therapy represents one of the O_2 therapy alternative modalities. HFNC is a supportive O_2 therapy device using a specially designed HFNC warmed and moisturizer, resulting in a stable flow rate. The physiological mechanisms of HFNC make its efficacy better than other O_2 therapy applications. Management of COVID-19 focuses on preventing disease worsening, and HFNC can optimize the outcome of therapy. Further studies are necessary to evaluate the benefits of HFNC in hypoxemia management. It is also important to clarify its contraindications and factors associated with HFNC failure.

Introduction

Coronavirus disease-2019 (COVID-19) is an infectious disease caused by Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2). SARS-CoV-2 is a new type of coronavirus identified in humans, first reported in Wuhan, China, on December 31st, 2019. COVID-19 has spread to various parts of the world, and on March 11th, 2020, WHO declared a COVID-19 pandemic (1,2).

A preliminary report from the Chinese Centers for Disease Control and Prevention estimated that most confirmed cases of SARS-CoV-2 infection were 81% mild-moderate, 14% severe, and 5% in critical condition (3). Severe SARS-CoV-2 infection

is a critical condition for the occurrence of acute respiratory distress syndrome (ARDS), and even respiratory failure (4).

Patients with COVID-19 who are accompanied by pneumonia have respiratory problems hindering the maintenance of O_2 needs. Respiratory disorders can range from respiratory distress to respiratory failure. Respiratory distress is the increase and worsening of breathing effort. Respiratory failure is a disorder of one or more respiratory functions and is a life-threatening condition. COVID-19 patients will experience respiratory failure in severe and critical conditions (2).

O_2 therapy is the first-line therapy for respiratory distress with hypoxemia due to COVID-19. The method for providing O_2 therapy varies and must follow the severity of the disease (2). The goal of O_2 therapy is to maintain O_2 (SpO_2) saturation >90% (2). High flow nasal cannula (HFNC) is a modality for O_2 therapy in COVID-19 with mild to moderate ARDS. HFNC is an option to treat hypoxia (2). The most important rational approach to O_2 therapy with HFNC in respiratory distress patients with hypoxemia is to treat hypoxia as soon as possible to reduce the risk of intubation. However, HFNC therapy requires close monitoring, and if there is no improvement, an immediate switch to another therapeutic modality is needed, such as intubation with mechanical ventilation.

Differences between O_2 support with low flow and high flow nasal cannulas

O_2 support therapy is an intervention used by clinicians in acute hypoxia treatment. One of the supportive O_2 therapies is the use of a conventional low flow nasal cannula (LFNC). However, LFNC is only effective in providing O_2 support with a flow rate of 4-6 liters/minute or equivalent to the inspired oxygen fraction (FiO_2) of 37-45% (5). Furthermore, LFNC can cause dryness and irritation in the nasal mucosa, increasing the potential for bleeding during long-term use (5). Additionally, there are limitations to O_2 support with LFNC as it depends on the diameter of the nasal cannula used and the adult size is 6.1 mm (5). Furthermore, LFNC is an open O_2 supplementation approach with a high rate of gas leakage and delusions of O_2, limiting its efficacy (6).

Respiratory disorders require external support to provide O_2 and excrete CO_2 effectively. Additionally, a device that allows comfort, long-term tolerability, and oral nutrition while providing effective oxygenation and reducing the breathing work. This need has enabled experts to find a device named HFNC (7). HFNC therapy is an O_2 supplementation that can deliver O_2 warmed and humidified up to 100% with a flow rate of up to 60 liters/minute (7). The flow control ability of FiO_2 is greater than that of LFNC (5,6)

High flow nasal cannula structure and settings

The high flow O_2 therapy requires a specially designed cannula that is warmed and moisturized, resulting in a stable flow rate (8). HFNC fulfills inspiration needs by providing an air-O_2 mixture warmed to 37 ^oC with a relative humidity of 100% and can deliver 21-100% FiO_2 with a flow rate of up to 60-70 liters/minute (9). The flow rate and FiO_2 can be titrated independently based on patient needs (9).

HFNC has three essential components (7). First, an airflow generator produces flow rates of up to 60-70 liters/minute. Second, the O_2-air blender increases the control of FiO_2 regulation from 21 to 100%. Third, the humidifier sets an absolute humidity of 44 mmH_2O/liter and a heater sets the temperature between 35-37 ^oC (Figure 1). Also, the system includes a non-condensing water reservoir, a circuit system, and a special nasal cannula. The adult-sized cannula is 7.2 mm (8,10).

There are two main settings in HFNC, flow rates, and FiO_2. Flow rates can be set between 30 and 70 liters/minute (7). HFNC can provide air-O_2 mixtures with FiO_2 from 21% to almost 100% (7). Renda et al. (11), recommend the following practical settings for HFNC in Table 1.

Physiological effects of high flow nasal cannula

HFNC has several advantages over conventional O_2 treatments (12). O_2 therapy with HFNC has five physiological mechanisms that make the efficacy of HFNC better than other O_2 therapy supports, including;

1. CO_2 washout in the physiological death space,
2. Lowering the respiratory rate,
3. There is a positive final expiratory pressure (PEEP),
4. Increase tidal volume,
5. Increase the final expiratory lung volume (EELV) (8).

Anatomical dead space accounts for about one-third of the tidal volume of the breath (2). When ventilation is ineffective, there is CO_2 accumulation and retention in the anatomical dead space during inspiration. So that this condition can decrease O_2 diffusion in the alveoli (2).

High FiO_2 in HFNC increases the volume of gas in the alveoli more than the patient's volume ventilated physiologically (10). Increased ventilation allows washout or elimination of CO_2 with excess O_2 and can also increase mucus clearance in the airways (8). This results in creating a more significant O_2 diffusion gradient, potentially increasing oxygenation (8). HFNC could eliminate the residual volume of CO_2 in the upper airway and replace it with O_2-rich gas quickly and effectively (13).

HFNC reduces airway resistance in the nasopharynx and improves ventilation and oxygenation through positive pressure (7). Physiologically, the nasopharynx is a dynamic area that allows expansion or constriction of the airway. HFNC creates a positive pressure area, pressing the interior of the nasopharynx outward, thus causing dilation of the nasopharyngeal diameter and reducing airway resistance (7). Reduced airway resistance increases the potential for ventilation and oxygenation.

Additionally, HFNC creates positive alveolar pressure at the end of expiration to reduce the respiratory rate and the distribution of alveolar ventilation to be homogeneous throughout the lung region and improve tidal volume (11).

HFNC can also increase EELV (14). An increase in EELV indicates a positive alveolar PEEP. As a result, the PEEP in the lower airway increases (14). Positive pressure causes an increase in the alveolar surface area, so it does not collapse because of the increased pressure on the surface during expiration (14). Therefore, the ventilation process is improved and gas diffusion becomes more effective, resulting in better oxygenation. However, one thing that must be considered when using HFNC is that mouth must be closed to get optimal PEEP (15). The estimated amount of PEEP produced with the mouth closed at 1 cm H_2O at a 10 liters flow (15,16). It is a difficult challenge for patients to cover their mouths when experiencing respiratory distress (15).

The high flow nasal cannula mechanism in the treatment of respiratory distress

Administration of O_2 therapy with HFNC has a favorable mechanism for overcoming respiratory distress with hypoxemia (17);

1. High FiO_2 gas flow encourages the release of CO_2 gas in the anatomical dead space,

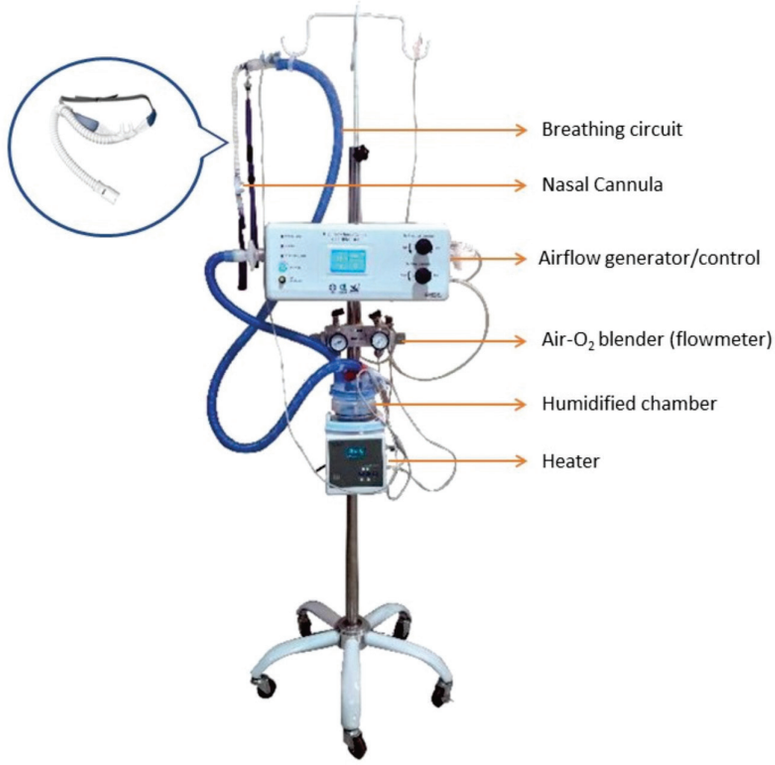

Figure 1. High flow nasal cannula components

Table 1. Recommended HFNC settings (11)

Prongs	Prongs should not totally occlude nostrils
Flow rate	Start at 30-40 liters min-1 and increase to meet the patient's demand
Temperature	Set at 37 °C
FiO_2	Increase the FiO_2 until satisfactory SaO_2 is achieved
Flow	Increase the delivered flow until a reduction in respiratory rate and stable SaO_2 are achieved
Water reservoir	Place as high as possible above the humidifier
Monitoring	Continuous monitoring of heart rate, respiratory rate, SaO_2
Positive response and weaning	Gas flow rate and FiO_2 adjusted according to the clinical response (expected within 1 h). Reduce FiO_2 by 5-10% and reassess after 1-2 h. Reduce the flow rate by 5 liters min-1 and reassess after 1-2 h. Consider weaning from HFNC with flow rates of 25 liters min-1 and FiO_2 <0.40.
Ineffective response	If there is no improvement after 60-120 min, treatment escalation must be considered.

HFNC: High flow nasal cannula, FiO_2: Inspired oxygen fraction

2. The high flow of FiO_2 gas in the alveolar increases lung volume,

3. Gas flow warmed to 37 °C and humidified by 100% can maintain mucociliary function for a good cleaning function,

4. The use of HFNC can reduce the airway resistance,

5. HFNC, in general, increases patient comfort compared to conventional O_2 or non-invasive positive pressure ventilation.

Clinical applications and indications for O_2 therapy with HFNC

The following are some clinical conditions and indications for the use O_2 therapy with HFNC (7,10);

1. Acute hypoxemic respiratory failure,

2. Hypercapnic respiratory failure (e.g., chronic obstructive pulmonary disease),

3. Impaired respiration due to acute heart failure/pulmonary edema,

4. Impaired respiration due to immune disorders,

5. Impaired pre-intubation and post-extubation respiration,

6. ARDS in COVID-19,

7. Patients who cannot be intubated,

8. Reducing suffering in patients at the end of life.

HFNC can be used effectively in moderate hypoxemic acute respiratory failure. Hypoxemic acute respiratory failure results from intrapulmonary shunt due to collapsed alveoli (7). It is refractory to O_2 supplementation (18). Increased alveolar-capillary hydrostatic pressure and permeability cause the alveoli to fill with blood due to bleeding and/or fluid in inflammatory conditions such as pneumonia (18).

O_2 therapy is essential, but it has maximum-positive benefits and potentially toxic effects. Increased levels of hyperoxia-induced reactive O_2 species (ROS) have an impact on the surrounding biological tissues. Increased levels of ROS cause hyperpermeability, coagulopathy, and collagen deposition within the alveolar space (19). In hyperoxia, multiple signaling pathways determine the pulmonary cellular response, including apoptosis, necrosis, and repair (19). Therefore, it is crucial to prevent alveolar damage caused by hyperoxia in patients requiring HFNC (19).

Contraindications to using O_2 therapy with HFNC

Contraindications to O_2 therapy with HFNC are divided into absolute and relative contraindications (2,10);

A. Absolute contraindications

1. Decrease in consciousness,

2. Cardiac arrest,

3. Respiratory arrest,

4. Conditions requiring emergency actions.

B. Relative contraindications

1. Cardiogenic shock,

2. Gastrointestinal bleeding,

3. Status epilepticus,

4. Airway obstruction (e.g., a large tumor pressing the airway),

5. Anaphylaxis that causes airway disorders.

Evaluation of therapy with HFNC

HFNC requires periodic evaluations to determine whether the patient is experiencing improvement or worsening. If HFNC is not closely monitored, it can result in delayed intubation. The criteria of HFNC evaluation (20):

1. PaO_2 <60 mmHg and O_2 saturation <90%, with an HFNC flow rate of 30 liters/minute and FiO_2 of 1.0,

2. Respiratory acidosis with a pH <7.25 and pCO_2> 50 mmHg,

3. Respiratory rate >30 times/minute (tachypnea) or unable to produce phlegm,

4. Increased thoraco-abdominal breathing occurring 15-30 minutes after the start of HFNC.

If there are two of the four criteria above, the respiratory condition is expected to worsen (20). The doctor must immediately switch HFNC to intubation with mechanical ventilation.

Respiratory rate and oxygenation (ROX) index

The ROX index, defined as the ratio of SpO_2/FIO_2 to respiratory rate is used to assess as a predictor of the need to intubate in patients who received HFNC O_2 therapy (21,22). ROX index can assess the success of using HFNC in COVID-19 patients with ARDS. The ROX index is used, with the following formula (23);

$$\text{ROX Index} = \frac{(SpO_2 / FiO_2)}{(\text{Respiration Rate})}$$

HFNC therapy is started at O_2 30-40 liters/minute, which can be increased according to the patient's need (4). The flow of O_2 increases until the inhalation rate decreases and a stable SaO_2 reach. In acute respiratory failure, up to 100% FiO_2 can be administered (21,22). The flow rate and FiO_2 can be adjusted according to the patient's clinical response (4).

After 1 h of HFNC, an evaluation should be performed if the patient shows clinical improvement and meets good ventilation criteria (24). Ventilation is accepted sufficient if the ROX index is ≥4.88 at 2, 6, and 12 h (25,26). This indicates that the patient does not need invasive ventilation. However, ROX index <3.85 indicates a failure to use HFNC and the patient is considered at high risk of immediate intubation (25,26). If the ROX index is between 3.85 and <4.88, scoring must be repeated every 1 to 2 h (Figure 2) (4,25,26).

Weaning off HFNC therapy

Weaning off HFNC can be started when the patient's condition is improved. Weaning is started by lowering the FiO_2 by 5-10% and re-evaluating it every 1-2 hours (11). If FiO_2 <0.4, the flow rate can be reduced by 5 liters/minute. HFNC can be removed when the flow rate reaches ≤25 liters/minute and FiO_2 <0.4 (11).

HFNC therapy in COVID-19

COVID-19 in critical condition with ARDS requires treatment in the intensive care unit (ICU). There are two types of respiratory failure; hypoxemic respiratory failure (type 1) and hypercapnic respiratory failure (type 2) (27). Type 1 respiratory failure occurs if the partial arterial O_2 pressure (PaO_2) is ≤60 mmHg in room air, and type 2 respiratory failure occurs when the partial arterial CO_2 pressure ($PaCO_2$) is ≥50 mmHg. COVID-19 cases with respiratory failure using a ventilator have a mortality of up to 80% (2,25).

COVID-19 with ARDS or respiratory failure requires supportive O_2 therapy. In this modern era, the choice of non-invasive O_2 support is the modality of choice using HFNC (17). O_2 support with HFNC can be administered to treat mild to moderate ARDS. The management of COVID-19 focuses on efforts to prevent the disease from worsening. Therefore, treatment management needs to be planned immediately to optimize the outcome of therapy. There are three essential steps in preventing disease worsening (2,25);

1. HFNC is preferred over non-invasive mechanical ventilation (NIV) in patients with ARDS,

2. Restricted fluid resuscitation, especially in patients with pulmonary edema,

3. Positioning the conscious patient in the prone position,

The European Respiratory Society provides recommendations for the use of HFNC in COVID-19 patients as follows (25,27):

1. In adults with COVID-19 and acute hypoxemic respiratory failure, HFNC is superior to conventional O_2 therapy (weak recommendation, low-quality evidence).

2. In adults with COVID-19 and acute hypoxemic respiratory failure, HFNC is superior to NIPPV (weak recommendation, low-quality evidence).

3. In adults with COVID-19 who receive NIPPV or HFNC, close monitoring of worsening respiratory status to make the decision of early intubation (best practice statement).

Combination of the prone and HFNC positions in COVID-19

A study by Xu et al. (28) reported COVID-19 patient with a P/F ratio ≤300 who was in the prone position early for more than 16 hours and combined with HFNC therapy have no worsening or received invasive ventilation and survived completely. This study also showed that HFNC could reduce the rate of endotracheal intubation in patients with respiratory failure. Additionally, the prone position can correct the ventilation-perfusion mismatch and open the airway, reducing pulmonary atelectasis with adequate sputum drainage (28).

The prone position combined with HFNC requires a patient with good mental status, communication, and stable hemodynamics (Figure 3). The patient should be briefed on the prone position maneuvers and ensure adequate HFNC placement. The vital and clinical signs are monitored during the prone position (28,29).

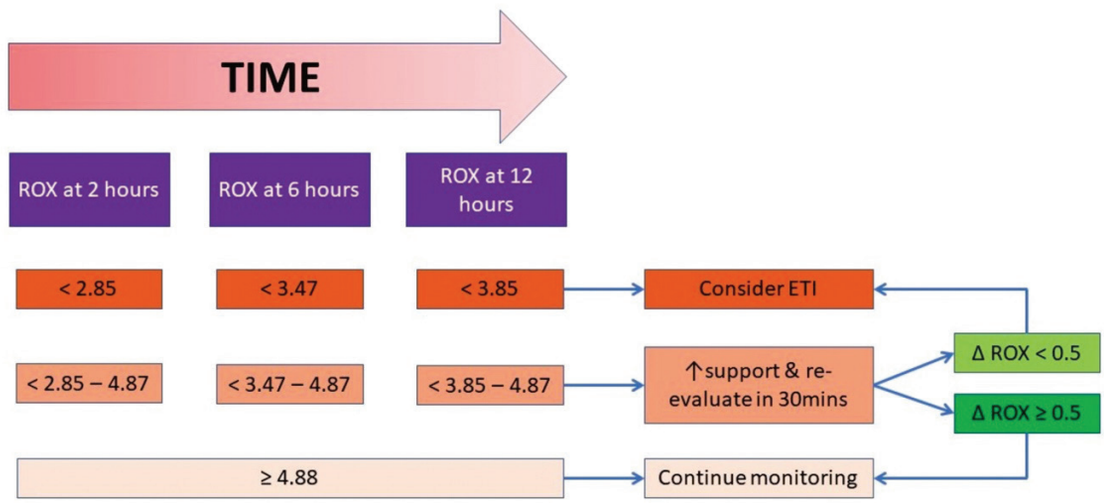

Figure 2. Evaluation of HFNC with the ROX index

HFNC: High flow nasal cannula, ROX: Respiratory rate and oxygenation

Advantages and limitations of HFNC in COVID-19 patients

The advantages of HFNC include ease of setup and use, such as the use of nasal prongs for patients. The low risk of skin irritation because of high O_2 flow makes the mucosa dry, but HFNC can keep nasal mucosa humidity. Patients can still eat, drink and communicate without removing the HFNC, facilitating the performance of medical personnel, and reducing the time spent on nasal tightness. Additionally, it is more stable and does not remove the equipment that triggers aerosols (30,31).

Like many other medical interventions, there are limitations and drawbacks to HFNC. One of the main drawbacks is the higher maintenance cost, the complexity of the HFNC device, training requirement, risk of gas leakage, and loss of positive pressure effects on the improper airway seal (7,10). Another limitation is the potential for delaying intubation and end-of-life decisions in patients with decreased consciousness, facial injuries, excessive secretions increasing the risk of aspiration, and hemodynamic instability (7,10). Additionally, the disadvantages of HFNC include that the sound of a loud instrument makes noise, patient's activities become limited, and complaints of altered smell in rare cases (20).

Aerosol effects and safety of HFNC

HFNC might increase some concerns about the risk of virus transmission. A study by Whittle et al. (32), is measuring the spread of aerosols on various breathing devices. Aerosol dispersion distances for nasal cannula range 3-40 cm, simple mask at all flows ≈ 30 cm, ventury mask range 33-40 cm, NRM at all flows <10 cm, HFNC ranges 4.8-17 cm, NIPPV ranges 85-95 cm, and nebulizers <80 cm (32). However, recent practice recommendations for COVID-19 patients indicate using a medical mask over the HFNC device to limit the particle dispersion due to exhaled gas flow (33). Whittle et al. (32) recommended that the use of HFNC in COVID-19 should be applied cautiously and that adequate personal protective equipment (PPE) and protective measures for healthcare workers should be provided. The Australian and New Zealand Intensive Care Society (ANZICS) guidelines on COVID-19 state that HFNC therapy in the ICU is the recommended therapy for hypoxia associated with COVID-19, as long as the staff wears PPE (34). The risk of airborne transmission to healthcare workers is low with optimal use of PPE and good infection control precautions (34). The use of a negative pressure room is recommended for patients receiving HFNC therapy (34,35).

Conclusion

HFNC has many advantages in COVID-19 patients. It gives warm and moisturize O_2 to increase the patient's comfort. It also reduces the need for inspiration, increases the functional residual capacity, reduces the dilution of O_2-air, and can wash out CO_2 in the anatomical dead space. An initial assessment

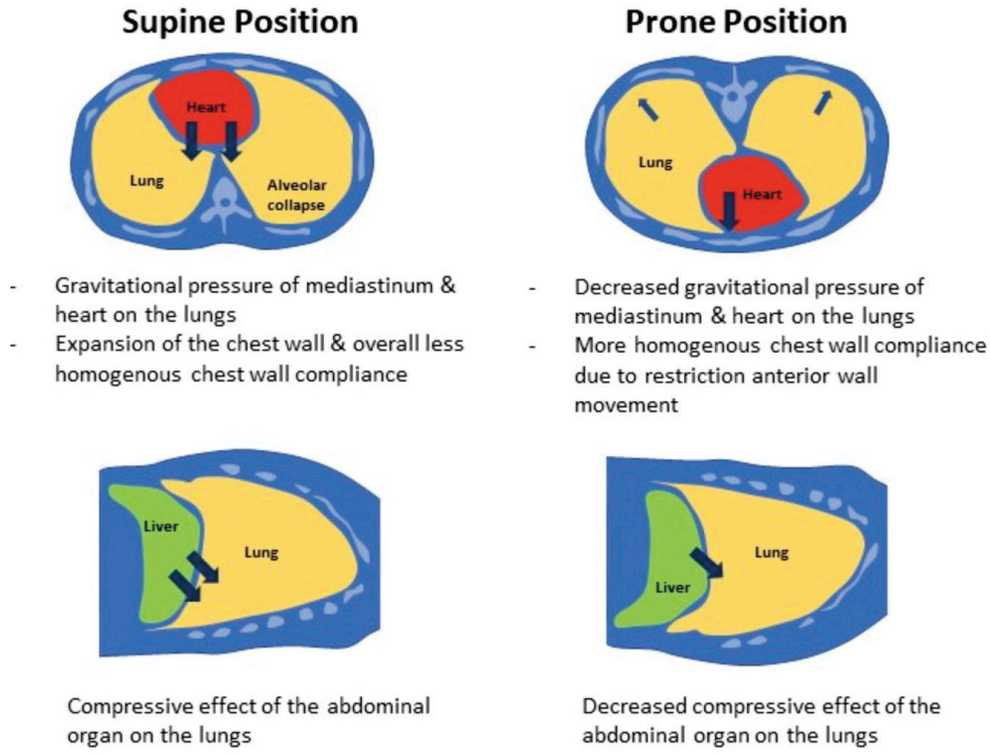

Figure 3. Comparison of the physiological effects of the supine and prone positions

is critical for the success of HFNC therapy. The ROX index can be used to predict the success or failure of HFNC therapy. Supportive O_2 therapy with HFNC is among the modalities for COVID-19 patients, having advantages over other conventional O_2 therapies. However, further studies are necessary to evaluate the benefit of HFNC in hypoxemia management. HFNC can be used in the ICU, operating room, emergency room, and ordinary wards. However, the use of HFNC in COVID-19 patients is recommended in rooms with negative pressure to minimize the risk of aerosol transmission.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.P.D., E.M., Concept: K.P.D., I.I., M.A., Design: K.P.D., E.M., Data Collection or Processing: K.P.D., Literature Search: K.P.D., I.P.D., Writing: K.P.D., I.P.D.

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

Financial Disclosure: The authors declared that this study received no financial support.

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Gulhane Med J 2022;64:136-43

Roles of angiotensin II, Olmesartan and PD123319 on proliferation, oxidative stress and TGF-β1 in HUVEC culture

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Date submitted:
15.10.2020
Date accepted:
22.02.2021
Online publication date:
15.03.2022

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Keywords: Angiotensin II, endothelium, HUVECs, oxidative stress

ABSTRACT

Aims: Angiotensin II (Ang II) causes endothelial cell damage. Oxidative stress is involved in the pathophysiology of cardiovascular diseases via transforming growth factor-beta 1 (TGF-β1). In most studies increases in Ang II and TGF-β1 levels in several cell types are bidirectional. The present study investigated the effects of Ang II on oxidative stress, cell proliferation, and TGF-β1 levels in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were treated with Ang II (0.1 μM), Ang II type 1 receptor (ATR1) antagonist Olmesartan (1 μM), and Ang II type 2 receptors (ATR2) antagonist PD123319 (1 μM) for 24 hours. Cell proliferation and viability were evaluated by the tetrazolium salt (MTT) assay. Total antioxidant capacity (TAC) and total oxidant capacity (TOC) were measured by spectrophotometer intracellularly and in the culture medium. The TGF-β1 level was measured by enzyme-linked immunosorbent assay (ELISA).

Results: The addition of 1 μM, 0.1 μM, and 0.01 μM Ang II increased proliferation in HUVECs. Cell proliferation increased significantly in both Ang II and Ang II+Olmesartan+PD123319 groups. However, Ang II+Olmesartan tended to decrease cell proliferation. In the control group TAC and TOC levels remained in the normal range in HUVEC extracts. In other all groups, TOC values increased compared to control. In HUVECs medium, TAC level was higher in the control, Ang II and Ang II+Olmesartan groups, but normal and tolerable in other groups whereas, TOC levels were elevated in control and other all groups. In HUVECs extracts, compared with the control, TGF-β1 level was significantly lower in the Ang II group, but increased in the Ang II+Olmesartan group. There was no significant difference in TGF-β1 levels between medium groups.

Conclusions: Ang II shows its proliferative effects through ATR1 activation, whereas stimulation of ATR2 seems to have a key role in the pathophysiology of oxidative stress.

Introduction

Endothelial cells lay the inner surface of all blood vessels as a single layer, and play an important role in the regulation of vascular homeostasis (1). The resting endothelial cells prevent thrombus formation in the blood-tissue interface and play a crucial role in the regulation of inflammation (2). They are also involved in the growth and proliferation of various cell types, especially the smooth muscle cells (3). Endothelial dysfunction is associated with adverse clinical outcomes since vascular wall injury is responsible for vascular diseases (4). Smooth muscle cell proliferation and matrix synthesis can lead to damaged vessel wall repair, but can also cause occlusion in the same lumen (5).

Angiotensin II (Ang II), the main effector molecule of the Renin-Angiotensin System, has physiological and pathological effects on the cardiovascular system (6). Ang II affects the vascular system via Ang II type 1 (ATR1) and type 2 receptors (ATR2) (7). It activates the phospholipase C enzyme by binding

to ATR1s (7,8). However, previous studies have reported that the vascular effects of Ang II may be related to the balance between ATR1-mediated nitric oxide (NO) function and reactive oxygen species (ROS) (8). ROS play role in the physiopathology of many diseases, including hypertension, diabetes and their long-term chronic complications (9).

It has been reported that oxidative stress occurs due to an imbalance in the production of reactive oxygen derivatives and antioxidant systems (10). Free radicals or ROS are the metabolic products that are formed by oxygen spent during the conversion of energy from nutrients in the body (11). Oxidative stress increases the proliferation of vascular cells and accelerates plaque formation (12). Besides, with increasing levels of ROS, organelle damage (e.g., mitochondria), DNA damage, and other disorders including wrong folding of proteins occur (13).

Transforming growth factor-beta (TGF-β) is a cytokine with many subtypes that has regulatory and physiological effects on cells (14). The role of TGF-β on cell growth, development, proliferation, extracellular matrix synthesis, immune system modulation, apoptosis, cell cycle, migration, and angiogenesis was also reported (15). TGF-β inhibits cell proliferation in some cell types (endothelial, epithelium, immune cells) depending on the environment in which it lives (16). TGF-β has also been shown to play a role in the transformation of mesenchymal cells into endothelial cells. TGF-β has many subtypes, including TGF-β1, TGF-β2 and TGF-β3 (16).

Some *in vivo* and *in vitro* studies found that Ang II increases TGF-β1 levels in several cells and they have a relation with each other (17,18). It has also been reported that Ang II and TGF-β subtypes play significant roles in the pathophysiology of many cardiovascular diseases. For instance, oxidative stress and TGF-β1 are important in the pathophysiology of hypertension (19,20). Various drugs such as Angiotensin Converting Enzyme and Ang II type 1 receptor blockers that inhibit RAS components are used in the treatment of many RAS-related cardiovascular diseases (6). Considering the information given above, we investigated the effects of Ang II, and its blockage with Olmesartan and PD123319 on proliferation, total antioxidant capacity (TAC), total oxidant capacity (TOC), and TGF-β1 levels in human umbilical vein endothelial cells (HUVECs).

Methods

Cell culture and treatments

HUVEC culture line was obtained from ATCC, USA. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, D6046, Sigma-Aldrich) with 20% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific), penicillin-streptomycin (PSA, P4333-100 mL, Sigma-Aldrich) at 37 °C in humid air with 5% CO_2. The culture medium was replaced every 72 h. The cells were obtained by passaging for the proliferation and were incubated with different doses of Ang II, ([Val5]-Ang II acetate salt hydrate, A2900-50 mg, Sigma-Aldrich, 1000, 100, 10, 1, 0.1, 0.01, and 0.001 μM), ATR1 blocker Olmesartan (Olmesartan, SML1394-50 mg, Sigma-Aldrich, 1 μM) and ATR2 blocker PD123319 (PD123319 di (trifluoroacetate) salt hydrate, P186-10 mg, Sigma-Aldrich, 1 μM) for 24 h. Chemicals were dissolved in a cell medium (DMEM+10% FBS+1% PSA). Cell proliferation, TAC, TOC, and TGF-β1 levels were measured in all groups.

Experimental groups were; 1: Control, 2: Ang II (0.1 μM), 3: Olmesartan (1 μM), 4: Ang II+Olmesartan, 5: PD123319 (1 μM), 6: Ang II+PD123319, 7: Olmesartan+PD123319, 8: Ang II+Olmesartan+PD123319.

Evaluation of cell proliferation

Cell proliferation, cytotoxicity, and viability were evaluated using a tetrazolium salt (MTT) assay. It is based on the measurement of cell metabolic activity. Briefly, HUVECs were plated in a 96-well plate with $5x10^3$-$1x10^4$ cells in each well. Cells were incubated for 24 h at 37 °C in a 5% CO_2 environment with Ang II, Olmesartan, and PD123319. The cell medium was changed and 100 uL fresh medium was added. After adding 10 μL MTT (5 mg/mL, Thiazolyl Blue Tetrazolium Bromide, M5655-1G, Sigma-Aldrich) solution to each well, the cells were incubated for 3-4 h. Then the solution was replaced with 100 μL DMSO (dimethyl sulfoxide, EMPLURA, Merck, M116743100) and incubated again for an additional 20-30 min. The pale yellow MTT dye was formed. The average absorbance for each group was calculated using a microplate reader (A570 nm-A630 nm, Molecular Devices Filter Max F5) (21).

Preparation of cell and medium extract

The cells were seeded in six-well culture plates following trypsinization. After waiting for 24-72 h. for cell proliferation, Ang II (0.1 μM), Olmesartan (1 μM), and PD123319 (1 μM) were applied. After 24 h, cell and medium extraction were performed. Cell media were taken into falcon tubes and the supernatants were taken after centrifuging 200xg at +4 °C for 10 min. For cell extraction, 100 μL 140 mM KCl solution was added to the wells, and the bottom of the wells was scraped. The cells were then broken down in the sonicator for a few minutes and centrifuged at 10.000xg for 15 min and supernatants were collected. Samples were kept at -80 °C until studied.

