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ReviewArticle

The enigma of regional anesthesia in enhanced recovery after total knee arthroplasty - A brief review

Ahmed Thallaj
College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia

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ABSTRACT

Enhanced recovery after primary total knee arthroplasty (TKA) depends on the type of anesthesia and post-operative pain control strategies that facilitate early mobilization and shorten the length of hospital stay of the patients. The use of new regional block ultrasound (US) guided fascial plane blocks as part of the multimodal analgesia technique is an essential part of enhanced recovery after TKA. The aim of this brief review is to describe the new US guided fascial plane blocks used in TKA in our setting, which is an integral part of TKA enhanced recovery program.

KEY WORDS: adductor canal block, total knee arthroplasty, ultrasound

INTRODUCTION

Total knee arthroplasty (TKA) is a common orthopedic procedure which requires fast and enhanced recovery after surgery. Acute postoperative pain in TKA was found to be an independent predictor of persistence of postsurgical pain following TKA[1].

Patient controlled analgesia (PCA) with morphine is associated with significant side effects that can prolong the length of hospital stay. Spinal anesthesia is our preferred anesthetic technique for TKA. Early mobilization and the initiation of physiotherapy as soon as possible mandate the use of fascial blockades as part of multimodal analgesia (MMA).

Multimodal analgesia (MMA) technique

MMA refers to the combination of non-opioid analgesics that are able to inhibit or modulate pain transmission at different sites of pain pathway. Non-opioid drugs according to World Health Organization ladder have different mechanism of action, their effects are synergistic, and include paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors[2]. Other adjuvants such as dexamethasone, with its anti-emetic and analgesic effects, is commonly used[3].

Regional anesthesia

Nerve blockade will significantly affect muscle strength with the risk of patient fall during early mobilization[4]. Two novel fascial plane block techniques for TKA have emerged recently as part of an MMA: the adductor canal block (ACB), and infiltration of local anesthetics (LA) within the fascial plane between the popliteal artery and the posterior border of the knee capsule. The aim is to selectively block the sensory nerves around knee joint capsule with motor sparing.

Adductor canal block (ACB)

The adductor canal border is described in Fig 1[5]. Under ultrasound (US) guidance, the needle tip is positioned anterior to the artery and deep to the posterior fascia of the sartorius muscle. A volume of 20 ml bupivacaine 0.25% or Ropivacaine 20 ml (0.5%) is injected (Fig 2). Intravascular injection, femoral artery puncture, local anesthestic systemic toxicity, nerve injury and infection are among the potential complications of ACB[6].

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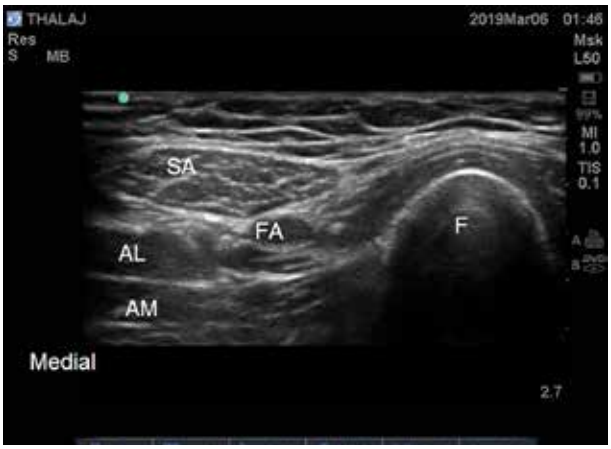

Fig 1. Short-axis US image of the adductor canal.
FA: femoral artery; SA: sartorius muscle; AL: adductor longus muscle; AM: adductor magnus muscle; VA: vastus medialis muscle; F: femoral shaft.

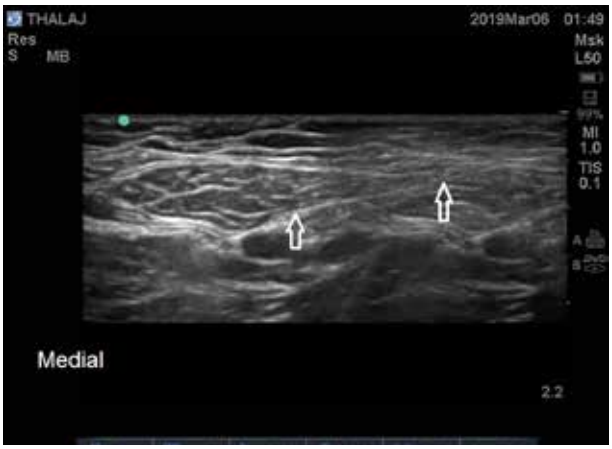

Fig 2. Adductor canal block. Arrows point at the needle tip and shaft.

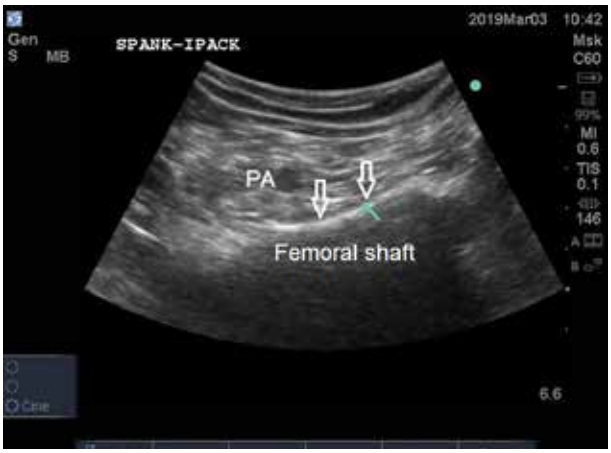

Fig 3. Short-axis US view at the popliteal area, 3 - 4 cm above the femoral condyles. Arrows point at the injection plane. PA: Popliteal artery.

Fig 4. Patient mobilization within 1 hour after discharge from post anesthesia care unit.

SPANK and IPACK blocks

Two fascial plane blocks were described in the literature, the Sensory Posterior Nerves of the Knee (SPANK) block and Infiltration between Popliteal Artery and Capsule of the Knee (IPACK) block, to selectively block the posterior sensory articular nerves of the knee joint with motor sparing effect[7,8]. Both techniques involve deposition of local anesthetic in a plane between the popliteal artery and the femoral shaft, 2 - 3 cm proximal to the femoral condyles (Fig 3). The technique originally describes needle insertion from the medial side between the vastus medialis and sartorius muscles under US guidance; our preference is needle insertion from the lateral side between the vastus lateralis and biceps femoris tendons to avoid vascular injury. Needle direction aims to contact femoral shaft and needle is then walked-off to the posterior border of the femur; 20 ml of bupivacaine 0.25% in injected under US control.

The patient received paracetamol (1g) and celecoxib (200 mg) orally on the day of surgery. Following spinal anesthesia with 2 ml hyperbaric bupivacaine 0.5%, US-guided ACB was performed. Infiltration of LA between the popliteal artery and posterior knee joint

capsule is performed under US guidance as described before. Propofol infusion at a rate of 25 mcg/kg/min is our preferred drug for sedation. Headphones are used to isolate patient from peripheral noise caused by electrical saw and hammering, and help to increase patient satisfaction.

After discharge from post-anesthesia care unit, physiotherapy team is informed to start physiotherapy and test motor strength. Once the patient recovers fully from spinal anesthesia, knee brace is removed and mobilization is started immediately (Fig 4). All patients will be on paracetamol and COX-2 inhibitors regularly, IV fentanyl is given through PCA to manage breakthrough pain. Second day post-operative, PCA fentanyl is usually stopped and patient is prescribed buprenorphine 10 mcg patch (BUTRANS); the analgesic effect of BUTRANS lasts for one week.

LITERATURE REVIEW

MMA using NSAIDs and acetaminophen is associated with decrease in post-operative opioid consumption. Peripheral nerve block and local infiltration is also associated with decreased post-operative opioid use for TKA[9]. Kuang *et al* showed in their meta-analysis that compared to femoral nerve block, ACB have resulted in better quadricep strength. There was no significant difference in other parameters such as pain score at rest or movement, opioid consumption and patient satisfaction[10].

IPACK and SPANK techniques are new blocks to control posterior knee pain post TKA that have not been well studied. Kandarian *et al* retrospectively reviewed data for TKA patients who received ACB with or without IPACK. On post-operative day 0, the lowest pain score was lower in IPACK group[11]. Thobhani *et al* analyzed data of 106 TKA patients comparing to other study groups, and supplementation of IPACK to ACB reduces opioid consumption and improves physiotherapy post-operatively[12].

CONCLUSIONS

The use of MMA technique together with ACB and the new novel regional blocks SPANK or IPACK, decrease perioperative opioid use, control postoperative pain, and facilitate functional recovery and early mobilization. These techniques should be part of a comprehensive perioperative enhanced recovery program for patients undergoing primary TKA.

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Original Article

Validity and reliability of the Public Health Literacy Knowledge Scale: The Turkish version

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ABSTRACT

Objectives: The identification of health literacy has a crucial role in raising the level of health and improving health consciousness. To measure health literacy based on the public health approach, Pleasant and Kuruvilla developed the Public Health Literacy Knowledge Scale (PHLKS). The availability of PHLKS in different societies will contribute to situational investigation and community awareness by public health professionals. The aim of this study was to evaluate the validity and reliability of the PHLKS in Turkish society.
Design: Cross-sectional study. PHLKS was translated into Turkish.
Setting: This study was conducted on a random sample of people from rural and urban family health center in Kütahya Province, located in the West Anatolia Region.
Subject: Data were collected by researchers via face-to-face interviews with participants.
Intervention: PHLKS was used as an intervention tool.
Main outcome measure: Not applicable
Results: According to factor analysis, variance in the one-dimensional structure was 52.8%, and the factor loads of 17 items in the scale ranged between 0.39 and 0.72. For internal consistency, Cronbach's alpha was found to be 0.673. The average scores of the public health literacy information level in the rural region were significantly lower than those in the urban region (Z: -3.167, p: 0.002).
Conclusion: Although PHLKS can be used as a valid and reliable scale for the Turkish society and culture, it will be beneficial if applied to larger and different sample groups.

KEY WORDS: literacy, public health, reliability, scale, validity

INTRODUCTION

Recently, health perceptions, lifestyles, and preferences of individuals have influenced health policies, especially in developed countries. These health policies focus more on issues such as chronic diseases, health promotion, and health literacy[1-3]. One of the aims of the Ministry of Health in our country is to improve health literacy among individuals to increase the responsibility of personal health. Thus, it will be possible for individuals to acquire the competency to reach, understand, and practice correct healthcare for the treatment of diseases. However, it should be noted that health literacy is the competence of healthy individuals to benefit from primary healthcare and should be acquired for primary protection.

At this point, the concept of public health literacy has emerged, and the ability of individuals to address public health subjects has been defined as the ability to assess useful and harmful interventions for public health[4,5]. The identification of public health literacy has a crucial role in raising the level of health and improving health consciousness[5].

To measure health literacy based on the public health approach, Pleasant and Kuruvilla developed the Public Health Literacy Knowledge Scale (PHLKS)[6]. The availability of this scale in different societies will contribute to situational investigation and community awareness by public health professionals. However, currently, there is no public health literacy scale available in our country.

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The aim of this study was to evaluate the validity and reliability of Turkish version of PHLKS.

SUBJECTS AND METHODS

Study Design

The study was conducted on a sample of people from rural and urban regions in Kütahya Province, located in the West Anatolia Region. There are "family health centers" in Kütahya, where preventive and primary health care is performed. This care is provided by 70 (25 urban, 45 rural) family health centers. The province was divided into two regions (rural and urban). Two family health centers from each region were selected randomly.

The field study was conducted in August - September 2017. It was a cross-sectional study involving individuals aged >18 years.

The approval of the local ethics committee (no:2015-KAEK-86/08-65) and necessary formal approvals were obtained for the research. Permission for using the scale was obtained by interviewing Pleasant. The participation in the study was based on volunteerism, and the identification of information of participants was not requested. Every step of the study was conducted according to Helsinki criteria. Sampling will not be performed for the study. A sample volume which is 20 times more than the number of questions in the measure will be used in accordance with the literature knowledge[7], which was determined as a minimum of 250 individuals.

Data Collection Tools

The prepared questionnaire and study data were collected by the researchers via face-to-face interviews with participants. The questionnaire included questions regarding sociodemographic characteristics of participants, PHLKS, Health Perception Scale (HPS), and Single-Item Health Literacy Screening Question.

PHLKS

Developed by Pleasant and Kuruvilla in 2008, PHLKS is a measure of 17 items concerning the core issues of public health[6]. Each item is answered as true or false, and 1 point is given for each correct answer. The minimum score is 0, and the maximum score is 17, with no cut-off value. The validity and reliability of the scale has been tested in China, Mexico, Ghana, and India. Cronbach alpha was 0.79 (0.67 - 0.89).

HPS

HPS is a Likert-type measure developed by Diamond *et al* in 2007[8]. It has 15 items and four subfactors titled "Center of Control," "Self-awareness," "Certainty," and "Importance of Health." Each item in the measure is answered in the form of "Strongly agree (5)," "Agree (4)," "Neither agree nor disagree (3)," "Disagree (2)," and "Strongly Disagree." Negative expressions in the measure are scored in reverse. The minimum score is 15, and the maximum is 75. The lowest scores for the subscales "Center of Control," "Self-awareness," "Certainty," and "Importance of Health" are 5, 3, 4, and 3, whereas the highest scores are 25, 15, 20, and 15, respectively. The validity and reliability of the scale in the Turkish population was evaluated by Kadioglu and Yildiz in 2012. Cronbach alpha was 0.70[9].

Single-Item Health Literacy Screening Question

It included the following question: "How often do you get help to read health instructions, brochures, or other written materials from your doctor or pharmacist?" It is a 5-point Likert scale, with 1 point for the answer "Never" and 5 for "Always." For individuals scoring >2 points, the screening is considered positive, indicating that the individual has difficulty in reading healthcare material[10].

Validity-Reliability Evaluation of the Scale

Independent language experts translated English into Turkish for cultural adaptation and language validity of the scale. Another expert then translated Turkish into English. The scale was translated into Turkish and back-translated into English. There was no discrepancy between the two translation results. The original instrument scale was assessed by public health experts for content compliance and clarity. The preliminary test was performed on 10 people, and the participants indicated that it was clear and understandable.

To test construct validity, confirmatory factor analysis was initially performed. Two hypotheses were then proposed to test the concurrent criterion validity.

Hypothesis 1: People with low levels of public health literacy have lower health perceptions. To test this hypothesis, HPS and PHLKS scores were compared.

Hypothesis 2: People living in rural areas have lower public health literacy levels than those living in urban areas. To test this hypothesis, participants in the rural and urban areas were compared using their PHLKS scores.

The correlation between the test-retest scores was determined to test the time-invariance of the scale. After 15 days from the application of the questionnaire, the scale was again applied to 50 individuals in the study group.

The Single-Item Health Literacy Screening Question was used as the gold standard to determine the predictive value of the scale. The regions where individuals scored <2 points for this question were predicted to have a higher score than PHLKS, and the cut-off score was calculated.

Table 1: Validity and reliability evaluation

Validity - Reliability	Validity - Reliability value	Scores and Cronbach's α value	Test value
Construct validity	Factor analysis	Variance: 52.8%	KMO* : 0.74
		Factor loads: 0.39 - 0.72	Bartlett test: 951.75 p <0.001
The concurrent criterion validity	Hypothesis 1	The mean score of PHLKS: 12.64 ± 2.32	r: 0.433
		The mean score of HPS: 50.32 ± 6.69	p <0.001
Reliability	Hypothesis 2	The mean score of rural PHLKS: 12.23 ± 2.12	Z: -3.167
		The mean score of urban PHLKS: 13.03 ± 2.44	p: 0.002
	Test-retest	The mean score of test: 12.42 ± 2.24	r: 0.849 p <0.001
Reliability		The mean score of retest: 12.21 ± 2.19	Z:-1.268 p: 0.205
	Internal consistency	Cronbach's α: 0.673	p <0.001

*Kaiser-Meyer-Olkin test; PHLKS: Public Health Literacy Knowledge Scale; HPS: Health Perception Scale

Statistical Analysis

SPSS version 21 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis. The number, percentage, mean, and standard deviation were used to evaluate the descriptive data. Mann-Whitney U test and Spearman's correlation were used to compare group averages because the data were not normally distributed.

Factor analysis and Spearman's correlation analysis were used to determine construct validity. Item total score correlation, internal consistency (Cronbach alpha), and test–retest correlation (Wilcoxon signed rank test) were used to assess the reliability of the scale. The estimated value of the scale was calculated using ROC analysis.

RESULTS

The study involved 285 participants, with 133 (46.7%) males and 152 (53.3%) females, of which 48.4% belonged to rural areas.

The mean score of PHLKS was 12.64 ± 2.32 (min – max: 5 - 17). The mean score for males was 12.64 ± 2.22 and for females was 12.65 ± 2.43. No statistically significant difference was found (p >0.05).

Validity analysis results

Confirmatory factor analysis was used to examine the construct validity of the scale. The Kaiser-Meyer-Olkin coefficient was 0.74, and the Bartlett test result was significant at the advanced level (X^2 = 951.75; p =

Table 2: Reliability analysis results of PHLKS

Items of PHLKS	Factor Extraction	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
For a healthy pregnancy and birth, all pregnant women should visit a health worker before the baby is born	0.42	0.27	0.65
Births that are not assisted by a skilled birth attendant are as safe as births that are assisted by a skilled birth attendant	0.43	0.29	0.63
It is normal if children below the age of 1 year weigh the same over a 2-month period	0.54	0.28	0.63
Children who are vaccinated are protected from dangerous diseases	0.39	0.29	0.66
Overall, vaccination has more risks than benefits	0.49	0.31	0.67
Children learn a lot by playing	0.53	0.32	0.67
Most injuries and accidents cannot be prevented	0.56	0.27	0.66
If a child is breathing rapidly or has difficulty breathing, the child should be taken immediately to a health-care provider	0.43	0.26	0.65
Many diseases can be prevented by washing hands before touching food	0.46	0.31	0.66
Using condoms when having sex can prevent the spread of AIDS	0.51	0.27	0.63
Using mosquito nets helps prevent malaria	0.66	0.41	0.69
Exercise helps prevent heart disease	0.72	0.34	0.66
Coughs and colds only get better with medicine	0.51	0.27	0.63
It is the father's gene that decides whether the baby is a boy or a girl	0.47	0.41	0.67
Antibiotics kill viruses as well as bacteria	0.50	0.31	0.63
Cigarette smoking causes lung cancer	0.72	0.33	0.67
All bacteria are harmful to humans	0.62	0.47	0.68

0.001). According to factor analysis, variance in the one-dimensional structure was 52.8%, and the factor loads of 17 items in the scale were found to range between 0.39 and 0.72.

Hypothesis 1 was adopted to test simultaneous criterion validity. There was a positive correlation between PHLKS and HPS scores ($r = 0.333$, $p < 0.001$).

Hypothesis 2 was accepted; the average scores of the public health literacy level were lower in rural areas (12.23 ± 2.12) than in urban areas (13.03 ± 2.44) ($Z = -3.167$, $p = 0.002$)(Table 1).

Reliability analysis results

The Cronbach alpha coefficient for internal consistency was found to be 0.673. On examining the change in scores with respect to time, there was no significant difference in the average scale scores of participants between the first (12.42 ± 2.24) and second (12.21 ± 2.19) interviews ($Z = -1.268$, $p = 0.205$). There was a positive correlation between test and retest scores ($r = 0.849$, $p < 0.001$).

Detailed reliability analysis results are presented in Table 2. The item total score correlation coefficient for the scale was found to be >0.20, with no negative related substance. The total correlations of the 17 items in the scale ranged between 0.26 and 0.47. When any of the items were subtracted, the Cronbach alpha coefficient, which ranged between 0.62 and 0.68, did not change significantly (Table 2).

Cut-off value

Receiver operating characteristic analysis revealed a cut-off point of 12, with 77% sensitivity and 70% specificity [Area under the curve = 0.721 (0.64 - 0.80), $p = 0.000$]. Health literacy level was considered to be inadequate for participants with a score of ≤12 on the scale (Figure 1).

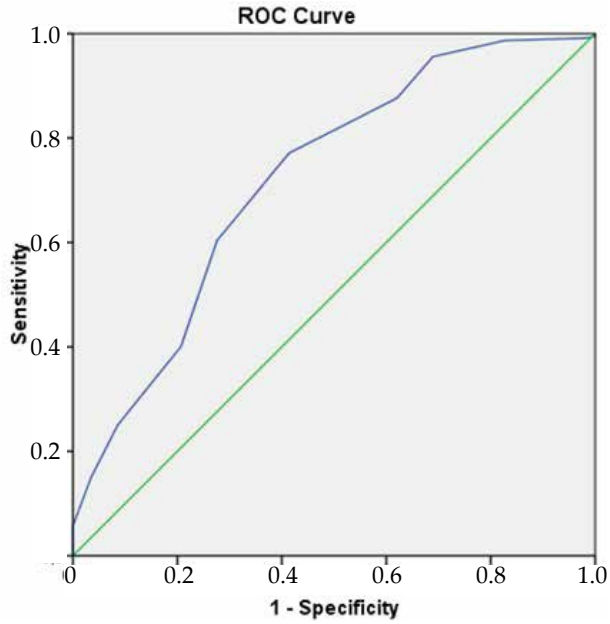

Fig 1. Cut-off value of PHLKS

DISCUSSION

Public health literacy is a concept necessary to understand and address the broad array of factors, such as health behavior, treatment and prevention that influence the public's health. The identification of health literacy for these factors has a crucial role in raising the level of health and improving health consciousness, but measuring health literacy presents particular challenge in public health because public health practices are broad and diverse[1,6,11-13]. Additionally, there is a marked scarcity of tested scale geared to public health[6,12]. According to our observations, there is no study in the literature about use of PHLKS.

In this study, we aimed to evaluate the validity and reliability of PHLKS developed by Pleasant and Kuruvilla for the Turkish society and its cultural adaptation.

In the preparation of the Turkish form of the scale, experts were consulted to ensure the language and cultural appropriateness of the instrument translation. Expert views and scales indicated that the form of expression, content, suitability, and coverage of the subject area were sufficient.

Factor analysis was used to determine the validity of the scale. Although Likert-type answers may be more appropriate for scales[14], factor analysis results were found to be appropriate, and the scale showed a one-dimensional structure as in the original. A total variance of 52.8% for this structure was an acceptable value[15]. In contrast, the process of determining the validity of a scale is the same as the process of a scientific theory development. Also, it has been reported that construct validity can be tested by forming testable hypotheses and by performing statistical evaluation of these hypotheses[14,16,17]. We tested the hypotheses to determine the validity of PHLKS and confirmed hypotheses 1 and 2 by statistical evaluation.

Inadequate levels of health literacy among individuals are associated with poor health information and negative health outcomes, leading to deterioration of health status[12,18]. A cross-sectional study investigating the relationship between health literacy and health perception found that 41% of people have low health literacy levels and that their health perceptions are low[18]. The study by Abel *et al* shows similar results [12].

There was a positive correlation between PHLKS and Health Perception Scale in our study. However, a high correlation coefficient[17] required for concurrent

criterion validity was obtained during the development of this scale.

In support of our hypothesis 2, there are studies showing that the level of health literacy in rural areas is lower than that in urban areas[19,20]. Taken together, we can propose that the expressions of PHLKS are appropriate for Turkish culture, and that PHLKS represents the area to be measured.

Reliability is defined as the accurate determination of the ability of the measurement tool to measure and provide consistent results[16]. The former is also explained as consistency among the answers obtained at the same time and is determined using the reliability coefficient Cronbach alpha. The higher the internal consistency coefficient, the more likely it is that the items on the scale are consistent with each other. The Cronbach alpha value is expected to be 0.60. The second criterion for reliability is consistency between responses obtained at different times[16,21].

In our study, Cronbach alpha for PHLKS was found to be 0.673. Cronbach alpha values differ for scales applied (original study) in different populations and cultures. For example, this value was 0.89 for the Mexican study, 0.67 for the Chinese study, and 0.79 for the original study[6]. These differences may be due to different sample sizes.

Also, the item total score correlation is important to show the relationship between scores obtained from the test items and the total test score. When this correlation is positive and high (>0.20), the materials exemplify similar behaviors, and the internal consistency of the test is high[16,17,21].The item total score correlation coefficient for PHLKS ranged between 0.26 and 0.47.

The correct response rate of all items of the research group (except item 15) was found to be >50%. The lowest percentage of correct responses was for the item "Antibiotics kill viruses as well as bacteria," which may be due to the fact that viruses and bacteria are considered to be harmful microorganisms belonging to the same genus. When the participants completed the questionnaire, interviewers had the opportunity to interact and impart knowledge regarding any areas of interest. In addition, face-to-face interviews with the participants helped us achieve outcome. Every item on the scale emphasizes the importance of basic and preventive health services and encourages individuals to think and be conscious about their health.

Attention should be paid to the use of an appropriate scale in determining the level of health literacy in society. It is expected that this scale will be high in reliability and appropriate for the cultural structure of the society and needs[12,13]. According to our study, PHLKS provides these criteria. That's why we can propose that PHLKS is appropriate for Turkish culture. The use of such scales, including clinical as well as public health approaches, contributes to public awareness, particularly in understanding and assessing health literacy.

CONCLUSION

Although PHLKS can be used as a valid and reliable scale in Turkish society and culture, we believe that it will be useful to apply this scale to larger and different sample groups. Furthermore, determining a cut-off value may make it easier for users to evaluate and compare the results with those of other studies.

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Original Article

The effectiveness of intra-articular bleomycin versus methotrexate in a chronic synovitis model

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ABSTRACT

Objective: To compare the effectiveness of methotrexate which was proven to be effective for rheumatoid arthritis and bleomycin in a synovitis model. Our aim was to show whether bleomycin could be used for chemical synovectomy purposes.
Design: Experimental study
Setting: Department of Orthopedics and Traumatology, Faculty of Medicine, Afyon Kocatepe University, Afyon, Turkey
Subjects: Fifteen mature New Zealand rabbits were studied. Synovitis was induced by repeated injections of lambda carrageenan.
Interventions: Knees of the subjects were grouped as sham, bleomycin and methotrexate. After synovitis occurred, sterile saline injected knees formed the sham group, bleomycin and methotrexate injected knees composed the study groups. In the 9th week of the study, animals were euthanised. Standard specimens were harvested from all knees. Immunoperoxidase staining was performed.
Main outcome measures: The intensity of synovitis was evaluated with thickness of the synovial cell layers, intensity of inflammatory infiltrate and iNOS expression in cells.
Results: Thickness of the synovial cell layers was greater in the sham group than in the study groups ($p < 0.05$). Intensity of inflammatory infiltrate, lymphoplasmacytoid infiltrate, and histiocytes in the sham group were greater than in the study groups ($p < 0.05$). iNOS expression in histocytes and plasma cells was significantly decreased in the study groups compared to sham operated group ($p < 0.05$). There was no statistically significant difference between bleomycin and methotrexate groups with respect to synovial cell layer thickness and lymphoplasmacytoid infiltrate.
Conclusions: According to these results, intraarticular bleomycin seems as effective as methotrexate in experimental synovitis model.

KEY WORDS: bleomycin, carrageenan, methotrexate, rheumatoid arthritis, synovitis

INTRODUCTION

Synovitis is the key and often triggering mechanism of many synovial joint pathologies. Synovitis may precede arthritis and subsequently lead to joint degeneration[1,2]. Rheumatoid arthritis (RA) is a well-known systemic, polyarticular disease that is characterized by chronic synovial inflammation and joint destruction. Other diseases such as psoriasis, systemic lupus erythematosus and Sjögren's Syndrome may cause similar systemic inflammatory polyarthritis.

A variety of methods have been described and applied both clinically and experimentally to treat synovitis, including surgical, chemical and radionuclide synovectomies. Research and clinical studies are still ongoing. Each method has inherent limitations. For example, corticosteroids interfere with articular cartilage and collagen metabolism[3,4]. Chondral damage has also been reported after osmic acid and radionuclide injections[5,6].

The reported clinical success rates of chemical and radionuclide synovectomies are relatively low, with most studies reporting 50% or lower clinical success at one-year follow-up[7,8].

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It has been well established in clinical and experimental studies that nitric oxide (NO) plays an important role in the pathogenesis of arthritis[9–11]. NO levels in serum and joints increase because of the increased release of inducible nitric oxide synthase (iNOS). iNOS, which can be upregulated by cytokines, is the main enzyme involved in the excess production of NO in arthritic disorders[11]. In this study, we therefore evaluated the severity of synovitis by measuring iNOS immunoreactivity.