Lowry method

Standards and samples (50 μL) were pipetted into 96-well dishes and left at room temperature for 45 min. The C reagent (150 μL) was added to the wells, which consists of a 100:1 mixture of A and B reagents. A reagent was 2% Na_2CO_3, 0.4% NaOH, 0.16% Na-tartrate; B reagent was 4% $CuSO_4.5H_2O$. Folin-Ciocalteu's reagent (3 μL) was added to all tubes after incubation. The absorbance values of the samples and standards were measured using a spectrophotometer at a wavelength of 660 nm. The protein amount of each sample was

obtained using BSA absorbances at the doses of 4, 2, 1, 0.5, 0.25, 0.125, 0.062, 0.031 and 0 mg/mL. The total amount of protein in cell extract and cell medium was calculated using the standard curve. The standard curve was created with bovine serum albumin absorbances and the total amount of protein in the cell extract and medium was calculated (22,23).

Biochemical analysis

TAC in cell and medium extracts was measured by Rel Assay commercial kit (Diagnostics Rel Assay Kit, Turkey). Results were expressed as mmol Trolox equivalent/L. TOC in cell and medium extracts was also measured by Rel Assay commercial kit (Diagnostics Rel Assay Kit, Turkey) and results were expressed as μmol H_2O_2 equivalent/L. The oxidative stress index (OSI), which is expressed as the percentage of the ratio of TOC levels to TAC levels, was calculated and the results were expressed as "arbitrary unit" (24).

Measurement of TGF-β1 level

The levels of TGF-β1 in the cell and medium supernatant were measured using an enzyme-linked immunosorbent assay kit (Invitrogen Immunoassay Kit/KAC1689, United States). The kit was used in accordance with the protocols specified. The plate was evaluated at 450 nm absorbance with a microplate reader (Molecular Devices Filter Max F5, USA).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences Statistics for Windows software, version 21.0 (IBM Corp., Armonk, NY). The results were expressed as mean±standard deviation. The normality of distribution was tested using the Shapiro-Wilk test. Dunnett and Tukey tests were performed as ANOVA post-hoc after ensuring homogeneous distribution. The statistically significant level was <0.05.

Results

Total protein measurement

Measurements of total protein in HUVECs cell and medium supernatant are shown in Table 1.

Effects of Angiotensin II doses on HUVEC proliferation

HUVEC proliferation was expressed as absorbance (Figure 1). We examined the viability of HUVECs at the concentrations of 1000, 100, 10, 1, 0.1, 0.01, and 0.001 μM of Ang II. Results showed that cell proliferation increased at concentrations of 1 μM (14%, p=0.008), 0.1 μM (14%, p=0.006), and 0.01 μM (25%, p<0.001) compared to the control group.

Effects of Angiotensin II, Olmesartan, and PD123319 on HUVEC proliferation

Ang II 0.1 μM administration increased cell proliferation by 12% compared to controls (p=0.006) (Figure 2). There was a decreasing trend (15%) in Ang II+Olmesartan group bu the difference was not significant. Compared with the Ang II+Olmesartan treated group, cell proliferation was significantly elevated in Ang II+Olmesartan+PD123319 treated group (73%), (p<0.001).

TOC and TAC levels

Concerning the TAC level (mmol Trolox Equiv./L), the following instructions were provided by the manufacturer of the

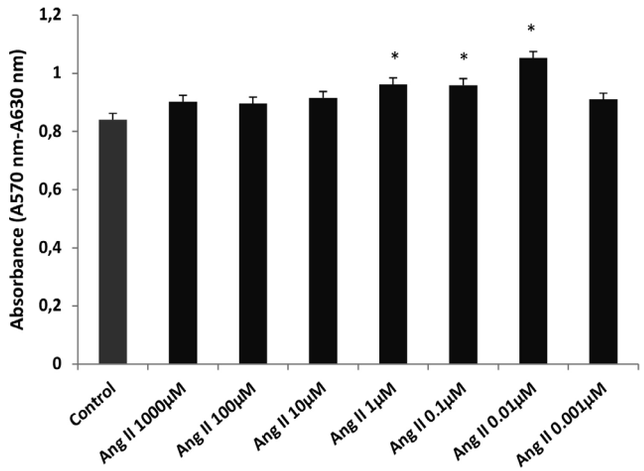

Figure 1. Angiotensin II dose study in human umbilical vein endothelial cells. The data are expressed as mean±standard deviation

*p<0.05 according to the control group. One-way ANOVA post-hoc Dunnett test (n=8-12)

Table 1. The total protein amount in cell and medium extract

Groups	Cell extract (mg/mL)	Cell medium (mg/mL)
1. Control	1.71	4.90
2. Ang II	2.71	5.15
3. Olmesartan	3.56	5.06
4. Ang II+Olmesartan	1.50	4.49
5. PD123319	3.46	4.24
6. Ang II+PD123319	2.37	6.22
7. Olmesartan+PD123319	1.22	6.81
8. Ang II+Olmesartan+PD123319	1.35	6.17

Ang II: Angiotensin II

kit used in the analysis; >2.0 very good, 1.45 to 2.00 normal, 1.20 to 1.45 tolerable, 1.00 to 1.20 low, and <1.20 very low antioxidant level. Concerning the TOC level (μmol H_2O_2 Equiv./L) the following instructions were provided; <5.00 very good, 8.00-5.00 normal, 12.00-8.00 high, and >12.00 very high oxidant level. In our measurements, in the control group TAC (1.75 mmol Trolox Equiv./L) and TOC levels (7.2 μmol H_2O_2 Equiv./L) remained in the normal range in HUVEC extracts. In other HUVEC extracts groups (Ang II+Olmesartan, Olmesartan+PD123319, Ang II+Olmesartan+PD123319), TAC values were normal and tolerable (1.3-1.7 mmol Trolox Equiv./L) and Ang II, Olmesartan, PD123319, and Ang II+PD123319 groups TAC values were higher (2.4-3.6 mmol Trolox Equiv./L). In cell extracts TOC values increased in Ang II, Olmesartan, Ang II+Olmesartan, PD123319, Ang II+PD123319, Olmesartan+PD123319, Ang II+Olmesartan+PD123319 groups compared to control (9.5-14.2 μmol H_2O_2 Equiv./L).

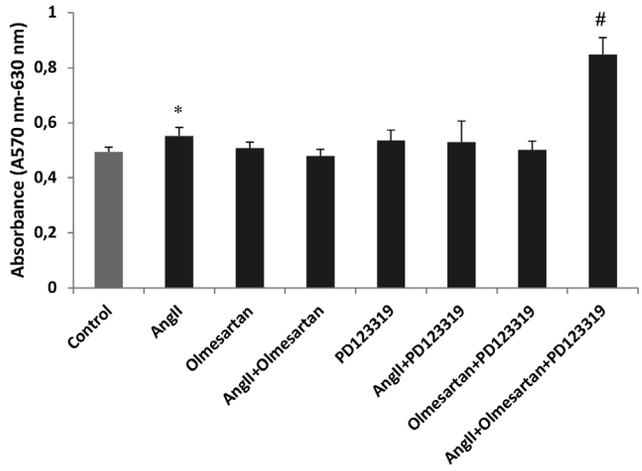

Figure 2. Angiotensin II, Olmesartan, and PD123319 proliferation study in human umbilical vein endothelial cells culture. Data are expressed as mean±standard deviation

*p<0.05 according to the control group, #p<0.05 compared to Ang II+Olmesartan group. One-way ANOVA post-hoc Tukey test (n=8-16)

Ang II: Angiotensin II

In the HUVECs medium, TAC levels were higher in the control (4.8 mmol Trolox Equiv./L), Ang II and Ang II+Olmesartan groups (2.8-2.86 mmol Trolox Equiv./L) but normal and tolerable in Olmesartan, PD123319, Ang II+PD123319, Olmesartan+PD123319 and Ang II+Olmesartan+PD123319 medium groups (1.41-1.68 mmol Trolox Equiv./L). Whereas, TOC levels increased in control (11.4 μmol H_2O_2 Equiv./L) and other all groups in HUVECs medium groups (10.8-14.4 μmol H_2O_2 Equiv./L). TAC, TOC, and OSI (TOS/TAC) values measured in the samples obtained from HUVEC extract groups are shown in Table 2 and 3.

Table 2. TOC, TAC, and OSI in HUVECs extracts

HUVEC groups (cell extracts)	TAC (mmol Trolox Equiv./L)	TOC (μmol H_2O_2 Equiv./L)	OSI (arbitrary unit)
1. Control	1.75	7.2	4.11
2. Ang II	2.8	9.5	3.39
3. Olmesartan	3.6	10.2	2.83
4. Ang II+Olmesartan	1.7	13.3	7.82
5. PD123319	3.5	11.4	3.25
6. Ang II+PD123319	2.4	14.2	5.91
7. Olmesartan+PD123319	1.3	12.6	9.69
8. Ang II+Olmesartan+PD123319	1.4	11.3	8.07

Equiv.: Equivalent, OSI: Oxidative stress index, TAC: Total antioxidant capacity, TOC: Total oxidant capacity, HUVECs: Human umbilical vein endothelial cells, Ang II: Angiotensin II

Table 3. TOC, TAC, and OSI in HUVECs medium extracts

HUVECs groups (medium extract)	TAC (mmol Trolox Equiv./L)	TOC (μmol H_2O_2 Equiv./L)	OSI (arbitrary unit)
1. Control	4.8	11.4	2.37
2. Ang II	2.8	10.8	3.85
3. Olmesartan	1.41	12.1	8.58
4. Ang II+Olmesartan	2.86	13.2	4.61
5. PD123319	1.53	11.9	7.77
6. Ang II+PD123319	1.33	14.4	10.82
7. Olmesartan+PD123319	1.42	12.6	8.87
8. Ang II+Olmesartan+PD123319	1.68	11.3	6.72

Equiv.: Equivalent, OSI: Oxidative stress index, TAC: Total antioxidant capacity, TOC: Total oxidant capacity, HUVECs: Human umbilical vein endothelial cells, Ang II: Angiotensin II

Table 4. TGF-β1 level in HUVECs extract and medium

Groups	Cell extract (pg/mg) (mean±SD)	Cell medium (pg/mg) (mean±SD)
1. Control	318±25.06	61.58±4.09
2. Ang II	206±14.88*	40.93±2.83
3. Olmesartan	162±14.35*	41.42±6.00
4. Ang II+Olmesartan	374±19.52#	40.55±6.46
5. PD123319	181±10.36*	42.85±9.57
6. Ang II+PD123319	231±17.66	31.58±6.11
7. Olmesartan+PD123319	396±32.93	28.17±3.92
8. Ang II+Olmesartan+PD123319	356±36.10#	24.84±2.65

Data are expressed as mean±SD. *p<0.05 relative to control, #p<0.05 relative to Ang II group. One-way ANOVA post-hoc Tukey test (each group worked 5 replicates). SD: Standard deviation, TGF-β1: Transforming growth factor-beta 1, HUVECs: Human umbilical vein endothelial cells, Ang II: Angiotensin II

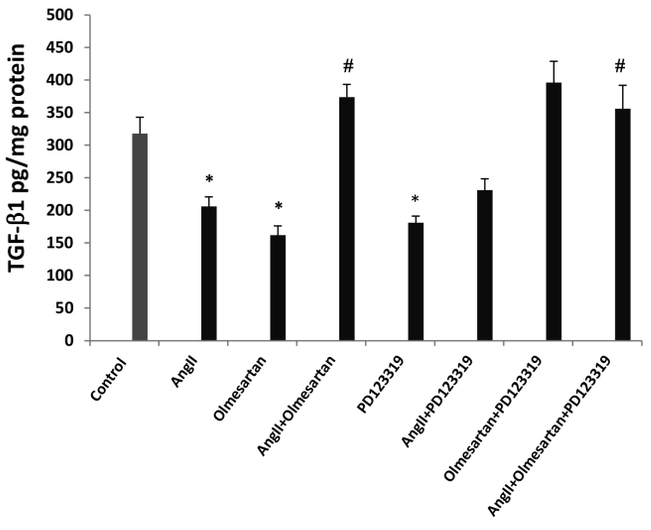

Figure 3. TGF-β1 levels in Angiotensin II, Olmesartan, and PD123319 treated groups in human umbilical vein endothelial cells culture. Data are expressed as mean±standard deviation

*p<0.05 compared to the control group, #p<0.05 compared to Ang II group. One-way ANOVA post-hoc Tukey test (n=5)

TGF-β1: Transforming growth factor-beta 1, Ang II: Angiotensin II

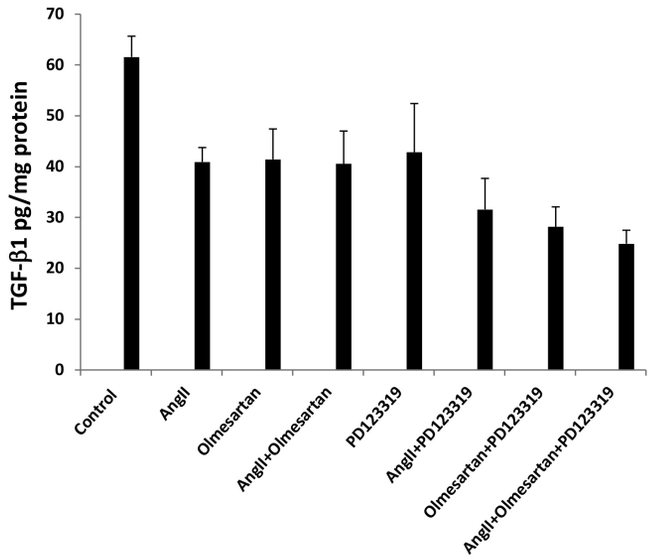

Figure 4. TGF-β1 levels in human umbilical vein endothelial cells medium in Angiotensin II, Olmesartan, and PD123319 applied groups. Data are expressed as mean±standard deviation

One-way ANOVA post-hoc Tukey test (n=6)

TGF-β1: Transforming growth factor-beta 1, Ang II: Angiotensin II

TGF-β1 levels in HUVECs extract and medium

TGF-β1 levels measured in cell extracts and medium in HUVEC groups are shown in Table 4. Compared with all HUVECs medium groups, TGF-β1 level in all HUVECs extract groups was significantly higher (p<0.001). In HUVECs extracts, compared with the control, TGF-β1 level was significantly lower in the Ang II group (p=0.030). Compared to the Ang II group, TGF-β1 level was significantly higher in the Ang II+Olmesartan group (p<0.001) and Ang II+Olmesartan+PD123319 group (p=0.001) (Figure 3). There was no significant difference in TGF-β1 levels between medium groups (Figure 4).

Discussion

Hypertension is one of the most important health problems worldwide (25). While many of the physiological mechanisms and intracellular molecular pathways that regulate blood pressure are known, some of the pathological mechanisms that lead to hypertension are still unknown (7,25). It was indicated that RAS components and receptors may be responsible for the physiopathology of hypertension (26). Drugs that inhibit RAS components and receptors are frequently used for treating this disease (6).

Ang II, the most important potent molecule of RAS, increases proliferation and oxidative stress in endothelial cells (8). 1 μM, 0.1 μM, and 0.01 μM Ang II treatment increased HUVEC proliferation in the current study. As the administration dose of Ang II reduces, cell proliferation appears to decrease in a dose-dependent manner. Therefore, the dose of Ang

II for maximum proliferative effect was chosen as 0.1 μM for combined applications with ATR blockers in our study. This finding is consistent with the studies performed with different cell types (27,28). In our previous study, enhanced proliferation was observed also with the same dose of Ang II in primary culture of vascular smooth muscle cells obtained from rat thoracic aorta (29). Dose-dependent cellular effects of Ang II are complex. Additionally, plasma and tissue levels of Ang II (differences in physiological dose) in humans and different species may differ (8). There is no adequate and comprehensive study on intracellular and extracellular Ang II measurement.

It is already known that Ang II may have some different cellular effects and responses (8). Studies have shown that ATR1 blockers reduce cell proliferation caused by Ang II in some different cell cultures (28,30). However, Olmesartan did not inhibit the proliferation of Ang II in HUVECs in our study. The reason may be the insufficient dose of Olmesartan (1 μM). However, the doses of Olmesartan (1 μM) and PD123319 (1 μM) were determined in accordance with the literature and chosen for co-administration (31,32). PD123319 was found to increase endothelial cell proliferation in this study. Forrester et al. (8) reported that ATR2 blockers increase cell proliferation compared to ATR1 blockers. It was considered that stimulation of ATR2s affects vascular cell physiology and exposes these effects by increasing NO release. Contrary to the current study, while 1 μM PD123319 increased proliferation, 1 μM Olmesartan reduced proliferation in vascular smooth muscle cells in our previous study (29). As a possible explanation, PD123319 and Olmesartan might activate different intracellular proliferative and non-proliferative signaling mechanisms in the endothelial cells.

TAC and TOC levels in the cell extract control group were determined within normal limits. Concerning OSI we observed that the oxidant and antioxidant processes were balanced in the control group, whereas both oxidants and antioxidants were augmented in the cell extract groups (Ang II, Olmesartan, PD123319, and Ang II+PD123319). This finding may indicate a potential cause of vascular diseases. Moreover, there was very high oxidative stress level in Ang II+Olmesartan, Olmesartan+PD123319, and Ang II+Olmesartan+PD123319 cell extract groups. These findings may be considered more serious and many pathologies can be suspected. In the literature, it has been reported that Ang II increases oxidative stress, while ATR1 blockers decrease oxidants on the vascular structure (8).

As Ang II can cause endothelial cell damage, inflammation, and even cell death (33), and ATR1 blockers can reverse these effects (6). However, there was no elevation in TAC levels in the Olmesartan group in our study. The reason for this finding may be the insufficient dose of Olmesartan. we also found that when the medium and cell extract control groups were compared, both the oxidant and antioxidant capacity of the medium were increased. This may be explained by the maintanece of balance by the cells as a physiological process. Except for the Ang II and Ang II+Olmesartan groups, higher oxidative stress was found in all treatment groups. Ang II may induce oxidative stress on the cardiovascular system through ATR2s. Nitric oxide is a free radical and plays a potent role in the physiological mechanisms of the cardiovascular system (34). Also, the stimulation of ATR2s increases nitric oxide synthesis (8). In addition, increased nitric oxide levels in cardiac cells may decrease the expression of ATR1. It can be thought that the blockade of ATR2s may reduce oxidative stress.

Cellular TGF-β1 level was higher inside the cell compared to the medium in our study. At the same time, Ang II and ATR1 blocker Olmesartan reduced TGF-β1 synthesis in endothelial cells. However, in an *in vivo* study, it was reported that Ang II administration to rat cardiovascular endothelial cells has augmented TGF-β1 levels and another ATR1 blocker losartan decreased plasma level of TGF-β1 (35). Although it is known that the TGF-β protein family increases cell proliferation and migration, its effects on some cell types may be the opposite (19). Our results showed that TGF-β1 may have proliferative effects on vascular cells and Ang II administration may reduce TGF-β1 levels. The proliferative effects of Ang II on cardiovascular cells are known and the induced TGF-β1 levels are reported to be related to proliferation (36,37). It has been suggested that TGF-b1 can affect intracellular signaling through cell type-dependent mechanisms (38,39). Therefore, the relationship between Ang II and TGF-β1 is important, which has been postulated in the physiopathology of cardiovascular diseases such as hypertension (19). In this respect, when all cellular mechanisms are evaluated as a whole, the fact that Ang II reduces TGF-β1 levels in our study and this result cannot be associated with proliferation. We expected an increase in TGF-β1 levels in correlation with the proliferation-enhancing effects of Ang II. Our fındings suggest that Ang II may affect different TGF-β subtypes that may be more effective in endothelial cell proliferation, and may activate different proliferation pathways.

Several limitations of the current study should be acknowledged. In contrast to other studies in different cell types, Olmesartan application did not decrease HUVEC proliferation in our study. This might have been caused by the use of only a single and low dose of Olmesartan (1 μM), as well as by the measurement of cell proliferation with a single method. As another limitation, only TGF-β1 could be measured as one of the cell proliferation pathways. Oxidative stress could be evaluated by using superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde, which lacked in our analyses.

Conclusion

The present study showed that Ang II and ATR2 blocker PD123319 increased HUVEC proliferation. While, Ang II

demonstrated most of its proliferative effects through ATR1 activation, stimulation of ATR2 seemed to have a more prominent role in oxidative stress processes in endothelial cells.

Ethics

Ethics Committee Approval and Informed Consent: Ethics committee approval was not obtained from the patient and a consent form was not obtained because it was a cell culture study. The HUVEC cell culture line used in the study was obtained from the Health Sciences University Stem Cell Research Center.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.Ç., K.A., A.Ş.İ., Concept: Z.Ç., K.A., A.Ş.İ., Design: Z.Ç., K.A., A.Ş.İ., Data Collection or Processing: Z.Ç., K.A., A.Ş.İ., Analysis or Interpretation: Z.Ç., K.A., A.Ş.İ., Literature Search: Z.Ç., K.A., A.Ş.İ., Writing: Z.Ç., K.A., A.Ş.İ.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.73745
Gulhane Med J 2022;64:144-51

Clinical findings and molecular diagnosis in children with Bardet-Biedl Syndrome in Turkey: Identification of novel variants

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Date submitted:
24.06.2021
Date accepted:
22.11.2021
Online publication date:
15.06.2022

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Keywords: Bardet-Biedl syndrome, next-generation sequencing, phenotype-genotype correlation, variants

ABSTRACT

Aims: Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy with multisystemic involvement and variable phenotypic features. A diagnosis is usually made through the clinical diagnostic criteria. However, the clinical diagnosis can be difficult due to the absence of clear phenotype-genotype correlation and overlapping findings with other ciliopathies. Next-generation sequencing (NGS) is a rapid and cost-effective diagnostic method for this group of diseases. Besides correct diagnosis, detection of novel variants also contributes to establishing new phenotype-genotype correlations and delineating the pathophysiology of the syndrome. Here, we aimed to present clinical and molecular findings of patients with BBS using NGS panel analysis to contribute to the genotype-phenotype correlation in this rare syndrome.

Methods: We retrospectively reviewed the medical records of patients with a suspicion of BBS admitted to the Pediatric Genetics Department in Umraniye Training and Research Hospital. Patients who met the BBS clinical diagnostic criteria were included in the study. Targeted NGS analysis, including 20 genes associated with BBS, was performed on an Illumina Next-Seq-500 platform.

Results: The final analyses included 6 patients (age, mean±standard deviation: 14.3±6.6 years, female: 50%). Rod cone dystrophy (100%), polydactyly (100%), and intellectual disability (100%) were the most common findings followed respectively by obesity (83%), renal anomalies (83%), liver anomalies (67%), dental problems (67%), metabolic problems (50%), genital anomalies (33%), psychiatric disorders (33%), and sleep apnea (33%). The 5th metatarsal shortness and camptodactyly were anomalies reported for the first time in BBS. Seven variants were detected in the BBS1, BBS7, BBS5, BBS9, and MKKS, two of which were novel. BBS7 was the most common gene.

Conclusions: Our study expanded the genotypic spectrum of the disease with two novel variants reported. Besides, by defining novel/rare clinical features, including camptodactyly, the fifth metatarsal, the 4th-5th metacarpal shortness, and nephrocalcinosis, it formed a source for the phenotype-genotype correlation trying to be established in the literature.

Introduction

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy with multisystemic involvement. Retinal rod-cone dystrophy, obesity, polydactyly, intellectual disability, hypogonadism, and renal disease are the characteristic findings of the syndrome. In addition to these findings, variable other neurological, gastrointestinal, endocrinological, and cardiovascular system involvements have been reported (1-5). The prevalence of the syndrome differs according to geographical regions, reported as 1/160.000 in Europe and rises to 1/13.500 in regions such as the Middle East, where the consanguinity rate is high (1,3,6).

In BBS, the key element in the pathophysiology is cilia dysfunction. Cilias are organelles that have functions primarily in the determination of cell polarity, regulation of the cell cycle, and mechano-sensation. Also, they take part in the

signaling pathways involved in vertebral development and organ differentiation in the embryological period (7). These widespread functions of the cilia are reflected in the phenotype as multi-systemic involvement, as in BBS. Bi-allelic/tri allelic loss of function mutations in 23 genes (BBS1-21) associated with cilia structure, biogenesis, and function, are reported in approximately 80% of patients with BBS (8).

The clinical diagnosis of the syndrome is made through the clinical diagnostic criteria presented in Table 1 (9,10). According to those criteria, four major features or the coexistence of three major and two minor features are adequate to establish the diagnosis (3,4,6,11,12). However, the diagnostic criteria may be insufficient when evaluating patients in early childhood, where some clinical findings have not yet emerged. Additionally, phenotypic variability is expected in BBS, and some patients may not meet the diagnostic criteria despite a confirmed molecular diagnosis. There is also no clear phenotype-genotype correlation in the syndrome, and an accurate clinical diagnosis can be difficult due to overlapping findings with other ciliopathies (6,13). Considering all these, it is undeniable that a molecular diagnosis is an essential tool for timely and accurate diagnosis. Next-generation sequencing (NGS) technologies, which have become widespread in clinical use recently, are a rapid and cost-effective diagnostic method in this group of diseases with genetic heterogeneity. Novel variants to be detected by this method will also contribute to establishing new phenotype-genotype correlations and delineating the pathophysiology in this rare group of diseases, apart from achieving accurate diagnosis in patients.

Here, we aimed to present the clinical and molecular findings of patients with BBS using NGS panel analysis to contribute to the genotype-phenotype correlation in this rare syndrome.

Methods

We retrospectively reviewed the medical records of patients with a suspicion of BBS admitted to the Pediatric Genetics Department in Umraniye Training and Research Hospital. Patients who met at least four major criteria or three major and two minor criteria according to the BBS clinical diagnostic criteria were included in the study (9,10). Beyond the demographic data, family history, clinical presentation; renal function test, urinalysis, liver function test, thyroid function test, abdominal-renal ultrasonography, echocardiography, and the examination results performed by the eye, eye-nose-throat, and cardiology departments were collected from the medical records of the patients. The local ethics committee approved the protocol of the study with an accession number of BS.GP.0.01/177. The study was conducted following the Declaration of Helsinki. Informed consent was obtained from each patient and their legal guardians for molecular analysis.

Targeted NGS analysis and variant interpretation

After obtaining written informed consent, peripheral blood samples were collected from all individuals. Following the standard protocols of the QIAAmp DNA Mini (Qiagen) kit, automatic DNA isolation was performed in EDTA-anticoagulated blood samples. Targeted NGS analysis was performed on the Illumina Next-Seq 500 platform using SOPHIA Clinical Exome Solution using Illumina V2 chemicals as outlined previously (14,15). The gene panel consisted of 20 genes associated with BBS; *ARL6* (NM_001278293), *BBS1* (NM_024649), *BBS10* (NM_024685), *BBS12* (NM_001178007), *BBS2* (NM_031885), *BBS4* (NM_001252678), *BBS5* (NM_152384), *BBS7* (NM_176824), *BBS9* (NM_001033604), *C8ORF37* (NM_177965.4), *CCDC28B* (NM_001301011), *CEP290* (NM_025114), *LZTFL1* (NM_001276378), *MKKS* (NM_018848), *MKS1* (NM_001165927), *SDCCAG8* (NM_006642), *TMEM67* (NM_001142301), *TRIM32* (NM_001099679), *TTC8* (NM_001288781), *WDPCP* (NM_001042692). Sequence analysis covers the coding regions of each gene, including all coding exons, +/− 10 base pairs of adjacent intronic sequences, and each nucleotide is read at a depth of at least 50X. Variants that fall outside these regions and exonic variants with a minor allele frequency of less than 10% were considered false positives and unanalyzed. Copy number variations were not examined.

Table 1. Clinical diagnostic criteria for Bardet-Biedl syndrome (9,17)

Major features	Minor features
Retinal cone-rod dystrophy	Neurologic abnormalities (ataxia, poor coordination, mild spasticity, speech delay)
Central obesity*	Olfactory dysfunction (anosmia, hyposmia)
Postaxial polydactyly	Oral/dental abnormalities (dental crowding, hypodontia, small roots, high arched palate)
Cognitive impairment	Gastrointestinal abnormalities (liver disease**, inflammatory bowel disease, celiac disease, Hirschsprung disease)
Hypogonadism	Cardiovascular and other thoraco-abdominal abnormalities (congenital heart diseases, situs ambiguous)
Renal disease	Endocrine/metabolic abnormalities (T2DM, DI, hypothyroidism, metabolic syndrome)

*Features associated with obesity (including endocrine/metabolic abnormalities and non-alcoholic fatty liver disease) are defined as minor features of BBS.
**Liver disease is considered as abnormalities in liver imaging and/or abnormal transaminase levels.
T2DM: Type 2 diabetes mellitus, DI: Diabetes inspidus, BBS: Bardet-Biedl syndrome

The DNA sequences were aligned to the NCBI Build37 (hg18) version of the human genome. Alignments were confirmed using the Integrative Genomics Viewer v.2.313. The Sophia-DDM-V5.2 bioinformatics analysis program performed variant calling and data analysis. The interpretation of the variants was performed according to the 2015 American College of Medical Genetics (ACMG) standards and guidelines (16). Iranome and GnomAD data were used as the control population. The variants' effects on protein function were investigated using *in silico* prediction programs such as SIFT, PolyPhen2, M-CAP, Mutation Taster, and MVP. The Human Gene Mutation Database and ClinVar and PUBMED databases were used to investigate mutations previously associated with BBS. Only variants of unknown significance (VUS), pathogenic (P), and likely-P variants were reported in the results section. Segregation analysis was performed by Sanger sequencing. Primer sequences and reaction conditions are available on request.

Results

Demographic and clinical characteristics

We identified 10 patients who were followed up with a suspicion of BBS. Four patients who did not fulfill the BBS clinical criteria were excluded and the final analyses included 6 patients (age, mean±standard deviation: 14.3±6.6 years, female: 50%). In the study group, while all major criteria were present in four of the patients, two had only four major criteria. Rod cone dystrophy (100%), polydactyly (100%), and intellectual disability (100%) were the most common findings in all patients. These findings were followed respectively by obesity (83%), renal anomalies (83%), gastrointestinal abnormalities anomalies (67%), dental problems (67%), endocrine/metabolic problems (50%), hypogonadism (33%), psychiatric disorders (33%) and sleep apnea (33%). Among the renal abnormalities, renal agenesis/hypogenesis (40%) and nephrocalcinosis (40%) were the most common abnormalities. All male patients had hypospadias regarding hypogonadism. Hepatosplenomegaly and elevated transaminase levels were the findings detected in all patients with liver abnormalities. In the dental anomalies group, malocclusion was the most common dental anomaly (75%); hypodontia was present in only one patient (Figure 1). Anxiety was the most common problem detected in 75% of patients with neuropsychiatric disorders. In the metabolic/endocrine finding group, diabetes mellitus (DM) was present in only one patient; however, hyperlipidemia (75%) stood out as the major metabolic problem. Cardiac abnormalities were present in only one patient with the atrial septal defect. Hearing loss and olfactory problems were not observed in the study group. Clinical features and the details of the anomalies are presented in Table 2.

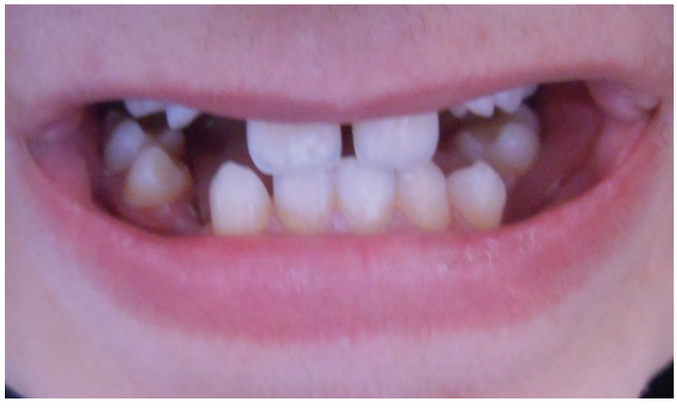

Figure 1. Image of the oral region showing the hypodontia in Patient 2

Molecular analysis

A molecular diagnosis was achieved in all the patients. Seven different variants were detected in the *BBS1, BBS7, BBS5, BBS9*, and *MKKS* genes. There were three frameshift (fs), two splice-site, and two missense variants, two of which were novel variants that were not previously reported in patients with BBS (Figure 2). The details of the variants are presented in Table 2.