Antineoplastic agents, mainly methotrexate (MTX), were proposed in previous studies to be used for the intraarticular treatment of chronic synovitis, although few clinical and experimental studies have been performed[12–14]. However, bleomycin has never been used for this purpose. Bleomycin is an antineoplastic antibiotic that inhibits DNA synthesis. Apart from bleomycin's cytotoxic effects, it is also a highly sclerosing agent that causes fibrosis and scarring. This sclerosing effect makes it very effective in the local treatment of symptomatic malignant pleural effusions, lymphangiomas and cystic hygromas[15,16].

RA is an autoimmune disease that primarily affects synovial joints. RA's main target of immunologic attack is the synovium. Once an immunologic process is started, the synovium becomes an aggressive tissue that can damage the entire joint. The synovial membrane becomes markedly hyperplastic, oedematous and infiltrated with inflammatory cells. This invasive tissue is known as pannus and causes cartilage and bone degradation via cytokines and enzymes.

In this study, one of our aims was to test whether bleomycin has a sclerosing effect on synovial tissue in a similar manner to its effect on pleura and vascular tumours. We considered that if such an effect existed it would be useful in treating synovitis. Another aim of this study was to compare the effectiveness of intraarticular bleomycin with MTX in a synovitis model.

SUBJECTS AND METHODS

This study was approved by the Ethical Committee of Afyon Kocatepe University. All procedures were in accordance with animal rights as dictated by the Guide for the Care and Use of the Laboratory Animals. Fifteen mature New Zealand rabbits were included in this study. Synovitis was induced by injecting a 1% solution of lambda carrageenan into both knees of the animals. Injections were performed under sterile conditions and at weekly intervals over a 4-week period.

Chronic synovitis was observed in all knees, which was evidenced by marked swelling above the knee. Ten knees of five animals were assigned to a sham group (group I). A second set of weekly injections was begun on experiment day 28, lasting again for a 4-week period. Both knees in group I animals received 1 ml sterile saline, while the right knees of the study group animals (group IIA) were injected with 1 ml (5 mg) of bleomycin solution (Onko, Turkey). The left knees of the study group animals (group IIB) were injected with 1 ml (25 mg) of MTX (Orna, Turkey). To easily observe the animals clinically, two different agents were injected into the knees of subjects. There is no agreement on an appropriate intra-articular dose of MTX in rabbits, and very few prior studies exist. We decided to administer 25 mg weekly because it has been shown that 30 mg/kg/i.m. weekly causes no adverse effects[17]. We also could not find an exact dose for bleomycin in rabbits. An experimental study in rabbits by Catravas *et al* showed that bleomycin could be given 5 mg/kg s.c. three times weekly without causing lung fibrosis[18]. Thus, we determined the intra-articular bleomycin dose as 5 mg/weekly. In the 9th week of the study, animals were euthanised with I.V. overdose pentothal. A medial parapatellar arthrotomy was performed in all knees, and three standard specimens were harvested:

1. Medial capsule and synovial layer, including the medial collateral ligament
2. Patella, extensor mechanism and the anterior capsule with fat pad
3. Lateral capsule and synovial layer, including the lateral collateral ligament

Synovial tissues were placed into buffered formalin solution. After 24 hours, they were processed and embedded in paraffin. Histopathologic sections were stained with haematoxylin and eosin and assessed with a light microscope. All sections were reviewed by the same pathologist blind to the study groups.

Immunohistochemistry

Immunoperoxidase staining was performed using avidine-biotinylated horseradish peroxidase complex. Formalin-fixed, paraffin embedded tissue sections were processed for microwave antigen retrieval in 0.01 M sodium citrate buffer (pH 6.0) for 10 minutes at maximum power. Endogenous peroxidase activity was blocked by treating the slides with 2% hydrogen peroxide for 20 minutes. The slides were then incubated for 30 minutes in blocking buffer (20% normal goat serum in PBS with 1% BSA) and the primary anti-iNOS monoclonal antibody (diluted at 1:100, Neomarkers, LabVision Coop., CA, U.S.A.) overnight at 4 °C. Slides were subsequently treated with biotinylated secondary antibody and avidin-biotin peroxidase complex (LabVision Coop., CA, U.S.A.) according to the manufacturer's instructions. Aminoethylcarbazol was used for visualization of the immunoreaction. Then, sections were counter stained with Mayer's haematoxylin.

Three sagittal sections from the lateral, medial

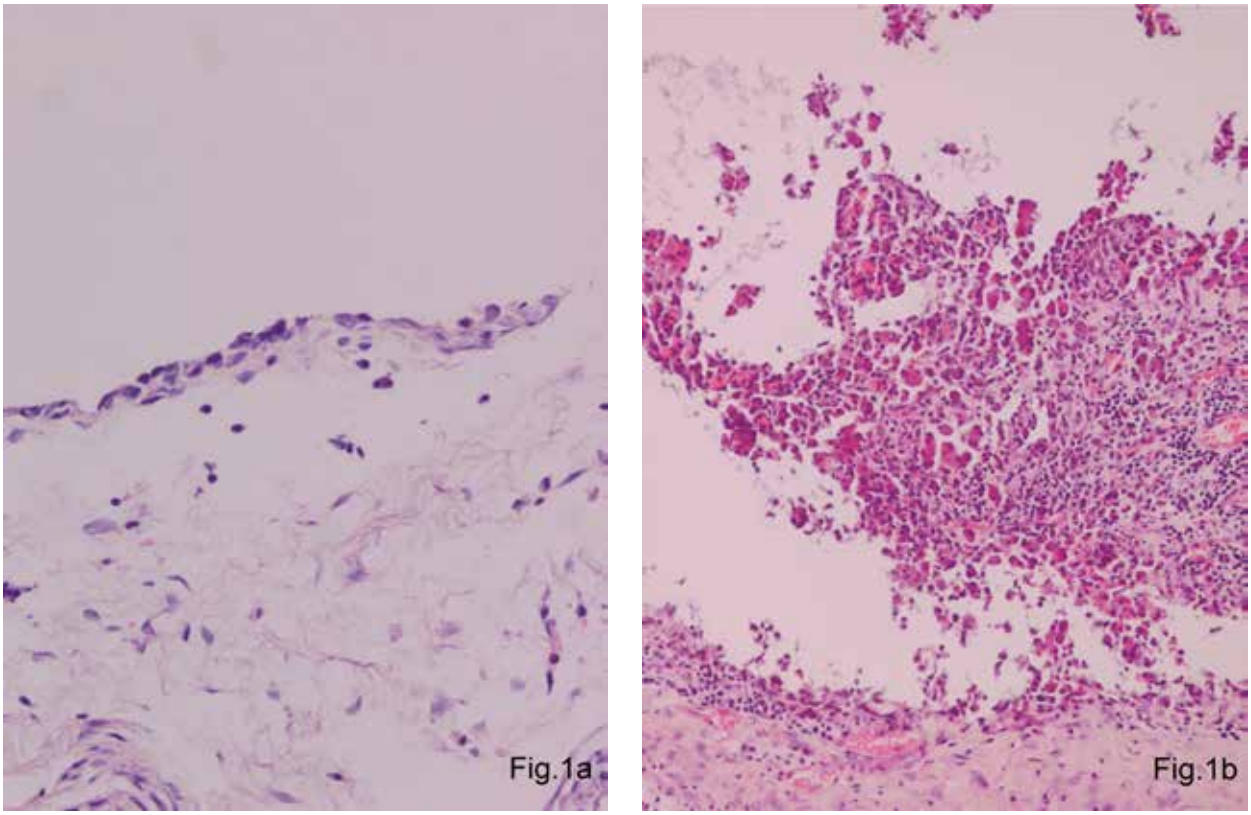

Fig 1 (a): Histologic appearance of grade 0 synovial hyperplasia (Hematoxyline eosine, x200), **(b).** Severe synovial lining layer hyperplasia and inflammatory change in the sham group (Hematoxyline eosine, x200).

and upper portion of the rabbit knee were selected to permit the measurement of changes in the thickness of the synovial cell layers in the synovial tissues. The thickness of synovial cell layers was graded as follows: grade 0, 1-3 layers; grade 1, 4-6 layers; and grade 2, 7 or more layers (Fig 1a, 1b).

Histopathologic changes were scored from 0 to 2 for acute inflammatory infiltrate, lymphoplasmacytic infiltrate and histiocytes. Fibrosis, necrosis and vascular dilatation were evaluated as positive or negative. The immunoreactivity of iNOS was evaluated in the synovial lining cells, histiocytes and plasma cells. The

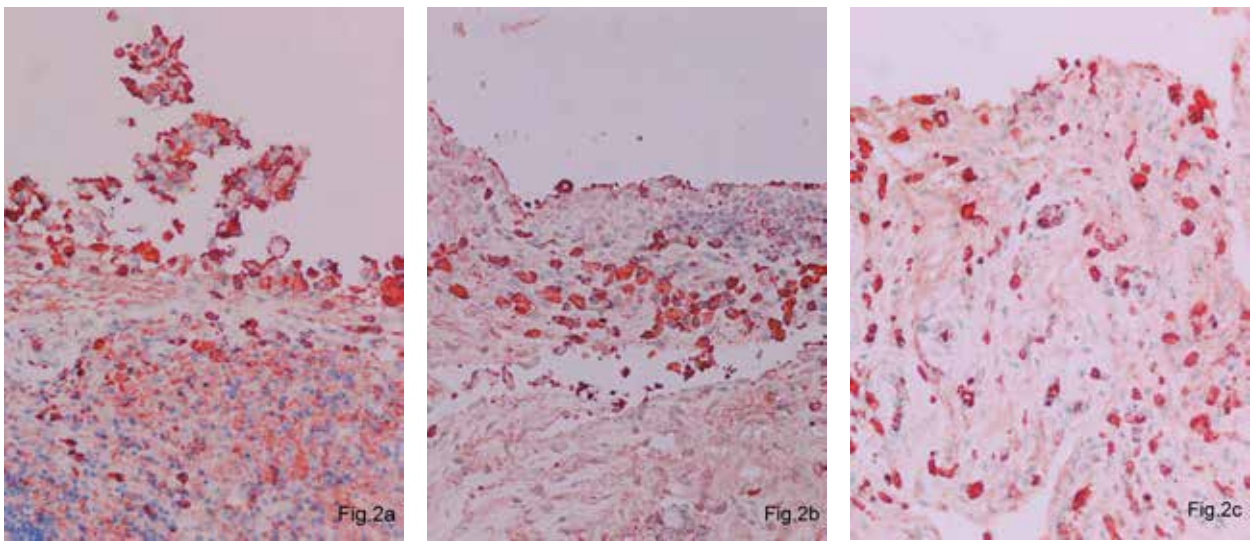

Fig 2 (a): iNOS immunoreactivity is increasingly expressed in the cytoplasm of the synovial lining cells in the induced-synovitis. iNOS expression in histiocytes **(b)** and plasma cells **(c)** are also observed (x200) .

staining intensity of iNOS was classified as follows: 2+, >50% of cells strongly stained; 1+, 10 - 50% distinctly stained or more cells weakly stained; -,<10% were stained.

Statistical Analysis

The results were decoded and statistically analysed. Data were expressed as the mean ± standard error of the mean. Statistical analysis was performed using the chi-square, Kruskal Wallis and Mann-Whitney U tests. A p-value <0.05 was considered significant.

RESULTS

One knee in the sham group was excluded because of septic arthritis secondary to injection. The results are summarised in Tables 1 and 2. Synovial cell layer thickness was greater in the sham group than in Group IIA and Group IIB ($p < 0.05$) knees. The intensity of the inflammatory infiltrate, lymphoplasmacytic infiltrate and histiocytes in the sham group was also significantly greater than in Group IIA and Group IIB ($p < 0.05$). There were no statistically significant differences between Group IIA and IIB with respect to synovial cell layer thickness and lymphoplasmacytic infiltrate. Fibrosis was observed in 88.8% of sham and 80% of Group IIA animals, but it was not observed in Group IIB ($p < 0.001$). Vascular dilatation was lower in Groups IIA and IIB than in the sham group ($p < 0.005$).

Table 1: iNOS staining intensity in sinovial tissue

Cell Type	iNOS positivity		
	Negative (%)	Weak (%)	Strong (%)
Synovial lining cells			
Sham	-	9 (100.0)	-
Group IIA	-	10 (100.0)	-
Group IIB	-	10 (100.0)	-
Histiocytes*			
Sham	-	6 (66.7)	3 (33.3)
Group IIA	10 (100.0)	-	-
Group IIB	10 (100.0)	-	-
Plasma cells*			
Sham	1 (11.1)	4 (44.4)	4 (44.4)
Group IIA	4 (40.0)	6 (60.0)	-
Group IIB	5 (50.0)	3 (30.0)	2 (20.0)

*p <0.05 Sham vs Group IIA and Group IIB

Positive iNOS expression was observed mainly in synovial lining cells (Fig 2a), histiocytes (Fig 2b) and plasma cells (Fig 2c). iNOS immunoreactivity was weakly positive in the synovial lining cells of all rabbit knees. However, the intensity of iNOS expression in histiocytes and plasma cells was significantly decreased in the study groups compared with the sham group ($p < 0.05$).

Table 2: Histopathologic changes in synovial tissue

The Criteria	0 (%)	1 (%)	2 (%)	Mean ± SEM
Thickness of synovial cell layers				
Sham	-	5 (66.6)	4 (44.4)	1.44 (1.57)*
Group IIA	-	10 (100.0)	-	1.0 (0.0)*
Group IIB	-	9 (90.0)	1 (10.0)	1.1 (1.0) *
Acute inflammatory infitrate				
Sham	1 (11.1)	1 (11.1)	7 (77.8)	1.67 (0.23)§
Group IIA	6 (60.0)	4 (40.0)	-	0.4 (0.16)§
Group IIB	6 (60.0)	4 (40.0)	-	0.4 (0.16)§
Lymphoplasmocytoid infiltrate				
Sham	1 (11.1)	2 (22.2)	6 (66.7)	1.56 (0.24)*
Group IIA	-	10 (100.0)	-	1.0 (0.0)*
Group IIB	-	6 (60.0)	4 (40.0)	1.4 (0.16)*
Histiocytes				
Sham	-	3 (33.3)	6 (66.7)	1.67 (0.17)*
Group IIA	-	9 (90.0)	1 (10.0)	1.1 (1.0)*
Group IIB	-	6 (60.0)	4 (40.0)	1.4 (0.16)*

*p <0.05 Sham vs Group IIA and Group IIB
§p <0.01 Sham vs Group IIA and Group IIB
SEM :standard error of the mean

DISCUSSION

iNOS immunoreactivity

The main finding of the present study was decreased iNOS immunoreactivity in both the MTX and bleomycin groups compared with sham animals. However, the intensity of iNOS positivity varied between the layers of the synovium. In synovial lining cells, there was weak staining in all the groups (sham and experimental groups)(Table 1).

However, in the subintimal layer, histiocytes of experimental groups showed negative staining, whereas the sham group showed weak or strong staining ($p < 0.05$) (Table 1). Similarly, subintimal plasma cells of the sham group showed weak-strong staining, whereas experimental groups showed negative-weak staining ($p < 0.05$) (Table 1).

It is clear that the subintimal layer of the synovium is a target of immunologic attack in RA and is often heavily infiltrated with macrophages, lymphocytes, neutrophils and mast cells[19–21]. Thickening of the synovial lining layer is seen as well. Based on our results, we hypothesize that inflammation occurs more severely in the subintimal layer than the synovial lining layer as expected in this model. iNOS positivity was weak in synovial lining cells in both the MTX and bleomycin groups. There was also no strong staining in the sham group. This means that a milder inflammatory reaction occurred in the synovial lining layer. Intense inflammation occurred mainly in the subintimal layer, and both MTX and bleomycin were effective in reducing inflammation in this area.

Garg NK *et al* showed that in an experimentally-induced RA animal model treated by delivering the drug into the joint through lipid carriers, MTX reduced the synovial fluid iNOS levels[22]. Lee SW *et al* investigated the effectiveness of transcutaneous and intraperitoneal MTX in a collagen-induced arthritis (CIA) model in mice[23]. They observed that both forms of the drug decreased the levels of iNOS in the inflamed joint. In our study, we also observed reduced iNOS positivity in histiocytes and plasma cells. Interestingly, bleomycin also reduced iNOS positivity in these cells.

Bleomycin is a cytotoxic agent that is used in the treatment of myriad malignancies, including lymphomas, squamous cell carcinomas, germ cell tumours and malignant pleural effusions. Bleomycin is used to create a chemical pleurodesis in malignant pleural effusions[15,16]. It is also useful when administered via intralesional injection in tumours such as lymphangiomas and cystic hygromas. Its efficacy is attributed to an inflammatory response that decreases fibrinolytic activity and stimulates fibroblast proliferation and fibrosis[16].

There are many studies about bleomycin-induced lung fibrosis. Cytokines such as IL-1, macrophage inflammatory protein-1, platelet-derived growth factor, and (TGF)-β are released from alveolar macrophages in animal models of bleomycin toxicity, resulting in fibrosis[15].

In this study, we found that bleomycin reduced iNOS immunoreactivity in a rabbit inflammatory arthritis model. This result seems contradictory to what was previously described in bleomycin-induced lung fibrosis models. However, a comparison of findings from two completely different tissues may lead to improper conclusions.

Although we do not know exactly how bleomycin suppresses iNOS activity, reduced leukocyte proliferation (Table 2) may contribute to a reduction in NO production and inflammation.

Histopathologic changes in synovial membrane

Synovial cell layer thickness was greater in the sham group compared with both experimental groups (Fig 1b). Both MTX and bleomycin reduced the thickness of the synovial cell layer. For MTX, our results were consistent with those observed in other experimental studies[22,24–26]. These studies showed that MTX reduced synovial hyperplasia in RA model. We found that bleomycin also reduced the synovial lining layer hyperplasia. In their study, to determine the effectiveness of cilostazol and MTX in RA, Kim HY *et al* evaluated these agents on synovial fibroblasts obtained from patients with RA using a mouse model of CIA[27]. They observed that MTX suppressed the proliferation of synovial fibroblasts in vitro, suppressed cytokine serum levels and inhibited macrophage recruitment.

In the subintimal layer, we evaluated the infiltration of different cell types. The grade of acute inflammatory infiltrate was higher in the sham group compared with both experimental groups ($p < 0.01$). Lymphoplasmacytic infiltrate was greater in the sham group than both experimental groups ($p < 0.05$). Histopathologic examination demonstrated a higher histiocyte infiltration in the sham group compared with the experimental groups ($p < 0.05$). There was no difference between the MTX group and bleomycin group in terms of acute inflammatory, lymphoplasmacytic and histiocytic infiltrate.

In prior literature, there is strong evidence that MTX inhibits synovial mononuclear and polymorphonuclear cell infiltration in animal models of RA[22,24–27]. Some authors explain the mechanism for the anti-inflammatory and immunosuppressive actions of MTX as pro-inflammatory cytokine inhibition, while others attribute this to increased extracellular adenosine levels. In contrast, bleomycin normally increases some pro-inflammatory and fibrotic cytokines, particularly TNF-α and TGF-β in the lungs. Collagen deposition and resultant fibrosis may be due to enhanced expression of these cytokines[28].

Prior work has shown that MTX suppresses inflammatory arthritis by reducing some pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, MMP-1, TNF-α and TGF-β[22,26,27,29]. In contrast, the main mechanism of action of bleomycin is DNA cleavage via oxidative damage caused by free radicals. Breaks in the DNA chain lead to cell death. This action increases the oxidative stress in affected cells. As mentioned above regarding iNOS immunoreactivity, we observed that bleomycin reduced the oxidative stress of synovial tissue. This suggests that a reduction in synovial hyperplasia, acute inflammatory and lymphoplasmacytic infiltrate and histiocytes after bleomycin administration is possibly caused by a direct apoptotic effect, not oxidative damage to DNA.

Fibrosis

In a clinical study[21], the histologic and immunopathologic features of the synovial membrane of 18 patients with RA and 12 patients with orthopaedic arthritis were examined. Patients were given different treatment protocols, which included NSAIDs, prednisone and MTX. They showed that MTX inhibited the development of fibrosis. They attributed this anti-fibrotic effect to the suppression of inflammatory mediators responsible for fibroblast activation.

In our study, fibrosis was found in the bleomycin and sham groups but not in the MTX group. Bleomycin induced lung injury and fibrosis is a well-known animal model. An overproduction of reactive oxygen species can lead to an inflammatory response that can cause pulmonary toxicity, fibroblast activation and subsequent fibrosis[30]. We did not observe an overexpression of iNOS in the synovial membrane of the bleomycin group. We believe that reduced iNOS activity and oxidative stress suppressed the requisite inflammatory reaction that would eventually result in fibrosis. We, therefore, did not see massive fibrosis as we expected.

During the study, we recorded experimental side-effects. We did not observe joint contracture in either experimental group. When the knees were removed for pathologic examination at the end of the study, we did not observe any difference between groups, and macroscopically, there was no evidence of arthrosis or chondral damage.

This is a preliminary histopathologic study, and we did not evaluate other parameters indicative of inflammation, especially pro-inflammatory cytokines (IL-1, IL-6, TNF-α, TGF-β etc.), in synovial fluid and tissue. This is the main limitation of our study that precludes us from making more precise comments on bleomycin's mechanism of action. Other limitations of this study are the relatively small number of subjects, and the standard drug dose administered to each animal.

Comparing lung fibrosis and RA synovitis models could lead us to draw improper conclusions. The deficiency of bleomycin hydrolase –which inactivates bleomycin– in normal lung tissue potentiates this drug's effect and leads to massive fibrosis. We do not know how bleomycin acts on the synovium. Decreased activity of iNOS and nitrite levels in the tissue seems to be important. Properly matched experimental studies are therefore needed to identify the mechanism of action of bleomycin on rheumatoid synovium.

CONCLUSION

In this preliminary study evaluating the effects of bleomycin on an experimental RA synovitis model, we found that bleomycin decreased iNOS activity and led to mild fibrosis of the synovium. Decreased iNOS activity may have reduced the severity of synovial inflammation and fibrosis. Bleomycin also reduced synovial inflammatory cell proliferation, which contributed to a reduction in oxidative status.

This preliminary data suggests bleomycin may have a beneficial effect for treatment of synovitis. Further experimental efficacy and safety studies are warranted.

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Original Article

Risk factors for Systemic Inflammatory Response Syndrome after percutaneous nephrolithotomy

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ABSTRACT

Objective: To evaluate the correlation between preoperative midstream urine culture, pelvic urine culture and stone culture findings during percutaneous nephrolithotomy (PCN)
Design: Retrospective study
Setting: Department of Urology, College of Medicine, Dumlupınar University, Turkey
Subjects: The data of all the patients who underwent PCN at our institution between September 2015 - December 2017 who were managed in the department of Urology at Dumlupınar University medical hospital
Intervention: Medical records of all the patients were reviewed and the data were collected retrospectively.
Main outcome measures: The preoperative parameters included any previous history of urinary tract information, complete blood workup, and noncontrast helical computed tomography. A preoperative midstream urine culture (MSUC) was obtained for all patients.
Results: A total of 248 consecutive patients were retrospectively recruited into the study. There were 36 (14.5%) patients in the Systemic Inflammatory Response Syndrome (SIRS) group (I) and 212 (85.5%) patients in the non-SIRS group (II). None of the patients in either group developed clinical septic shock and all patients recovered without sequelae. The factors affecting SIRS in multivariate analysis, namely BMI, post-operative residue and plasmacytoid urothelial carcinoma (PUC) were statistically significant.
Conclusion: We have shown that MSUC is a poor predictor of upper tract colonization. Intraoperative PUC is an important factor for detecting post-PCN SIRS risk.

KEYWORDS: percutaneous nephrolithotomy, postoperative sepsis, renal stone, urine culture

INTRODUCTION

Percutaneous nephrolithotomy (PCN) is one of the most common treatments for renal stones. Although it provides sterile conditions and preoperative antimicrobial prophylaxis, postoperative sepsis is one of the most common complications after PCN, with an incidence reported between 9.8% and 37%, while severe sepsis and septic shock occur in 0.3 - 4.7%, with risk of mortality[1-11]. Nevertheless, some patients with a preoperative sterile midstream urine culture (MSUC) develop postoperative urinary tract infection (UTI), suggesting that MSUC may be a poor predictor of the outcome after PCN. Charton *et al* demonstrated a 35% incidence of bacteriuria after PCN among patients with known struvite stones and sterile preoperative urine[12]. The aim of the present study was to evaluate the correlation between preoperative MSUC, pelvic urine culture (PUC) and stone culture (SC) findings during PCN and to investigate whether routine SC or PUC may help in choosing the appropriate antimicrobial treatment of patients with UTI or systemic inflammatory response syndrome (SIRS) after PCN.

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MATERIALS AND METHODS

The Institutional Review Board approved this study and waived informed consent requirements. The data of all the patients who underwent PCN at our institution between September 2015 - December 2017 were retrospectively analyzed. The preoperative parameters included any previous history of UTI, complete blood workup, and noncontrast helical computed tomography. A preoperative MSUC was obtained for all patients. Patients with a history indicative of an infective stone were treated preoperatively for a week before surgery according to the results of the most recent positive MSUC. Ten patients with positive preoperative MSUC were treated until the MSUC became negative before surgery. All patients with a negative preoperative MSUC received prophylactic antibiotics (750 mg cefuroxim) in accordance with the EAU Urological Infections Guidelines 2013.

After antiseptic preparation with chlorhexidine, a standard cystoscope with a working channel was introduced into the bladder and an ureteral catheter was placed. The ureteral catheter was stabilized to the urethral 16F Foley catheter by silk ties to prevent displacement while turning the patient from supine to prone position. The renal collecting system was imaged using retrograde contrast medium, diluted with saline about one to one. After the introduction of the 18-G needle, the guidewire was inserted through the needle into the renal collecting system. Urine was first aspirated from the pelvicaliceal system and sent as PUC. Renal parenchyma dilatation was performed up to 30F. The bulls-eye and triangulation techniques were established for puncture. In our clinic, amplatz dilators are generally preferred. During the operation, ureteral catheter was removed at the earliest opportunity and the guide wire was introduced through it. After the guide wire is inserted through the ureter catheter, the ureter catheter is removed. The guide wire is used to place a re-entry malecot catheter through the entrance to the kidney. Immediately after the operation is finished, the re-entry malecot catheter is placed over the wire and then the guide wire is removed. This is the standard method for all percutaneous nephrolithotomy operations. Re-entry malecot catheter is removed on post-operative first day.

When the operation was completed, re-entry Malecot catheter was mostly placed in such a way that it fits into the renal pelvis. For those patients with hemorrhage, the nephrostomy was maintained clamped, until they are transferred to bed. Urinary catheter was generally removed on the post operative first day for all the patients. As for the stable patients without hematuria, nephrostomy was also removed on the post operative first day. Stone fragments were collected to be processed for culture. A sample of fragmented stones was collected, and the surface contaminants were washed off by using the standardized Stamey method[13]. Crushed stones were cultured on thiosulfatecitrate bile salt sucrose and MacConkey's agar. Urine cultures were done simultaneously. Urine samples were inoculated onto 5% blood agar and Eosin-Methylene Blue agar with 0.01 ml calibrated loops by semi-quantitative technique. Urine and stone culture plates were incubated for 18 - 24 hours at 37 ^{o}C aerobically. Isolated bacteria were identified by standard laboratory techniques[14] or automated (VITEK2, bioMerieux, France), as required.

The data were divided according to MSUC, PUC, SC colonization; the association between different groups was assessed by using the chi-square test. The sensitivity, specificity and positive and negative predictive values of MSUC to detect SC and PUC colonization were calculated.

In addition, we analyzed the data of all patients by postoperative SIRS. Postoperative SIRS was defined according to the International Sepsis Definitions Conference of 2001[15] as the presence of a source of infection with the SIRS, satisfying at least two or more of the following conditions: temperature greater than 38 °C or less than 36 °C, heart rate greater than 90 beats/min, respiratory rate greater than 12 breaths/min or arterial carbon dioxide pressure less than 32 mm/Hg, and white blood cell count greater than 12,000 or less than 4000/mm^3. The difference between the initial MSUC, PUC and SC findings, the relative risk of SIRS when SC, PUC or MSUC were positive, and the change in antibiotic treatment in this group were assessed.

All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) 15.0 for Windows. Chi-square test was used to group and to define clinical parameter importance. Independent risk factors were determined by employing univariate and multivariate Cox regression analysis. Concordance was determined by Kendall correlation analysis. P-values below 0.05 were considered statistically significant.