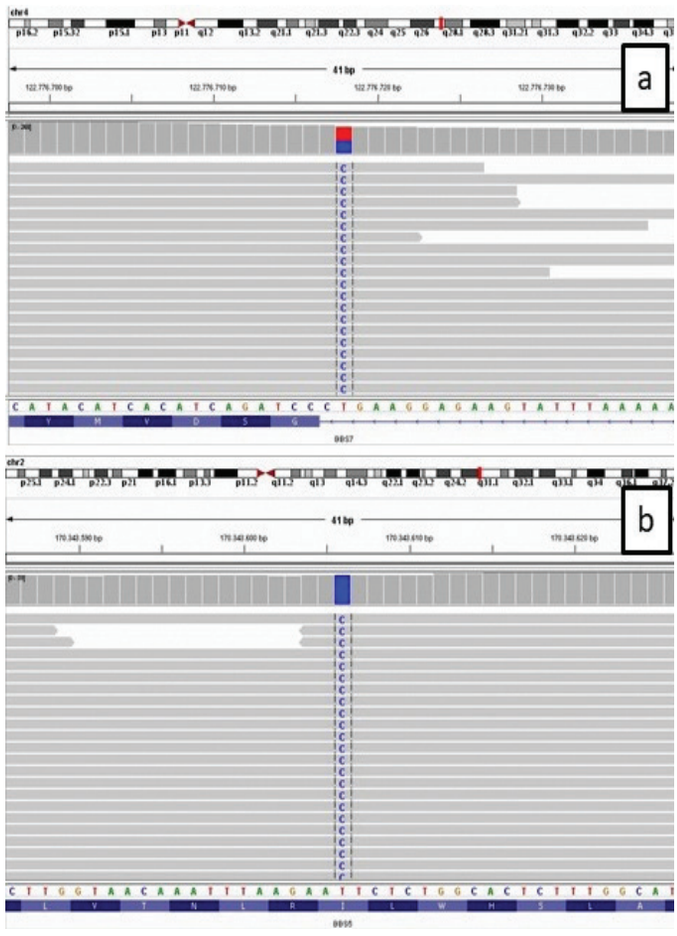

Figure 2. Integrative genomics viewer images of the novel variants reported in Patient 1 (BBS7 c.529-2A>G) (a) and Patient 2 (BBS5 c.170T>C) (b)

Table 2. Clinical and molecular findings of the patients

Patient ID	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Percentage
Gene	*BBS7/BBS7*	*BBS5*	*BBS7*	*MKKS*	*BBS9*	*BBS1*	
NM number	NM_176824	NM_152384	NM_176824	NM_018848	NM_001033604	NM_024649	
Depth	215/213	47	125	726	47	110	
50x	99.11%	98.87%	99.92%	99.98%	86.77%	84.83%	
Mutation type	Frameshift/Splice	Missense	Splice	Missense	Frameshift	Frameshift	
cDNA	c.712_715delAGAG/ c.529-2A>G	c.170T>C	c.849+1G>T	c.541G>A	c.310del	c.1232_1235del	
Protein	p.Arg238Glufs*59/p.(?)	p.Ile57Thr	p.(?)	p.Ala181Pro	p.(Cys104Valfs*20)	p.(Gly411Glufs*12)	
Reference	Reported/Novel	Novel	Reported	Reported	Reported	Reported	
Zygosity	Het/Het	Hom	Hom	Hom	Hom	Hom	
ACMG class	P/P	VUS	P	LP	P	P	
ACMG evidence	PVS1, PP5, PM2/PVS1, PM2	PM2, PP2, PP3	PVS1, PM2, PP3	PM2, PP2, PP3	PVS1, PM2, PP3, PP5	PVS1, PM2, PP3, PP5	
Age (years)	18	17	2	14	14	21	
Gender	M	F	M	F	F	M	
BMI (kg/m²)*	29.4	30.5	28.1	42.3	34.5	43	
CDC BMI-for-age percentiles	(95^{th} -97^{th})	(95^{th} -97^{th})	(>97^{th})	(>97^{th})	(>97^{th})	(>97^{th})	
Rod cone dystrophy	Yes	Yes	Yes	Yes	Yes	Yes	100
Limb abnormalities	Yes	Yes	Yes	Yes	Yes	Yes	100
Polydactyly	Yes	Yes	Yes	Yes	Yes	Yes	
Syndactyly	Yes	No	No	No	No	No	
Others	No	5^{th} metatarsal, 4^{th}-5^{th} metacarpal shortness	No	No	No	Camptodactyly Brachydactyly	
Intellectual disability	Moderate	Mild	NA	Moderate	Mild	Mild	83
Developmental delay	Yes	Yes	Yes	Yes	Yes	Yes	100
Renal abnormalities	No	Left renal agenesis Right renal cyst	Grade 2 pelvic ectasia Bilateral medullar nephrocalcinosis	Bilateral renal cyst	Nephrocalcinosis Proteinuria	Bilateral renal hypogenesis Renal insufficiency	83
Genital abnormalities	Hypospadias	No	Hypospadias	No	No	Hypospadias	50

Table 2. Continued

Patient ID	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Percentage
Liver abnormalities	No	No	No	Hepatosplenomegaly Hepatosteatosis High transaminase levels	No	Hepatosplenomegaly High transaminase levels	33
Diabetes mellitus	No	No	No	Yes	No	No	17
Dyslipidemia	Yes	No	Yes	Yes	No	Yes	67
Dental abnormalities	Malocclusion	Multiple caries Erosion Hypodontia	No	Malocclusion	Malocclusion	No	67
Neuropsychiatric problems	Anxiety	Anxiety	No	OCD/seizures	Anxiety	No	67
Sleep apnea	No	No	No	Yes	No	Yes	33
Hearing loss	No	No	No	No	No	No	0
Anosmia	Yes	No	No	No	No	No	17

*CDC BMI-for age charts (2-20 years) were used to determine the weight status (underweight: <5th percentile, healthy weight: 5th-85th percentile, overweight: 85th-95th percentile, obesity: >95th percentile).
Hom: Homozygous, Het: Heterozygous, P: Pathogenic, LP: Likely pathogenic, VUS: Variant of insignificance, M: Male, F: Female, BMI: Body mass index, CDC: Centers for disease control and prevention, OCD: Obsessive-compulsive disorder

One of the novel variants was the *BBS7* c.529-2A>G, a null variant detected in Patient 1. We predicted that this variant would cause exon skipping by affecting the acceptor site of the *BBS7*. This was not previously reported in community databases such as GnomAD and Iranome. The variant was classified as P with the evidence of PVS1 and PM2.

The other novel variant was the homozygous missense c.170T>C variation in *BBS5*. It was not reported in the GnomAD and Iranome databases. Most *in silico* prediction tools (M-CAP, MutationTaster, and SIFT) showed that the variant had a damaging effect on the protein. The variant was classified as VUS with the ACMG evidence of PM2 and PP3.

Discussion

BBS is characterized by both phenotypic and genotypic heterogeneity (2). Thus, identifying new variants and deep phenotyping in patients is vital in elucidating this rare syndrome's pathophysiology and advancing therapeutic research. Our study is the third study conducted for this purpose in patients with BBS in Turkey (1,11). We managed to detect the underlying molecular pathology in all of our patients using the targeted NGS panel. Although the targeted panel analysis yielded a diagnostic rate up to 86% in previous studies, we achieved the molecular diagnosis in all of our patients (1,2). We suggest this finding may be related to the inclusion of patients who only fulfilled the BBS clinical diagnostic criteria in our study.

BBS1 and *BBS10* stand out as the genes with the most common disease-associated variants in the patient cohorts reported so far (5,17). Consistent with these findings, *BBS10* and *BBS1* were reported as the most common genes in the largest series conducted in our country (1). However, in our study, while no variant was detected in *BBS10*, a *BBS1*-related variant was detected in only one patient. Also, with three variants detected, *BBS7* stood out as the most common gene. Variations in *BBS7* are defined as a rare cause of the syndrome, with a frequency of 1.5-3% in multiethnic groups (18-21). This rate was up to 15% in studies conducted in our country (1,11). When evaluated with the data from our research, one can suggest that *BBS7*-associated BBS is more frequent in the Turkish population than in other ethnic groups.

Missense variations were reported as the most common type of variation in BBS-related genes (2,22). Unlike this finding, null variants (fs, splice site) were detected more frequently in our study, as in the other two studies conducted in Turkey (1,11). We can suggest that this finding may be related to Turkish ethnic origin and geographical region; however, studies involving more patients from Turkey are needed to make an accurate assessment.

We contributed to the BBS-related genotypic spectrum with the two novel variants detected. The first of these was the heterozygous *BBS7* c.529-2A>G variant. The fact that it was a

null variant unreported in population databases was important evidence to be considered P associated with BBS. Furthermore, we thought that this variant would explain the clinical findings related to BBS if it was found in a trans with the previously reported *BBS7* c.712_715delAGAG, fs variant detected in the same patient. However, although we assumed that these two variants were found to be compound heterozygous based on clinical findings, we could not conduct a segregation analysis in terms of compound heterozygosity because the parents were deceased, and we could not confirm this situation. The other novel variant was the *BBS5* c.170T>C variant. It was absent in population databases and determined as damaging according to most of the *in silico* prediction tools. These findings provide important evidence for associating the variant with the disease phenotype. Additionally, the interspecies conservation score of the genomic position (PhyloP100way score: 6.026, GERP score: 5.5) was also high. The patient's clinical findings had met five of the major and two minor diagnostic criteria of BBS. Therefore, although it was classified as VUS according to ACMG, we thought the variant explained the patient's phenotype. However, it was also kept in mind that functional studies should be conducted, or the same variant should be reported in another patient to prove the pathogenicity of the variant.

In terms of phenotypic findings, rod-cone dystrophy, polydactyly, developmental delay, and intellectual disability, which are the major features of the syndrome, were present in all of our patients. Rod-cone dystrophy has been reported in approximately 80-90% of patients in previous studies and is usually detected after three years of age, at a mean age of 8.5 years (1,2,4). In this study, all but one patient was diagnosed with rod-cone dystrophy in adolescence, in line with these data. The youngest patient with rod-cone dystrophy in our study was a two-year-old girl diagnosed at the age of 1.5 years when examined for nystagmus (Patient 3). She had a homozygous null variant in the *BBS7* gene, so we conclude that the retinal pathology observed at such an early age may be related to the null variant. Nystagmus has only been reported in two patients with BBS (1,23). Since they both had variants of *BBS7*, authors have suggested that there is a relationship between *BBS7*-related variants and nystagmus (1,23). Although the number of reported cases is scant, our patient appears to be a case that supports this correlation.

Polydactyly has been reported in 60-80% of patients with BBS (1,2,6). In addition to polydactyly; syndactyly, brachydactyly, and clinodactyly are among the other extremity anomalies reported in BBS. Among these anomalies, syndactyly and brachydactyly were also detected in two patients in our study group. Moreover, isolated 5th metatarsal, 4th, and 5th metacarpal shortness in the patient with the homozygous missense variant in *BBS5* and camptodactyly in the patient with homozygous fs variant in *BBS1* were the extremity anomalies that we reported for the first time in association with BBS (Patient 2 and Patient 6) (Figure 3).

Developmental delay is reported in approximately 90% of patients with BBS while varying degrees of intellectual disabilities are described in 60% of patients (1,2,24). While there is no clear genotype-phenotype correlation in terms of intellectual disability, milder intellectual disability is reported with *BBS1* and *BBS12* variants (2). Mild intellectual disability was observed in most patients, and moderate intellectual disability was found in only two of the patients with *BBS7* and *MKKS* variants in our study (Patient 1 and Patient 4). However, there is no data in the literature to support this phenotype-genotype correlation considering moderate intellectual disability.

Another major feature, obesity, was found in 67% of the patients in our study group, similar to the rate of 70-90% reported in the literature (1,3,4). DM and liver abnormalities that are reported to be associated with obesity were also present mainly, in obese individuals in the study group (2,8). However, contrary to DM and liver abnormalities, it has been suggested that dyslipidemia can also be detected in patients with BBS without obesity (2). Dyslipidemia observed in 18-year-old and 2-year-old individuals who were not obese but overweight may support this view (Patient 1 and Patient 3). Therefore, it is crucial to follow the patients from an early age in terms of these metabolic problems to minimize the related complications.

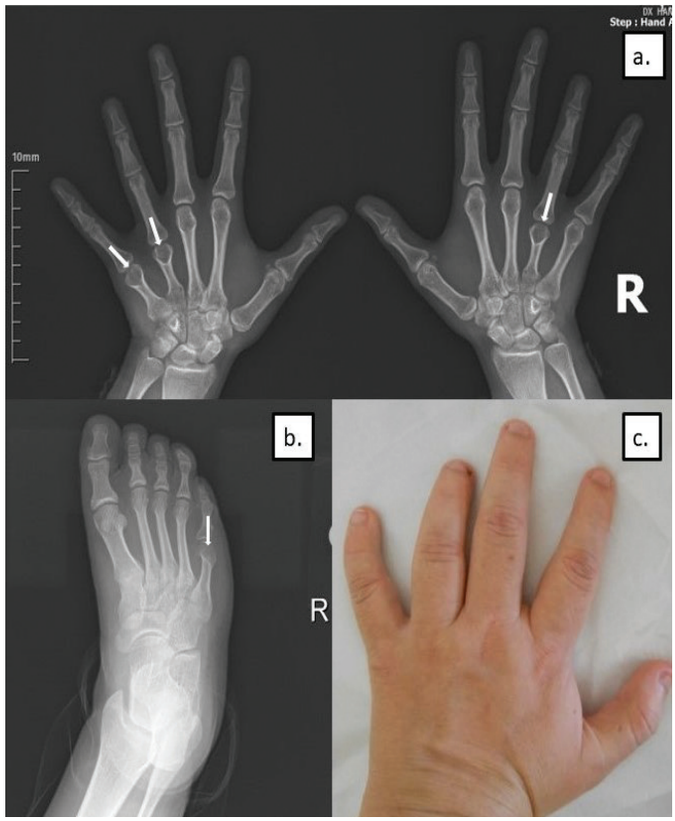

Figure 3. Foot and hand X-rays of Patient 2 and hand image of Patient 6. Note the 4th and 5th metacarpal shortness in left, 4th metacarpal shortness in right hand (a), metatarsal shortness in right foot (b), camptodactyly in third and fourth fingers in the left hand (c)

Renal anomalies are reported in 50-80% of individuals with BBS and cause severe morbidity and mortality with advancing age, particularly if not properly managed (1,2,25,26). Renal involvement was present in 67% of patients, and it stood out as the most common organ involvement causing morbidity in this study. The patient with the most severe renal involvement was a 21-year-old male followed up for end-stage renal disease (Patient 6). It is thought that the presence of the homozygous null variant in *BBS1* in this patient may have led to a severe clinical course. Nephrocalcinosis, an infrequent renal finding in individuals with BBS (27), had been observed in two patients with variants of *BBS7* and *BBS9*, respectively in our cohort (Patient 3 and Patient 5). However, more cases are needed to establish a genotype-phenotype correlation for nephrocalcinosis.

Among the non-classical findings, psychiatric problems observed in 66% of the study group were remarkable. These psychiatric disorders, primarily including autism, obsessive-compulsive disorder, and anxiety, have been defined in 30-50% of individuals with BBS and can affect the functionality of a patient's daily lives (1,2,11,24). Especially in patients with severe multisystemic involvement, psychiatric problems may remain in the back and have been ignored by the patient and the family. So it is of great importance that health professionals do not overlook these problems but make appropriate interventions in the early period (2,24).

This study has some limitations. First, the number of patients and the heterogeneity of the detected genes prevents us from establishing any widespread phenotype-genotype correlations in the study. However, in this rare syndrome, the phenotype and genotype information reported in the literature are of great value. Second, since a wide phenotypic heterogeneity is defined in BBS, the use of strict clinical criteria for inclusion in the study group may have resulted in some patients being missed. The enrollment of patients in the study with looser criteria would have allowed broadening of the disease's genotypic/phenotypic spectrum.

Conclusion

Our study expanded the genotypic spectrum of the disease with two novel variants reported. Besides, by defining novel/rare clinical features, it formed a source for the phenotype-genotype correlation trying to be established in the literature.

Ethics

Ethics Committee Approval: Ethical approval was obtained at the Health Sciences University, Istanbul Umraniye Training and Research Hospital Local Clinical Research Ethics Committee (approval number: B.10.1.TKH.4.34.H.GP.0.01/177, date: 27.05.2021).

Informed Consent: Retrospective study.

Peer-reviewed: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ö.A.D., Design: Ö.A.D., N.B.A, Data Collection, or Processing: Ö.A.D., N.B.A., Analysis, or Interpretation: Ö.A.D., N.B.A., Literature Search: Ö.A.D., N.B.A., Writing: Ö.A.D.

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

Financial Disclosure: The authors declare that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.46036
Gulhane Med J 2022;64:152-8

Evaluation of 25-hydroxyvitamin D levels in obese individuals

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Date submitted:
05.04.2021
Date accepted:
12.06.2021
Online publication date:
15.06.2022

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Keywords: Adult obesity, vitamin D deficiency, 25(OH)D, parathyroid hormone, body composition

ABSTRACT

Aims: Although 25-hydroxyvitamin D [25(OH)D] deficiency is common, only a few studies have focused on the 25(OH)D level in obese people in Turkey. Therefore, this study assessed the relationship between 25(OH)D status and obesity in the Kayseri region in Turkey.

Methods: This cross-sectional study included otherwise healthy individuals aged 20 to 49 years. Obesity was defined as body mass index (BMI) ≥30. A bioelectrical impedance analyzer was used to measure the body composition. The short form of the "International Physical Activity Questionnaire" was used to assess the physical activity level. Blood 25(OH)D and parathyroid hormone (PTH) levels, dietary intake, body composition, and potential factors associated with the 25(OH)D status were determined.

Results: The study included 268 adults (mean age: 31.4±9.5 years, 69.5% were women). The proportion of vitamin D deficiency (<20 ng/mL) was 84.3% and 83.5% in the obese and normal-weight individuals, respectively. Obese individuals had lower 25(OH)D level [11.6 (8.1-17.9) vs. 13.5 (9.7-18.3) ng/mL, p=0.069] and higher PTH level [44 (35.0-63.0) vs. 36 (28.0-47.0) pg/mL, p<0.001] compared with their normal-weight counterparts. Dietary vitamin D and calcium intake were similar, whereas phosphorous intake was higher in the obese subjects [1052.7 (754.9-1118.4) vs. 945.7 (754.9-1118.4) mg/day p=0.015]. 25(OH)D levels correlated with BMI (p<0.001, r=-0.170), body fat mass (p<0.01, r=-0.179), and muscle mass (p<0.001, r=0.251).

Conclusions: 25(OH)D deficiency is common among obese and normal-weight individuals in this large middle Anatolian city of Turkey. This study found a relationship between serum 25(OH)D levels and BMI, body fat, and muscle mass in the study sample.

Introduction

Vitamin D and parathyroid hormone (PTH) are indispensable elements of calcium homeostasis and bone metabolism (1). Cumulative evidence indicates that vitamin D has a critical function in extra-skeletal health and diseases such as diabetes mellitus, cancers, cardiovascular diseases, and autoimmune disorders (2-4). The best indicator of vitamin D status is the serum 25-hydroxyvitamin D [25(OH)D] levels. Unfortunately, as is known today, most populations worldwide suffer from vitamin D insufficiency (1). Obesity potentially increases the incidence of vitamin D insufficiency since obese people have lower levels of 25(OH)D than the non-obese (5,6).

It has been found that throughout one's life, opposite connections exist among body weight, body mass index (BMI), or body fatness measures with vitamin D status (3,5,7).

Age, race, geography, skin color, habitual dressing style, and sun exposure are listed as some factors leading 25(OH)D levels to be different (8). Additionally, there is an inverse relationship between vitamin D status and BMI, body weight, and body fat. Although Turkey is located in a sun-soaked climate zone, sunlight is not exploited enough for several reasons: dietary habits, skin color, cultural factors, and clothing style. Apart from the study by Aypak et al. (9) and Tosunbayraktar et al. (10), most of the studies in Turkey analyze the 25(OH)D levels of postmenopausal women and the elderly.

Based on the previous studies (3,5,7), it was hypothesized that higher adiposity would lead to lower vitamin D levels. We particularly aimed to (a) evaluate the correlations of serum 25(OH)D with body composition measurements, (b) determine the link between vitamin D and PTH levels with obesity, and (c) compare serum 25(OH)D and PTH levels related to obesity in Kayseri/Turkey.

Methods

Study design

This cross-sectional study was conducted in the Endocrinology Outpatients Clinic of Erciyes University Faculty of Medicine. Individuals were classified according to their BMI as normal-weight and obese. The assignment was made using several factors: wearing a headscarf, sociodemographic features, and physical activity level. A general questionnaire was administered through face-to-face interviews. Biochemical parameters [25(OH)D, PTH], 24-hour dietary intake for three consecutive days (two weekdays and one weekend day), and physical activity levels (PAL) were identified, anthropometric measurements were taken, and bioelectrical impedance analysis was performed.

Written consent was obtained from all participants at the beginning of the study, which was approved by the Ethics Committee of the Faculty of Medicine, Erciyes University, Kayseri, Turkey (approval number: 2013/659, date: 22.11.2013).

Inclusion criteria

Inclusion criteria were as follows: (1) Being between 20 and 49 years of age, (2) applying to the endocrine outpatient clinic at Erciyes University Faculty of Medicine, (3) having a low physical activity level (4) volunteering to give written consent, (4) agreeing to give blood samples.

Exclusion criteria

Individuals with any of the following conditions that could affect vitamin D metabolism were excluded from the study: being under 20 and more than 49 years of age, pregnancy, lactation, postmenopausal status, being underweight (BMI<18.5 kg/m^2), taking dietary vitamin D supplements, alcohol consumption above 50 g/day, more than 3% change in body weight or taking drugs, which could affect body weight in the previous three months, and having comorbid diseases.

Anthropometric measurements

A dietitian performed anthropometric measurements according to the criteria suggested by the World Health Organization (WHO) (11). A bioelectrical impedance analyzer (TANITA MC 780 MA, Tanita Corp., Tokyo, Japan) was used to measure body composition. Participants were instructed to avoid food or liquid intake and vigorous exercise 4 h before the measurement and not wear any metallic objects. Height was measured using a stadiometer with subjects standing barefoot, keeping their shoulders relaxed, arms hanging freely, and the head in Frankfort horizontal plane (12).

Waist and hip circumferences were measured while the individuals were standing, arms were open on both sides, and feet were closed. Waist circumference was measured with a tape measure between the iliac crest and the lowest ribs (mid-point crossing circumference) while the individual was exhaling. The tape measure was positioned horizontally, parallel to the floor, and the measurement was carried out, paying attention not to apply pressure on the skin. Hip circumference was determined from the highest point of the side of the hip (12).

Based on participants' BMI values calculated, individuals were classified as normal-weight (BMI=18.5-24.9 kg/m^2), and obese (BMI≥30 kg/m^2), according to WHO criteria (13).

Assessment of biochemical parameters

After 8-hour fasting overnight, blood samples were collected between 08.30-10.30 am. Then the serum was separated by centrifugation and stored at -80 °C until it was shipped on dry ice to Düzen Laboratory (accredited) in Ankara, where 25(OH) D and PTH analyses were performed. 25(OH)D was measured using Liquid Chromatography and Mass Spectrometry (LC-MS/MS), and PTH (Intact) was measured using a Test Roche Cobas e601 equipment by ECLIA method. Vitamin D status was considered deficient if the serum 25(OH)D level was 20 ng/mL or lower (≤50 nmol/L); inadequate if it was between 21 and 29 ng/mL (52-72 nmol/L); and adequate if it was 30 ng/mL and higher (≥75 nmol/L) (14).

Dietary assessment

The dietary intake was evaluated based on their food consumption frequencies and 24-hour dietary assessment (filled in three consecutive days, including two weekdays and one weekend). The participants were asked to record all the foods and beverages they consumed before the study. A Nutrient Database (BeBiS, Ebispro for Windows, Germany; Turkish version/BeBiS 7) was used to determine participants' energy and nutrient intakes (15,16). Portion sizes were estimated with 2-dimensional food models and a food atlas, including 3 to 5 portion size images of 120 foods (17).

Determination of physical activity levels

The short form of the "International Physical Activity Questionnaire" was used to determine physical activity levels. This form consisted of seven questions and provided information about the time spent sitting, walking, and moderate to vigorous activities. The total score included the duration (minutes) and frequency (days) of the activities performed. Physical activity levels were classified as physically inactive (<600 MET -min/week), low physical activity (600-3000 MET -min/week), and

adequate physical activity (with health benefits) (>3000 MET -min/week) (18). Individuals with low activity levels were included in the current study.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY) was used for statistical analysis. A minimum of 240 samples was evaluated with a 95% confidence interval in NCSS-PASS software, and vitamin D deficiency was estimated to be 32% in obese adults (19). The normality of the data distribution was determined via the histogram, Shapiro-Wilk test, and q-q graphics. Descriptive analysis [mean±standard deviation, median (25 percentile-75 percentile), and frequencies (%)] was performed. Independent Sample t-test and Mann-Whitney U test were used to compare continuous variables, whereas chi-square analysis was used for categorical variables. The Spearman rank correlation test was performed, and scatter plot graphs were drawn to investigate the relationship between quantitative data. $p<0.05$ was noted to be statistically significant.

Results

Two hundred sixty-eight adults (83 men and 185 women) aged 20-49 years were recruited and classified as normal weight and obese. The measurements of anthropometric and bioelectrical impedance analysis and daily energy and some nutrient intake, and biochemical findings of participants, classified based on BMI values, are presented in Table 1. According to Table 1, 40.6% (BMI=18.5-24.9 kg/m^2, n=109) of the individuals were normal-weight, whereas 59.4% of them were obese (BMI ≥30 kg/m^2 n=159). The mean ages of the normal-weight and obese individuals were 26.2±7.3 and 35.0±9.2 years, respectively ($p<0.001$). Mean body weight and height for the normal-weight individuals were 63.0±10.6 kg and 166.6±10.2 cm, and 93.7±16.5 kg and 164.4±10.5 cm for the obese individuals. Additionally, the median waist circumference for normal weight and obese subjects was 79.0 cm (71.0-88.0) and 106 cm (99.0-114.0), respectively ($p<0.001$, Table 1). However, basal metabolic rate, body fat percentage, body fat mass, lean body mass and total body water of the obese participants [1734.0 (1547.0-2071.0) kcal/day, 36.6±6.3%, 56.3±10.6 kg, 55.8 (50.5-68.8) kg, 40.1 (36.1-49.1) kg, respectively] were statistically higher than their normal-weight counterparts [1379.0 (1284.0-1673.0) kcal/day, 23.7±5.4%, 45.7±9.3 kg, 45.2 (41.1-56.4) kg, 32.6 (29.5-40.3) kg, respectively].

Energy intakes of the normal-weight and obese individuals were 1911.7 (1420.3-2233.8) kcal/day and 1639.9 (1320.7-1879.0) kcal/day, respectively ($p<0.05$). Carbohydrates, protein, and fat percentages were not statistically different between the

Table 1. Body composition, daily energy intake, nutrient intake, and biochemical parameters of the participants

	Obese (n=159, 59.4%)	Normal-weight (n=109, 40.6%)	p
Age (year), mean±SD	35.0±9.2	26.2±7.3	**<0.001***
Body weight (kg), mean±SD	93.7±16.5	63.0±10.6	**<0.001***
Height (cm), mean±SD	164.4±10.5	166.6±10.2	0.085*
BMI (kg/m^2), median (25 percentile-75 percentile)	33.4 (30.8-36.8)	23.0 (20.9-24.1)	**<0.001****
WC (cm), median (25 percentile-75 percentile)	106 (99.0-114.0)	79 (71.0-88.0)	**<0.001****
HC (cm), mean±SD	119.9±10.5	97.3±6.1	**<0.001***
BMR (kcal/day), median (25 percentile-75 percentile)	1734.0 (1547.0-2071.0)	1379.0 (1284.0-1673.0)	**<0.001****
Body fat (%), mean±SD	36.6±6.3	23.7±5.4	**<0.001***
Body fat mass (kg), mean±SD	56.3±10.6	45.7±9.3	**<0.001***
LBM (kg), median (25 percentile-75 percentile)	55.8 (50.5-68.8)	45.2 (41.1-56.4)	**<0.001****
TBW (kg), median (25 percentile-75 percentile)	40.1 (36.1-49.1)	32.6 (29.5-40.3)	**<0.001****
Energy (kcal/day), median (25 percentile-75 percentile)	1911.7 (1420.3-2233.8)	1639.9 (1320.7-1879.0)	**0.001****
Protein (%), median (25 percentile-75 percentile)	15.0 (13.0-16.0)	15.0 (13.0-18.0)	0.120**
Fat (%), mean±SD	35.8±6.3	35.9±5.7	0.868*
Carbohydrate (%), mean±SD	49.3±7.1	48.7±6.6	0.518*
Calcium (mg/day), median (25 percentile-75 percentile)	627.7 (467.5-833.8)	603.3 (468.9-777.3)	0.395**
Phosphorus (mg/day), median (25 percentile-75 percentile)	1052.7 (754.9-1118.4)	945.7 (754.9-1118.4)	**0.015****
Vitamin D (mcg/day), median (25 percentile-75 percentile)	1.3 (0.9-2.1)	1.2 (0.7-1.9)	0.179**
25(OH)D (ng/mL), median (25 percentile-75 percentile)	11.6 (8.1-17.9)	13.5 (9.7-18.3)	0.069**
PTH (pg/mL), median (25 percentile-75 percentile)	44 (35.0-63.0)	36 (28.0-47.0)	**<0.001****

*Student's t-test, **Mann-Whitney U test.
WC: Waist circumference, HC: Hip circumference, BMR: Basal metabolic rate, LBM: Lean body mass, TBW: Total body water, SD: Standard deviation, PTH: Parathyroid hormone, BMI: Body mass index

normal-weight and obese subjects (p>0.05). Moreover, dietary intake of vitamin D and calcium was similar between the normal-weight and obese counterparts (p>0.05). However, the dietary phosphorous intake of the obese participants [1052.7 (754.9-1118.4)] mg/day was higher than that of those with normal-weight participants [945.7 (754.9-1118.4)] mg/day (p<0.05) (Table 1).

Although it was statistically insignificant, 25(OH)D levels of the normal-weight individuals were higher than those of the obese [13.5 (9.7-18.3) ng/mL and 11.6 (8.1-17.9) ng/mL, respectively]. The PTH levels of the obese subjects [44 (35.0-63.0) pg/mL] were statistically higher than those of the normal weight [36 (28.0-47.0) pg/mL, p<0.001] (Table 1).

Vitamin D levels of the participants according to BMI classification and gender are shown in Table 2. Vitamin D deficiency was 84.3% in the obese individuals and 83.5% in their normal-weight subjects (<20 ng/mL). Additionally, vitamin D insufficiency (20-29.9 ng/mL) was detected in 14.4% of the obese participants and 14.7% of the normal-weight participants.

Correlations between 25(OH)D and BMI, body fat mass, and body muscle mass are shown in Figures 1, 2, and 3. A negative, weak, and statistically significant relationship was found between 25(OH)D, BMI (p<0.001, r=-0.170), and body fat mass (p<0.01, r=-0.179). Additionally, a positive, weak, and statistically significant relationship was found between muscle mass (p<0.001, r=0.251) and 25(OH)D (Figures 1, 2, 3).