RESULTS

A total of 248 consecutive patients were retrospectively recruited into the study. Table 1 lists the demographic characteristics of the patients and their operative findings. The mean age was 46.5 ± 26.7 years (ranging from 19 to 72 years), and 164 (66.1%) of the patients were male. The mean body mass index (BMI) was found as 27 (± 3.8) kg/m^2. Postoperative residual stone (> 4 mm) was found in 91 (36.7%) patients, while per-operative complications (bleeding and/or perforation of the collecting system) were observed in 64 (25.8%) patients. There were 36 (14.5%) patients in the SIRS group (I) and 212 (85.5%) patients

Table 1: Patients' demographics data in two groups

Patient data	All patients (N = 248)	Group 1 (n = 36)	Group 2 (n = 212)	Univariate Analysis	Multivariate Analysis
Mean age	46.5	35.4	48.4	<0.0001	0.280
Gender				<0.0001	0.783
Male (%)	164	13 (7.9)	151 (92.1)		
Female (%)	84	23 (27.3)	61 (72.7)		
BMI (std deviation)	27 (3.8)	24.6	27.4	<0.0001	0.004
Operation time (min)	85.2	92.9	83.9	0.223	
Mean stone load (mm^2)	23.3	26.30	22.80	0.003	0.755
Access				0.133	
Single (%)	223	30 (13.4)	193 (86.6)		
Multiple (%)	25	6 (24)	19 (76)		
Per-operative complication				0.019	0.440
Yes (%)	64	17 (26.5)	47 (73.5)		
No (%)	184	19 (10.3)	165 (89.7)		
Post-operative residue				<0.0001	0.005
Yes (%)	91	3 (3.2)	88 (96.8)		
No (%)	157	33 (21)	124 (79)		
Number of stones				0.015	0.245
Single Stone (%)	149	12 (8)	137 (92)		
Multiple Stones/Staghorn (%)	99	54 (54.5)	45 (45.5)		
Hydronephrosis				0.227	
Yes (%)	93	16 (17.2)	77 (82.8)		
No (%)	155	20 (13)	135 (87)		
Midstream Urine Culture				<0.0001	0.729
Positive (%)	29	20 (69)	9 (31)		
Negative (%)	219	16 (7.3)	203 (92.7)		
Pelvic Urine Culture				<0.0001	<0.0001
Positive (%)	25	20 (80)	5 (20)		
Negative (%)	223	16 (7.1)	207 (92.9)		
Stone Culture				<0.0001	0.074
Positive (%)	124	32 (25.8)	92 (74.2)		
Negative (%)	124	4 (3.2)	120 (96.8)		

in the non-SIRS group (II). None of the patients developed clinical septic shock in either groups and all patients recovered without sequelae. The correlation between the risk factors and SIRS is shown in Table 1. In univariate analysis, mean age, gender, BMI, mean stone burden, perioperative complications (bleeding, collector system perforation), postoperative residue, number of stones, MSUC, PUC and SC were statistically significant between the two groups. Factors affecting SIRS, namely BMI, postoperative residual stone and PUC were found to be statistically significant in multivariate analysis.

Preoperative MSUC was positive in 10 cases (4%), which were treated with culture specific antibiotics before surgical intervention. PUC was positive in 25 cases (10%), of which 3 (46.6%) had negative MSUC. Concordance between the species identified on PUC and MSUC was 78%. SC was positive in 124 cases (50%), where all the 124 (100%) cases had negative UC. Concordance between species identified on SC and MSUC was 26%. The culture results of the individuals are shown in Table 2. The most common pathogen was *E. coli*, followed by *Enterococcus* and *Klebsiella*. The most frequently used antibiotics for treatment according to culture results (alone or in combination) were as follows: beta-lactam/beta-lactamase inhibitors (n = 5), third generation cephalosporins (n = 5), glycopeptides (n = 8), kinolon (n = 3) and carbapenems (n = 15) in SIRS patients. In our study, BMI, postoperative residual stone and PUC were strongly correlated with the development of SIRS ($p < 0.001$) (Table 1). MSUC, PUC, and SC were positive in 93.7%, 80%, and 25.8% of the patients in group I, but only in 6.3%, 20%, and 74.2% of the patients in group

Table 2: Culture results

Culture results	Bladder Urine	
	Positive	Negative
Stone positive		
Pelvic urine positive	16	9
Pelvic urine negative	0	103
Stone negative		
Pelvic urine positive	0	3
Pelvic urine negative	0	124

Table 3: Predicting SIRS using various specimens

Variables	Bladder urine culture	Pelvic urine culture	Stone culture
Sensitivity %	63	69	90
Specificity %	99	97	86
PPV	97	87	28
NPV	90	92	98

II, for the corresponding specimens, respectively. For the detection of post-PCN SIRS risk, the calculated MSUC, PUC, and SC sensitivity values were 63%, 69%, and 90% and specificity values were 99%, 97%, and 86%, respectively (Table 3). The sensitivity of MSUC and SC for indicating pelvic urine culture positivity were found to be 73% and 100%, and specificity to be 69% and 100%, respectively (Table 4).

Table 4: Predicting infected pelvic urine with bladder urine culture and stone culture

Variables	Bladder urine culture	Stone culture
Sensitivity %	73	100
Specificity %	100	69
PPV	64	20
NPV	96	55

PPV: positive predictive value; NPV: negative predective value

DISCUSSION

Post-operative fever and sometimes sepsis can be seen despite the provision of sterile urine and using appropriate prophylactic antibiotics preoperatively for the procedure of percutaneous nephrolithotomy. Urosepsis and shock have been found to occur in direct proportion to the duration of the procedure, urine bacterial load, severity of obstruction by stone and infection in the stone[13,16]. O'Keefe *et al* retrospectively reviewed a series of 700 patients undergoing upper tract manipulation[11]. Dogan *et al*[8], who investigated the infectious complications in 338 patients undergoing PCN, showed that 66% of 82 patients with SIRS had negative preoperative urine culture. In another study, SIRS after PCN was observed in 25.5% of patients[16]. In our study, however, post-operative SIRS was observed in 14.5% of patients. The possible explanation for PCN related bacterial dissemination (urosepsis) includes: (i) bacterial colonization of stone and release of endotoxins during the fragmentation of infected stone; (ii) systemic absorption of irrigation fluid containing bacteria and endotoxin via the venous system or lymphatic channels due to increased positive pressure through the nephroscope (nephrostomy tract) during surgical procedure[7]; (iii) many patients with renal stones, especially struvite stones, have been treated for recurrent urinary tract infections, consequently the potential for antibiotic resistance becomes high.

Proposed risk factors for postoperative sepsis can be listed as the duration of surgery, larger amount of irrigation fluid, high pressure in the collecting system, larger stone burden, multiple access tracts, bacterial load in the urine, indwelling ureteral stent or nephrostomy tube, severity of obstruction and presence of infection in the stone[4,5,17-20]. In our study, positive PUC, BMI and post-operative residual stone were significant risk factors for postoperative SIRS in multivariate analysis. No association was found between postoperative SIRS and age, gender, stone burden, presence of hydronephrosis, number of stone, multiple accesses, operation time, peroperative complications, MSUC, PUC and SC.

In their study, Sharifi Aghdas *et al* have reported higher incidence of fever in women than in men[6]. Similarly, postoperative fever was observed more in women than in men in our study, but it was not statistically significant. The result of our study showed that only 13 (7.9%) of the 164 male patients developed SIRS after PCN. The higher incidence of infected stone in women may be explained by the fact that they are more likely than men to develop UTI and therefore they are at an increased risk to form an infected stone and to develop SIRS[6,21].

One of the parameters that we considered in our study was the BMI. To our knowledge, the effect of BMI on postoperative SIRS in patients who underwent PCN has not been evaluated previously. However, obesity was shown to influence post-operative infections and complications negatively in general surgical procedures[22]. In the study by Tjeertes *et al*, obesity has been shown to cause surgical site infection, higher amount of blood loss and extension of the operation time[22]. BMI was found significantly higher in our study group that developed SIRS. Possible mechanisms regarding the effects of obesity on postoperative infection are considered to be the deterioration of immunity and increased level of blood glucose[22-25].

In their study evaluating the factors affecting infection after PCN, Margel and Erdil have shown that postoperative residual stones exhibit no effect on the development of postoperative SIRS[26,27]. In contrast to these studies, we determined in our study that postoperative residual stones can be effective in the development of postoperative SIRS. We think this is the result of the continuation of bacterial colonization in the residual stone, thus causing infection in the postoperatively compromised immune system.

Prolongation of operation time causes the collector system to remain open to the ambient for a longer time and more irrigation fluid passing into the venous

system. Therefore, extension of the operation time can be considered among the factors that may affect SIRS after PCN. In the study by Gönen *et al*, the operation time has been shown to effect post-PCN SIRS. These results were also supported by some other studies[20,28]. On the contrary, in the studies by Margel and Erdil, the operation time did not influence the post-PCN SIRS[26,27]. In this present study however, we also observed that the operation time is not a factor influencing post-PCN SIRS.

Sometimes more than one access is needed to clear all stones during PCN. This in turn involves multiple incisions and thus, may cause infection. However, in our study as well as in some other studies, the number of access was shown not to effect post-PCN SIRS[26,27].

Larger stones are more likely to be triple phosphate stones and they have been found to harbor infection. Shigeta *et al* found infected stones in 10% of their study subjects and they noted that bacteriuria was more prevalent in stones greater than 30 mm in diameter[29]. Contrary to this situation, Mariappan *et al* have shown in their study that the size of the stone is not correlated with urosepsis[2]. However, we observed that stone burden was not correlated with post-PCN SIRS.

In our study, retrograde contamination of renal pelvic urine was excluded since bladder urine specimens were negative in all the patients with infected pelvic urine and there was a clear discrepancy between the types of microorganisms cultured. The sterile technique practiced and the rigorous washing of stones ensured no cross-contamination and interpretation of bacteria in the stone core.

In some studies, positive culture was shown in the bladder urine cultures of 21.5 - 11% of patients, in the pelvis cultures of 20.4 - 4.7% of patients and in stone cultures of 35.2 - 9% of patients[2,16,26]. In our study, however, positive MSUC was determined in 6.4% of patients, positive PUC in 10% of patients and positive SC in 50% of the patients.

However, a weak correlation has been reported betweeen stone culture as well as bladder urine culture and pelvic urine culture[2,30,31]. Also in our study, the correlation between bladder urine culture and stone culture was determined to be at a lower level of 12.5%. Lewi *et al* analyzed stones, pelvic urine and bladder urine from 63 patients, and found that urine culture and sensitivity was positive in 29%, stones were infected in 38% and pelvic urine was infected in 30%[32].

In numerous studies conducted on this topic, more positive cultures have been reported for urine obtained from the pelvis compared to the bladder cultures and that, the pelvic urine cultures were more correlated with sepsis[2,31,32]. This higher incidence could be explained by the fact that these studies included the patients with obstructing upper tract stones. In another study, pelvic urine cultures of patients with preoperative pelvicaliectasis or hydronephrosis were shown to be positive at a higher rate than patients without dilation. In our study, pelvic urine culture was positive in 80% of patients with post-PCN SIRS and this was statistically significant.

The preoperative prediction of urosepsis is ideal and some groups at high risk can be identified, such as patients with staghorn calculi, abnormal anatomy and diabetes, and immunocompromised patients. In this series, we also determined BMI, post-operative residual stone and PUC as possible predictors of SIRS. It may also be judicious to aspirate the pelvic urine percutaneously in patients who are more prone to sepsis and determine the treatment according to the result of the culture.

This study adds to data from previous studies that have demonstrated the importance and support the routine use of PUC during PCN[2,4,7,26].

CONCLUSION

We have shown that MSUC are poor predictors of upper tract colonization. Intraoperative PUC is an important factor for detecting post-PCN SIRS risk. We recommend collecting PUC and SC to identify the offending organism and guide the treatment accordingly because intraoperative cultures may be essential in directing the antibiotic regimen postoperatively and should be routinely used to prevent progression to clinical septic shock in patients with post-PCN SIRS.

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Original Article

Effect of Orem's self-care model on quality of life and complications in the patients with cutaneous ureterostomy after radical cystectomy

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ABSTRACT

Objective: To investigate the effect of Orem self-care program on quality of life (QoL) and complications of patients with cutaneous ureterostomy (CU) after radical cystectomy (RC)
Design: Single-center blind randomized controlled trial
Setting: Patients with CU after RC suffer from physical, psychological, social, and economic complications; we consider that maintaining and improving their health is important.
Subjects: Eighty-six patients who underwent RC with CU urinary diversion due to bladder cancer were enrolled in the study and randomly allocated into the experiment and control groups (each with 43 patients).
Intervention: The experimental group adopted the self-care deficit needs based self-model in addition to receiving the usual treatment; control group performed only routine nurse care of the clinic.
Main outcome measure: Demographic information, Functional Assessment of Cancer Therapy-Bladder (FACT-G) and The Hospital Anxiety and Depression Scale (HADS) and complications before and three months after intervention.
Results: The analysis was finally conducted on 40 experimental and 41 controls. The demographic variables of the two groups were not significantly different. All dimensions of QoL, containing FACT-G and HADS, in the experimental group improved significantly after three months intervention when compared to those of the control group. Additionally, there were fewer complications in the experimental group than in the control groups.
Conclusion: Orem's self-care nursing model can make a significant improvement in the function and oval QoL of patients with CU after RC. Therefore, it is necessary and helpful to delineate Orem self-care program and implement it as a part of the treatment program.

KEYWORDS: cutaneous ureterostomy, Orem's self-care, quality of life, radical cystectomy

INTRODUCTION

Bladder cancer (BC) is a common neoplasm in China with particular prevalence among 50 - 70 year old individuals[1]. Most of the cases are transitional cell carcinoma. BC is divided into two types, non-muscle invasive and muscle invasive. The gold standard treatment of muscle invasive BC is radical cystectomy (RC) with urinary diversions, such as cutaneous ureterostomy (CU), Bricker conduit and continent reservoir[2]. CU is less preferred compared with other urinary diversions, but it is more appropriate for patients with elderly age, poor performance status, and when an intestinal segment cannot be used to produce the internal reservoir[3]. Furthermore, when CU is successfully conducted, it is as efficient as the other types of incontinent diversions[4]. Nevertheless, CU may result in a series of complications, including sepsis, urine leakage, dermal papilla, pyelonephritis, and wound infection, even dehiscence[5]. In addition, it also changes the original way of urination with losing the normal micturation ability, which leads to psychological, social complications, such as anxiety, depression, low self-esteem, short of family and friends' attention and support[6]. These complications negatively influence these patients' quality of life (QoL) [7,8]. Thus far, after patient's discharge, QoL in stoma

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care is a priority. Recently, it has been proposed that QoL is an important parameter to assess individual health, determine and evaluate the overall health index of the community, and it also helps to discover the main problems of personal life on different aspects[9]. Focusing on QoL will help the nurses to understand the patients' needs and improve QoL of the patients. Nurses, as a member of the health care team, play a critical role in helping the patients accommodate their ways of life to the new situation, which leads to changes in their lives due to urinary diversion and the related complications.