Discussion

The most notable finding in the current study was that serum 25(OH)D levels appeared to be associated with BMI, body fat, and muscle mass. Although no statistically significant difference was observed, median 25(OH)D levels of the obese were lower than those of the normal-weight in the current study. Accordingly, 84.3% of obese individuals were found to have vitamin D deficiency. Although they had higher levels of vitamin D deficiency than those with normal body weight (83.5%), no statistically significant difference was found between the groups. Previous studies have stated that vitamin D deficiency may be a risk factor for obesity. However, the mechanisms behind lower levels of 25(OH)D and higher PTH have not been fully understood. Several hypotheses have been proposed about this relationship (6,9,20,21). Aypak et al. (9) stated that an increase in BMI causes 25(OH)D levels to decrease and that obese individuals are at risk of 25(OH)D deficiency. In Parikh et al.'s (20) study, that serum 25(OH)D levels of the obese (23.5±12.2 ng/mL) were significantly lower than that of the normal weight individuals (31±14.4 ng/mL). However, Karlsson et al. (21)

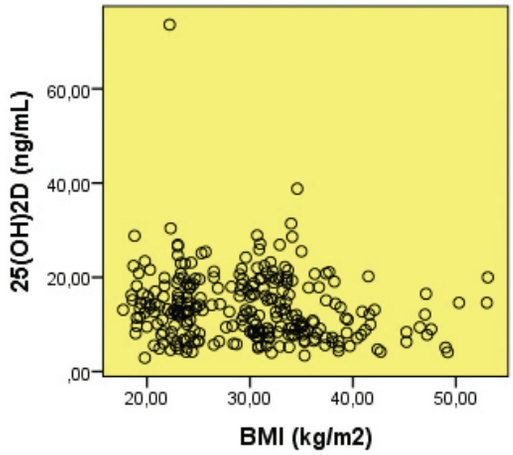

Figure 1. Correlation between 25(OH)D level and BMI. A negative, weak and statistically significant relationship was found between 25(OH)D, and BMI (p<0.001, r=-0.170)

BMI: Body mass index

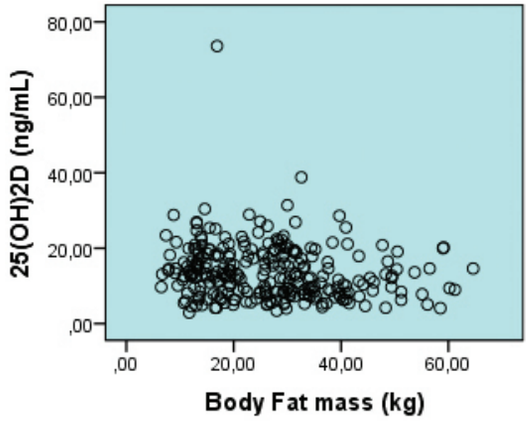

Figure 2. Correlation between 25(OH)D level and body fat mass. A negative, weak and statistically significant relationship was found between 25(OH)D, and body fat mass (p<0.01, r=-0.179)

Table 2. Vitamin D levels of the participants according to BMI categories and gender

	Obese (n=159, 59.4%)			Normal weight (n=109, 40.6%)		
	Men	Women	Total	Men	Women	Total
Deficiency (<20 ng/mL), n (%)	38 (70.3)	96 (91.4)	134 (84.3)	17 (58.6)	74 (92.5)	91 (83.5)
Insufficiency (20-29.9 ng/mL), n (%)	15 (27.8)	8 (7.6)	23 (14.4)	12 (41.4)	4 (5.0)	16 (14.7)
Normal (≥30 ng/mL), n (%)	1 (1.9)	1 (1.0)	2 (1.3)	-	2 (2.5)	2 (1.8)
	p=0.001*			p<0.001*		

*Pearson chi-square test; BMI: Body mass index

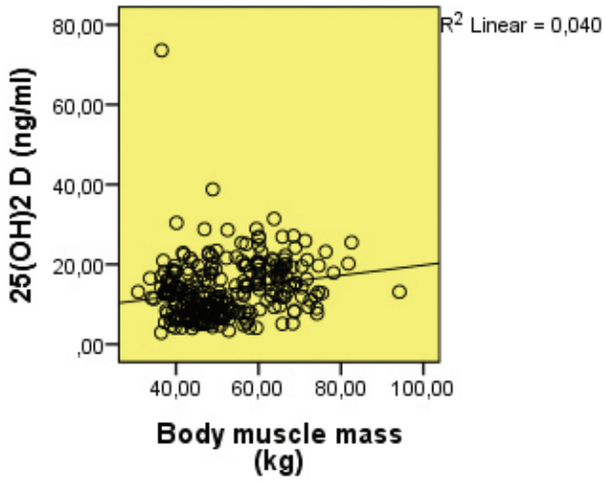

Figure 3. Correlation between 25(OH)D level and body muscle mass. A positive, weak and statistically significant relationship was found between 25(OH)D, and body muscle mass ($p<0.001$, $r=0.251$)

determined that although obese pregnant women received a greater amount of dietary vitamin D than normal-weight women, the former had lower levels of serum 25(OH)D. Likewise, Mai et al. (6) identified a negative relationship between low serum 25(OH)D levels and BMI in the Trøndelag health study (HUNT) on 26.616 adults between the ages of 19-55. In contrast, Karlsson et al. (21) found similar results on the dietary vitamin D levels of the obese and normal-weight individuals.

In this study, the median serum PTH levels of the obese were significantly higher than that of the individuals with normal body weight, which was in concordance with other studies (22,23). The current explanation for this phenomenon is the increased sequestration of 25(OH)D in excess subcutaneous fat, ultimately decreasing the bioavailability of vitamin D for calcium absorption. The diminished availability of serum 25(OH) D causes a compensatory increase in PTH secretion to maintain serum calcium concentrations (22,23). We think that our results confirm this statement.

Moy and Bulgiba (24) identified that women have lower levels of 25(OH)D and a higher frequency of 25(OH)D deficiency. This study also found, in compliance with the literature, that both obese (91.4%) and normal-weight (92.5%) women had significantly higher vitamin D deficiency (<20 ng/mL) than obese (70.3%) and normal-weight men (58.6%). It is speculated that the intersexual difference in 25(OH)D levels originates from the fact that women are less exposed to exposure sunlight exposure than men because of cultural reasons such as different dressing styles. Previous studies have demonstrated that low 25(OH)D levels of the obese were associated with lifestyle factors such as physical activity, alcohol consumption, and smoking status (25,26). Although individuals were assigned to groups based on several factors (such as wearing a headscarf, sociodemographic features, and physical activity level), there were no differences in vitamin D levels between obese and normal-weight individuals. Moreover, studies have stated that low 25(OH)D levels of the obese were associated with dietary factors such as low dietary vitamin D and calcium (25,26). In contrast to these study results, dietary vitamin D and calcium intake were similar between normal and obese subjects.

The finding of lower serum 25(OH)D concentration in higher fat mass in the current study agrees with several other observational studies (27-29). Prior studies largely support a positive association between higher serum 25(OH)D concentration and better muscle mass (30,31). Similarly, this study generated a positive and significant relationship between 25(OH)D levels and muscle mass.

There are some strengths and limitations of this study. First, only a few studies have investigated the link between vitamin D and PTH levels and obesity in Turkey. Second, the author conducted all the interviews, thus ensuring a consistent technique and interpretation of the answers given. However, bias may arise because of the study design and the type of sampling as the work was designed as a pilot study, and therefore, the sample chosen may not represent the general population, and the conclusions cannot be generalized. Future studies should consider multiple centers. Due to the differences in demographic features, geographic regions, assessment methods of 25(OH)D level, and threshold values, it is not easy to compare vitamin D status found in different studies.

Conclusion

This study showed that 25(OH)D deficiency is widespread in obese and normal-weight people and that the differences are not significant. Further studies are warranted to investigate the potential pathological mechanisms and establish optimal strategies to improve vitamin D status in obese and normal-weight populations.

Acknowledgments

We would like to thank the physicians involved in data collection and the women who participated in the study.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Faculty of Medicine, Erciyes University, Kayseri, Turkey (approval number: 2013/659, date: 22.11.2013).

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.Ü., Concept: G.K., N.İ., Design: G.K., N.İ., E.B., Data Collection or Processing: G.K., E.B., K.Ü., Analysis or Interpretation: G.K., N.İ., E.B., Literature Search: G.K., N.İ., E.B., K.Ü., Writing: G.K., N.İ.

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

Financial Disclosure: This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) 3001 Research & Development Projects Funding Program (approval number: 114S043).

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DOI: 10.4274/gulhane.galenos.2021.00719
Gulhane Med J 2022;64:159-63

Geographical prevalence of dens invaginatus in the upper lateral teeth of young adult Turkish men

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Date submitted:
08.05.2021
Date accepted:
11.08.2021
Online publication date:
15.06.2022

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Keywords: Dens invaginatus, dental anomaly, lateral incisors

ABSTRACT

Aims: Dens invaginatus, a malformation of teeth caused by infolding of the dental papilla during development or invagination of layers of the enamel organ in dental papillae. Its aetiology is unclear. The present study was conducted to investigate the characteristics of the patients with dens invaginatus anomaly.

Methods: A single-center, cross-sectional, retrospective study was conducted on male subjects aged 18 to 24 years. Dens invaginatus in maxillary lateral incisors were identified using periapical radiographs. The evaluation was performed on patients with and without abnormalities.

Results: A total of 316 records were evaluated and 296 individuals were eligible for the analysis. No abnormality was detected in 210 (70.9%) individuals, whereas 86 individuals (29.1%) had images of dens invaginatus. The abnormalities were bilateral in 59 (19.9%) and unilateral in 27 (9.1%) individuals. Among the 59 individuals with bilateral dens invaginatus, most subjects were from the Marmara region (27.1%). Unilateral dens invaginatus was most frequently detected in the Marmara, Black Sea, and Eastern Anatolia regions.

Conclusions: This study showed that up to one-third of young adult males had images of dens invaginatus on an untargeted screening. The prevalence of dens invaginatus varied across different geographical regions of Turkey.

Introduction

There have been numerous epidemiological studies worldwide to assess the prevalence of different types of dental anomalies. Dental anomalies occur during the embryological and developmental life of the tooth and usually involve a single tooth germ (1,2). Changes in the number, size, shape, structure, and eruption of teeth are associated with dental anomalies (3). While the disorders observed after the teeth complete their normal formation are called “acquired dental anomalies”, the disorders that occur during the formation process of the teeth are defined as “developmental dental anomalies” (3-5). Developmental anomalies are generally linked to genetic factors, mutations, metabolic disorders, and environmental factors including physical, chemical, and biological insults (5).

One of the dental anomalies is dens invaginatus (6). Floquet’s anomaly in a whale tooth was first noticed in 1794 (7). While efforts have been made recently to explain the cause of dens invaginatus, the underlying cause of this malformation is not exactly known (6). It is generally considered that abnormal pressure by the surrounding tissues on the tooth germ, infections occurring during tooth development, insufficient local development of enamel, stimulation of the tooth buds, epithelial developing disorders, as well as hereditary factors may play a role in the formation of dens invaginatus (7). It is commonly accepted that various genetic and environmental factors may play a role in the development of dens invaginatus (6,8,9). The interaction between mesenchymal and epithelial tissue cells during tooth development are also among the proposed mechanisms (10-12).

This interaction is regulated by different signalling proteins such as fibroblast growth factors, morphogenic bone proteins, tumour necrosis factors, Wnts and sonic hedgehog (13,14). Variations in the genes involved in these signalling pathways affect tooth formation and tooth morphogenesis (13,15,16). A 7q32.25 chromosomal deletion was also reported in a patient with dens invaginatus and multiple tooth anomalies. Although it has been suggested that dens invaginatus develops with focal excessive cell proliferation of the inner enamel epithelium and abnormal growth of the dental papilla. Affected teeth show a deep fold of enamel and dentin that begins at the foramen coecum and even the tip of the tubercles and may extend deep into the root. The most commonly affected teeth are the maxillary lateral incisors, and bilateral occurrence is not uncommon (6,17), external forces from the adjacent teeth, trauma and infections may also contribute to the cause of dens invaginatus (6,8).

Family members of the patients with dens invaginatus are also affected (18) and these lesions are associated with other genetically inherited anomalies, suggesting the heritability of the anomaly (19-21). Given the potential role of the hereditary factors that play an important role in gingiva development, a higher prevalence was observed in people with Down syndrome (22). The lowest prevalence is known to exist in Caucasians (23). Further supporting such associations, an individual lacking chromosome 7q32 was reported to present with dens invaginatus in addition to other dental anomalies such as hypodontia (24).

Dens invaginatus has been reported at a frequency of 0.04% to 10% in the general population. The variations in the prevalence may be associated with the geographical differences, unstandardized diagnostic criteria, and the methods of investigation (25). It occurs mostly in the maxillary lateral sections (42.2%), followed by the maxillary central incisors, canines, premolars, and molars (6,26). Any tooth in the maxillary and mandibular arch may be affected by dens invaginatus, but the maxillary lateral incisors are most affected (6).

Most reports of dens invaginatus are case based in the literature. Only a few studies have assessed the prevalence of dens invaginatus (27,28). Moreover, dens invaginatus malformations are usually detected accidentally on radiographs ordered for other causes (29). It is generally not discovered unless the clinical signs appear, such as an acute dentoalveolar or sinus tract abscess. Currently, the general characteristics of subjects remain weakly described among Turkish young adults.

The aim of this study was to investigate the prevalence and geographical differences of dens invaginatus among subjects admitted to a tertiary outpatient clinic.

Methods

A cross-sectional retrospective, single-centre study was conducted using the radiographs and patient files. The study included 316 subjects initially, and the final analysis included 296 patients between the ages of 18 and 24 years admitted to the Gulhane Military Hospital outpatient dental clinic from January through September 2010. The sample size was calculated as a minimum of 191 individuals at medium effect level (0.03), 80% power and 0.05 significance level. The study protocol was approved by the Institutional Review Board (50687469-1491-144-16\1648-453).

Subjects with incomplete records or low-quality radiographs were excluded. Individuals with carious, restored, and fractured teeth, function apical foramen formation, undetectable furcation, and fused roots were also excluded. The presence of dens invaginatus in the maxillary lateral teeth was investigated on periapical radiographs. Radiographs were examined independently by two experienced dentists in a dark room, using an X-ray viewer. Each radiograph exhibiting dens invaginatus was re-examined carefully by both examiners, and a decision was made by consensus.

For each patient with dens invaginatus, demographic variables including age, sex, birth of place within the seven geographical regions of Turkey were retrieved from the medical records.

Statistical Analysis

Descriptive statistics were used to display the prevalence (proportion of subjects) of dens invaginatus in the overall sample and subanalyses.

Results

Of the 296 individuals studied, no abnormalities were detected in 210 (70.9%) individuals, dens invaginatus was found in 86 individuals (29.1%). These included bilateral abnormalities in 59 (19.9%) individuals and unilateral abnormalities in 27 (9.2%) individuals (Table 1).

When the geographical origin of individuals without dens invaginatus were evaluated, most (21.9%) originated from Eastern Anatolia, while the lowest occurrence was 4.3% in the Aegean region (Table 1). Among the 59 individuals with bilateral dens invaginatus, most subjects were from the Marmara region (27.1%), while central Anatolia (8.5%), the Mediterranean region (8.5%), and South-Eastern Anatolia (8.5%) had the lowest frequency of bilateral dens invaginatus (8.5%; Table 1). Unilateral dens invaginatus was most frequently detected in the Marmara (18.5%), Black Sea (18.5%), and Eastern Anatolia (18.5%) regions. The lowest unilateral prevalence was in the South-Eastern Anatolia region, with a ratio of 3.7% (Table 1).

Taken together, dens invaginatus occurred most frequently in the Marmara region, and least prevalence was in South-Eastern Anatolia (Table 1).

Table 1. Prevalence of dens invaginatus anomaly in maxillary lateral teeth according to different regions of Turkey

Region		In upper lateral teeth dens invaginatus			Total
		Without DI	Bilateral DI	Unilateral DI	
Marmara	N	38	16	5	59
	% within teeth	18.1	27.1	18.5	19.9
Black Sea	N	35	9	5	49
	% within teeth	16.7	15.3	18.5	16.6
Central Anatolia	N	33	5	3	41
	% within teeth	15.7	8.5	11.1	13.9
Aegean	N	9	9	4	22
	% within teeth	4.3	15.3	14.8	7.4
Mediterranean	N	24	5	4	33
	% within teeth	11.4	8.5	14.8	11.1
Eastern Anatolia	N	46	10	5	61
	% within teeth	21.9	16.9	18.5	20.6
South-Eastern Anatolia	N	25	5	1	31
	% within teeth	11.9	8.5	3.7	10.5
Total	N	210	59	27	296
	% within teeth	70.9	19.9	9.2	100.0

DI: Dens invaginatus

Discussion

The cause of dens invaginatus is uncertain, however, it appears that both genetic and environmental factors play a role. Although the findings from previous studies that examined the influence of genetic factors have suggested that primitive races had fewer anomalies than "civilized" humans, and higher prevalence of dens invaginatus was observed in ancient Chinese teeth 2000 years ago than in modern humans (30). Researchers have also identified that dental anomalies varies by race, with a higher prevalence in Mongoloid people, lower prevalence in Negroid people, and a very low frequency in Caucasians (30).

Oehlers has suggested that it dens invaginatus is the result of external forces exerting an effect on the tooth germ during development (23). Such forces could originate from the adjacent tooth infections, for example, the central incisor or canine, which develop at least 6 months before the lateral incisor (31). Other external factors, such as trauma and infection, have also been suggested as potential causes (6,32).

Very few studies have addressed the prevalence of dens invaginatus in different populations, most of which have been case reports. Overall, the prevalence of dens invaginatus varies across different populations. An earlier study has reported worldwide prevalence of dens invaginatus as 7.74% ranging from 0.04% to 10% (33). Cakici et al. (29) and Kirzioğlu and Ceyhan (34) reported a 1.3% and 12% prevalance of dens invaginatus, respectively, among Turkish dental patients. Hamasha and Alomari (27) reported the prevalence of dens invaginatus 0.65% per individual and 43.2% for all teeth among Jordanian individuals. In our study we observed dens in 29.1% of the participants in the maxillary lateral incisors of 296 Turkish men. The teeth in both the maxillary and mandibular arches may be affected by dens invaginatus, but the permanent maxillary lateral incisors are the most involved teeth (35,36). In the study by Cakici et al. (29) dens invaginatus was observed in 13 (1.3%) of 1012 teeth on anterior teeth and in 16 of 2011 (0.8%) maxillary lateral incisors. Additionally, in the study by Boyne (37), the prevalence of teeth with dens invaginatus was 0.3% in 1000 maxillary lateral incisors (38).

When only the anterior teeth are considered, the prevalence of dens invaginatus has been reported by 0.02% (39). This number was considerably lower than the findings by Cakici et al. (29) (0.8%) and by Kirzioğlu and Ceyhan (34) (0.8%) among the Turkish population. The rate of dental invaginatus of anterior teeth among Iranian subjects was also found higher by 5.8% in a study by Poyton and Morgan (40), whereas much lower prevalence of 0.25% was reported by other authors in the same population (41). These variations in the prevalence of dens invaginatus in different populations may be due to ethnic variations but may also be influenced by differences in the criteria used for interpretation of dens invaginatus, as well as the specific teeth examined, and geographical differences (25,38,42). According to previous studies conducted in the Turkish population, dens invaginatus occurs equally in men and women (29,33).

In the literature, there are numerous studies indicating that the dens invaginatus abnormality is generally observed under

a bilateral condition (26). The appearance of symmetric dens invaginatus was considered as a common finding by some authors. However, it has also been reported that bilateral dens invaginatus may be related to other dental anomalies, such as germination, gemination, fusion, and taurodontism (43). Colak et al. (39) found that bilateral dens invaginatus occurred in 25% (3 of 15) of cases, and Çakıcı et al. (29) observed that it was 23.1% among the Turkish population and 24.5% among the Jordanian population (27). In the current study, dens invaginatus was observed bilaterally in 59 of 296 individuals (19.9%). The variation may be explained by marked differences in the sample size, case selection, and the methods used. Thus, further investigations are necessary to clarify this issue.

Our results have shown that there are regional and ethno-racial variations in the prevalence of dens invaginatus anomalies. The highest prevalence among the 59 affected individuals was detected in the Marmara region (27.1%). Central Anatolia, the Mediterranean region, and South-Eastern Anatolia showed a lower frequency of bilateral prevalence (8.5%). When we examined the rate of unilateral dens invaginatus, a higher rate was observed in the Marmara region (18.5%), Black Sea (18.5%), and Eastern Anatolia (18.5%), when compared with the other regions. The lowest rate in unilateral dens invaginatus rate was in the South-Eastern Anatolia (3.7%) region, like the prevalence of bilateral dens invaginatus. Marmara region is a developed and densely populated region of Turkey. On the other hand, the potential causes of higher rates of dens invaginatus in this region need to investigated.

This study was limited by its retrospective design, single center design, small sample size, and poor generalizability of results to a larger population.

Conclusion

This study showed that up to one-third of young adult males had images of dens invaginatus on an untargeted screening. The prevalence of dens invaginatus varied across different geographical regions of Turkey. On the other hand, due to the relatively small sample size of this study, the prevalence of dens invaginatus should be considered with caution, as they may not be representative of the overall Turkish population. Further large-scale multicentre studies are therefore required to assess its prevalence in the general population.

Ethics

Ethics Committee Approval: The study protocol was reviewed and approved by the Academy Research Ethics Committee (50687469-1491-144-16\1648-453).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.A.O., A.A., Design: E.A.O., Data Collection or Processing: S.K., N.A., A.A., B.E., Analysis or Interpretation: N.A., Literature Search: E.A.O., A.A., Writing: E.A.O., S.K., N.A., B.E.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.46220
Gulhane Med J 2022;64:164-8

Clinical outcomes and feasibility of laparoscopic cholecystectomy in elderly patients

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Date submitted:
14.05.2021
Date accepted:
09.07.2021
Online publication date:
15.06.2022

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Keywords: Laparoscopic cholecystectomy, adult patient, elderly patient, feasibility, cholelithiasis

Presented in: This work was orally presented in "International Aegean Symposiums on Natural & Medical Sciences-II" on September 18-20, 2020.

ABSTRACT

Aims: Laparoscopic cholecystectomy (LC) is considered to be the gold standard treatment of symptomatic cholelithiasis and acute cholecystitis. However, there is still a controversy about this approach in elderly patients. In some cases, surgeons tend to postpone the LC in elderly patients due to a higher risk of complications. This study evaluated the feasibility of LC in elderly patients, in a comparative approach with adult age patients.

Methods: We retrospectively analyzed patients who were operated on with a diagnosis of cholelithiasis or acute cholecystitis at Samsun Training and Research Hospital, General Surgery Unit between December 2015 and December 2019. The patients were divided into two groups: adult age (<65 years of age) and elderly (≥65 years of age). Basic characteristics, American Society of Anesthesiologists (ASA) scores, preoperative ultrasonography (USG) findings and laboratory results, preoperative history of acute cholecystitis or pancreatitis attack, surgery type, complications, length of hospital stay, intensive care unit (ICU) admissions, operation time and mortality were compared between the groups.

Results: The study included 620 patients (age, mean±standard deviation; 52±14.9, female 73%), of whom 481 were in the adult group (age, mean±standard deviation; 46±11.5, female 74.7%) and 139 (age, mean±standard deviation; 71±5.8, female 67.7%) were in the elderly group. Patients in the elderly group had higher ASA scores compared with the adult age group (ASA 3; 27.3% vs. 5.8%, p<0.001). Preoperative acute cholecystitis findings on USG (3.7% vs. 7.1%, p=0.08) and history of previous cholecystitis/pancreatitis (19.1% vs. 26.6%, p=0.055) were comparable between the groups. Postoperative complication rates (2.5% vs. 5.7%, p=0.055) and severity of complications according to the Clavien-Dindo (CD) classification (CD 1-2; 1.6% vs. 2.8%, p=0.456) were similar in the two groups. The length of hospital stay (2.5±1.6 days vs. 3.2±2.2 days, p=0.028) and the rate of ICU admission (2.9% vs. 20.8%) were significantly higher in the elderly group compared with the adult age patients.

Conclusions: LC is overall a safe and feasible treatment strategy with low complication rates in elderly patients. However, the risk of longer hospital stay and ICU admission were found higher than adult age patients in the current study.

Introduction

Laparoscopic cholecystectomy (LC) is one of the most common abdominal surgeries in the world. It is superior to less pain and faster recovery times than primary open surgery. It also offers a safe and cost-effective procedure compared with open cholecystectomy (1). After German surgeon Erich Mühe performed LC for the first time in 1985, French surgeon Philip Mouret performed the first LC in 1987 (2). With the advances in surgical skills and technology, LC has become the gold standard surgery for gallstone diseases and acute cholecystitis (3,4).

The incidence of cholelithiasis and acute cholecystitis increases with age (4,5). The population of the world is aging, necessitating improvements in healthcare services and quality of life. While the global population is still growing, the number of people aged 65 or over is estimated to more than double between 2019 and 2050 (6). It is suggested that due to the comorbidities in the elderly patients, more complications can be

observed especially when performing a laparoscopic operation (7). Age-related comorbidities are considered the most important factors that may increase postoperative morbidity and mortality (8). Also, there is still controversy about the feasibility of LC in elderly patients in the literature (9). This study investigated the feasibility of LC in elderly patients compared with adult age patients.

Methods

This retrospective study enrolled patients aged 18 years or older who underwent cholecystectomy (open, laparoscopic, or converted to open) in University of Health Sciences Türkiye Samsun Training and Research Hospital, General Surgery Unit between December 2015 and December 2019. A total of 661 records were initially identified. Patients who underwent primary open cholecystectomy (n=32) and patients with missing data (n=9) were excluded. The study protocol was approved by the Non-invasive Investigation Ethics Committee of our hospital.

The screened indications for surgery were acute cholecystitis, biliary pancreatitis, symptomatic cholelithiasis, and gallbladder polyposis. The patients who underwent emergency surgery due to gallbladder perforations and gangrenous cholecystitis were also included in the study. The patients who were operated on 72 h after emergency admission were categorized as the emergency surgery group. Patients who underwent percutaneous cholecystostomy were excluded from this study. The diagnosis of acute cholecystitis was made by clinically right upper quadrant pain, Murphy sign, and 38 °C or higher body temperature, leukocytosis, gallbladder wall thickening, gallbladder distention, and pericholecystic fluid collection on ultrasonography (USG) or computed tomography.

The participants were classified into two groups: <65 years of age (adult group) and ≥65 years of age (elderly group). Age, gender, American Society of Anesthesiologists (ASA) scores, preoperative USG findings (acute cholecystitis, cholelithiasis, and gallbladder polyposis), preoperative laboratory tests [complete blood counts, aspartate aminotransferase (AST), alanine aminotransferase, albumin, and bilirubin levels], comorbidities, type of surgery (laparoscopic and conversion to open), complications, length of hospital stay, intensive care unit (ICU) admission and mortality were recorded. Clavien-Dindo (CD) classification was used to determine the severity of complications (10). CD score ≥3 was compared with a CD score <3 in the two groups.

LC technique applied in our surgery clinic in the examined period of the study was the standard North American four ports technique (11). Pneumoperitoneum in this approach is established using a Veress needle or Hasson cannula according to the surgeon's choice and the patient's history of past abdominal surgery. After careful dissection, the cystic duct and artery are clipped and cut. An epigastric port site is used to retrieve the gallbladder. Routine intraoperative cholangiography is not used. The use of drain tubes is chosen by the surgeon on demand. Dense adhesions, evidence of common bile duct injury, and uncontrollable hemorrhage are the main reasons for conversion to open surgery. When conversion to open cholecystectomy is needed a subcostal incision is used. Prophylactic antibiotics are used in all operations.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY) was used for all statistical analysis. Quantitative variables were presented as means±standard deviation (SD) and medians (1st quartile-3rd quartile). Categorical variables were expressed as percentages. When comparing two groups the chi-square test was used to compare nominal variables. The Student's t-test was used for normally distributed data and the Mann-Whitney U test for non-normally distributed data. P<0.05 was considered the level of statistical significance.

Results

A total of 620 patients (age, mean±SD; 52±14.9, female 73%) were eligible for the study. Of these, 481 (77.5%) (age, mean ± SD; 46±11.5, female 74.7%) were adult patients and 139 (22.5%) (age, mean±SD; 71±5.8, female 67.7%) were elderly (Table 1). There was no gender difference between the two groups. A significant difference was observed in terms of ASA classification. While the proportion of ASA grade 3 and grade 4 in the elderly patient group was 27.3% and 1.4% respectively, this rate was 5.8% and 0.2% in the adult age group. Similar to this finding, the number of of comorbidities was higher in the elderly group. The most common comorbidity was hypertension in both groups (5.4% vs. 15.1%, respectively).

The proportion of patients with preoperative acute cholecystitis on USG was similar between the groups (p=0.08) (Table 1). Preoperative white blood cell count, hemoglobin, AST, and direct bilirubin levels were also similar (Table 2).

Postoperative complication rate was higher in the elderly patient group, however, the difference was marginally nonsignificant (2.5% vs 5.7%, p=0.055). There was no significant difference in the degree of complications between the groups (Table 3). The list of complications are listed in Table 4. While postoperative bleeding was more frequent in the adult age group, bile leakage, wound infection, and other surgery-related complications were similarly recorded in the two groups.

Most operations were completed laparoscopically in both groups (98.3% versus 95.6%). However, 8 (1.7%) patients in the adult age group and 6 (4.4%) patients in the elderly group required conversion to open cholecystectomy, mainly because of the difficulty in gallbladder exposure with unclear anatomy and safety issues. The conversion rate between the groups was statistically similar (p=0.064). No deaths were recorded in the whole sample.

Table 1. Basic demographic and clinical characteristics

	Age <65 years (n=481)	Age ≥65 years (n=139)	p
Age, years, mean ± SD	46.39±11.57	71.82±5.82	<0.001
Sex			
Female, n (%)	359 (74.7)	94 (67.7)	0.101
ASA classification, n (%)			
ASA1	174 (36.1)	32 (23)	**<0.001**
ASA2	278 (57.7)	67 (48.2)	
ASA3	28 (5.8)	38 (27.3)	
ASA4	1 (0.2)	2 (1.4)	
Preoperative USG findings, n (%)			
Acute cholecystitis	18 (3.7)	10 (7.1)	0.08
Cholelithiasis	421 (87.5)	127 (91.3)	
Gallbladder polyps	42 (8.8)	2 (1.4)	
Previous cholecystitis/pancreatitis, n (%)	92 (19.1)	37 (26.6)	0.055
Comorbidities, n (%)			
Hypertension	26 (5.4)	21 (15.1)	**<0.001**
CAD/CHF	17 (3.5)	19 (13.6)	**<0.001**
Diabetes mellitus	16 (3.3)	10 (7.1)	**0.044**
COPD/asthma	22 (4.5)	6 (4.3)	0.898

SD: Standard deviation, ASA: American Society of Anesthesiologists, USG: Ultrasonography, CAD: Coronary arterial disease, CHF: Congestive heart failure, COPD: Chronic obstructive pulmonary disease

Table 2. Preoperative laboratory results

	Age <65 years (n=481)	Age ≥65 years (n=139)	p
WBC count (1,000/mL)	7.4 (6.2-8.8)	7.0 (6.0-8.9)	0.971
Hb value (g/dL)	13.3 (12.2-14.4)	13.0 (12.1-14.1)	0.161
Albumin (g/dL)	4.2 (4.0-4.5)	4.0 (3.8-4.3)	**<0.001**
AST (U/L)	22 (18-27)	22 (19-27)	0.841
ALT (U/L)	20 (15-30)	18 (14-26)	**0.049**
Total bilirubin (mg/dL)	0.5 (0.4-0.7)	0.6 (0.4-0.8)	**0.047**
Direct bilirubin (mg/dL)	0.1 (0.1-0.1)	0.1 (0.1-0.2)	0.102

Values are presented as median (1st quartile-3rd quartile).
WBC: White blood cell, Hb: Hemoglobin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 3. Surgical outcomes

	Age <65 years (n=481)	Age ≥65 years (n=139)	p
Surgery type, n (%)			
Laparoscopic	473 (98.3)	133 (95.6)	0.064
Conversion to open	8 (1.7)	6 (4.4)	
Complication, any, n (%)	12 (2.5)	8 (5.7)	0.055
Clavien-Dindo classification, n (%)			
Grade 1-2	8 (1.6)	4 (2.8)	0.456
Grade 3-5	4 (0.8)	4 (2.8)	
Length of hospital stay, days, mean±SD*	2.5±1.6	3.2±2.2	**<0.001**
Intensive care unit requirement, n (%)	14 (2.9)	29 (20.8)	**<0.001**
Operation time, minutes, median (1st quartile-3rd quartile)	49 (39-59)	49 (39-60)	0.450
Mortality, n (%)	0	0	

SD: Standard deviation

Table 4. Major complications

Complications, n (%)	Age <65 years (n=12)	Age ≥65 years (n=8)
Bile duct injury	2 (16.6)	0
Bile leak	2 (16.6)	2 (25.0)
Postoperative bleeding	3 (25.0)	1 (12.5)
Wound infection	2 (16.6)	2 (25.0)
Surgery related to other complications (ileus, trocar hernia)	2 (16.6)	2 (25.0)
Pulmonary complications (atelectasis, pneumonia)	1 (8.3)	1 (12.5)

Discussion

Although LC is considered the gold standard method for treating gallbladder diseases, it remains a challenge for surgeons in elderly patients. Owing to the high-risk comorbidities and reduced physiological reserves, elderly patients are likely to have more complications after surgery (7,12-15). Recent publications have reported almost 30% of elderly patients who have gallbladder diseases are not operated due to the fear of age-dependent comorbidities (16). However, it is also well known that non-operative treatment options may cause recurrent biliary problems.