If the patients' self-care is promoted, they will better control their daily lives and handle social performance, which will improve their QoL in turn[10]. It can improve self-management and reduce complications through empowering the self-care strategies to the patients. Therefore, it is important and meaningful for the patients to learn the related knowledge and skill in making decisions and solving disease-related problems. Fortunately, Orem self-care model can contribute to self-care and include the behaviors that individuals identify and perform for their own sake in order to keep their lives and health. Consequently, it usually makes the patients have well-being feelings[11]. Orem self-care nursing model is designed in three categories: wholly compensatory, partly compensatory, and supportive developmental[12].

Based on the condition that patients with CU after RC suffer from physical, psychological, social, and economic complications, we consider that maintaining and improving the patients' health is very important. In addition, as far as we know, there has been no study in investigating the effect of Orem self-care program on QoL and complications of these patients. In this regard, we conduct the present study to solve the above issues.

SUBJECTS AND METHODS

Subjects

We enrolled 86 consecutive patients who underwent RC with CU urinary diversion due to BC in Urology Department of our hospital between September 2010 and March 2016. The inclusion criteria included the patients with an American Society of Anesthesiologists score >2, received RC with CU urinary diversion and have the ability of reading comprehension and writing. The exclusion criteria were other urinary diversions, such as ileal conduit and continent reservoir, failure to perform the intervention appropriately, palliative cystectomy for massive bleeding, and unwillingness to participate in this study. This study was performed according to the Declaration of Helsinki and approved by the institutional review boards of The First Affiliated Hospital of Nanjing Medical University (#JSHP2010-575, approved 8/07/2010). All subjects signed an informed consent.

All the subjects underwent collection of general information (demographic characteristics and pathological results of the surgical specimens), a general version of Functional Assessment of Cancer Therapy-Bladder (FACT-G), Hospital Anxiety and Depression Scale (HADS) and postoperative complications. FACT-G has been validated and used in some types of cancers, and it consists of four domains: physical well-being (PWB), social/family well-being (SWB), emotional well-being (EWB), functional well-being (FWB)[13,14]. Well-being is assessed according to the patient's response to the statement with a five-level scale, which ranges from "not at all" to "very much". HADS is designed for self-assessment and comprises seven items dealing with anxiety and seven referring to depression[15].

The patients were randomly grouped into the experimental group and the control group. Patients were assigned to each group with 1:1 matching (each group with 43 patients). Then, the experimental group adopted the self-care deficit needs based self-model, which is in addition to receiving the usual treatment, while the control group performed only routine nurse care of the clinic.

The steps for Orem's Self-Care nursing model in the experimental group are as follows. Firstly, the experimental group evaluated and identified the CU urinary diversion and established self-care needs to design the self-care model. The self-care needs pertain to cleaning and changing urinary drainage bags, nursing the skin surrounding the stoma, observing the color of urine, stress control methods, nutrition, regular return visit, activity, and sleep improvement; Secondly, to adapt the patient to the "self-care", the contents mentioned in the first step must be provided. Therefore, four theoretical and practical sessions of 35 - 40 minutes were conducted for demonstrating the self-care program in the experimental group per day for one week. According to the education and understanding level of the participants and their needs, we prepared each training session with target contents. Moreover, all the points in the training session were edited in the pamphlet and provided to the patients. The patients were asked to comply to the self-care program for three months. Finally, necessary consolations were provided with the aim that the patients gain enough knowledge and skills about self-care. For the sake of knowing how the patients care for themselves, we gave the patients the self-care checklists at the end of each session and tabulated them every time. At the last session of the training, the patients were asked to follow the program as above and record their everyday actions in the checklist for three months to improve

Table 1: Demographic characteristics of patients in the two groups

Demographic parameters	Experiment group (n = 40)	Control group (n = 41)	p-value
Age (years)	68.2 ± 11.4	66.9 ± 10.9	0.591
Sex n(%)			0.404
Male	31 (77.5%)	35 (85.3%)	
Female	9 (22.5%)	6 (14.7%)	
Education level n(%)			0.579
Graduate	12 (30.0%)	14 (34.1%)	
High school	21 (52.5%)	17 (41.5%)	
None or primary school	7 (17.5%)	10 (24.4%)	
Occupation status			0.710
Housekeeper	7 (17.5%)	5 (12.2%)	
Employee	25 (62.5%)	29 (70.7%)	
Other jobs	8 (20.0%)	7 (17.1%)	
Economic status n(%)			0.496
Weak	4 (10.0%)	2 (4.79%)	
Moderate	27 (67.5%)	26 (63.4%)	
good	9 (22.5%)	13 (31.7%)	
Duration of hospitalization period (days)	8.9 ± 2.6	8.2 ± 2.3	0.628
Histological type			0.326
Transitional cell carcinoma	36 (90.0%)	38 (92.7%)	
Adenocarcinoma	2 (5.0%)	3 (7.3%)	
Squamous cell carcinoma	2 (5.0%)	0 (0.0%)	

their QoL. During this period, we monitored the experiment patients through phone calls once a week and answered the patients' questions, considered their problems and provided advice and support for better care.

In the end, after 3 months, the self-care checklists and the form FACT-G and HADS of the experiment and control group were completed and collected again. Additionally, the complications were recorded.

Statistical analysis

The data are shown as the means ± standard deviation when variables are normally distributed and compared by comparative t-test between two groups. Chi-square or Fisher' exact test was used for categorical variables. Additionally, a paired Student's t-test was applied to compare the values before and after application within each group. A two-sided $p < 0.05$ was considered statistically significant. The data was analyzed with SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) statistical software.

RESULTS

Characteristic of the subjects

During the study, totally five patients, including three in the experimental group and two in the control group, were not included in this study due to scheduling/employment conflicts, unwillingness to continue and newly diagnosed cancer recurrence. Finally, 81 patients (94.2%) completed the program and were analyzed. Table 1 presents demographic and clinical characteristics of the included patients. No significant differences were found between the groups in age, gender, education level, occupation and economic status, duration of hospitalization period and histological type ($p > 0.05$, all).

Changes in QoL outcome measures

From the results of self-care checklists (Table 2), we found that related knowledge of stoma and nursing methods were mastered remarkably better in the experimental group in comparison with the control group. Participants reported QoL as measured by FACT-G and HADS. The mean scores of PWB, SWB, EWB, and FWB were respectively 19.8 ± 5.1, 20.8 ± 4.4, 18.4 ± 3.4, and 17.3 ± 5.2 in the experiment group; and respectively 19.5 ± 6.1, 21.2 ± 5.3, 17.9 ± 4.1, and 16.8 ± 5.8 in the control group. There were no statistically significant differences in the PWB, SWB, EWB, and FWB between the two groups. However, after the intervention, compared with the pre-intervention values, significant increased scores of PWB, SWB, EWB, and FWB were achieved for those patients in the experiment group ($p = 0.041$, 0.032, 0.021, 0.012, respectively, Figure 1). The mean score increased by 4.4, 3.7, 4.0 and 5.8, respectively. However, there were even decreased trends of PWB, SWB, EWB, and FWB scores after three months in the control group, although there were no statistic differences ($p = 0.432$, 0.501, 0.212, 0.398, respectively). Figure 1 also showed the comparison results of the constituents of FACT-G scores in patients with CU after RC prior to the intervention and three months later in the experimental and the control group. Similarly, HADS was obviously improved after three months

Table 2: Comparison of the patients' related knowledge of stoma and nursing methods after the intervention between the two groups

Items	Experimental group (n = 40)	Control group (n = 41)	χ^2	p-value
Drainage bag cleaning and replacement	37 (92.5%)	21 (51.2%)	16.969	<0.001
Stoma care	38 (95.0%)	12 (29.3%)	37.029	<0.001
Urine observation	38 (95.0%)	24 (58.5%)	14.993	<0.001
Diet choice	35 (87.5%)	12 (29.3%)	28.188	<0.001
Appointment and indications of catheter replacement	36 (90.0%)	11 (26.8%)	33.173	<0.001

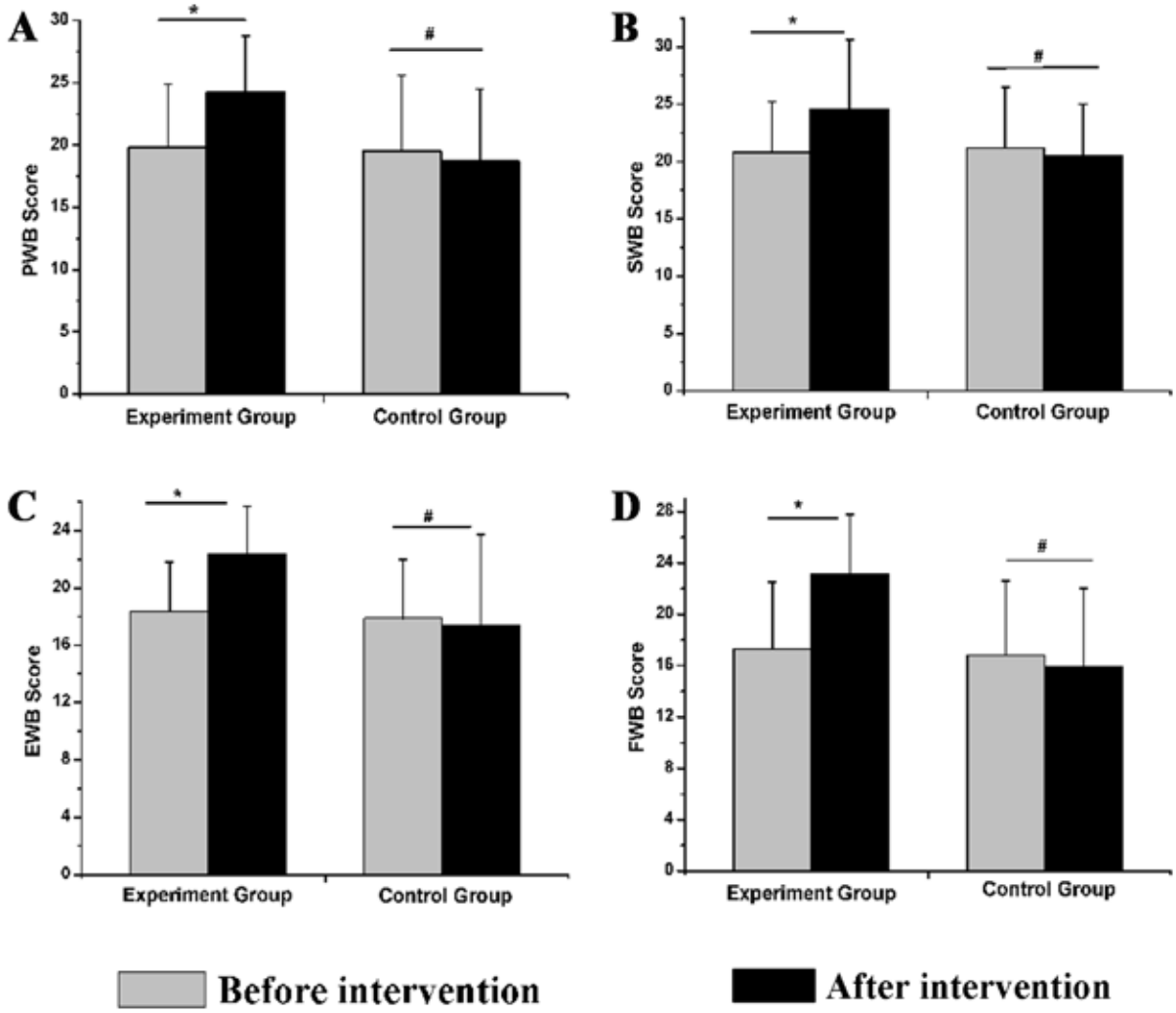

Fig 1. Comparison of quality of life scores before and after intervention in the experiment and control groups of patients with cutaneous ureterostomy after radical cystectomy. **A:** physical well-being (PWB); **B**: social/family well-being (SWB); **C**: emotional well-being (EWB); **D**: functional well-being (FWB).
*p <0.05 and #p <0.05.

intervention in the experiment group (12.8 ± 5.7 versus 8.5 ± 3.2, p <0.001). There were no significant changes in those scores of HADS in the control group (13.1 ± 6.2 versus 14.2 ± 7.2, p = 0.142).

Comparisons of the incidence of complications

In the experiment, six patients in total had complications, including one patient with urine leakage, one with stenosis of the external orifice, one with ureterectasia, one with pyelonephritis, and two with stoma infection. A total of 15 patients in the control group underwent complications, including five patients with stoma infections, one with nipple atrophy, one with terminal necrosis, one with ureterctasia, two with urine leakage, two with stenosis of the external orifice, and three with uronephrosis. The incidence rate of complications in the experiment and control group was 17.5% and 31.7%, respectively. The complication incidence during the intervention in the experiment group was significantly lower than that in the control group (χ^2 = 4.912, p = 0.027).

DISCUSSION

The results have suggested a significant increase in QoL (FACT-G and HADS) of the patients with CU after RC after performing Orem's self-care model in the experimental group when compared with that of the control group. In addition, the patients in the experimental group had fewer complications than those in the control group after hospital discharge.

RC with various types of urinary diversions is the gold standard care for muscle-invasive bladder

cancer. Compared with other urinary diversions, CU has some advantages with shorter operative time and lower risk of bowel and metabolic complications. As a consequence, it is suitable for elderly patients with advanced disease[6]. However, it brings about many negative influences on different aspects of patients' QoL, such as psychosocial functioning, micturition status, day life (physical and sexual) activities, and depression related to body image[16,17]. The more the problems are, the lower the QoL of the patients will be. Nowadays, cure or control of cancer is no longer the only pursuit for malignancy therapy; support in social, psychological, and sexual activities of the patients are also concerned. The QoL concept has been well established in clinical research and often applied to evaluate status changes after surgical or medical interventions. In this study, we took FACT- G and HADS instruments into account for the treatment-related impact of QoL. FACT- G instrument is cancer-specific and intended to measure four dimensions (physical, social, emotional and functional well-being) of QoL[14]. Meanwhile, HADS is a domain-specific instrument, which composed of seven items dealing with anxiety and seven referring to depression[15].