In this study, we observed significantly higher ASA scores in the elderly patients who underwent LC. However, preoperative USG findings, sex distribution, and preoperative history of acute cholecystitis or pancreatitis were similar between the adult age and elderly groups. These findings were comparable with the literature (5,7,14,17).

When the adult age and elderly groups were compared for the postoperative outcomes, the length of hospital stay and the rate of ICU admission were higher in the elderly group. However, conversion to open surgery, operation time and the rates of complications were similar in our study. In parallel to our findings, Bhandari et al. (18) found no significant differerences in the mean operation time and complication rates between the age groups. On the other hand, despite the similarities in overall complication rates, elderly patients had slightly more severe complications (grade 3 or higher) than adult patients according to the CD classification. The severity of complications and preoperative comorbid conditions could be linked to longer length of hospital stay and higher ICU admission rates in the elderly group.

In a retrospective study, van Heesewijk et al. (3) found more complication rates in the elderly patients. In our study, we also found more complication rates in elderly patients (2.5% vs 5.7%) but the difference was not statistically significant. According to the CD classification system, low severity complications (grade 1-2) were more common in our total study group. This finding is consistent with the existing literature (13,16).

The reported conversion rates in previous studies varied from 1.5% to 20% in elderly patients (16). We found a 4.4% conversion rate in the older age group, which was not significantly different from the adult age group. In our study, we did not analyze the patients with primary open cholecystectomy mainly to demonstrate the safety of laparoscopic cholecystectomy in elderly patients. On the other hand, Fukami et al. (14) included primary open cholecystectomies in their study and reported a higher rate of primary open cases in the octogenarian group. They stated that in high-risk patients (i.e., severe cardiac disease) their choice was not LC.

Previous studies have reported mortality rates between 0 and 1.6% after LC in elderly patients (8,19). In octogenarian patients, the rate of mortality increases beyond 6% (3,14,18,20). However, no mortality was recorded in our study.

This study has some limitations. First, the design of this study was retrospective. Therefore, a selection bias and missing data may not be neglicable. Second, we excluded patient who were treated with percutaneous cholecystostomy. This treatment method is not commonly used in our surgery department due to the lack of experienced interventional radiologists. And lastly, we did not analyze the long-term effects of complications, as we focused on early complications. Bile duct injury and bile leaks can lead to further operations and interventions like endoscopic retrograde cholangiopancreatography (ERCP) and nasobiliary tube placement. These patients may have benign biliary strictures or past cholangitis events. Therefore, their quality of life may have been impaired.

Conclusion

This study revealed statistically similar rates of postoperative complications and conversion rates between elderly and adult patients who underwent LC. The results overall suggest that LC in elderly patients is safe and feasible with acceptable complication rates.

Ethics

Ethics Committee Approval: The study was approved by the Samsun Training and Research Hospital of Non-invasive Investigation Ethics Committee (protocol number: GOKA/2019/3/12, date: 25.12.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.B.Ç., A.C.S., Concept: A.B.Ç., Design: A.B.Ç., Data Collection or Processing: A.B.Ç., S.O., A.B.Ç., Analysis or Interpretation: A.B.Ç., S.O., A.B.Ç., Literature Search: A.B.Ç., A.B.Ç., Writing: A.B.Ç.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.07379
Gulhane Med J 2022;64:169-77

Evaluation of the effects of the COVID-19 pandemic on pain, stress, sleep and quality of life in patients with chronic musculoskeletal pain

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Date submitted:
13.06.2021
Date accepted:
02.12.2021
Online publication date:
15.06.2022

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Keywords: COVID-19, chronic pain, anxiety, sleep

ABSTRACT

Aims: The study aimed to evaluate the association between the novel Coronavirus disease-2019 (COVID-19) pandemic and the level of pain, stress, sleep quality, and quality of life in patients with chronic musculoskeletal pain.

Methods: This cross-sectional study included patients aged 18-65 years with chronic musculoskeletal pain. Age, gender, body mass index, systemic diseases, dominant pain area, duration of pain, analgesic use, and exercise habit before and after the pandemic were recorded. Participants were asked to evaluate their pain severity and global assessments in the last month and the pre-pandemic period with a visual analog scale (VAS). The Perceived Stress Scale (PSS), The Pittsburgh Sleep Quality Index (PSQI), and The Nottingham Health Profile (NHP) were administered

Results: The study included 100 participants (age, mean ± SD: 42.8±12.5 years, female: 69%). Compared with the pre-pandemic period, an increase in pain and global assessment scores, and poorer sleep hygiene in the last month were reported by 82%, 67%, and 66%, respectively. Pain severity VAS score (pre-pandemic: 3.9±2 vs. post-pandemic: 5.9±2.1, $p<0.001$), global assessment VAS score (pre-pandemic: 3.8±2 vs. post-pandemic: 5.6±2.3, $p<0.001$), the proportion of patients using routine analgesics (pre-pandemic: 44% vs. post-pandemic: 52%, $p=0.008$), and the number of routine analgesics (pre-pandemic: 10.4±18.6 vs. post-pandemic: 15.3±24, $p<0.001$) in the last month was significantly more compared to the pre-pandemic period. There were no significant differences in pain score, patient's global assessment score, the number of routine analgesics, PSS-14, PSQI, NHP-1, and NHP-2 scores between subjects with and without a history of COVID-19.

Conclusions: In patients with chronic musculoskeletal pain, an increase in pain severity, analgesic use, and deterioration in general well-being were observed during the COVID-19 pandemic. The pronounced changes were not found to be dependent on the history of COVID-19.

Introduction

Chronic pain is defined as persistent or recurrent pain that lasts longer than three months or exceeds the normal tissue healing time (1). It is evaluated in 7 categories as primary, cancer-related, posttraumatic/post-surgical, neuropathic, visceral, musculoskeletal, and head/orofacial pain (2). Chronic musculoskeletal pain is one of the most common complaints in routine clinical practice (3). The prevalence of chronic pain in the general population is around 20%, and it is a common worldwide condition that causes limitations in daily living activities, disability, and a decrease in quality of life (4-7). It is a clinical picture with high personal, social, and economic burdens (8,9).

Chronic pain is a biopsychosocial model with biological, cognitive, affective, emotional, and social problems (10,11). Many factors, such as demographic factors (such as age and occupation), lifestyle-related factors (such as alcohol use,

smoking, and physical activity), mental health conditions (such as past pain experiences, and traumatic events), physical factors, and genetic factors are associated with the development of chronic pain (11,12).

The novel Coronavirus disease-2019 (COVID-19) pandemic has had negative impacts on chronic pain conditions in many ways (3,5,11,13-16). One of the first conditions associated with COVID-19 is chronic pain that occurs as a component of the postviral syndrome and has been linked to virus-associated organ damage (11). Although COVID-19 infection mainly affects the lungs and internal organs, musculoskeletal system damage is also prevalent (3,15). Pain, particularly myalgia and low back pain, is among the early symptoms of COVID-19 infections (16). Additionally, since most chronic pain patients are at an advanced age and have comorbidities, the risk of COVID-19 is considered to be increased (13,15,16). Restricting the admission to healthcare services due to fear of infection is another situation that supports chronicity in painful situations (16). Other conditions associated with chronic pain in the COVID-19 pandemic are worsening of pre-existing physical or mental complaints and emerging chronic pain due to pandemic-related risk factors such as poor sleep, inactivity, fear, anxiety, and depression in people with no previous chronic pain and a history of COVID-19 (11).

Infection control strategies for pandemics around the world support home isolation (16). Many elective patient examinations and surgical procedures have been postponed, and access to non-emergency healthcare services has been restricted (5,13,16,17). It has become more difficult than ever for patients with chronic pain to access appropriate treatment time in a reasonable time (3,5,13,14,16). Disruptions in the follow-up and treatment of the patients can increase pain, decrease functionality, increase analgesic use, and deteriorate the quality of life (18). Difficulties in access to medical care and isolation can not only have a negative impact on pain management and psychological conditions but also increase the burden of patients with chronic pain (5,16,18). However, life-related psychosocial stressors such as decreased interpersonal relationships, isolation, fear of illness, future anxiety, and financial difficulties brought about by the pandemic may cause pain to exacerbate in patients with chronic pain (19). Thus, pain management is considered particularly important in the COVID-19 pandemic (3).

The aim of this study aimed to evaluate the effects of the COVID-19 pandemic on pain, stress, sleep, and quality of life in patients with chronic musculoskeletal pain and the relationships between them.

Methods

Study design and participants

In this single-center, cross-sectional study, patients aged 18-65 years who presented with chronic (more than three months) musculoskeletal pain were consecutively enrolled, between January 2021 and March 2021. The exclusion criteria had a history of trauma and surgical intervention in the last three months, inflammatory rheumatic disease, chronic severe systemic or neurological disease, and moderate to severe impairment of cognitive status determined by the Mini-Mental State test (20). Ethics committee approval was obtained from the institutional review board (Ethics Committee of AYBÜ Yenimahalle Training and Research Hospital, Turkey, decision number/date: 2020-3-16/16.12.2020). The participants provided informed consent, and the study conformed to the principles of the Helsinki Declaration principles were followed (ClinicalTrials.gov Identifier: NCT04878900/04.05.2021).

Data collection

The primary outcome measures of the study were; general pain severity and global well-being assessment with the visual analog scale (VAS) (21) in the pre-pandemic period and the last month, the Perceived Stress Scale (PSS) (22), the Pittsburgh Sleep Quality Index (PSQI) (23), and the Nottingham Health Profile (NHP) (24) scale scores. Age, gender, body mass index (BMI), and systemic diseases of the participants were recorded as demographic data. Dominant pain area in the musculoskeletal system, duration of pain, routine use of analgesics, and exercise habits before and after the pandemic were recorded. Information about the personal history of COVID-19 or the relatives of the participants was also collected. Then, the participants were asked to evaluate their general pain severity and global well-being assessment with the VAS in the pre-pandemic period and the last month. The patients marked their pain severity on a line of 10 centimeters (cm) with the starting point (0) expressing no pain, and the endpoint (10) indicating the most severe pain experienced in life. On the patient's global assessment scale, the patients marked their global assessment on a line of 10 centimeters (cm) with the starting point (0) expressing very good and the endpoint (10) indicating very bad. The distance between the point marked by the patient and the starting point was measured. The higher the measured value meant the greater the severity of pain and the worse the patient's global assessment (21).

The Perceived Stress Scale

The PSS is a scale developed by Cohen et al. (22) to evaluate how stressful some situations in life are perceived by the individual. A Turkish validity and reliability study of the scale was conducted by Eskin et al. (25). The long form of the scale consists of 14 items. The situations given in each item are evaluated with a 5-point Likert-type scale (0=never, 1=almost never, 2= sometimes, 3= fairly often, 4= very often). Seven items with positive statements are scored in the reverse. The scale has two short forms consisting of 10 and 4 items. The total score ranges from 0-56 for PSS-14, 0-40 for PSS-10, and

0-16 for PSS-4. The higher the scores, the greater the person's perception of stress (22,25).

The Pittsburgh Sleep Quality Index

The PSQI is a scale developed by Buysse et al. (23) designed to evaluate sleep quality and disorders. A Turkish validity and reliability study of the scale was conducted by Agargun et al. (26). These consisted of 24 questions. Nineteen questions are self-assessment questions, and five questions are assessment questions made by their spouse or roommate. Questions about the evaluations made by the spouse or roommate are not taken into consideration in the score calculation. Eighteen question items were used for scoring. The scale consists of 7 components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction. While some components consist of a single question item, some are formed by grouping several items. Each component is scored between 0 and 3 points, and the sum of the component scores gives the total score of the scale. The higher the total score which can vary between 0 and 21, the worse the sleep quality. A total score greater than 5 indicates poor sleep quality (23,26).

The Nottingham Health Profile

The NHP is a general health status scale developed by the European Group for Quality of Life and Health Measurement that evaluates perceived problems in physical, emotional, and social areas and how these problems affect daily activities (24). The adaptation study of the scale to Turkish was published by Kücükdeveci et al. (27). The questions in the first part of the scale are about the health status of the people, and the questions in the second part are about the effects of their health status on daily life. The first part consists of 38 items in which each item is marked yes or no. This section has 6 sub-dimensions: pain, emotional reactions, sleep, social isolation, and physical mobility and energy. Each question in the sub-dimensions has a different score weight, and each sub-dimension is scored between 0 and 100. The sum of all sub-scores gives the total score of the first part of the scale. The higher the score, the worse the perception of quality of life regarding health status. The second part consists of 7 items that question whether there are problems in the daily life areas that are most probably affected by the health condition of the person, such as work-life, housework, social life, interpersonal relationships, sexual life, hobbies, and holidays, each item is marked yes or no (24).

Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY). The conformity of the variables to normal distribution was examined by visual (histogram and probability charts) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's) and the homogeneity of variances using the Levene test. In descriptive analyses, continuous variables were expressed as mean and standard deviation, and categorical variables as numbers and percentages. In comparisons between dependent groups, the dependent groups' t-test was used for numerical data that met parametric test conditions, the Wilcoxon test for data that did not, and McNemar's test for categorical data. Mann-Whitney U test was used for numerical data, and the chi-square test was used for categorical data in comparisons between independent groups. To examine the relationships between variables, Pearson correlation analysis (two-tailed) was used for variables that both conformed to the normal distribution, and the Spearman test (two-tailed) was used for variables, at least one of which did not conform to the normal distribution. Possible risk factors for increased pain severity, patients' global assessment, and poor sleep quality were analyzed using logistic regression analysis. The model fit assessment was made using the Hosmer-Lemeshow test. The statistical significance level was accepted as p=0.05.

Results

Basic characteristics

The study included 100 participants (age, mean ± SD: 42.8±12.5 years, female: 69%). In the last month, compared with the pre-pandemic period, 82% of the patients had an increase in their pain scores, 67% of the patients had an increase in the patients' global assessment scores, and 65.9% of the patients who used routine analgesics (29% of all patients) had an increase in the number of analgesics they used routinely. The body areas where the pain was most dominant were low back in 31, neck in 20, lower extremity in 19, upper back in 17, and upper extremity in 13 patients. While 16% of the patients had a personal history of COVID-19 and 13% of their relatives, 71% were free of COVID-19 history. There was a history of systemic disease in 28% of the patients. There was asthma in 5% (n=5), diabetes mellitus in 8% (n=8), hypertension in 13% (n=13), and coronary artery disease in 3% (n=3). The basic characteristics are shown in Table 1.

Outcome analyses

In the last month, compared to the pre-pandemic period, the pain severity VAS score ($p<0.001$), the patient's global assessment VAS score ($p<0.001$), the number of patients using routine analgesics ($p=0.008$), and the number of routine analgesics ($p<0.001$) were significantly higher. The number of patients who exercised regularly in the pre-pandemic period was significantly higher than the number of patients who exercised regularly in the last month ($p=0.02$). The comparison results of the variables in the pre-pandemic period and the last month are given in Table 2.

In 66% of the patients, the PSQI score was above five, which indicates poor sleep quality. Descriptive results for the scales included in the study are given in Table 1.

There were no statistically significant differences between patients with and without a personal history of COVID-19 or in their relatives in terms of age, gender, BMI, pain duration,

Table 1. Basic characteristics and descriptive results of the scales

Age-years, mean±SD (range)	42.8±12.5 (23-65)
Gender, n (%)	
Female	69 (69)
Male	31 (31)
BMI-kg/m^2, mean±SD (range)	26.1±5.3 (18.5-48.8)
Systemic disease history, n (%)	
Yes	28 (28)
No	72 (72)
Pain duration-months, mean±SD (range)	52.6±51.8 (12-240)
COVID-19 history, n (%)	
No	71 (71)
COVID-19 history in the person	16 (16)
COVID-19 history in the person's relatives	13 (13)
Increase in the pain score, n (%)	
Yes	82 (82)
No	18 (18)
Increase in the patient's global assessment score, n (%)	
Yes	67 (67)
No	33 (33)
Increase in the amount of routine analgesics, n (%)	
Yes	29 (29)
No	15 (15)
PSS, mean±SD (range)	
- PSS-14	25.1±7.7 (6-46)
- PSS-10	18±5.9 (4-34)
- PSS-4	6.9±2.8 (0-14)
PSQI, mean±SD (range)	
- Subjective sleep quality	1.4±0.7 (0-3)
- Sleep latency	1.5±1 (0-3)
- Sleep duration	1±0.8 (0-3)
- Habitual sleep efficiency	0.5±0.8 (0-3)
- Sleep disturbance	1.6±0.6 (0-3)
- Use of sleep medication	0.2±0.7 (0-3)
- Daytime dysfunction	1.1±0.9 (0-3)
- Total	7.4±3.3 (1-17)
PSQI >5, n (%)	
- Yes	66 (66)
- No	34 (34)
NHP-1, mean±SD (range)	
- Pain	40.6±30.9 (0-100)
- Emotional reactions	28.2±32.3 (0-100)
- Sleep	30±31 (0-100)
- Social isolation	15.8±25.7 (0-100)
- Physical mobility	20.1±21 (0-88.5)
- Energy	34.6±39.4 (0-100)
- Total	168.7±125.3 (0-479.8)
NHP-2, mean±SD (range)	1.5±1.9 (0-7)

BMI: Body mass index, COVID-19: Coronavirus disease-2019, PSS: Perceived Stress Scale, PSQI: Pittsburgh Sleep Quality Index, NHP: Nottingham Health Profile, SD: Standard deviation

last month vs. pre-pandemic differences in pain score, patient's global assessment score, the number of routine analgesics, PSS-14 score, PSQI total score, NHP-1, and NHP-2 scores. Among patients without a personal history of COVID-19 or in their relatives, PSQI-sleep time (p=0.05), PSQI-sleep disorder (p=0.05), NHP-1-sleep duration (p=0.02), and NHP-1-physical (p=0.02) sub-scores were significantly higher compared to the participants any history of COVID-19. There were no statistically significant differences between other sub-scores of the scales between the groups (all p>0.05). The number of participants with a history of systemic disease was statistically significantly higher than the group without a personal history of COVID-19 or in their relatives compared to the participants with any history of COVID-19 (p=0.02). The results of the comparison of variables between the groups with and without a personal history of COVID-19 or in their relatives are given in Table 3.

There were strong positive correlations between the differences in pain score and patient's global assessment score (r=0.71, p<0.001). A moderate positive correlation between NHP-1 total score and NHP-2 score (r=0.60, p<0.001); age and BMI (r=0.53, p<0.001); PSS-14 score and NHP-1 score (r=0.58, p<0.001) and PSQI total score and NHP-1 total score (r=0.52, p<0.001) were also observed. Other correlations between the evaluated variables were low or insignificant level or statistically insignificant (Table 4).

Table 2. The comparison of variables between the pre-pandemic period and the last month

	Pre-pandemic period	Last month	p
Pain severity VAS score, mean±SD (range)	3.9±2 (0-7)	5.9±2.1 (1-10)	**<0.001***
Patient's global assesment VAS score, mean±SD (range)	3.8±2 (0-10)	5.6±2.3 (0-10)	**<0.001***
Presence of using routine analgesics, n (%) Yes No	 44 (44) 56 (56)	 52 (52) 48 (48)	**0.008***
Number of analgesics/month, mean±SD (range)	10.4±18.6 (1-90)	15.3±24 (1-120)	**<0.001***
Regular exercise, n (%) Yes No	 32 68	 22 78	**0.020***

*Statistical significance level p=0.05.
VAS: Visual analog scale, SD: Standard deviation

Table 3. Comparisons of variables between the groups with and without a personal of COVID-19 or in their relatives

	COVID-19 history (+)	COVID-19 history (+)	p
Age-years, mean±SD (range)	39.5±12.4 (23-65)	44.1±12.4 (23-65)	0.06
Gender, n (%) Female Male	 18 (62.1) 11 (37.9)	 51 (71.8) 20 (28.2)	0.34
BMI-kg/m^2, mean±SD (range)	24.8±3.1 (18.5-31.3)	26.6±5.9 (18.7-48.8)	0.19
Presence of systemic disease history, n (%) Yes No	 13 (44.8) 16 (55.2)	 15 (21.1) 56 (78.9)	**0.02***
Pain duration-months, mean±SD (range)	51.3±56.8 (12-240)	53.1±50 (12-240)	0.88
Difference in pain scores, mean±SD (range)	1.3±1.9 [(-4.3)-(4.4)]	2.3±2.2 [(-2.7)-(7)]	0.09
Difference in patient's global assessment scores, mean±SD (range)	1.2±1.9 [(-4.2)-(6)]	1.9±2.3 [(-2)-(8)]	0.22
Difference in the amount of routine analgesics, mean±SD (range)	9.3±13.6 (0-30)	3.2±5.6 (0-30)	0.89
PSS-14, mean±SD (range)	25.6±7.7 (10-40)	25±7.7 (6-46)	0.71
PSQI total, mean±SD (range)	6.8±2.7 (2-13)	7.6±3.5 (1-17)	0.33
NHP-1, mean±SD (range)	135.9±116.5 (0-382.7)	182.1±127.1 (0-479.8)	0.07
NHP-2, mean±SD (range)	1.4±2.1 (0-7)	1.5±1.8 (0-7)	0.27

*Statistical significance level p=0.05.
COVID-19: Coronavirus disease-2019, BMI: Body mass index, PSS: Perceived Stress Scale, PSQI: Pittsburgh Sleep Quality Index, NHP: Nottingham Health Profile, SD: Standard deviation

Table 4. Correlation analysis among the study variables

	Age	BMI	Difference in pain scores	Difference in patient's global assessment scores	Difference in the amount of routine analgesics	PSS-14 score	PSQI total score	NHP-1 total score	NHP-2 total score
Age		**r=0.53 p<0.001****	r=0.09 p=0.39	r=0.04 p=0.66	r=0.004 p=0.98	r=-0.09 p=0.35	r=-0.09 p=0.39	r=0.12 p=0.21	r=0.19 p=0.06
BMI	**r=0.53 p<0.001****		r=0.14 p=0.16	r=0.06 p=0.56	r=-0.19 p=0.21	r=-0.07 p=0.46	r=0.01 p=0.89	**r=0.21 p=0.04***	r=0.13 p=0.19
Difference in pain scores	r=0.09 p=0.39	r=0.14 p=0.16		**r=0.71 p<0.001****	r=0.29 p=0.05	**r=0.20 p=0.05***	r=0.13 p=0.20	**r=0.26 p=0.008***	**r=0.22 p=0.03***
Difference in patient's global assesment score	r=0.04 p=0.66	r=0.06 p=0.56	**r=0.71 p<0.001****		**r=0.32 p=0.03***	**r=0.27 p=0.006****	**r=0.21 p=0.04***	**r=0.29 p=0.003****	**r=0.26 p=0.009****
Difference in the amount of routine analgesics	r=0.004 p=0.98	r=-0.19 p=0.21	r=0.29 p=0.05	**r=0.32 p=0.03***		r=0.16 p=0.30	r=0.29 p=0.05	r=0.21 p=0.17	**r=0.33 p=0.03***
PSS-14 score	r=-0.09 p=0.35	r=-0.07 p=0.46	**r=0.20 p=0.05***	**r=0.27 p=0.006****	r=0.16 p=0.30		**r=0.38 p<0.001****	**r=0.58 p<0.001****	**r=0.40 p<0.001****
PSQI total score	r=-0.09 p=0.39	r=0.01 p=0.89	r=0.13 p=0.20	**r=0.21 p=0.04***	r=0.29 p=0.05	**r=0.38 p<0.001****		**r=0.52 p<0.001****	**r=0.28 p=0.004****
NHP-1 total score	r=0.12 p=0.21	**r=0.21 p=0.04***	**r=0.26 p=0.008***	**r=0.29 p=0.003****	r=0.21 p=0.17	**r=0.58 p<0.001****	**r=0.52 p<0.001****		**r=0.60 p<0.001****
NHP-2 total score	r=0.19 p=0.06	r=0.13 p=0.19	**r=0.22 p=0.03***	**r=0.26 p=0.009****	**r=0.33 p=0.03***	**r=0.40 p<0.001****	**r=0.28 p=0.004****	**r=0.60 p<0.001****	

Statistical significance level of the correlation. *p=0.05 (2-tailed) and **p=0.01 (2-tailed).
BMI: Body mass index, PSS: Perceived Stress Scale, PSQI: Pittsburgh Sleep Quality Index, NHP: Nottingham Health Profile, SD: Standard deviation, COVID-19: Coronavirus disease-2019

Logistic regression analyses indicated that the increase in the patient's global assessment score was a significant risk factor [Odds ratio: (OR): 2.7 95% confidence interval (CI): 1.5-5, p=0.001] for the increase in pain score (in any amount) in the last month compared with the pre-pandemic period (Table 5). Age, gender, BMI, presence of systemic disease, history of COVID-19, duration of pain, decrease in exercise (reported by the patient), PSS-14 score, and PSQI total score were not independently associated with increased pain score in the post-pandemic period. Additionally, the increase in the pain score was a statistically significant risk factor [OR: 2.1 (95% CI): 1.5-3, p<0.001] for the increase in the patient's global assessment score (in any amount) in the last month compared to the pre-pandemic (Table 5). Age, gender, BMI, presence of systemic disease, history of COVID-19, duration of pain, decrease in exercise, PSS-14 score, and total PSQI score were not independently associated with an increased patient's global assessment score in the post-pandemic period. Also, advanced age was a statistically significant risk factor [OR: 0.95 (95% CI): 0.90-1, p=0.04] for poor sleep quality, which was determined by the total PSQI value greater than 5 (Table 5). Gender, BMI, presence of systemic disease, COVID-19 history, pain duration, decrease in exercise, PSS-14 score, increases in the pain severity score, and the patient's global assessment score in the last month was not independently associated with poor sleep quality.

Discussion

In this study, among patients with chronic musculoskeletal pain, we found an increase in pain scores and global assessment scores by 82% and 67%, respectively, in the last month compared to the pre-pandemic period. Moreover, 65.9% of the patients who used routine analgesics showed an increase in the number of analgesics they used routinely. The pain severity VAS scores the patient's global assessment VAS score, and routine analgesics use were significantly higher in the last month compared to the pre-pandemic period. The PSQI score was above 5, which indicated poor sleep quality in 66% of the participants. Several authors have emphasized that the pandemic and

Table 5. Logistic regression analysis

	Increase in pain score		Increase in the patient's global assessment score		Poor sleep quality	
Risk factors	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Increase in pain score	-	-	2.1 (1.5-3.0)	**<0.001**	-	-
Increase in the patient's global assessment score	2.7 (1.5-5.0)	**0.001**	-	-	-	-
Age	-	-	-	-	0.95 (0.90-1.0)	**0.04**

OR: Odds ratio, CI: Confidence intervals (only the significant associates are shown)

quarantine measures leading to social isolation have brought an increase in pain, sedentariness, mood disorders, fatigue, and the need for analgesics in patients with chronic pain (28-32). Among patients with rheumatic pain, 37.4% had worsening disease activity assessed by pain, stiffness, and fatigue, and 75.7% had emotional disturbances during the COVID-19 pandemic. Similar to our study, when only non-inflammatory conditions are considered, worsening in pain was reported in 50% and mood disturbances in 74% in cases of osteoarthritis/osteoporosis, whereas worsening in pain was reported in 63.6%, mood worsening in 87.7% in cases of fibromyalgia (33). In another study, Nieto et al. (34) reported that 70.8% of chronic pain patients had an increase in general pain severity, 79.1% experienced deterioration in sleep, and 79.9% had deterioration in physical activity during the pandemic period. Lacasse et al. (30) evaluated the effects of the COVID-19 pandemic on pharmacological, physical, and psychological pain treatments in patients with chronic pain and demonstrated that 38.3% of the patients made some changes in their pharmacological treatments, and in 40.5% of these individuals, the underlying cause was reported as COVID-related conditions. The most common reported cause (in 11.5%) was worsening of pain. We observed that there was an increase in routine analgesic use during the pandemic period compared with the pre-pandemic period. Similarly, in a study by López-Medina et al. (33), non-steroidal anti-inflammatory drug use was significantly higher in the group with worsening rheumatic disease activity. In summary, when the results of our study and the sample studies mentioned are evaluated together, it can be said that the pandemic process has negative effects on the chronic pain picture.

Lacasse et al. (30) reported that restricting access to exercise opportunities in patients with chronic pain was the most common reason for the change in physical therapy components of patients with chronic pain (30). Similarly, in our study, the proportion of patients who exercised regularly in the pre-pandemic period was significantly higher than the proportion of patients who exercised regularly in the last month. In other words, it can be said that the pandemic process affects exercise habits negatively.

We observed that the only statistically significant risk factor for the pain score increase in the last month compared to the pre-pandemic period was the patient's global assessment score increase, for the patient's global assessment score increase in the last month compared to the pre-pandemic period was the pain score increase, for the poor sleep quality was advanced age. Age, gender, BMI, presence of systemic disease, history of COVID-19, duration of pain, decrease in exercise, PSS-14 score, and PSQI total score were not found to be significant risk factors for the pain score increase, the patient's global assessment score increase,and the poor sleep quality. Different results have been reported in the literature regarding the relationships between factors associated with chronic pain. In the study of Nieto et al. (34), it was reported that there were relationships between sleep problems and physical activity change, general well-being, mood, changes in pain, and disability; but there were no significant relationships between sleep problems and age, pain duration. López-Medina et al. (33) stated that, when examined, the factors related to disease activity in rheumatic pain, lack of exercise, anxiety, or feeling sad were statistically significant risk factors, while age and gender were not significant risk factors. In the study by Lacasse et al. (30), they reported that the change in pain severity during the pandemic period, the presence of psychological stress symptoms, and the change in physical treatment modalities including exercise practices were related to the change in pharmacological pain treatments, while pain duration, COVID-19 history, age, and gender were not found to be significant risk factors.

In our study, we observed that high perceived stress and low sleep quality were not significant risk factors for an increase in chronic pain. Similarly, in the study of Nieto et al. (34), it was reported that while stress and sleep disorders were reported more frequently as pain triggers in the pre-pandemic period, future anxiety, feeling of insecurity, negative thoughts, sadness, loneliness, sedentariness, and fear of infecting COVID-19 were reported more frequently as pain triggers during the pandemic period. In contrast, Shevlin et al. (35) reported that COVID-19-related anxiety was positively correlated with general anxiety and the severity of somatic symptoms, including pain and fatigue. The reason why we observed that they did not have significant effects on pain increases during the pandemic process may be that sleep and stress disorders are already common in chronic painful conditions.

A review by Clauw et al. (11) emphasized that the COVID-19 pandemic would inevitably increase chronic pain because it is a stressful life event. Life-related psychosocial stressors such as reduction in interpersonal relationships, isolation, fear of illness, anxiety for the future, and financial difficulties, which are the consequences of the pandemic, may cause exacerbation of pain in patients with chronic pain (19). In our study, we observed that there were no significant differences between the participants with or without a personal history of COVID-19 or in their relatives in terms of the pain score difference, patient's global assessment score, and the number of routine analgesics differences between the last month and the pre-pandemic period. This suggests that psychosocial stressors that come with the pandemic process, rather than the COVID-19 disease history, are the main factors affecting chronic painful conditions.

Our study has some limitations. First, the retrospective evaluation of the pain severity and global assessment of the participants for the pre-pandemic period may have reduced the reliability of the relevant data. Second, among the problems related to the mood that may affect chronic pain, only anxiety was evaluated. Finally, the evaluation of general anxiety, not COVID-19-specific anxiety, is another limitation of the study. There is a need for studies that will more comprehensively evaluate the psychological and socio-economic problems brought about by the COVID-19 pandemic. These studies will be able to provide clearer information about the points to be considered in pain management in patients with chronic pain during the pandemic period.