Our study has indicated that the overall mean score of FACT-G of the patients after RC was 75.9 ± 10.1 (76.3 ± 9.4 in the experiment group and 75.4 ± 10.7 in the control group). Meanwhile, the mean score of the two groups after three months intervention was 92.9 ± 11.2 and 72.5 ± 8.3, respectively. These results show that patients after RC have a lower quality of life, which is consistent with previous studies[18-20]. In the experiment group, the mean score of FACT- G and HADS was respectively increased and reduced in the last three months, this indicate that applying Orem's self-care model could significantly improve the QoL of patients with CU. On the contrary, the FACT- G and HADS scores were respectively reduced and increased in the control group. Although the change was not significant, it was noteworthy. This may be related to more complications. Additionally, the complications in the experiment group occurred considerably less than those in the control group, which may account for the fact that the patients get more elated knowledge of stoma and nursing methods after Orem's self-care model. The results of the present study are in accordance with those of other studies, and they further demonstrate that Orem's self-care program plays a positive role in promoting QoL of patients with different chronic diseases.

The above results confirm that designing and performing Orem's self-care theory-based training programs, which have considered the needs of patients with CU after RC, and this is effective in improving the patients' QoL with regular follow-ups. Hence, appropriate programs on the basis of the patients' needs can enhance their quality of care, reducing their dependency and complications, which will make their health optimal. Therefore, medical staff, such as nurses, can prevent the patients from suffering from mental and psychosocial problems by designing and training a self-care program as a part of treatment. Also, fewer complications could bring higher QoL for the patients.

Limitations

There are several limitations in our study. Indeed, the effects of Orem self-care programs on the patients' QoL have been assessed from the three month short-term outcomes. Due to the time constraints, we think that the long-term effects of Orem self-care programs need to be observed for determining the extent reliability of these interventions. Another limitation of this study is the sample size with a relatively small number of patients in each group, and external validation of these data is required.

CONCLUSION

This comparative study demonstrated that QoL of the patients with CU after RC who participate in the self-care program got significantly better than that of those who did not. Thus, to improve QoL and reduce complications for the patients, it is worth performing and implementing Orem self-care programs, which are designed in the light of the patients' needs. In other words, Orem self-care programs could be conducted as a part of the treatment program for the patients. To improve QoL of the patients, it is necessary and helpful to delineate Orem self-care program and implement it as a part of the treatment program.

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Competing financial interests

The authors declare no competing financial interests.

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Original Article

Progesterone treatment preference in heavy menstrual bleeding: Per Os or Levonorgestrel releasing intrauterine device

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ABSTRACT

Objectives: To find out the most satisfactory progesterone treatment method in women who have heavy menstrual bleeding
Design: Open-label, randomized, parallel group, therapeutic study
Setting: The study was carried out at Istanbul Medeniyet University Göztepe Education and Research Hospital gynecology and obstetrics department. Random sequence methods were used; randomization was undertaken using computational random-number generators.
Subjects: This study covered 192 women aged between 32 and 50 years.
Intervention: Ninety-seven patients were applied levonorgestrel intrauterine device (LNG-IUD) and 95 patients were given oral norethisterone acetate 10 mg (5 mg two times daily) during the cycle of 5 - 25 days. LNG-IUD was applied in the first 10 days of the menstrual cycle.
Main outcome measures: The patients in both groups were called for the control on the first visit and on the following 6th and 12th months. In LNG-IUD group, on the level of hemoglobin in the sixth and twelfth months, significant increase was seen and this increase was higher than oral progesterone group ($p < 0.01$). The ratio of decrease on the level of visual bleeding score in the sixth and twelfth month of the study in LNG-IUD group was significantly higher than the oral progesterone group. Due to discontinuation of treatment because of irregular drug usage, LNG-IUD is one step forward.
Results: By using LNG-IUD, we can prevent needless hysterectomy and endometrial ablation in premenopausal women and so minimize postoperative mortality and morbidity. The cost-effectiveness of LNG-IUD is lower than surgery.
Conclusions: At the end of the study, we can say LNG-IUD is a more satisfying alternative treatment method than oral progestogens for overcoming the problem of heavy menstrual bleeding due to its ease of use.

KEYWORDS: anemia, heavy menstrual bleeding, LNG-IUD, menorrhagia, oral progesterone

INTRODUCTION

Heavy menstrual bleeding (HMB), or menorrhagia, is defined as excessive menstrual blood loss that occurs alone or in combination with other symptoms and has a negative impact on a woman's physical, social, emotional, and material quality of life[1]. HMB is menstrual blood loss of 80 ml or greater[2]. Approximately 30% of women are negatively affected by menorrhagia during their reproductive years[3,4]. Conventional medical treatment (mefenamic acid, tranexamic acid, norethindrone, medroxyprogesterone acetate injection, or combined oral contraceptive pills) in patients with menorrhagia were included. Although endometrial ablation is a less invasive surgical alternative to hysterectomy, it does not eliminate surgical risk and is followed by further surgery within 4 years in up to 38% of the women who undergo this treatment[5,6]. Hysterectomy is often used to treat women with this complaint, but medical therapy may be a successful alternative. The intrauterine coil device was originally developed as a contraceptive, but the addition of uterine relaxing hormones, progestogens,

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to these devices resulted in a large reduction in menstrual blood loss[7,8].

In the study, we have compared the use of levonorgestrel intrauterine device (LNG-IUD) and the most frequently used oral progesterone in respect to the effectiveness for minimizing the present symptoms, patient satisfaction and the side effects.

SUBJECTS AND METHODS

The study was carried out at Istanbul Medeniyet University Göztepe Education and Research Hospital gynecology and obstetrics department between November 30, 2011 and December 31, 2013. This study covered 192 women aged between 32 and 50 years. It was designed as an open-label, randomized, parallel group, therapeutic study. The two medical treatment methods were suggested to the women who refused any kind of surgery. Some of them didn't want to have an intrauterine device. Random sequence methods were used; randomization was undertaken using computational random-number generators. Consequently, not all patients who attended the hospital during the study period were included in the trial, even if they met the inclusion criteria. All women participated voluntarily and involved in the study based on their polyclinic application number. Each patient was examined by ultrasound. They gave their written informed consent before entry into the study. The study was granted ethical approval from Istanbul Medeniyet University Göztepe Education and Research Hospital Ethics Committee.

The criteria applied for the inclusion of the patients in the study are the following:

1. Heavy menstrual bleeding
2. The size of the uterus is less than 10 weeks
3. No presence of the endometrial polyp, submucosal myomas, endometrial atypical hyperplasia and malignancy
4. Being decisive not to have children anymore

Unknown reason for vaginal bleeding, having gynecologic malignancy including ovary and breast cancer, liver illness, diabetes mellitus, pelvic inflammatory disease and septic abortus in the last three months are our criteria to exclude from the study.

All women were asked about their gynecological history, and general and pelvic examination was done for each of these patients. Transvaginal ultrasound was performed to assess the uterus, adnexa and endometrial line. To exclude cervical pathology, PAP smear was done with pipelle and endometrial biopsy was done by pipelle not requiring general anesthesia.

To discriminate between menorrhagia and normal menstrual blood loss, a simple visual assessment technique was used as described by Jannsen *et al*[9]. Information on menstrual bleeding was obtained by interview prior to entering the study using a pictorial chart form to describe the degree to which the sanitary wear was soiled. A score was calculated by multiplying the number of slightly, moderately and heavily soiled pads and tampons by 1, 5 and 20 for pads, 1, 5 and 20 for tampons, according to their degree of staining (Figure 1). A score of 185 was used as a cutoff point as this score has a predictive value of 85% to be consistent with menorrhagia.

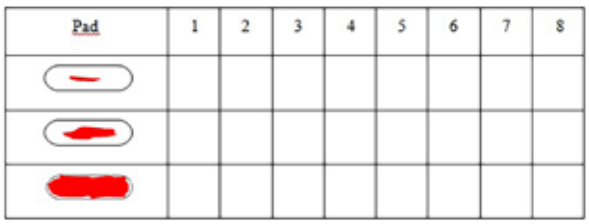

Fig 1. Menstrual bleeding measurement with pads

Another main outcome measure was health-related quality of life, measured by the SF-36 instruments. Women were followed-up at the first visit, and at the 6th and 12th months following insertion of LNG-IUD. During follow-up, women were interviewed about their bleeding patterns and any side effects or adverse reactions.

The patients were divided into two groups. Ninety-seven patients were applied LNG-IUD and 95 patients had been given oral norethisterone acetate (NETA) 10 mg (5 mg two times daily) during the cycle of 5 - 25 days. LNG-IUD was applied in the first 10 days of the menstrual cycle.

The patients in both groups were called for the control on the first visit and on the following 6th and 12th months. They were asked to bring their menstrual pictorial chart on their visits, performed by transvaginal ultrasound, required cell blood count, and their filled-up SF-36 questionnaire. In this way, we assessed the patients' view on treatment, the complaints and the possible side effects.

The findings of the study were assessed by SPSS (Statistical Package for Social Sciences) for Windows 15.0 programmer. Besides descriptive statistical methods (mean, standard deviation); comparing quantitative data for normal distributed parameters into two groups were assessed by Student t test and not normal distributed parameters into two groups were assessed by Mann Whitney U test. Paired Samples T Test was applied within the group for the parameters which are normally distributed. Wilcoxon Signed Ranks test was applied within the group for the parameters which are not normally distributed. For the comparison of qualitative data, we used Chi square and Fisher's Exact Chi square tests. The

Table 1: VBS evaluation

Visual Blood Score	Treatment		+p-value
	Oral Progesterone Average ± SS (median)	LNG-IUD Average ± SS (median)	
Inception	400.21 ± 105.58 (390)	491.86 ± 126.02 (560)	
6th month	169.92 ± 163.13 (90)	73.95 ± 103.36 (65)	0.001**
12th month	129.91 ± 104.06 (90)	64.21 ± 86.91 (60)	0.001**
Inception - 6th month (%) change	-55.03 ± 44.55 (-75)	-83.37 ± 25.12 (-87.17)	0.001**
Inception - 12th month (%) change	-64.49 ± 29.36 (-75.57)	-85.54 ± 21.47 (-88.19)	0.001**
6th Month - 12th month (%) change	23.89 ± 166.02 (-11.1)	-13.21 ± 6.33 (-12.5)	0.001**
Inception - 6th month ++p	0.001**	0.001**	0.950
Inception - 12th month ++p	0.001**	0.001**	
6th Month - 12th month ++p	0.041*	0.001**	

+Mann-Whitney U test; ++Wilcoxon Sign test
*p <0.05; **p <0.01

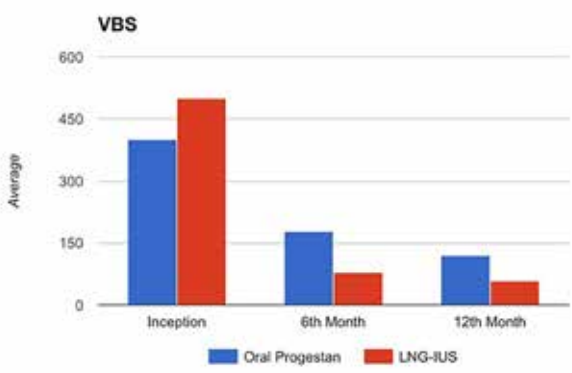

Fig 2. VBS levels distribution

significance value is p <0.05. The results are taken from all the patients who continued to participate in the study.

RESULTS

This study was carried out between November 30, 2011 and December 31, 2013 on 192 patients; 97 in LNG-IUD group and 95 in NETA group. The age of the patients ranged from 32 to 50 years, the average age was 40.47 ± 4.17 years in LNG-IUD group, parity 2.42 ± 1.03 and in NETA group the average age was 40.20 ± 4.28 years and the parity was 2.54 ± 0.93.

The level of visual blood score (VBS) in the sixth and twelfth month of study in oral progestogens was significantly higher than in LNG-IUD group (p <0.01); the VBS in LNG-IUD at the beginning was significantly higher than oral progesterone group (p <0.01) (Figure 2).

Table 1 summarizes the ratio of decrease in the level of VBS in the sixth and twelfth month of the study in LNG-IUD group, which was found to be significantly higher than the oral progesterone group.

Table 2 shows hemoglobin levels were significantly increased (p <0.01) in oral progesterone group in the sixth and twelfth months. In LNG-IUD group, a significant increase was seen in the level of hemoglobin in the sixth and twelfth months, and this increase was higher than oral progesterone group (p <0.01).

At the beginning, heavy menstrual bleeding was seen in patients in both groups. At the sixth month

Table 2: Hemoglobin evaluation

Hemoglobin	Treatment		+p-value
	Oral Progesterone Averages (median)	LNG-IUD Averages (median)	
•Inception	10.56 ± 0.81	11.03 ± 0.99	0.001**
•6th month	10.97 ± 0.77	11.74 ± 0.96	0.001**
•12th month	11.35 ± 0.67	12.77 ± 0.56	0.001**
+Inception - 6th month (%) change	3.83 ± 10.55 (3.77)	7.62 ± 10.65 (6.94)	0.031*
+Inception - 12th month (%) change	7.76 ± 9.56 (7.31)	18.0 ± 10.88 (17.99)	0.001**
+6th Month - 12th month (%) change	3.57 ± 8.86 (2.56)	9.66 ± 8.39 (8.29)	0.001**
Inception - 6th month ++p	0.013*	0.001**	
Inception - 12th month ++p	0.001**	0.001**	
6th Month - 12th month ++p	0.008**	0.001**	

•Student t test; +Mann-Whitney U test; ++Paired Samples t test
*p<0.05; **p<0.01

of the study, there was no statistical difference in their menstruation situation in both groups (p >0.05). However, there was a significant statistical difference in menstruation situations at the twelfth month of the study. The ratio of amenorrhea in LNG-IUD was 40.2% and in the oral progesterone group was 5.3% at the end of the twelfth month (p <0.01). The ratio of menorrhagia at the twelfth month was 12.2% in LNG-IUD and 35.1% in oral progesterone group (p <0.01). Normal and oligomenorrhea situation had no statistical difference in both groups (p >0.05).

Table 3 summarizes the side effects. In looking for the side effects, there was a significant statistical difference at the end of six and twelve months. In oral progesterone group, edema ratio was 16.9% and in LNG-IUD it was 4.7% (p <0.05). In sixth-month side effects, the other side effects had no significant difference in both groups. The depression ratio was 1.2% in LNG-IUD group, and 8.8% in oral progesterone group at the twelfth month of treatment (p <0.05). The ratio of edema was 10.5% in oral progesterone group and 1.2% in LNG-IUD at the end of the twelve months (p <0.05). The other twelfth month side effects had no statistical difference in both groups (p >0.05).