Conclusion

In patients with chronic musculoskeletal pain, there was an increase in pain severity and analgesic use and deterioration in general well-being during the COVID-19 pandemic. These effects were independent of the history of COVID-19 in patients with chronic musculoskeletal pain.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Institutional Review Board (Ethics Committee of AYBÜ Yenimahalle Training and Research Hospital, Turkey, decision number/date: 2020-3-16/16.12.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B.G., M.Ç.Ö., A.U., S.A., Concept: E.B.G., M.Ç.Ö., A.U., S.A., Design: E.B.G., M.Ç.Ö., A.U., S.A., Data Collection or Processing: E.B.G., M.Ç.Ö., A.U., S.A., Analysis or Interpretation: E.B.G., M.Ç.Ö., A.U., S.A., Literature Search: E.B.G., M.Ç.Ö., A.U., S.A., Writing: E.B.G., M.Ç.Ö., A.U., S.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.83703
Gulhane Med J 2022;64:178-88

Factors associated with treatment outcome of tuberculosis in Bale Robe General Hospital, Southeastern Ethiopia: A retrospective study

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Date submitted:
04.08.2021
Date accepted:
11.11.2021
Online publication date:
15.06.2022

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Keywords: Tuberculosis, treatment outcome, treatment success rate, Ethiopia

ABSTRACT

Aims: This study investigated the treatment success rate and the associated factors in patients with different forms of tuberculosis (TB) at Bale Robe General Hospital (BRGH), southeastern Ethiopia from 2012 to 2016.

Methods: An institution-based retrospective study was conducted. The study population was patients with TB registered at BRGH. The inclusion criteria were TB diagnosis with Ziehl-Neelsen staining for acid-fast bacillus and/or radiography and having treatment initiated from 2012 through 2016. Subjects with incomplete information were excluded.

Results: Among 807 patients with TB, 665 (82.4%) had successful treatment outcome (cure: 25.5%, treatment completion: 56.9%) and 142 (17.6%) patients had unsuccessful treatment outcome. In multivariate logistic regression analysis, age 65 years or older, being unemployed, having treatment after a previous failure, and receiving treatment during 2014 were associated with a reduced probability of treatment success. TB patients who were HIV-negative and treated during 2015 had higher treatment success. In multinomial logistic regression analysis, patients younger than 65 years of age showed a lower risk of death, whereas HIV-positive patients and patients treated during 2012 were more likely to experience death. New patients with TB were less likely to have treatment failure, but patients with TB who had treatment after failure were more likely to be transferred.

Conclusions: This study showed that TB out treatment success rate was satisfactory but below the minimum target set by the World Health Organization (85%). Age 65 and over, being unemployed, having treatment after failure and HIV co-infection were associated with unsatisfactory treatment outcomes.

Introduction

Tuberculosis (TB) is the tenth leading cause of death worldwide and the leading cause of death from a single infectious agent, ranking above HIV/AIDS (1). According to the World Health Organization (WHO) Global TB Report, TB caused an estimated 1.3 million deaths among HIV-negative people and 300,000 deaths in HIV-positive people (1). The highest TB morbidity and mortality rates occur in Africa (2). The fatality rate exceeds 50% in some African countries with high HIV infection rates (3).

Ethiopia is listed among the top 30 countries with a high TB burden, TB-HIV co-infection (TB/HIV), and multidrug-resistant TB (MDR-TB). Together, TB, TB/HIV, and MDR-TB accounted for 84% and 90% of the global burden during 2015 and 2016, respectively (4,5). Ethiopia is also a country achieving treatment success rates (TSRs) above 70% (6).

In 1992, WHO promoted the Directly Observed Treatment, Short-Course (DOTS), a treatment scheme where the physicians observe patients while the patients take prescribed TB medications. This strategy was subsequently adopted by nearly

all the countries (6). In Thailand, for instance, DOTS provided by health care workers has improved the treatment success of TB compared to self-administered therapy (SAT), consolidating the relevance of DOTS over SAT (7). Using the DOTS strategy, the highest treatment success of 95% was attained in Anqing, China (8). DOTS remains a core element of its successor, the Stop TB strategy (9), for which the WHO set targets of 84% case detection rate and 87% TSR by 2015.

In Ethiopia, DOTS was started in 1992 as pilots in the Arsi and Bale zones (10). The DOTS strategy was scaled up and implemented at the national level in 1997, and almost in all public, private, and non-governmental health facilities (11). However, the treatment success for patients with TB treated with DOTS varies from one country to another, and TSRs below the WHO target of 85% for cases of drug-susceptible TB are regularly reported by WHO's 194 member states, including Ethiopia (12-15). The DOTS program could not be optimized in Ethiopia due to poor treatment-seeking behavior; incomplete treatment or poor compliance; and treatment interruption or default, a phenomenon that contributes to prolonged infectiousness and increasing risk of drug resistance, relapsed TB, and death (16). Additionally, low literacy levels, discriminatory behavior of healthcare professionals, self-denial due to stigma, long treatment duration, inaccessibility of public health facilities, and shortage of drugs jeopardize successful treatment outcomes (17,18).

Previous studies conducted in different parts of Ethiopia revealed socio-demographic and clinical factors affecting the treatment outcomes of TB (19-21). Except for a single cohort study of TB treatment outcomes between 2007 and 2012 in Bale Robe town (14), information on treatment outcomes is lacking. Therefore, the purpose of this study was to determine the treatment outcome of all forms of TB cases and to identify associated factors for all types of TB cases involved in the DOTS program reported from 2012 to 2016 at Bale General Regional Hospital (BRGH).

Methods

Patients

The study was conducted at BRGH located in Robe Town in Bale Zone from January 2012 to December 2016. The town is located 430 km Southeastern of Addis Ababa, Ethiopia. Currently, the BRGH delivers clinical services to more than half a million urban and rural people. The hospital also serves as a training center for Madda Walabu University and provides clinical services to students, prisoners, and students of private and public colleges in the town. The Hospital's DOTS clinic operates within the National TB and Leprosy Control Program (NTBLCP) of Ethiopia (10,11,14). The DOTS clinic provides basic treatment and diagnostic services for all forms of TB through clinical diagnosis using the Ziehl-Nielsen staining method for acid-fast bacilli (AFB) and chest radiographs.

Study design, period, and data collection

An Institutional-based retrospective design was used for studying the TSR of TB and associated factors. The research participants were drawn from all patients with TB registered at the BRGH DOTS clinic in Bale Robe. The inclusion criteria were all TB cases diagnosed with Ziehl-Neelsen Staining for AFB and/or radiography and initiated TB treatment at the BRGH DOTS clinic from January 2012 through December 2016; cases with incomplete information were excluded. Data on various variables, including socio-demographic data (patient's gender, age, residence, and occupation), HIV serology status, TB types, TB patient category, outcomes of each study participant's NTBLCP-recommended treatment (10,11), and year of treatment, were recorded by trained nurses.

TB types, patient category, and treatment outcome definitions

Type of TB, patient category and treatment outcomes were defined according to the NTBLCP guideline (10) and Guidelines for Clinical and Programmatic Management of TB, TB/HIV, and Leprosy (11) adapted from the WHO. Types of TB were defined as the site of the lesions as either smear-positive pulmonary TB (PTB+) or smear-negative pulmonary TB (PTB-) or extra-pulmonary (EPTB). Patients were defined as having PTB+ if they had one of the following 3 conditions: (i) at least 2 initial sputum smears positive for AFB by direct microscopy, (ii) one initial smear positive for AFB by direct microscopy and positive culture, or (iii) one initial smear positive for AFB by direct microscope and radiographic abnormalities consistent with active TB. Patients were defined as having PTB- if they met one of the following 3 conditions: (i) had symptoms suggestive of TB with at least 3 initial smears negative for AFB by direct microscopy and no response to a course of broad-spectrum antibiotics; (ii) had 3 negative smears by direct microscopy, radiological abnormalities consistent with PTB, and a decision by a clinician to treat with a full course of anti-TB therapy; or (iii) had a diagnosis based on culture positive for *M. tuberculosis* but 3 initial smears negative by direct microscopy. EPTB is TB in organs other than the lungs, diagnosed by one culture-positive specimen from an extra-pulmonary site, histopathological evidence from a biopsy, or strong clinical evidence consistent with active EPTB and the decision by a physician to treat with a full course of anti-TB therapy.

Patients with TB fell into the following six case categories. New case: A patient who never had treatment for TB, or has been on anti-TB treatment for less than four weeks in the past; Relapse: A patient declared cured or treatment completed of any form of TB in the past, but who reports back to the health service and is now found to be AFB smear-positive or culture-positive; Treatment after failure: A patient who, while on treatment, is smear-positive at the end of the fifth month or

later, after starting. Treatment after failure includes patients who were initially sputum smear-negative, but who became smear-positive during treatment at 2 months or later; Default: A patient previously recorded as default from treatment and returns to the health facility with smear-positive sputum. Transfer-in: A patient who is transferred from a particular treatment unit with continued treatment after starting treatment in another treatment unit for at least four weeks; Other: A patient who does not fit into any above categories.

Treatment outcomes are defined as the following. Cured: A patient whose sputum smear or culture was positive at the beginning of the treatment but who was smear- or culture-negative in the last month of treatment and on at least one previous occasion; Treatment completed: A patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion; Treatment failure: A patient whose sputum smear or culture is positive at 5 months or later during treatment or patients found to harbor a MDR strain at any point of time during the treatment, whether they are smear-negative or -positive; Defaulter: A patient who has been on treatment for at least four weeks and whose treatment was interrupted for eight or more consecutive weeks; Died: A patient died from any cause during the course of treatment; Transferred out: A patient who started treatment and has been transferred to another reporting unit and for whom the treatment outcome is not known at the time of evaluation of treatment results. Treatment success is the sum of cured patients and patients who complete treatment. The TSR is defined as the ratio, expressed as a percentage, of the sum of cured patients and patients who completed treatment to the total number of patients studied.

Data quality assurance

Data were collected after training was provided to data collectors (trained nurses in the DOTS clinic of BRGH). This pretest was conducted at BRGH. The completed data were supervised by the principal investigator for completeness, and 5% of the samples were randomly selected and validated against the registration book. Data entry was done to the Epi-Info version to check data consistency.

All procedures were accomplished following the Declaration of Helsinki. Approval was received from the Institutional Research Ethics Committee of the College of Natural and Computational Science of Madda Walabu University (ref. no. CNCS/234/2017; September 26, 2017). Permission to conduct the research was obtained from the Robe Hospital administration before data collection. Because of the retrospective nature of the study, informed consent from the study participants was not needed not requested. Patient records were identified before analysis and kept confidential to ensure the confidentiality of patient information.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows, version 21.0, IBM. Corp., Armonk, NY, 2012). Univariate analysis and multivariate logistic regression analyses were used to analyze factors associated with TSR in patients with TB. The independent variables were checked for the presence of multicollinearity using the variance inflation factor. The goodness of fit of the employed model was evaluated using the Hosmer-Lemeshow test, which compares observed with expected frequencies of the outcome and computes a test statistic that is distributed according to the chi-squared distribution. A p-value >0.05 implies there is no evidence that the observed and expected frequencies differ and the model fits well to run the multivariate logistic regression analysis. Marginal variables with p≤0.2 in univariate analysis were exported into a multivariate logistic regression model to detect independent predictors associated with TSR of patients with TB. In multivariate logistic regression analysis, the independent variables with a 95% confidence interval (CI) excluding one were significantly associated with TSR of TB (p<0.05). Multinomial logistic regression analysis was performed to evaluate independent predictor factors associated with treatment outcomes (dependent variables). In unadjusted multinomial logistic regression “crude relative risk ratio (CRRR)”, independent variables with p-value <0.05 and the 95% CIs, excluding one were exported to adjusted multinomial logistic regression “adjusted relative risk ratio (ARRR)”. Six outcome variables were used; the cure was used as the reference category and the remaining five variables (completed, failure, default, death, and transferred out) as separate variables. In ARRR, independent factors whose 95% CIs excluding one were significantly associated with the treatment outcome and the strength was significant at a p-value <0.05.

Results

Socio-demographic and clinical characteristics

The demographic and clinical characteristics of the study participants are shown in Table 1. A total of 807 patients with TB were registered at the DOTS clinic of BRGH from January 2012 to December 2016. Most were males (57.4%) and lived in urban areas (80.8%). More than three-fourths (75.8%) of the patients were 15-44 years old, mean age was 31.8 years (standard deviation ± 10.7 years). The study participants were students, self-employed, employed, unemployed, prisoners, and farmers. The majority were new cases (79.6%). Of the study subjects treated for TB, 79.2% were HIV-negative and 20.8% were HIV-positive. The patients with TB were PTB- (43.5%), PTB+ (28.0%) and EPTB (28.5%) cases. New patients with TB had the highest percentage of all TB forms (Table 1).

Treatment outcome of the patients

The treatment outcomes for patients who were registered in the DOTS program at the BRGH from January 2012 through December 2016 are shown in Table 2. Among the patients, 25.5% were cured and 56.9% completed their treatment. However, 2.1%, 3.3%, 6.2%, and 5.9% of the patients with TB had treatment failure, defaulted, died, or were transferred, respectively. The highest and lowest percentages of cured patients were recorded in pairs of patients with TB in the age groups 15-24 years and 54-64 years, in PTB+ and EPTB patients, and during 2015 (29.8%) and 2014 (21.1%).

Treatment failure was considerably higher in treatment after failure (28.6%) compared to the other TB patient categories. Treatment failure (3.9%), defaulters (10.6%), and transferred-out (13.3 %) cases had generally higher rates in 2014. More deaths were recorded for patients with TB ≥65 years (21.2%), PTB- patients (8.3%), relapsed cases (8.3%), and HIV-positive patients with TB (10.8%) and in 2012 (12.1%) compared to their corresponding counterparts (Table 2).

Treatment success rate

The TSR of the TB patients treated during the study period was 82.4% (Table 3). The TSRs of males (82.5%) and females (82.3%), and of PTB+ (84.1%) and EPTB patients (83.9%) were similar. The TSR of TB was higher in HIV-negative patients with TB (83.7%) than in HIV-positive patients with TB (77.8%). TSR increased or decreased inconsistently over the years of study; the lowest was in 2014 (66.7%) and the highest in 2015 (90.1%) (Table 3).

Factors associated with TSR in TB

Results of the univariate analysis and multivariate logistic regression are summarized in Table 3. In univariate analysis, variables significantly associated with TSR were age group, occupation, HIV status, and year of treatment of patients with TB (all at $p<0.05$), and patient residence and TB patient category (at $p\leq0.2$). These variables were fitted to the multivariate logistic regression analysis. In multivariate logistic regression, age ≥65 years [Odds ratio (OR): 0.17 (95% CI: 0.06-0.45)], being unemployed [OR: 0.86 (95% CI: 0.44-0.97)], having treatment after failure [OR: 0.37 (95% CI: 0.15-0.91)], and receiving treatment during 2014 [OR: 0.47 (95% CI: 0.23-0.96)] were associated with reduced probability of treatment success. However, HIV-negative patients with TB [OR: 1.62 (95% CI: 1.13-2.34)] and patients treated during 2015 [OR: 2.29 (95% CI: 1.32-3.98)] had a higher odds of treatment success than their counterparts (Table 3). Sex and residence of the patients and TB type had no significant association with treatment success ($p>0.05$) (Table 3).

Factors associated with treatment outcome of TB

Independent variables that were significantly associated with the treatment outcome in CRRR analysis were fitted to the ARRR model to determine the treatment outcome of TB. In the ARRR analysis, patients below 65 years of age had a significantly lower relative risk of death than the reference category (Table 4). However, HIV- positive patients with TB [ARRR: 2.18 (95% CI: 1.18-4.04)] and patients treated during 2012 [ARRR: 2.70 (95% CI: 1.14-6.39) had a higher relative risk ratio of death to HIV-negative patients with TB and patients treated during 2016, respectively. Patients with TB with PTB+ had a significantly lower relative risk ratio of completion, and transferring-out than those in the reference category (EPTB patients, $p<0.05$). New patients with TB also had a significantly lower relative risk of failure than patients in the reference category [ARRR: 0.10 (95% CI: 0.015-0.72)]. Patients with TB who were treated in 2014 had a significantly higher relative risk of default [ARRR: 11.46 (95% CI: 4.16-31.54)], and being transferred out [ARRR: 3.01 (95% CI: 1.35-6.72)] than those treated in 2016 (the reference year). Unemployed patients with TB had a significantly higher relative risk of failure [ARRR: 7.97 (95% CI: 1.13-55.8)] and being transferred out [ARRR: 4.27 (95% CI: 1.25-14.61)] than patients with TB who were farmers (the reference occupation) (Table 4).

Discussion

This study found satisfactory TSR of TB (82.4%) in BRGH. Patients with TB aged 65 and above, unemployed, having treatment after failure, TB-HIV co-infection, and receiving treatment during 2014 significantly reduced the probability of successful treatment of TB. Death from TB was considerably higher in patients with TB aged 65 and above, TB-HIV co-infection, and those having treatment during 2012. The treatment failure was significantly lower in new patients with TB, but transfer out was higher in patients having treatment after failure and treated during 2014.

The percentage of male patients with TB was higher than that of females (Tables 1). The underuse of the health facility by female patients due to socio-economic and cultural factors or gender-based biological differences accounted for differences in susceptibility to active disease (22,23). Most patients with TB were in the productive age group (15-44 years), in agreement with patients with TB attending other health facilities in Ethiopia (78%) (12) and Dilla University Referral Hospital (79.4%) (24). TB-associated morbidity and mortality occur mainly in the economically productive age (25). This may be due to the high mobility and interaction of the study population of the productive age group and thereby exposing them to TB infection.

Among patients with TB, PTB- was higher than PTB+ and EPTB (Table 1). A previous study in the BRGH (14) also reported higher percentages of PTB- (40.7%) than PTB+ (33.3%) and EPTB (26%). The higher percentage of PTB- than other TB types might be related to a diagnosis failure in the hospital where the clinicians rush to designate TB cases, as PTB- cases are also

more common in patients with HIV (4,5,26). A higher prevalence of PTB- (44.8%) and PTB+ (37.0%) was also reported from surrounding communities in Gambella Regional State (27).

We found a significantly higher prevalence of all forms of TB in urban areas than in rural areas (Table 1), contrasting with findings from a study in southern Ethiopia that reported more patients with TB in rural (66%) than in urban areas (44%) (12). This discrepancy might be due to high-density housing and associated exposure risk in urban areas. The rate of TB-HIV co-infection (Table 1) was higher than in a study in Debre Tabor (12.7%) (28) but slightly lower than that reported by BRGH (22.2%) (14).

The results of our analysis revealed that the percentage of cured patients (Table 2) was higher than the rate in the BRGH (19.7%) (14) but lower than the result in a recent systematic review and meta-analysis for Ethiopia (33.9%) (29). Most of the patients with TB in BRGH had completed the treatment outcome in agreement with similar studies in Ethiopia (12,15,20). The rate of treatment failure was slightly higher than the results in BRGH for 2007-2011 (1%) (14) and Asella Teaching Hospital (0.2%) (30). The rate of defaulters in our study was lower than the previous results in BRGH (6%) (14) and South Africa (9.8%) (22). The percentage of dead patients was similar at Asella Hospital (6%) (30) but higher than that at Dilla University Referral Hospital (3.4%) (24) and lower than at BRGH (9%) (14). This indicates a considerable improvement in the rate of dead patients with TB in our study compared to a previous study in the same hospital (14), which might be related to the improvements in DOTS service in the BRGH. The lower percentage of failure and defaulted patients might be due to the deployment of health extension workers who provide treatment and prevention services to the public in all zones in Ethiopia, including BRGH (31).

The TSR in our study (Table 3) was similar to that reported in a study of global TSRs (83%) in a 2015 cohort) (4) but slightly higher than the TSR in the BRGH (78.2%) (14). The TSR of PTB-, PTB+ and EPTB patients in our study was slightly higher than the rate in BRGH (PTB-, 79.5%; PTB+, 76.1% and EPTB, 79.3%) (14). The TSR of new cases in our study was higher than the rate in 2007-2012 in BRGH (78.9%) (14) but lower than the national TSR in new patients registered during 2016 (90%) (5). The TSR of patients with TB among HIV-negative and HIV-positive patients was similar to the TSRs at Asella Teaching Hospital (83.4% in HIV-negative and 79.8% in HIV-positive) (30), but higher than the TSRs in the BRGH (80.8% in HIV-negative patients with TB and 67.1% among HIV-positive patients) (14). Variation in DOTS performance in various study areas, socio-economic characteristics of the patients, geographic setting, sample size, study period, and the TB clinic management may have contributed to differences in TSR for patients with TB (20).

Table 1. Characteristics of tuberculosis patients at Bale Robe General Hospital, Ethiopia (n=807)

Age, mean±SD	31.8±1.07
Age groups, n (%)	
0-14	56 (6.9)
15-24	300 (37.2)
25-34	204 (25.3)
35-44	108 (13.4)
45-54	60 (7.4)
55-64	27 (3.3)
≥65	52 (6.4)
Sex, n (%)	
Male	463 (57.4)
Female	344 (42.6)
Residence, n (%)	
Urban	652 (80.8)
Rural	155 (19.2)
Job status, n (%)	
Student	246 (30.5)
Self-employee	258 (32)
Employed	91 (11.3)
Unemployed	104 (12.9)
Prisoner	30 (3.7)
Farmer	78 (9.7)
TB type, n (%)	
PTB-	351 (43.5)
PTB+	226 (28.0)
EPTB	230 (28.5)
TB patient category, n (%)	
New	642 (79.6)
Relapse	36 (4.5)
Defaulted	14 (1.7)
Failure	7 (0.9)
Transfer-in	89 (11.0)
Others	19 (2.4)
HIV status, n (%)	
Positive	168 (20.8)
Negative	639 (79.2)
Treatment year, n (%)	
2012	140 (17.3)
2013	149 (18.5)
2014	180 (22.3)
2015	151 (18.7)
2016	187 (23.2)

TB: Tuberculosis, PTB: Pulmonary tuberculosis, PTB-: Smear negative pulmonary tuberculosis, PTB+: Smear positive pulmonary tuberculosis, EPTB: Extra-pulmonary tuberculosis; SD: Standard deviation

Table 2. Treatment outcomes in tuberculosis patients at Bale Robe General Hospital, Ethiopia

	Cured	Completed	Failure	Defaulted	Death	Transferred out	Total
Age groups, n (%)							
0-14	7 (12.5)	45 (80.4)	0 (0)	2 (3.6)	0 (0)	2 (3.6)	56
15-24	99 (33.0)	157 (52.3)	7 (2.3)	14 (4.7)	8 (2.7)	15 (5.0)	300
25-34	51 (25.0)	111 (54.4)	4 (2.0)	6 (2.9)	16 (7.8)	16 (7.8)	204
35-44	24 (22.2)	67 (62.0)	3 (2.8)	0 (0)	7 (6.5)	7 (6.5)	108
45-54	15 (25.0)	28 (46.7)	2 (3.3)	3 (5.0)	7 (11.7)	5 (8.3)	60
55-64	3 (11.1)	19 (70.4)	0 (0)	1 (3.7)	1 (3.7)	3 (11.1)	27
≥65	7 (13.5)	32 (61.5)	1(1.9)	1 (1.9)	11 (21.2)	0 (0)	52
Sex, n (%)							
Male	107 (23.1)	275 (59.4)	8 (1.7)	14(3.0)	33 (7.1)	26 (5.6)	463
Female	99 (28.8)	184 (53.5)	9 (2.6)	13 (3.8)	17 (4.9)	22 (6.4)	344
Residence, n (%)							
Urban	168 (25.8)	365 (56.0)	16 (2.5)	22 (3.4)	43 (6.6)	38 (5.8)	652
Rural	38 (24.5)	94 (60.6)	1 (0.6)	5 (3.2)	7 (4.5)	10 (6.5)	155
Job status, n (%)							
Student	88 (35.8)	130 (52.8)	4 (1.6)	9 (3.7)	2 (0.8)	13 (5.3)	246
Self-employee	59 (22.9)	150 (58.1)	5 (1.9)	7 (2.7)	22 (8.5)	15 (5.8)	258
Employed	21 (23.1)	49 (53.8)	2 (2.2)	5 (5.5)	8 (8.8)	6 (6.6)	91
Unemployed	13 (12.5)	65 (62.5)	4 (3.8)	5 (4.8)	7 (6.7)	10 (9.6)	104
Prisoner	8 (26.7)	19 (63.3)	1 (3.3)	0 (0)	2 (6.7)	0 (0)	30
Farmer	17 (21.8)	46 (59.0)	1 (1.3)	1 (1.3)	9 (11.5)	4 (5.1)	78
TB type, n (%)							
PTB-	43 (12.3)	239 (68.1)	8 (2.3)	13 (3.7)	29 (8.3)	19 (5.4)	351
PTB+	148 (65.5)	42 (18.6)	5 (2.2)	8 (3.5)	11 (4.9)	12 (5.3)	226
EPTB	15 (6.5)	178 (77.4)	4 (1.7)	6 (2.6)	10 (4.3)	17 (7.4)	230
TB patient category, n (%)							
New	159 (24.8)	373 (58.1)	10 (1.6)	23 (3.6)	44 (6.9)	33 (5.1)	642
Relapse	18 (50.0)	11 (30.6)	1 (2.8)	0 (0)	3 (8.3)	3 (8.3)	36
Defaulted	5 (35.7)	8 (57.1)	0 (0)	1 (7.1)	0 (0)	0 (0)	14
Treatment after failure	2 (28.6)	2 (28.6)	2 (28.6)	0 (0)	0 (0)	1 (14.3)	7
Transfer-in	20 (22.5)	53 (59.6)	3 (3.4)	2 (2.2)	2 (2.2)	9 (10.1)	89
Others	2 (10.5)	12 (63.2)	1 (5.3)	1 (5.3)	1 (5.3)	2 (10.5)	19
HIV status, n (%)							
Positive	43 (25.7)	87 (52.1)	2(1.20)	2 (1.2)	18 (10.8)	16 (9.6)	168
Negative	163 (25.5)	372 (58.2)	15 (2.3)	25 (3.9)	32 (5.0)	32 (5.0)	639
Treatment year, n (%)							
2012	38 (27.1)	76 (54.3)	2 (1.4)	1 (0.7)	17 (12.1)	6 (4.3)	140
2013	36 (24.2)	91 (61.1)	2 (1.3)	3 (2.0)	10 (6.7)	7 (4.7)	149
2014	38 (21.1)	82 (45.6)	7 (3.9)	19 (10.6)	10 (5.6)	24 (13.3)	180
2015	45 (29.8)	91 (60.3)	1(0.7)	2 (1.3)	9 (6.0)	3 (2.0)	151
2016	49 (26.2)	119 (63.6)	5 (2.7)	2 (1.1)	4 (2.1)	8 (4.3)	187
Total	206 (25.5)	459 (56.9)	17 (2.1)	27 (3.3)	50 (6.2)	48 (5.9)	807

TB: Tuberculosis, PTB: Pulmonary tuberculosis, PTB-: Smear negative pulmonary tuberculosis, PTB+: Smear positive pulmonary tuberculosis, EPTB: Extra pulmonary tuberculosis

Table 3. Univariate and multivariate analysis of treatment success rate

Variables	Treatment success n (%)	Univariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
Age in groups					
0-14	52 (92.9)	1		1	
15-24	256 (85.3)	0.68 (0.27-1.67)	0.403	0.53 (0.20-1.43)	0.214
25-34	162 (79.4)	0.56 (0.29-1.05)	0.073	0.48 (0.22-1.01)	0.055
35-44	91 (84.3)	0.77 (0.44-1.36)	0.381	0.71 (0.36-1.40)	0.326
45-54	43 (71.7)	0.51 (0.29-0.89)	0.019	0.54 (0.27-1.09)	0.087
55-64	22 (81.5)	1.18 (0.60-2.31)	0.618	0.99 (0.47-2.08)	0.990
≥65	39 (75.0)	**0.23 (0.94-0.56)**	**0.001**	**0.17 (0.06-0.45)**	**0.001**
Gender					
Male	382 (82.5)	1		-	-
Female	283 (82.3)	0.984 (0.74-1.30)	0.909	-	-
Residence					
Urban	533 (81.7)	1	1		
Rural	132 (85.2)	1.28 (0.88-1.85)	0.188	1.16 (0.7 -1.74)	0.460
Occupation					
Student	218 (88.6)	1		1	
Self-employed	209 (81.0)	0.26 (0.70-2.24)	0.430	0.94 (0.99-3.81)	0.052
Gov. employed	70 (76.9)	0.98 (0.60-1.62)	0.950	1.17 (0.65-2.10)	0.590
Unemployed	78 (75)	**0.54 (0.32-0.91)**	**0.021**	**0.86 (0.44-0.97)**	**0.036**
Prisoner	27 (90.0)	1.40 (0.80-2.44)	0.240	1.89 (0.99-3.61)	0.051
Farmer	63 (80.8)	0.46 (0.17-1.25)	0.130	0.74 (0.25-2.20)	0.593
TB type					
PTB-	282 (80.3)	1		-	-
PTB+	190 (84.1)	1.27 (0.91-1.78)	0.155	-	-
EPTB	193 (83.9)	0.98 (0.67-1.44)	0.950	-	-
TB patient category					
New	532 (82.9)	1		1	
Relapse	29 (80.6)	0.21 (0.04-1.17)	0.076	0.20 (0.36-1.17)	0.075
Defaulted	13 (92.9)	0.67 (0.24-1.89)	0.457	0.56 (0.18-1.66)	0.297
Treatment after failure	4 (57.1)	0.58 (0.25-1.32)	0.195	**0.37 (0.15-0.91)**	**0.030**
Transfer-in	73 (82.0)	2.10 (0.46-9.44)	0.330	2.35 (0.44-12.61)	0.318
Others	14 (73.7)	0.61 (0.24-1.52)	0.294	0.48 (0.18-1.28)	0.145
HIV status					
Positive	130 (77.8)	1		1	
Negative	535 (83.7)	**1.50 (1.08-2.08)**	**0.014**	**1.62 (1.13-2.34)**	**0.009**
Year of treatment					
2012	114 (81.4)	1		1	
2013	127 (85.2)	2.01 (1.24-3.26)	0.350	1.52 (0.96-3.03)	0.200
2014	120 (66.7)	**0.93 (0.53-2.50)**	**0.046**	**0.47 (0.23-0.96)**	**0.038**
2015	136 (90.1)	**4.42 (2.86-6.82)**	**0.001**	**2.29 (1.32-3.98)**	**0.003**
2016	168 (89.8)	0.97 (0.57-1.65)	0.920	1.73 (0.88-4.40)	0.445

TB: Tuberculosis, PTB-: Smear negative pulmonary tuberculosis, PTB+: Smear positive pulmonary tuberculosis, EPTB: Extra pulmonary tuberculosis, HIV: Human immune deficiency virus, OR: Odds ratio, CI: Confidence interval

In multivariate logistic regression, TSR was significantly lower in patients with TB ≥65 years of age (Table 3), in concordance with similar studies in Ethiopia (21,27,30). Individuals at a higher age experience increased co-infections with other diseases and develop immune-compromised situations that might contribute to poorer treatment outcomes (18,30). Unemployed patients with TB exhibited a significantly lower TSR than the students (the reference category) (Table 3), in agreement with studies in the Tigray Region (20) and Anqing, China (8). Unemployed patients with TB may interrupt the DOTS treatment for socio-economic reasons and thereby reducing the TSR of TB. Patients with TB with treatment after failure exhibited lower TSR than new patients (Table 3). Other studies linked a previously treated TB with MDR (6,32) and unsuccessful treatment outcomes (18). HIV-negative patients with TB had significantly higher TSR than HIV-positive counterparts (Table 3), in agreement with related studies in Ethiopia (29,33). HIV co-infection increases the risk of latent TB reactivation 20-fold (33). Patients with TB treated

Table 4. Multinomial logistic regression analysis of factors associated with treatment outcomes

Variable	Completed	Failure	Default	Death	Transferred out
Age (years)					
0-14	1.32 (0.34-5.06)	0.28 (0.01-6.01)	1.16 (0.15-8.97)	**0.042 (0.006-0.269)**	1.20 (0.13-10.54)
15-24	0.83 (0.26-2.62)	1.20 (0.14-9.86)	1.38 (0.23-8.28)	**0.078 (0.020-0.275)**	1.79 (0.26-12.25)
25-34	0.98 (0.31-3.14)	1.44 (0.17-11.79)	1.17 (0.19-7.18)	**0.097 (0.028-0.330)**	4.23 (0.63-28.39)
35-44	0.88 (0.26-2.97)	2.02 (0.23-17.84)	0.41 (0.054-3.19)	**0.065 (0.017-0.240)**	2.92 (0.40-21.28)
45-54	0.71 (0.20-2.51)	1.91 (0.21-17.20)	1.73 (0.26-11.1)	**0.13 (0.035-0.480)**	2.97 (0.40-22.0)
55-64	1.21 (0.25-5.88)	0.34 (0.008-14.18)	1.57 (0.15-16.10)	**0.80 (0.011-0.628)**	4.73 (0.50-44.88)
>65 (Ref.)	1	1	1	1	1
Occupation					
Student	0.76 (0.32-1.82)	0.96 (0.12-7.45)	1.48 (0.32-6.7)	0.33 (0.10-1.08)	1.21 (0.36-4.00)
Self-employee	1.23 (0.56-2.73)	1.71 (0.26-11.08)	1.89 (0.045-7.95)	1.33 (0.52-3.34)	1.08 (0.36-3.22)
Gov. employee	1.02 (0.40-2.61)	2.19 (0.27-17.54)	4.33 (0.92-20.45)	1.40 (0.46-4.22)	1.57 (0.43-5.68)
Unemployed	1.55 (0.57-4.22)	**7.97 (1.13-55.8)**	2.55 (0.57-11.41)	1.02 (0.32-3.23)	**4.27 (1.25-14.61)**
Prisoners	0.76 (0.21-2.65)	1.85 (0.17-19.9)	0.50 (0.02-5.92)	0.84 (0.16-4.20)	0.39 (0.04-3.74)
Farmers (Ref.)	1	1	1	1	1
TB type					
PTB-	0.62 (0.35-1.08)	1.06 (0.37-3.03)	1.10 (0.49-2.45)	1.28 (0.62-2.64)	0.59 (0.29-1.20)
PTB+	**0.34 (0.02-0.61)**	1.29 (0.54-4.36)	1.75 (0.76-5.24)	1.14 (0.86-1.27)	**0.069 (0.032-0.14)**
EPTB (Ref.)	1	1	1	1	1
TB patient category					
New	0.71 (0.15-3.23)	**0.10 (0.015-0.72)**	0.33 (0.047-2.41)	0.69 (0.09-5.28)	0.23 (0.04-1.31)
Relapse	0.28 (0.05-1.67)	0.12 (0.01-1.51)	0.10 (0.05-1.82)	0.49 (0.05-4.89)	0.28 (0.038-2.204)
Default	0.29 (0.036-2.30)	0.21 (0.06-6.76)	0.67 (0.05-8.97)	0.12 (0.04-4.18)	0.54 (0.01-2.22)
Treatment after failure	0.17 (0.08-1.06)	0.1 (0.08-1.34)	0.05 (0.01-1.07)	0.4 (0.03-1.07)	**2.91 (1.25-13.63)**
Transfer in	1.16 (0.23-5.84)	0.40 (0.05-3.31)	0.28 (0.033-2.54)	0.71 (0.07-6.61)	0.78 (0.12-4.91)
Others (Ref.)	1	1	1	1	1
HIV Status					
HIV(+)	0.79 (0.47-1.31)	0.41 (0.62-0.199)	0.49 (0.20-1.18)	**2.18 (1.18-4.04)**	1.77 (0.94-3.32)
HIV (-) (Ref.)	1	1	1	1	1
Year of treatment					
2012	0.58 (0.30-1.10)	0.46 (0.11-1.8)	0.71 (0.17-2.97)	**2.70 (1.14-6.39)**	0.73 (0.28-1.90)
2013	0.88 (0.47-1.65)	0.49 (0.12-1.95)	1.21 (0.35-4.19)	1.54 (0.61-3.84)	0.92 (0.37-2.27)
2014	0.58 (0.308-1.09)	1.23 (0.40-3.71)	**11.46 (4.16-31.54)**	1.48 (0.58-3.72)	**3.01 (1.35-6.72)**
2015	0.61 (0.33-1.14)	0.24 (0.05-1.06)	0.70 (0.18-2.65)	1.40 (0.50-3.52)	0.33 (0.11-1.93)
2016 (Ref.)	1	1	1	1	1

TB: Tuberculosis, CI: Confidence interval, ARRR: Adjusted relative risk ratio in multinomial logistic regression analysis. NB. Ref.: Reference category for each independent factor used in multinomial logistic regression analysis. Values indicated in bold are significantly associated with treatment outcome at $p<0.05$

during 2014 had lower TSRs, but those who were treated during 2015 had higher TSRs than the reference year (Table 3). The Federal Ministry of Health assessment in 2013/2014 showed that the coverage of the DOTS program reached 79% in health centers and 98.4% in hospitals nationwide (34), indicating a large gap in the TSR among health institutions in Ethiopia. A global TB report showed that the TSR for TB in Ethiopia during 2014 was 89% (1) and lower than that during 2012 (91%) (2). Therefore, the national TSR of TB was impeded by some unexplained factors that might have affected the TSR in BRGH during 2014.

The ARRR analysis revealed that death from TB was lower in patients with TB in the <65 years of the age group (Table 4). Higher death rates from TB have also been reported in people of older age groups (18-21,35). Age-related co-infections and physiological deterioration may lead to poor treatment adherence, which, with less access of older people to health facilities, may lead to poor outcomes (30,36). We also found more deaths among PTB- patients than among PTB+ and EPTB patients. Diagnosis and treatment delays and HIV infection among PTB- cases were suggestive of higher death in PTB- TB cases than in PTB+ and EPTB (21,26). The relative risk ratio of treatment failure was lower in new patients with TB than in their counterparts (Table 4). Drug resistance due to the prevalence of MDR-TB in Ethiopia was low (1.6%) among new cases and relatively higher (12%) among retreatment cases (10,11). The relative risk of death in patients with TB was significantly higher in HIV-positive than in HIV-negative patients with TB. This finding was in concordance with other studies in Ethiopia (30) and South Africa (37), which found strong associations between HIV infection and TB mortality. The reduction in death and improvement of the success rate of TB has been in connection to antiretroviral treatment in TB-HIV co-infected individuals (38). In the adjusted multinomial logistic regression analysis, patients with TB who were treated during 2012 had a higher relative risk of death. At the global level, an estimated 8.6 million people developed TB and 1.3 million died of the disease in 2012 (9). A higher death rate was also reported for patients with TB who were treated during September 2012-May to 2013 in southern Ethiopia than in other years (24). A similar study in Ethiopia reported a higher rate of transfer for patients with treatment failure (12).

This study has several limitations. Because of its retrospective design, several socio-demographic characteristics such as educational status, income, family size, and distance from the treatment center were not available. Additionally, data on clinical characteristics were missing, including the presence of co-morbidities, complications related to the treatment, drug resistance, and patients' awareness levels. Therefore, the interpretation of the results should cautiously be made. Despite these limitations, the large sample size and follow-up of internationally accepted ethical guidelines were the strengths.

Conclusion

This study showed that the rate of cure in patients with TB was 25.5%, and the rate of treatment completion was 56.9%. The rate of patients who died of TB was 6.2%, transferred was 5.9%, treatment failure was 2.1%, and defaulted was 3.3%. The TSR in all forms of TB in BRGH (82.4%) was satisfactory but below the minimum target (85.0%) established by the WHO. Age, occupation, treatment category, HIV status, and year of treatment were independently associated with treatment success of TB. Age and HIV status were independently associated with the risk of death, whereas occupation of the patients with TB was significantly associated with the risk of treatment failure and transfer. TB type was associated with the risk of treatment completion and transfer; and the year of treatment was associated with the risk of treatment outcomes such as default, death, and transfer. Based on the findings of this study, continuous monitoring of patients with unsuccessful treatment outcomes and TB-HIV co-infection, supervision of the implementation of the DOTS program, and targeted interventions may be recommended.

Acknowledgments

Madda Walabu University and the Oromia Education Office are highly acknowledged for facilitating the master's study of YB. Health professionals working in the DOTS clinic of BRGH are highly acknowledged for their genuine organization and provision of retrospectively registered data related to treatment outcomes of patients with TB. We also thank Ann Byers for reading the manuscript.

Ethics

Ethics Committee Approval: An approval was received from the Institutional Research Ethics Committee of the College of Natural and Computational Science of Madda Walabu University (ref. no. CNCS/234/2017; date: 26.09.2017).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.A.T., Y.B.G., Design: A.A.T., Y.B.G., H.K., Data Collection or Processing: A.A.T., Y.B.G., Analysis or Interpretation: A.A.T., Y.B.G., H.K., Literature Search: A.A.T., Y.B.G., Writing: A.A.T., Y.B.G., H.K.

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

Financial Disclosure: The authors declare that this study received small financial support from Madda Walabu University (MWU/SGS/2016/17).

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DOI: 10.4274/gulhane.galenos.2021.80299
Gulhane Med J 2022;64:189-96

Low serum zinc and total antioxidant capacity levels in individuals with premenstrual syndrome

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Date submitted:
04.09.2021
Date accepted:
16.11.2021
Online publication date:
15.06.2022

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Keywords: PMS, antioxidant capacity, serum zinc level

ABSTRACT

Aims: The aim of this study was to evaluate serum zinc (Zn) level, total antioxidant capacity (TAC) level, and nutritional status in premenstrual syndrome (PMS).

Methods: This cross-sectional, single-center study and case-control study enrolled university students 18 to 28 years old. Serum TAC analysis was performed via the enzyme-linked immunosorbent assay. Serum Zn level was measured using atomic absorption spectrometry. Nutritional status was assessed using the 24-hour dietary recall.

Results: The study population consisted of 110 individuals (mean±Sstandart deviation age: 20.3±1.9 years), 48 subjects with PMS, and 62 subjects without PMS. Individuals with PMS had lower serum Zn levels (28.04±31.40 µmol/L vs. 11.67±6.42 µmol/L, respectively), TAC levels (1.60±0.42 mmol/L vs. 1.47±0.10 mmol/L), and higher body mass index (22.30±02.71 kg/m^2 vs. 23.73±3.44 kg/m^2) than individuals without PMS. Individuals with PMS had lower fiber, polyunsaturated fatty acid (PUFA), vitamins E, vitamin B1, vitamin B2, vitamin B6, vitamin C, folate, and magnesium, and higher carbohydrate and sodium intake than those without PMS but the differences did not reach statistical significance. Serum TAC level, dietary antioxidant, and PUFA intake level were positively correlated ($p<0.05$), whereas serum TAC level was negatively correlated with dietary intake of protein, lipid, and carbohydrate intake ($p<0.05$).

Conclusions: This study showed that serum Zn and TAC levels were lower in PMS, and serum TAC levels were correlated with dietary antioxidant intake.

Introduction

Premenstrual syndrome (PMS) is a common condition that causes physical, emotional, and behavioral symptoms in women of reproductive age (1,2). It starts in the late luteal phase of the menstrual cycle, and the symptoms subside shortly after the onset of menstruation (3). The quality of life and reproductive health are significantly affected in affected individuals (2,4).

The Royal College of Obstetricians and Gynecologists has reported that 40.0% of women have premenstrual symptoms (5). The lowest incidence was reported in France (12.0%). The highest incidence was reported in Iran (98.0%) (6). In Turkey, the incidence has been reported by 66.0 to 91.8%, more commonly in young individuals (7,8).

The symptoms of PMS have been explained through reduced levels of progesterone and the progesterone metabolite

allopregnanolone, which act as agonists to the gamma-aminobutyric acid-A (GABA-A) receptor in the luteal phase (9). Progesterone and allopregnanolone exert antioxidant and sedative actions via the GABA-A receptor and do not cause mood changes in healthy individuals (10). However, they may cause hormonal imbalance, increased pro-oxidant activity, oxidative stress, and the disrupted GABAergic system, leading to PMS symptoms (11).

The oxidant/antioxidant balance is disrupted in PMS, associated with the symptom occurrence (12). Psychiatric problems, usually major depression and anxiety, are among the symptoms that frequently occur in individuals with PMS (13), and increased levels of serum superoxide dismutase and malondialdehyde, as well as decreased levels of ascorbic acid, levels have been noted in major depression (14). Additionally, impaired immune functions and decreased cytokine levels have been reported in individuals with anxiety, associated with increased cortisol secretion and increased oxidative stress (15).

Sleep disturbance and poor sleep quality are also frequently observed in individuals with PMS, particularly at the end of the luteal phase (16). Additionally, PMS has been associated with high levels of malondialdehyde, which is considered an indicator of oxidative stress, and low glutathione and glutathione peroxidase levels (11). However, it has been reported that oxidative stress markers are not altered in healthy individuals with sleep disorders (17).

It has been emphasized that zinc (Zn) metabolism should be taken into account in PMS because of its antioxidant properties and roles in progesterone binding, prolactin secretion, opiate effect, gonadal secretion, and menstrual cycle regulation (18). Long-term insufficient Zn intake decreases Zn levels in the body and results in impaired glucocorticoid production. This is often associated with neurological symptoms such as irritability, emotional instability, and depression, common in PMS (11).

PMS or premenstrual symptoms have been associated with the inflammatory response (19). In women of childbearing age, the levels of inflammatory mediators increase after ovulation and reach the highest levels during menstruation (20). Elevated C-reactive protein levels lead to more severe PMS symptoms, particularly mood and pain symptoms (21). Zn is also defined as an anti-inflammatory agent, and it has been suggested to relieve PMS symptoms by regulating the levels of high-sensitivity C-reactive protein (22). It has also been reported to exhibit antidepressant activity by upregulating the gene expression of neurotrophic factors (23). In a previous study, 30 mg/day Zn supplementation for 12 weeks had favorable effects on physical and psychological symptoms and total antioxidant capacity (TAC) in individuals with PMS (22).

The relationship between Zn levels/deficiency and TAC levels has not been evaluated sufficiently in individuals with PMS. Only one study reported favorable effects of 30 mg/day Zn supplementation for 12 weeks on symptoms and TAC in PMS (22). Therefore, more research on serum Zn and TAC in PMS are necessary to find relationships between these and symptoms.

This study aimed to determine the serum TAC levels, Zn levels, and their relationship with nutritional intake in PMS.

Methods

This case-control study was conducted between May and July 2019 on university students aged between 18 and 28 years. The exclusion criteria included the use of antidepressants, receiving hormone support therapy, and oral contraceptives, receiving nutritional support, and a diagnosis of menstrual irregularity and polycystic ovary syndrome.

The study was approved by the Zekai Tahir Burak Clinical Research Ethics Committee (38/2019 - 14/05/2019). All procedures were performed in compliance with the Helsinki Declaration. Additionally, all patients were informed about the study, and written consent was obtained.

Data collection

Survey form

Menstrual cycle characteristics (e.g., first menstrual age, length of the menstrual cycle, and menstrual pattern), eating habits (number of meals, increased/decreased food consumption) related to the menstrual period, and preference of any food/drink for relaxation of the symptoms were evaluated by face-to-face interviews using a survey form.

Anthropometric measurements

The body weight was measured on an empty stomach, without shoes, and in light clothes. Height was measured using a stadiometer with the individual standing feet closed, head in the Frankfurt plane (eye triangle and top of auricle aligned, parallel to the ground). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (meter). Individuals with a BMI of 18.5 to 24.9 kg/m² were classified as normal weight, 25.0 to 29.9 kg/m² as overweight, 30.00-34.99 kg/m^2 as type 1 obese, and 35.00-39.99 kg/m^2 as type 2 obese (24).

Premenstrual syndrome scale

The PMS status was evaluated using a PMS scale developed and validated by Gençdoğan (25) in Turkey according to The Diagnostic and Statistical Manual of Mental Disorders-III (DSM) and DSM-IV-R criteria. It is a five-point Likert-type scale that consisted of 44 items. Items are scored 1 for a response of “not any/never’’, 2 for “very little”, 3 for “sometimes”, 4 for “frequently” and 5 for “most times’’. The highest score is 220 and scores of 111 or higher indicate PMS.

International physical activity questionnaire (short form)

The international physical activity questionnaire-short form (IPAQ-SF) is a validated tool to evaluate physical activity levels in our study (26).

IPAQ-SF quantifies physical activity during the last 7 days in four categories: Vigorous intensity, moderate intensity, walking, and sitting. In addition to intensity, frequency and duration of physical activity are assessed. The sum of duration (minutes) and frequency (days) of walking, moderate-intensity, and vigorous activity is used to calculate the total score. A standard metabolic equivalent (MET) value is calculated and the required energy is calculated using the MET-minute scores. The following MET values were considered in the current study: Walking=3.3 METs, moderate physical activity=4.0 METs, vigorous physical activity=8.0 METs, and sitting=1.5 METs. Daily and weekly physical activity levels are evaluated using these values.

Biochemical analyses

Blood samples were taken on the 21st day of the menstrual cycle. Serum Zn level was measured using a commercial test kit (Relassay, Turkey) with atomic absorption spectrometry. The Zn in the sample changes color from the red-orange color of 5-Br-PAPS to light pink under alkaline conditions. The change in absorbance at 548 nm is proportional to the total Zn level in the sample. The calibration was performed with Zn sulfate dissolved in deionized water. A serum Zn level <10.7 µmol/L was considered Zn deficiency (27).

Serum TAC level was measured using a commercial kit (Relassay, Turkey). The antioxidants in the sample convert the dark blue-green ABTS radical solution to the colorless ABTS form. Thus, the change in absorbance at 660 nm is proportional to the total number of antioxidants. The kit was calibrated using a stable antioxidant standard called Trolox equivalent, similar to vitamin E. The results were expressed in mmol/L Trolox equivalents (28).

Evaluation of nutritional status

Twenty-four-hour dietary recall was used to determine the daily nutrient intake. Food consumption records were evaluated using the Nutritional Information System Package Software (Bebispro for Windows, Stuttgart, Germany; Turkish version, 2010), and the mean daily dietary intake of energy and macro- and micronutrients were determined (29).

Determination of total dietary antioxidant capacity

A food consumption frequency form was used to determine the amounts of dietary antioxidant intake calculated using the antioxidant database of foods created by the ferric reducing antioxidant power (FRAP) assay method by Carlsen et al. (30). The FRAP method is simple, fast, and inexpensive. Moreover, it ensures the optimization of the extracts in the determination of both lipophilic and hydrophilic antioxidants (30).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics for Mac, version 22.0 (IBM Corp., Armonk, NY) was used for the statistical analysis. The normality distribution was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics were presented as number (n), percentage, and mean±standard deviation (SD). Student's t-test was used to evaluate differences between age, BMI, menarche age, duration/length of menstruation, serum TAC and Zn levels, and physical activity MET values of individuals with and without PMS. Pearson's chi-square test was used to compare the difference in the incidence of Zn deficiency between the two groups. The effect of nutrient intake on serum TAC and Zn levels was evaluated using multiple linear regression analysis. The level of significance was determined to be $p<0.05$.

Results

The study population consisted of 110 individuals (mean±SD age: 20.3±1.9 years), 48 subjects with PMS, and 62 subjects without PMS. Mean BMI was 21.8±3.1 kg/m^2, age at menarche was 13.3±1.1 years and the duration of menstruation was 5.7±1.4 days in the whole sample. Of the study sample, 8.4% were underweight, 74.8% were normal weight, 15.0% were overweight, and 1.8% were obese. Mean serum TAC and Zn levels were 1.5±0.3 mmol/L vs. 20.9±25.2 µmol/L, respectively.

Compared with the group without PMS, individuals with PMS had higher BMI (23.7±2.7 vs. 21.3±3.4 kg/m^2, $p<0.05$) and lower serum TAC level (1.4±0.1 vs. 1.6±0.4 mmol/L, $p<0.05$), Zn level (11.6±6.4 vs. 28.0±31.4 µmol/L, $p<0.05$) and physical activity MET values (1051.5±931.3 vs. 1315.5±825.1 min, $p<0.05$). There was no difference between the two groups in terms of age, age at menarche, and duration of menstruation (Table 1).

Most individuals in both groups (92.3% with PMS vs. 91.8% without PMS) consumed two meals and two snacks a day. However, when changes in eating habits (food consumption increased or decreased) during the menstrual period were evaluated, 42.7% of them stated a change in eating habits, and it was more common in individuals with PMS than without PMS (54.2% vs. 33.9%, $p<0.05$) (Table 2).

As was also shown in Table 2, 52.1% of the individuals with PMS and 33.8% of those without PMS preferred consuming a particular food during their menstrual period for symptom relief, and 67.4% of them reported chocolate intake. Also, 25.0% of the individuals with PMS and 17.7% of the individuals without PMS consumed a particular drink during the menstrual period for symptom relief, and 56.5% of them preferred herbal tea. Additionally, the percentage of individuals with PMS taking a nutritional supplement during the menstrual period was higher than without PMS (40.3% vs. 29.2%, $p<0.05$) (Table 2).

Individuals with PMS had a lower intake of energy, protein, fat, fiber, polyunsaturated fatty acids (PUFA), vitamin E, vitaminB_1,

vitamin B_2, vitamin B_6 and vitamin C, folate, magnesium (Mg), and higher intake of carbohydrate (CHO) and sodium than individuals without PMS, but the differences were not significant (Table 3).

In individuals with PMS, serum TAC level was correlated with dietary antioxidant and PUFA intakes and negatively correlated with the percentages of dietary energy from CHO, protein, and fat (Table 4).

No significant relationship was found between serum TAC level and nutrient intake in individuals without PMS.

Zn deficiency was observed in 34.5% of the individuals, which was higher in individuals with PMS than without PMS (47.9% vs. 24.2%, respectively, $p<0.05$). Additionally, serum Zn levels were negatively correlated with the percentages of dietary energy from CHO, protein, and fat in individuals with PMS (Table 5).

Discussion

The exact pathophysiology of PMS remains unknown. It has been suggested that micronutrients improve PMS symptoms through neurotransmitters and hormones, but only a few studies have been performed in this field (31,32). Therefore, we hypothesized that individuals with PMS could have lower serum Zn and TAC.

Conditions such as sleep disorders, increased anxiety, and depression that are commonly reported in obese individuals, are among the symptoms observed in individuals with PMS, and that obesity is a potential risk factor for PMS (33). Furthermore, studies have shown that the prevalence of PMS in obese individuals is twice as high as in non-obese and the importance of appropriate body weight for treating PMS has been demonstrated (33-35). In this study, a significant difference in BMI was observed based on the PMS status and the BMI values of individuals with PMS were higher, which is consistent with the literature.

Table 1. General characteristics of the individuals based on PMS

Variables		n	$\bar{X}$	Levene's F-test	Levene's P-test	p value (two-tailed)
Age (years)	PMS (+)	48	20.6441	6.835	0.010	0.245
	PMS (-)	62	20.1823			
Body mass index (kg/m^2)	PMS (+)	48	23.7317	0.207	0.650	**0.036**
	PMS (-)	62	21.3073			
Age at menarche (years)	PMS (+)	48	13.4029	1.989	0.161	0.345
	PMS (-)	62	13.2378			
Duration of menstruation (days)	PMS (+)	48	5.6678	4.413	0.038	0.701
	PMS (-)	62	5.7574			
Serum TAC (mmol/L)	PMS (+)	48	1.4671	7.469	0.007	**0.019**
	PMS (-)	62	1.6021			
Serum Zn level (µmol/L)	PMS (+)	48	11.6792	14.766	<0.001	**<0.001**
	PMS (-)	62	28.0435			
Physical activity MET value (min)	PMS (+)	48	1051.51	1.450	<0.001	**0.020**
	PMS (-)	62	1315.56			

MET: Metabolic equivalent, TAC: Total antioxidant capacity, Zn: Zinc, PMS: Premenstrual syndrome

Table 2. Eating habits of individuals

Variables	PMS (+)				PMS (-)				p
	Yes		No		Yes		No		
	n	%	n	%	n	%	n	%	
Do your eating habits change during your menstrual period?	26	54.2	22	45.8	21	33.9	41	66.1	**0.033**
Is there a particular food you prefer to consume during your menstrual period for relaxing symptoms?	25	52.1	23	47.9	21	33.8	41	66.2	0.453
Is there a particular drink you prefer to consume during your menstrual period for relaxing symptoms?	12	25.0	36	75.0	11	17.7	51	82.3	0.444
Is there any nutritional support you take during your menstrual period?	25	40.3	37	59.7	14	29.2	34	70.8	**0.017**

PMS: Premenstrual syndrome

We observed that changes in eating habits during the menstrual period were more common in individuals with PMS than those without PMS. In the PMS group, 80.1% indicated that their food intake increased during the premenstrual period. Our finding that CHO consumption was higher in this group also confirms this data. Accordingly, increased BMI was previously proposed as a risk factor for PMS (36), and our findings showed that food intake might also increase in individuals with PMS, which may also contribute to this risk. Most individuals (86.0%) stated that chocolate was a particular food they preferred during the menstrual period. The increased desire to consume chocolate may be due to the increased need for substances such as Mg and serotonin due to the physiological changes during this period or its effect like creating a sense of pleasure through the endocannabinoid system (37). It has also been suggested that food intake increases depending on the change in serum steroid level and basal metabolic rate during this period (38). In PMS, nutritional consultation seems to be a crucial component of maintaining appropriate body weight and improvement of symptoms.

One of the most critical findings of individuals with PMS is the change in oxidative balance. Many hypotheses have been proposed regarding the underlying mechanism. Although there are contradictory studies, it has been confirmed that estrogen, which has pro-oxidant properties, causes serum TAC levels to decrease and oxidant levels to increase in individuals with PMS (39-41). In a previous study, the levels of oxidant F2-isoprostane were higher in PMS which were linked to symptom severity (42).

We observed lower serum TAC levels in individuals with PMS, and serum TAC levels were correlated with dietary antioxidants and PUFA intake. Individuals with PMS should pay attention

Table 3. Mean nutrient intake of individuals based on PMS

Variable		n	$\bar{X}$	SD	p value
Energy (kcal/day)	PMS (+)	48	949.4	434.01	0.752
	PMS (-)	62	972.9	311.61	
Protein (g/day)	PMS (+)	48	43.8	15.31	0.928
	PMS (-)	62	44.0	9.78	
CHO (g/day)	PMS (+)	48	118.5	42.64	0.421
	PMS (-)	62	112.5	34.87	
Fat (g/day)	PMS (+)	48	47.5	14.98	0.067
	PMS (-)	62	58.5	38.94	
Fibre (g/day)	PMS (+)	48	14.4	6.26	0.316
	PMS (-)	62	24.1	66.65	
PUFA (g/day)	PMS (+)	48	13.0	20.01	0.466
	PMS (-)	62	18.4	48.27	
Vitamin E (mg/day)	PMS (+)	48	6.6	2.85	0.076
	PMS (-)	62	8.0	4.89	
Vitamin B_1 (mg/day)	PMS (+)	48	1.5	1.26	0.331
	PMS (-)	62	4.3	20.03	
Vitamin B_2 (mg/day)	PMS (+)	48	0.8	0.48	0.606
	PMS (-)	62	0.9	1.01	
Vitamin B_6 (mg/day)	PMS (+)	48	17.1	21.29	0.321
	PMS (-)	62	57.9	282.58	
Vitamin C (mg/day)	PMS (+)	48	97.1	185.93	0.616
	PMS (-)	62	118.6	245.44	
Folate (mcg/day)	PMS (+)	48	183.3	81.28	0.517
	PMS (-)	62	205.6	226.60	
Magnesium (mg/day)	PMS (+)	48	244.0	308.88	0.771
	PMS (-)	62	264.5	403.01	
Sodium (mg/day)	PMS (+)	48	2059.2	798.45	0.312
	PMS (-)	62	1921.5	623.75	

CHO: Carbohydrate, PUFA: polyunsaturated fatty acid, PMS: Premenstrual syndrome, SD: Standard deviation

to taking fresh vegetables and fruits with higher antioxidant content in their diet. In addition, the importance of the adequacy of vegetable oils, oil seeds, and oilseeds should be emphasized as they can increase the endogenous antioxidant enzyme activity. Moreover, serum TAC levels are associated with the dietary pattern, and serum antioxidant capacity decreases as the percentages of dietary energy from CHO, protein, and fat increase in individuals with PMS. These findings should be considered in the food patterns of individuals with PMS.

It has been shown that as the percentage of energy derived from CHOs increases, adverse mood and behavioral changes occur more often in healthy women with a regular menstrual cycle (22).

Some micronutrient deficiencies may also be associated with PMS (43). Moreover, it has been indicated that serum Zn levels change during the menstrual cycle and are significantly lower in individuals with PMS than in individuals without PMS (22). This may lead to irregular glucocorticoid production and some neuropsychological symptoms (44).

In the current study, 34.5% of the individuals had Zn deficiency, which was significantly more common in individuals with PMS than in those without PMS. Furthermore, it was found that serum Zn levels were negatively correlated with the dietary percentage of carbohydrate. This finding may be explined thorough the lower zinc content of foods with high carbohydrate content.

In our findings, individuals with PMS also had lower MET values and lower levels of physical activity than individuals without PMS. A study found that 8-week aerobic exercise effectively reduces PMS symptoms (45). The incidence of PMS

Table 4. Multiple linear regression analysis of the effect of nutrient consumption on serum TAC level based on PMS status

Variable	PMS status	Beta	t	p	95% confidence interval	
Energy (kcal/day)	PMS (+)	−2.683E−005	−0.462	0.647	0.000	0.000
	PMS (-)	−2.991E−005	−0.105	0.917	−0.001	0.001
CHO (%)	PMS (+)	−0.029	−2.625	**0.013**	−0.052	−0.007
	PMS (-)	0.004	.299	0.766	−0.023	0.032
Protein (%)	PMS (+)	−0.029	−3.008	**0.005**	−0.049	−0.010
	PMS (-)	−0.011	−0.222	0.825	−0.110	0.088
Fat (%)	PMS (+)	−0.029	−2.047	**0.048**	−0.057	0.000
	PMS (-)	0.004	0.253	0.802	−0.026	0.033
PUFA (g/day)	PMS (+)	0.010	2.488	**0.017**	0.002	0.019
	PMS (-)	−4.620	0.000	1.000	−0.004	0.004
Dietary antioxidant amount (mmol/day)	PMS (+)	0.011	2.060	**0.046**	0.000	0.021
	PMS (-)	0.037	1.750	0.086	−0.005	0.079

CHO: Carbohydrate, PUFA: Polyunsaturated fatty acid, PMS: Premenstrual syndrome, TAC: Total antioxidant capacity

Table 5. Multiple linear regression analysis of the effect of nutrient consumption on serum Zn levels based on PMS status

Variable	PMS status	Beta	t	p	95% confidence interval	
Energy (kcal/day)	PMS (+)	0.004	1.103	0.277	−0.003	0.010
	PMS (-)	−0.41	−3.250	**0.002**	−0.066	−0.016
CHO (%)	PMS (+)	−0.861	−1.255	**0.008**	−1.487	−0.235
	PMS (-)	1.324	1.349	0.183	−0.646	3.295
Protein (%)	PMS (+)	−0.205	−0.540	0.592	−0.972	0.562
	PMS (-)	−2.032	−0.936	0.353	−6.387	2.322
Fat (%)	PMS (+)	−0.693	−1.946	0.059	−1.413	0.028
	PMS (-)	−0.616	−0.836	0.407	−2.094	0.862
Dietary Zn intake (mg/day)	PMS (+)	−0.421	−0.459	0.649	−2.276	1.434
	PMS (-)	−5.972	−2.779	**0.008**	−10.286	−1.659

CHO: Carbohydrate, Zn: Zinc, CHO: Carbohydrate, PMS: Premenstrual syndrome

also increases in individuals who engage in low physical activity levels, suggesting exercise as a potential treatment option (46).

Study Limitations

The limitations of this study include the use of a single daily record of 24-h food consumption on the 21st day of menstruation to evaluate the overall effect of diet on PMS. A longer record of food consumption (3-5 days) could provide more accurate information. Additionally, although ovarian hormones play a crucial role in the pathogenesis of PMS and reduced TAC levels (41), blood hormone measurement was not available in the current study.

Conclusion

This study found lower serum Zn and TAC levels and higher BMI in subjects with PMS. Serum TAC levels were related to dietary antioxidant intake and dietary protein, lipid, and CHO intake. The results suggest that nutritional counseling can be a part of PMS management.

Ethics

Ethics Committee Approval: The study was approved by the Zekai Tahir Burak Clinical Research Ethics Committee (numbered: 38/2019, dated: 14/05/2019).

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.K.C., Ö.M., A.G., H.Z., B.Ö., S.A., Concept: T.K.C., Design: T.K.C., Data Collection or Processing: T.K.C., D.A., Ö.M., A.G., H.Z., B.Ö., Analysis or Interpretation: T.K.C., D.A., Literature Search: T.K.C., D.A., Writing: T.K.C., D.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.1531
Gulhane Med J 2022;64:197-200

Multiple rare neoplasms arising from the nevus sebaceous of the scalp: A case report

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Date submitted:
03.12.2020
Date accepted:
22.02.2021
Online publication date:
15.06.2022

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Keywords: Nevus sebaceous Jadassohn, scalp, syringocystadenoma papilliferum

ABSTRACT

Nevus sebaceous of Jadassohn (NSJ) is a cutaneous hamartoma of pilosebaceous origin that differentiates into multiple neoplasms, most commonly trichoblastoma/basal cell carcinoma and syringocystadenoma papilliferum (SCAP). Malignant transformation of NSJ is rare and usually observed in the elderly. However, a diagnosis of more than three separate tumors concurrent to multiple malignancies developing from a single NSJ is extremely rare. We here report a case of a 72-year-old male patient with five distinct tumors arising from NSJ of the scalp, including SCAP, tubular apocrine adenoma, eccrine poroma, apocrine cystadenoma, and cutaneous apocrine carcinoma.

Introduction

Nevus sebaceous of Jadassohn (NSJ), first described by Joseph Jadassohn in 1895, is an organoid nevus of pilosyringosebaceous and adnexal origin that combines sebaceous gland abnormalities with anomalies of the epidermal, follicular, and apocrine glands (1,2).

NSJ typically presents at birth as linear/ovoid plaque most commonly on the face and scalp and progresses through self-differentiation into multiple neoplasms in late adulthood (3-5). However, reports of more than three simultaneous tumors from a single nevus and their malignant transformation are extremely rare (6,7). Here, we describe the features of multiple synchronous benign and malignant neoplasms arising from a long-standing nevus sebaceous of the scalp.

Case Presentation

A 70-year-old male patient was admitted with a complaint of a painful slow-growing ulcer in the right buccal mucosa and swelling in the scalp, which had grown slowly over years to its current size with occasional itching. The patient had a history of chronic tobacco use and betel quid chewing. On physical

examination, a lesion that was irregularly surfaced, ulcerated, and proliferating onto the mandibular alveolar process was detected along with asymptomatic solitary dome-shaped nodular growth measuring approximately 7.5x4.5 cm over the left parietooccipital region of the scalp (Figure 1). The lesion was hyperpigmented and soft to firm. An incisional biopsy of the oral lesion was performed and the histopathological evaluation was consistent with a well-differentiated squamous cell carcinoma (stage IVa). The intra-oral lesion was treated by wide excision with hemimandibulectomy; nerve and vein sparing type of neck dissection and reconstruction of the defect were carried out with pectoralis major myocutaneous flap.

Fine-needle aspiration of the lesion on the scalp was reported as syringocystadenoma papilliferum (SCAP). The lesion on the scalp was surgically excised and the defect was covered with a split-thickness skin graft.

Histopathological examination of the lesion revealed multiple lesions in the primary NSJ, namely SCAP, tubular apocrine adenoma (TAA), eccrine poroma (EP), apocrine cystadenoma (ACA), cutaneous apocrine carcinoma (CAC) and cutaneous apocrine carcinoma, with invasive areas (Figure 2A-G). Immunohistochemical studies ruled out basal cell carcinoma and malignant melanoma. S100 for nerve sheath tumors and malignant melanoma, CK 17 and cytokeratin screening for epithelial origin tumors, and CD 99 for mesenchymal tumors/ soft tissue sarcomas were studied during histopathological evaluations.

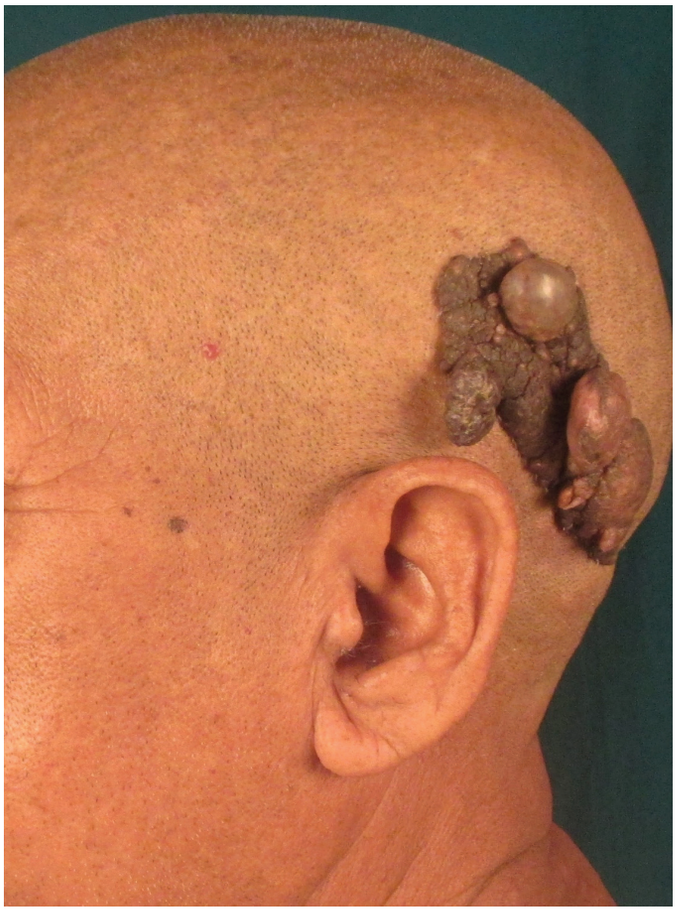

Figure 1. Preoperative lesion on the scalp

Discussion

NSJ follows three phases of its natural history designated by Mehregan and Pinkus (8) as infancy, pubertal stimulation, and post-pubertal histological degeneration; usually appears at birth (0.1-0.3% among newborns) or early childhood as a well-circumscribed, smooth, slightly raised, yellowish-brown alopecic patch that progresses through puberty, assuming a more verrucous texture, possibly due to the expression of various hormonal receptors (4). There have been only a few reported cases in the literature describing the simultaneous development of 5 or more neoplasms in a solitary lesion (2).

SCAPs are the most common hamartoma tumors associated with nevus sebaceous, which originate from the apocrine sweat glands (4). Stavrianeas report an incidence of 5 to 19% of the nevi complicated with SCAP (1). Section from our patient's slides revealed a stratified squamous epithelium with immature sebaceous glands and areas of basaloid hyperplasia along with immature hair structures and dilated ductal infundibulum in the dermis (Figure 2B).

TAA is a minor variant of SCAP consisting of a benign appendage tumor of apocrine origin. TAA and SCAP rarely develop together in the nevus sebaceous, and only a few such cases had been reported in 2004 (9). Sections of our slides showed a well-circumscribed dermal neoplasm comprising lobules of dilated tubules lined by two rows of cuboidal to columnar epithelial cells with eosinophilic cytoplasm and round bland nuclei (Figure 2C).

EP is a benign tumor originating from the epidermal sweat glands and occurs in middle-aged individuals on the sole and hands. However, on the scalp is extremely rare, with 18 cases reported until the year 2012 (10). Sections of our slides showed a downward proliferation of basaloid cells from the epidermis with delicate fibrovascular stroma in the form of anastomosing cords and nests with pigmentation (Figure 2D).

ACA is a rare, benign, solitary adenomatous cystic tumor of the skin. Sections of our slide revealed cystic areas in the dermis lined by columnar cells with small round basal nuclei and abundant eosinophilic cytoplasm arranged in a papillary pattern with a fibrovascular core (Figure 2E).

CAC is a rare malignancy with unknown etiology and only eight cases are reported in 2012 (6). We found many layers of pleomorphic cells with irregular hyperchromatic nuclei, prominent nucleoli, and abundant granular eosinophilic cytoplasm (Figure 2F). There was the presence of atypical mitoses, decapitation secretion, and tumor tissue invasion into the papillary and reticular dermis (Figure 2G).

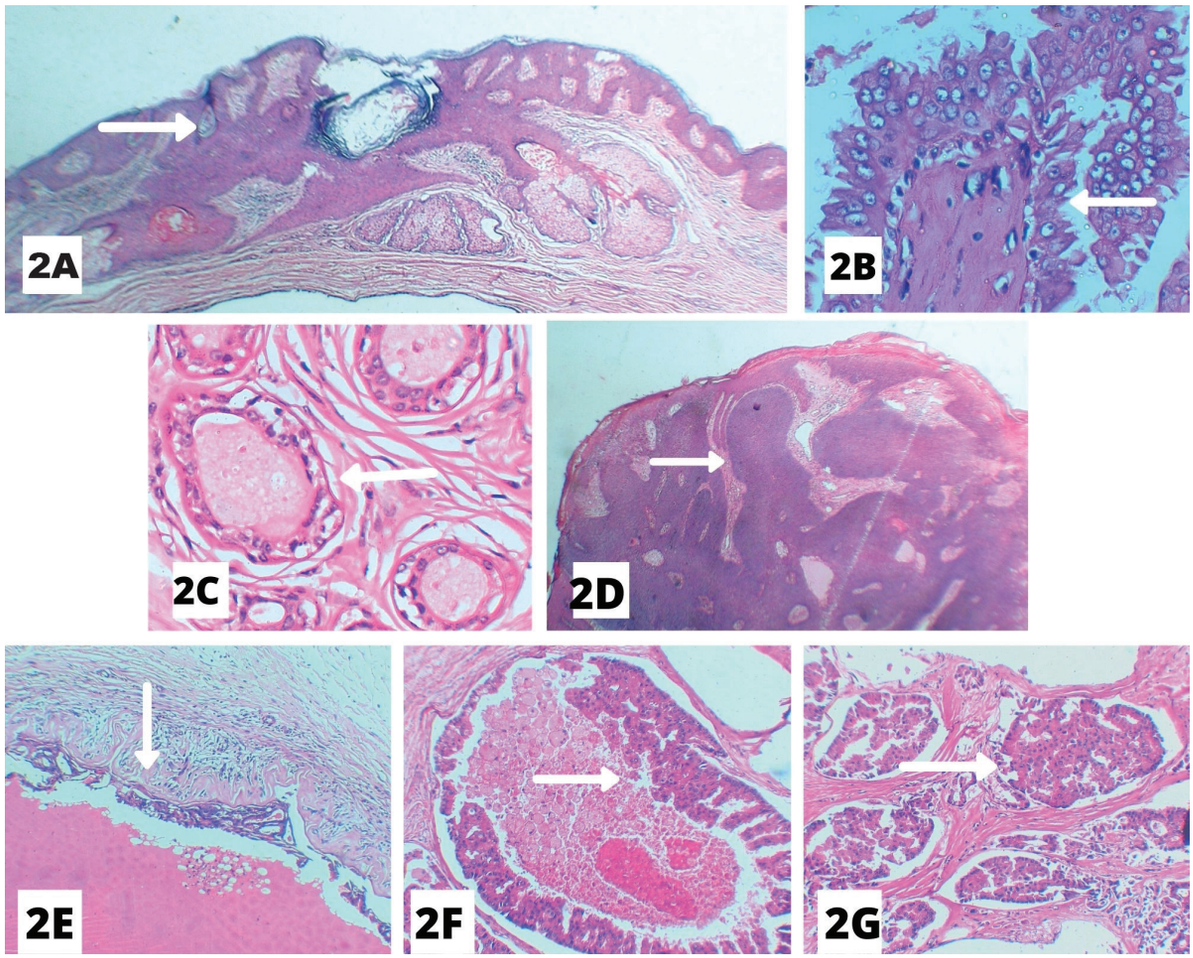

Figure 2. Multiple neoplasms arising from nevus sebaceous of Jadassohn. A: Nevus sebaceous of Jadassohn: [Haematoxylin and eosin (H&E)-stained photomicrograph 30x magnification] of nevus sebaceous of Jadassohn showing immature sebaceous glands, multiple heterotopic apocrine glands, defective hair follicles, and epithelial papillomatosis with hyperkeratosis. B: Syringocystadenoma papilliferum (SCAP): (H&E-stained photomicrograph 100x magnification) of SCAP showing connective tissue composed of glandular and ductal papillary proliferation and dense plasma cell infiltration. C: Tubular apocrine adenoma (TAA): (H&E-stained photomicrograph 100x magnification) of TAA comprised of variably sized intradermal nodules with tubular structures lined by 1-2 layers of cuboidal cells with eosinophilic cytoplasm and round bland nuclei. D: Eccrine poroma: (H&E-stained photomicrograph 30x magnification) of eccrine poroma showing dense basaloid proliferation into the dermis and connective tissue with reactive vessels and chronic inflammatory exudates. Islands of ductal structures were also noted. E: Apocrine cystadenoma (ACA): (H&E-stained photomicrograph 30x magnification) of ACA showing abundant intradermal cystic areas and papillary apocrine metaplasia. The immature glandular structures appear to be lined by a layer of columnar cells with abundant eosinophilic cytoplasm with decapitation secretion noted in multiple areas. F: Cutaneous apocrine carcinoma (CAC): (H&E-stained photomicrograph 100x magnification) of CAC: showing irregular layers of pleomorphic cells with an irregular hyperchromatic nucleus and abundantly granular cytoplasm. Atypical mitoses also were noted. G: CAC: (H&E-stained photomicrograph 100x magnification) with invasive areas and invasion into deeper layers of the dermis

Conclusion

The current case is in agreement with most authors who have suggested that NSJ should be surgically excised, histologically examined, and should be kept on a close follow-up to prevent malignant transformation.

Ethics

Informed Consent: Informed written consent has been obtained from the patient for publication.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.S., A.D., N.K., D.U.S., A.A., Concept: D.S., A.D., N.K., D.U.S., Design: D.S., A.D., Data Collection or Processing: D.S., A.A., S.B., Analysis or Interpretation: D.S., A.D., N.K., D.U.S., A.A., Literature Search: D.S., S.B., Writing: D.S., S.B.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.02996
Gulhane Med J 2022;64:201-3

Treatment of pediatric patients with Down syndrome, acute lymphoblastic leukemia, and hepatitis C infection by direct-acting antivirals

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Date submitted:
22.02.2021
Date accepted:
29.07.2021
Online publication date:
15.06.2022

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Keywords: Direct-acting antiviral, Down syndrome, hepatitis C infection, children

ABSTRACT

Millions of people worldwide suffer from hepatitis C, which can lead to hepatitis, cirrhosis, and hepatocellular carcinoma. The risk of hepatitis C virus (HCV) infection is increased in patients with leukemia due to the frequent need for blood transfusion. Owing to their proven effectiveness in treating HCV infection, direct-acting antivirals (DAAs) have become a new treatment approach in children older than 12 years. The patient present in this article is a 9-year-old boy who had acute lymphoblastic leukemia and Down syndrome, who was diagnosed with hepatitis C infection and was successfully treated with a combination of DAA glecaprevir (250 mg/day) and pibrentasvir (100 mg/day) over 8 weeks.

Introduction

Hepatitis C virus (HCV), a small, enveloped, single-stranded positive-sense RNA virus from the Flaviviridae family, is one of the chronic viral hepatitis agents in humans (1). Severe complications, such as portal hypertension, cirrhosis, and hepatocellular cancer, may occur over time in patients with chronic hepatitis C infection (2).

Down syndrome is the most common chromosomal disease characterized by three copies of chromosome 21 and the frequency of leukemia in patients with Down syndrome is higher compared to the general population (3). The risk of HCV infection is increased in patients with leukemia due to the frequent need for blood transfusion and immunosuppressive treatments (3).

According to the classical guidelines, the first-line treatment of hepatitis C in children is the combination of pegylated

interferon and ribavirin (4). Since these drugs have dreadful side effects, such as aggravated cytopenia causing deep immune dysfunction, bone marrow suppression, hepatotoxicity, and the treatment response rates are relatively low, nowadays direct-acting antivirals (DAAs) have started to be administered for treating adult patients with hepatitis C infection. Recently, the possible side effects of DAAs have been minimized and cure rates have reached 90-97% (5). The Food and Drug Administration approved the use of the combination of ledipasvir/sofosbuvir and the combination of sofosbuvir and ribavirin for the treatment of adolescents with chronic HCV infections (12-17 years). Although trials with DAAs are ongoing for younger children, the only available treatment in the US and Europe for those <12 years is still the dual therapy of pegylated interferon and ribavirin. The next generation, ribavirin-free, DAA combinations (i.e. sofosbuvir/velpatasvir and glecaprevir/pibrentasvir) demonstrate high efficacy with a shorter duration of treatment (6).

In this article, we present a 9-year-old boy who had Down syndrome, leukemia, and HCV infection and whose HCV infection was successfully treated with DAAs including a combination of glecaprevir and pibrentasvir.

Case Presentation

A 9-year-old boy with had Down syndrome and ALL was on maintenance therapy with methotrexate and 6-mercaptopurine and his treatment was interrupted for 2 weeks due to the detection of high transaminase levels at a routine examination. However, despite the interruptions of treatment, transaminase elevations continued in the examinations. There was no known history of heart, liver disease, and hypothyroidism. There was a history of multiple blood product transfusions because of leukemia-related problems.

The patient was referred to the pediatric gastroenterology department for the evaluation and treatment of hypertransaminasemia. After detailed investigations, he was diagnosed with hepatitis C infection. Anti-HCV antibody was positive and HCV RNA was 20.790.000 IU/mL. Abdominal ultrasound revealed enlarged liver and grade 2 hepatosteatosis. Liver biopsy was performed for assessing the stage and degree of activity in hepatitis C. Liver biopsy showed prominent secondary hemochromatosis, moderate lymphocyte infiltrates scattered to sinusoids, and reactive changes in hepatocytes, apart from partial degeneration and diffuse apoptosis. Genotype analysis showed genotype 3a. Other tests used for detecting causes of transaminase elevation yielded negative results.

The patient received glecaprevir (250 mg/day) and pibrentasvir (100 mg/day) combination for 8 weeks in October-November 2019 to treat HCV infection. Serum transaminase levels decreased to normal values two weeks after the onset of treatment, and the virus became undetectable in the blood after 8 weeks.

At the last follow-up visit, 10 months after completion of DAA treatment, the patient continued to show negative HCV RNA. An off-label prescription was approved for the use of DAAs and an informed consent form was obtained from the patient's family.

Discussion

There are currently approximately 11 million children under the age of 15 with HCV infection worldwide (7). Approximately 20% of infected people have self-limited acute hepatitis, but in the remaining 80%, the virus is not cleared, which leads to a chronic HCV infection (8).

Until recently, the treatment of pediatric patients with HCV infections was controversial because pegylated interferon and ribavirin used in the treatment of HCV cause major side effects such as severe neutropenia, immune system dysfunction, growth impairment, hepatotoxicity, and poor tolerability (7,9). Both the side effects and the low success rates of the treatment have led to the development of new-generation drugs. Currently, DAAs show a success rate of over 95% in the treatment of hepatitis C. DAAs target specific nonstructural proteins of the virus, resulting in the disruption of viral replication and thereby infection (8).

Although DAAs have been routinely used in adult patients, data on the use of these drugs is very limited in children, especially among those younger than 12 years of age. The investigation of the efficacy of DAAs for treating pediatric patients older than 12 years with HCV infection yielded similar results with adult patients (6). DAA therapy has several advantages over pegylated interferon therapy and it is used orally for up to 8 weeks (8).

There are no definitive guidelines for treating children 3-11 years of age with HCV infections. In children younger than 12 years of age, the decision to start treatment should be individualized to patients based on the HCV genotype, the severity of liver disease, the potential for side effects, the possibility of response, and the presence of co-morbidities. The possible off-label use of DAAs may be considered for treating children younger than 12 years with chronic HCV infection (6). We treated our patient successfully with a DDA over 8 weeks, which is a fixed-dose combination of an HCV NS3/4A protease inhibitor (glecaprevir) and an HCV NS5A inhibitor (pibrentasvir).

Conclusion

Our experience shows that treatment with DAAs may be used off-label in HCV-infected children under the age of 12 years. The advantages of treatment with DAAs over pegylated interferon and ribavirin therapy include shorter duration of treatment, higher effectiveness, and a lower rate of side effects.

Ethics

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.G., N.B., Concept: A.S., O.G., N.B., Design: M.A., O.G., N.B., Data Collection or Processing: A.S., Analysis or Interpretation: A.S., M.A., Literature Search: A.S., M.A., Writing: A.S., M.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

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DOI: 10.4274/gulhane.galenos.2021.96168
Gulhane Med J 2022;64:204-7

Diagnosis and treatment of constrictive pericarditis: From chest X-ray to pericardiectomy or anti-inflammatory therapy

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Date submitted:
25.08.2021
Date accepted:
18.10.2021
Online publication date:
15.06.2022

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Keywords: Constrictive pericarditis, pericardiectomy, transient constriction, anti-inflammatory drug therapy

ABSTRACT

Constrictive pericarditis (CP) consists of pericardial fibrotic thickening and calcification and causes the impaired diastolic filling of the ventricles. Echocardiography and other imaging modalities and left-right cardiac catheterization are used for diagnosis. The standard treatment for chronic CP is pericardiectomy. However, in the transient form of the disease, anti-inflammatory therapy should be the primary treatment option. In two cases of CP, the diagnostic process and the importance of distinguishing the chronic form of the disease from the transient form, and the difference in the two treatment modalities have been highlighted.

Introduction

Constrictive pericarditis (CP), a serious, often a difficult diagnosis that causes heart failure, is associated with thickening and calcification of the pericardial layers, impairing the diastolic functions of the heart (1). Chest X-ray, transthoracic echocardiography (TTE), cardiac computed tomography (CT) and magnetic resonance imaging (MRI), and left-right cardiac catheterization are used for diagnosis (1).

The standard treatment for chronic CP is pericardiectomy (1). In the transient form of the disease, anti-inflammatory therapy is the primary treatment option (2). In these two case reports, we want to pay attention to the diagnostic process and the importance of distinguishing the chronic form of the disease from the transient form, and the difference between the two treatment modalities.

Case Presentations

Case 1

A 32-year-old male patient was admitted with shortness of breath. Physical examination revealed pretibial edema, and increased jugular venous pressure. On admission, the electrocardiogram showed atrial fibrillation (AF), and the chest X-ray revealed pericardial calcification (Figure 1A). TTE examination showed normal biventricular function, moderate to mild mitral and tricuspid regurgitation, the diastolic bounce of the interventricular septum, and >25% variation in mitral inflow with respiration, mitral annular septal-lateral é reversal, and increased pericardial thickness (Figure 1B, 1C). Cardiac CT showed diffuse calcification of the pericardium, and the calcific plaques were 6 mm at the thickest part (Figure 1D). The patient was diagnosed with CP, but he refused surgery. On a phone

visit 6 months later, the patient was learned to have undergone pericardiectomy and was clinically better.

Case 2

A 50-year-old female patient was admitted with dyspnea and palpitations. We learned that the patient was a refugee and had a history of pericarditis and pericardiocentesis 6 months ago in his own country. In laboratory examination, hepatic and renal function and albumin levels were normal but brain natriuretic peptide 950 pg/mL, C-reactive protein 58 mg/L, white blood cell count 12.000/μL. The TTE of the patient revealed normal biventricular function, moderate atrial dilatation, marked dilation of the inferior vena cava (25 mm), and increased thickness of the pericardium (Figure 2A). Also, diastolic bounce of the interventricular septum, >25% variation in mitral inflow with respiration, and mitral annular septal-lateral é reversal, as indicators of ventricular interdependence, were observed (Figure 2B, 2C). Cardiac CT showed increased pericardial thickness and minimal calcification (Figure 2D). In the catheterization, left ventricular and right ventricular end-diastolic pressures equalized and increased, right atrial pressure increased, X and Y descent became evident, and there was respiratory discordance (Figure 2E, 2F). Since the patient had a previous history of pericarditis and pericardiocentesis and pericardial calcification was more limited, a follow-up was planned with anti-inflammatory therapy to rule out a transient constriction. In addition to standard medical therapy, the patient was prescribed oral corticosteroid. The patient showed clinical and TTE improvements at the 3-month follow-up.

Discussion

In these two case reports, we intended to draw attention to some significant differences in the diagnosis and treatment of CP. In particular, we emphasized that, after the diagnosis of CP is made, the transient form of the disease should be distinguished. In addition, CP can successfully be treated with anti-inflammatory therapy without the need for pericardiectomy.

CP is characterized by abnormal diastolic filling patterns and low cardiac output. The most common clinical findings are shortness of breath, edema, and increased jugular venous pressure. Also, hepatomegaly, pleural effusions, ascites, and hypoalbuminemia may occur in more advanced cases.

Chest X-ray, TTE, cardiac CT and MRI, and left-right cardiac catheterization are used for the diagnosis of CP (1). Chest X-ray can be an initial diagnostic test that can detect pericardial calcifications (3). The essential pathophysiologic mechanism in CP is the dissociation of intrathoracic and intracardiac pressures and ventricular interdependence within a fixed space.

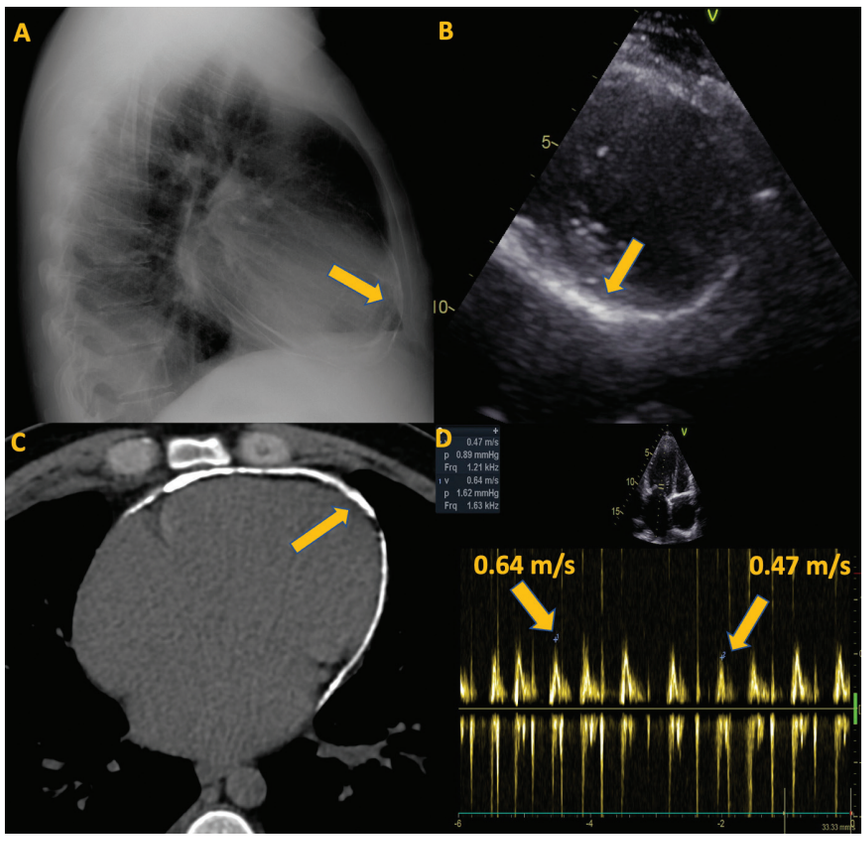

Figure 1. A-C: Chest X-ray, computed tomography, and echocardiographic images of increased pericardial thickness and calcification. D: >25% variation in E mitral inflow wave with respiration on echocardiography

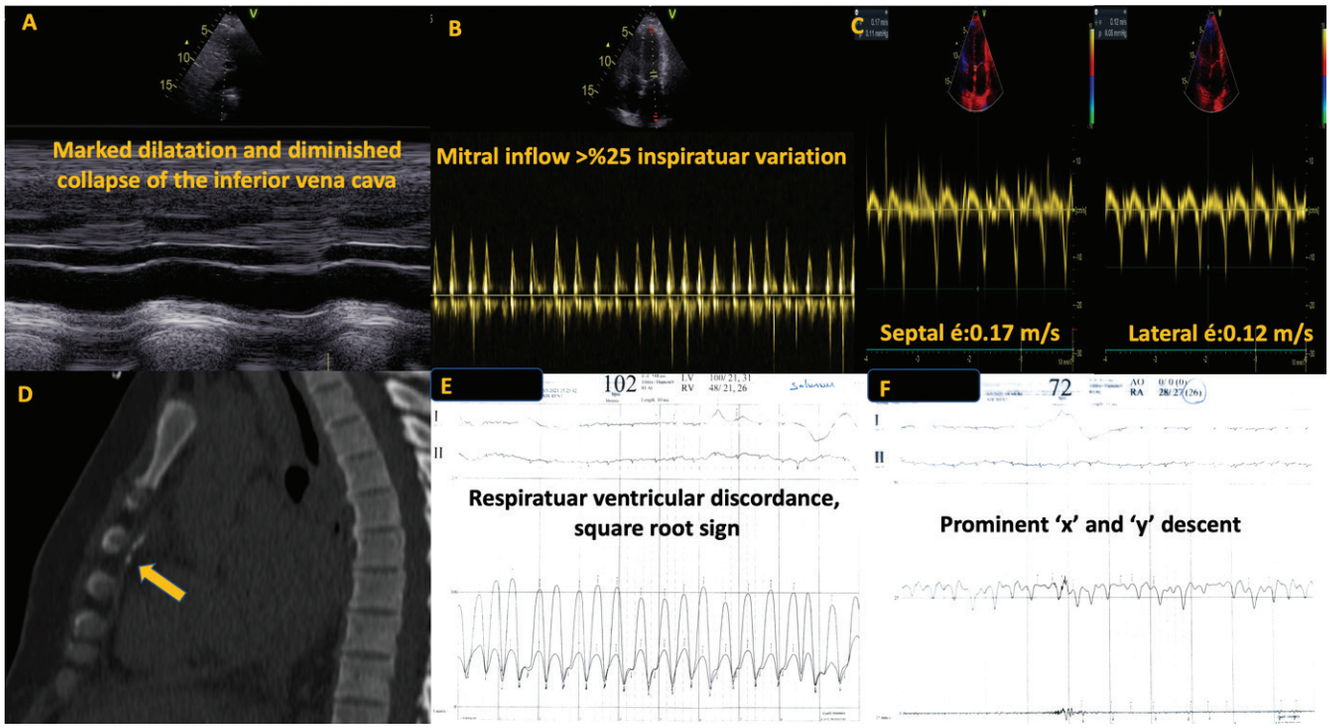

Figure 2. A, B: Transthoracic echocardiography showed >25% variation in mitral inflow with respiration, dilation of the inferior vena cava, and diminished collapse. C: Mitral annular septal-lateral é reversal. D: Thorax computed tomography showed increased pericardial thickness and minimal calcification. E, F: In the left and right heart catheterization, left ventricular and right ventricular end-diastolic pressures equalized and increased, right atrial pressure increased, X and Y descent became evident, and respiratory discordance

Through these pathophysiologic mechanisms in TTE, the abnormal septal motion might have increased the respiratory variation of mitral inflow velocity, and also reversed the mitral annulus septal-lateral velocity (4). In addition to TTE, CT is a valuable imaging modality, especially in the evaluation of pericardial calcifications (5). With the development of newer imaging methods, there has been a shift to invasive imaging tools, particularly when CP is suspected. However, cardiac catheterization remains a diagnostic tool, particularly when other imaging tools are inconclusive (6).

While it is critical to distinguish the disease from restrictive cardiomyopathy before the diagnosis of the disease, it is important to distinguish the disease in its transient form after the diagnosis of CP. The transient constriction is a typical clinical course that implies the presence of acute inflammatory pericarditis with constriction due to inflammation, which resolves once the inflammatory process is treated. Thus, conservative treatment may be conducted for 2 to 3 months before recommending pericardiectomy in the absence of signs that the problem is chronic (for example, cachexia, AF, hepatic dysfunction, or pericardial calcification) (2). Also, CT and MRI can be used to detect pericardial inflammation to diagnose transient constriction (5).

The standard treatment for chronic CP is pericardiectomy (1). However, pericardiectomy has significant perioperative mortality ranging from 6 to 12% (1,7). Therefore, pericardiectomy should be carefully evaluated in patients with left ventricular systolic dysfunction, severe renal and hepatic failure, cachexia, hypoalbuminemia, and patients with radiation-induced CP (1,2). Considering this information, we recommended pericardiectomy in our first case because the patient was young, did not have cachexia, and had low comorbidities.

In the transient form of the disease with concomitant evidence of pericardial inflammation and increased inflammatory markers, empiric anti-inflammatory therapy can be considered (1). While the combination of non-steroidal anti-inflammatory drugs and colchicine is recommended as initial treatment, corticosteroids can be chosen in resistant cases (1). In our second case, corticosteroid treatment was started due to increased inflammatory markers in the blood tetsts, resistance of the disease to the fşrst line treatment and the absence of findings indicating the chronic form of the disease such as hypoalbuminemia and pericardial calcification.

Conclusion

The diagnosis and treatment steps of two different cases of CP are discussed in this case report. The evaluation of patients

for transient constriction, especially before pericardiectomy can be recommended, which could prevent unnecessary and high-risk surgery.

Ethics

Informed Consent: Informed consent forms were obtained from all patients for the case reports here.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.A., S.E., Design: S.A., M.Ç., E.M., Data Collection, or Processing: S.A, E.M., Analysis, or Interpretation: S.A., M.Ç., H.K.K., Literature Search: S.A., S.E., Writing: S.A.

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

Financial Disclosure: The authors declare that this study received no financial support.

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