Table 3: Side effect evaluation

Month	Side Effect	Treatment		p-value
		Progesterone n (%)	LNG-IUD n (%)	
	None	20 (30.8%)	39 (45.3%)	
	•Acne	3 (4.6%)	4 (4.7%)	1.000
	•Headache	4 (6.2%)	5 (5.8%)	1.000
6th month	Depression	3 (4.6%)	2 (2.3%)	0.652
	•Hirsutism	5 (7.7%)	3 (3.5%)	0.291
	•Weight Gain	8 (12.3%)	6 (7.0%)	0.263
	•Mastalgia	7 (10.8%)	9 (10.5%)	0.952
	•Edema	11 (16.9%)	4 (4.7%)	0.013*
	Ovarian Cyst	2 (3.1%)	8 (9.3%)	0.189
	Pelvic Pain	2 (3.1%)	6 (7.0%)	0.467
12th month	None	26 (45.6%)	62 (75.6%)	
	Acne	2 (3.5%)	2 (2.4%)	1.000
	Headache	3 (5.3%)	2 (2.4%)	0.401
	Depression	5 (8.8%)	1 (1.2%)	0.042*
	Hirsutism	3 (5.3%)	1 (1.2%)	0.305
	Weight Gain	5 (8.8%)	2 (2.4%)	0.123
	Mastalgia	5 (8.8%)	3 (3.7%)	0.272
	Edema	6 (10.5%)	1 (1.2%)	0.019*
	Ovarian Cyst	1 (1.8%)	5 (6.1%)	0.401
	Pelvic Pain	1 (1.8%)	3 (3.7%)	0.644

Treatment discontinuations in oral progesterone group were 31.6% at the sixth month because of irregular drug usage (22 patients), edema (4 patients), depression (2 patients), mastalgia (1 patient) and hirsutisms (1 patient). At the end of the twelve months, there were 65 patients in oral progesterone group; 8 of them dropped out of the study (5 patients with irregular drug usage, 1 with patient acne, 1 with patient edema and 1 with patient headache). The discontinuation in LNG-IUD group was 11 patients (11.3%) at the sixth month and 4 patients (4.6%) from the remaining 86 patients at the twelfth month. In the 11 patients who discontinued at the sixth month, three patients had pelvic pain, three of them had mastalgia and three of them had ovarian cyst. At the end of twelve months, of the four that discontinued, two of them had mastalgia and one each had pelvic pain and hirsutisms.

DISCUSSION

HMB is one of the most common gynecological disorders affecting women of reproductive age, accounting for 20% of all gynecological visits to general practitioners[10]. HMB is associated with a lower quality of life, loss of productivity and increased healthcare expenses[3,11] and in usual practice, it is initially treated pharmacologically[12], with tranexamic acid and norethisterone believed to be the most effective[12]. Surgical treatment for HMB often follows unsuccessful or ineffective medical therapy; however, hysterectomy is a major surgical procedure with significant physical and emotional complications, in addition to the social and economic cost. Various minimally invasive surgical techniques, such as thermal balloon ablation, the LNG-IUD, transcervical resection of the endometrium, microwave ablation, diffused laser energy ablation, bipolar impedance-controlled ablation, cryoablation, hot saline instillation[13,14], and various methods of endometrial ablation have been developed, with the purpose of improving menstrual symptoms, and these have achieved great success.

The LNG-IUD was developed in Finland during the 1980s and is an intrauterine device that releases 20 μg levonorgestrel every 24 hours over 5 years. Although initially licensed as a contraceptive, in 1990, the LNG-IUD was tested and reported to be effective in the treatment of menorrhagia, as a non-contraceptive benefit[15].

In 2013, the Society of Gynecologic Surgeons Systematic Review Group published a review of 22 studies comparing non-surgical therapy for the treatment of abnormal uterine bleeding presumed due to endometrial dysfunction. The authors concluded that regarding reduction of menstrual bleeding, LNG-IUD (71 - 95% reduction), combined oral contraceptive pills (OCP) (35 - 69% reduction), extended cycle oral progestins (87% reduction), tranexamic acid (26 - 54% reduction), and nonsteroidal anti-inflammatory drugs (NSAID, 10 - 52% reduction) were all effective treatments. The LNG-IUD, combined OCPs, and antifibrinolytics were all superior to luteal-phase progestins. The LNG-IUD was the best treatment,

and it has been shown that it is superior to combined OCPs and NSAID. Antifibrinolytics were superior to NSAIDs. The authors have also underlined the lack of data about the impact of such treatment on the quality of life. Once again, according to American and Canadian guidelines, surgical treatments (hysterectomy and endometrial ablation) should be restricted to the failure of medical therapy, inability to utilize medical therapies, significant anemia, impact on quality of life, and concomitant uterine pathology.

Intrauterine devices were initially introduced as contraceptives, but after the addition of progestogen (LNG-IUD) these devices also reduce menstrual bleeding effectively. The local release of levonorgestrel in the uterine cavity suppresses endometrial growth. A systematic review of the effectiveness of the LNG-IUD in heavy menstrual bleeding concluded that the reduction of menstrual blood loss was 79 - 96% in the LNG-IUD group[16,17]. In women with heavy menstrual bleeding who presented to primary care providers, the LNG-IUD was more effective than usual medical treatment in reducing the effect of heavy menstrual bleeding on quality of life[19]. However, up to 60% of women discontinue LNG-IUD within 5 years because of unscheduled bleeding, pain, and/or systemic progestogenic side-effects[19]. In the Royal College of Obstetricians and Gynecologists guideline on heavy menstrual bleeding, the use of the LNG-IUD is the first therapeutic option when drug treatment has failed. This is not based on proven cost-effectiveness[19].

The main problem for progesterone is poor attendance of the patients. In the study carried out by Andrew M and his friends in the sixth month for LNG-IUD group, the ratio of attendance to the medical treatment was 77%; for NETA group this ratio goes down to 22%[20]. In this study, the drop out ratio of the patients using oral progesterone was 31.6% at the end of the sixth month. The drop out ratio for LNG-IUD was 11.3% at the end of the sixth month.

One hundred and sixty-five women were randomized (82 LNG-IUD / 83 medroxyprogesterone acetate). Increase in median hemoglobin levels from baseline to Cycle 6 (7.5% vs. 1.9%; p <0.001); baseline median hemoglobin levels were 12.4 g/ld. with the LNG-IUD and 12.2 g/dl with oral medroxyprogesterone acetate (MPA), respectively. At Cycle 6, the corresponding medians were 13.4 g/dl with the LNG-IUD and 12.6 g/dl with MPA. At Cycle 6, the proportion of women who rated their bleeding as 'improved' was higher with the LNG-IUD than with oral MPA, both according to investigator assessment (93.6% vs. 61%) and self-assessment (93.6% vs. 67.1%), parallel to our findings[21]. In our study, median hemoglobin levels at the beginning and at the end of six months in oral progesterone group were 10.56 ± 0.81 and 10.97 ± 0.77 respectively; in LNG-IUD group, the hematocrit levels at the beginning and at the end of six months were 11.03 ± 0.99 and 11.74 ± 0.96 respectively. In oral progesterone group, the sixth and twelfth month hemoglobin levels were significantly increased (p <0.01). In LNG-IUD group, on the level of hemoglobin in the sixth and twelfth months, a significant increase was seen, and this increase was higher than oral progesterone group (p <0.01).

Spotting is the most frequent side effect in the usage of LNG-IUD in the literature[22]. The reason of spotting is levonorgestrel diffusion in the endometrium was not homogeneous in the first months. This makes atrophy center patches and vessel variable changes in the endometrium, and so irregular endometrial exfoliating occurs[22,23]. In LNG-IUD group, 22.1% of the patients at the end of the six months and 13.4% at the end of the twelve months applied because of spotting. In oral progesterone group, the spotting ratio was 30.8% at the end of the six months and 29.8% at the end of the twelve months.

The two most important long-term effects of LNG-IUD were amenorrhea and oligomenorrhea. If patients were not informed clearly on this subject, this could be a reason to discontinue the treatment. The contraceptive effect of LNG-IUD is reversible, and fertility returns soon after the LNG-IUD was removed[24-26]. In our study, 40.2% amenorrhea and 18.3% oligomenorrhea were seen after twelve months in LNG-IUD group. In oral progesterone group, these ratios were 5.3% amenorrhea and 15.8% oligomenorrhea after twelve months. In both groups, nobody gave up their treatments.

While LNG-IUD was used, the levonorgestrel excessing to the blood had some side effects. They are mastalgia, weight gain, edema, hirsutisms, acne, headache and ovarian cysts[27]. In this study after six months, 31.6% in oral progesterone group and 11.3% in LNG-IUD group gave up the treatment. In oral progesterone group, the reasons for discontinuation were 22 with irregular drug usage, 4 with edema, 2 with depression, 1 with mastalgia and 1 with hirsutisms. In the 11 discontinued patients of the LNG-IUD group, 3 of them had pelvic pain, 3 had ovarian cysts and 4 had mastalgia.

However, none of the patients had stopped the medical treatment; functional ovarian cysts is mentioned to be one of the most frequently seen adverse effects in the literature (10%)[23,28]. In our study, five patients (6.1%) had functional ovarian cysts in LNG-IUD group at the end of the twelve months. These cysts are smaller than 4 cm, simple and do not give pain. In oral progesterone group at

the end of the twelve months, one patient (1.8%) had functional ovarian cysts. All these cysts have naturally disappeared during the controls.

CONCLUSION

LNG-IUD is one of two hormonal IUDs with Food and Drug Administration approval. The other is Skyla, which prevents pregnancy for up to three years. LNG-IUD can prove to be an effective treatment for menorrhagia. This is because levonorgestrel is a very potent blocker of estrogen activity on the endometrium. The effect of LNG in the uterine cavity is that it gradually reduces the thickness and vascularity of the endometrium over the initial 3 - 6 months of use. Due to this suppression of the endometrium, most women experience a reduction of blood loss, but its therapeutic significance is greatest in women with menorrhagia.

Since LNG-IUD has reversible effects in women of reproductive age, especially for those who want to keep their fertilities, it is an effective, noninvasive method in heavy menstrual bleeding treatment which provides long-term efficacy. By using LNG-IUD ,we can prevent needless hysterectomy and endometrial ablation in premenopausal women, and so we minimize postoperative mortality and morbidity. The cost-effectiveness of LNG-IUD is lower than surgery.

LNG-IUD is a more satisfying alternative treatment method than oral progestogens for overcoming the problem of heavy menstrual bleeding due to its ease of use.

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Ayse Kavasoglu conceived, designed and did statistical analysis, edited the manuscript, did data collection and manuscript writing. Professor Dr. Ahmet Gocmen did the review and final approval of manuscript takes the responsibility and is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. No potential conflict of interest was reported by the authors.

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Original Article

Sleep problems among parents of children with autism spectrum disorders in Oman: A case control study

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ABSTRACT

Objectives: Studies largely from Western countries have indicated a high rate of sleep problems among parents of children with autism spectrum disorder (ASD). This study was conducted in Oman, a Middle Eastern Arab country, in order to compare indices of sleep behavior and sleep quality among parents of children with presently operationalized control intellectual disabled (ID) and typically developing children (TD).
Design: Case control study
Setting: The quality of sleep was quantified using Arabic version of Pittsburgh Sleep Quality Index (PSQI). The highest performance in PSQI signifies poor quality of sleep.
Subjects: Parents with ASD children (n = 220), ID (n = 109), and TD (n = 125)
Main outcome measure: Overall, the performance of PSQI among parents with ASD are higher compared to control group (6.6, 6.0 vs. 5.5; p-value 0.001) indicating that parents with ASD endorsed more sleeping problems than parents with ID and TD children.
Results: In terms of performance in subscale of PSQI, parents of children with ASD endorsed themselves poorly in indices of subjective sleep quality, sleep latency and duration, compared to controls. Risk of having poor sleep quality was significantly higher among parents of ASD and ID children compared to TD children (p-value <0.05).
Conclusions: This study provides preliminary evidence of quality of sleep among parents caring for children with ASD, ID and TD. This study is in consonant with the trend from elsewhere, indicating that parents of children with ASD have poor quality of sleep and all the consequences this may entail.

KEYWORDS: autism, Oman, parents, sleep problems

INTRODUCTION

Many studies have reported that children with autism spectrum disorders (ASD) tend to have deranged integrity of circadian rhythm[1-4]. Park explored the repercussions of poor sleep in children with ASD in South Korea, and found that compared to typically-developed (TD) children, children with ASD tend to exhibit more propensity towards bedtime resistance, insomnia, and the resultant daytime sleepiness[5]. Blader examined the impact of disrupted sleep problem among elementary school children in a defined region in the USA[6]. The study found that children with disrupted sleep tend to exhibit social deficit during waking hours. Other studies are congruent with such view[7-10]. Diomedi observed that children with ASD exhibit abnormalities in sleep architecture based on a polysomnography study, suggesting that social deficits are likely to be underlined by an abnormality in the brain rather than just being attention seeking behavior[11].

Complementing aforementioned link between ASD and disturbance of quality and quantity of sleep[12-14], there are a plethora of studies suggesting that the majority of parents of children with ASD are marked with deprived quality and quantity of sleep[15-16]. This has led to interventions to mitigate disturbed sleep in parents with ASD children[17]. As there is a rising tide of ASD in different corners of the world[18], it

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remains to be seen whether sleep problem in parents of children with ASD is a universal problem or whether it is limited to certain societies.

There are limited number of publications exploring sleep behaviors and quality of sleep among family caregivers in the Arab/Islamic countries. In order to fill the gap in the available literature, the aim of this study is to compare the performance on indices of sleep behavior and quality of sleep among family caregiver of children with ASD, intellectual disabled (ID), and TD in Oman.

The Sultanate of Oman is an Arab/Islamic country located in Southwest Asia laying on the Southeast Coast of the Arabian Peninsula, covering 309,500 sq km with a population of approximately 3.3 million. Overall, Oman's population is characterized by large family size and consanguineous marriage which is thought to trigger many developmental anomalies including those that are likely to render one with social and intellectual incompetency[19]. Al-Farsi estimated the prevalence of diagnosed cases of ASD in Oman to be 1.4 per 10,000 Omani children (aged 0-14 years) in their catchment area[20]. Al-Farsi surveyed the perceived burden for caring among caregivers of children with ASD in an urban setting in Oman, and concluded that most caregivers in Oman tend to incur serious social and financial burden by having to care for children with ASD[21].

SUBJECTS AND METHODS

The present case-control study was designed to determine sleep quality and sleep-wake patterns among family caregivers of children with and without diagnosis of ASD. The study was conducted over the period from November 2013 to June 2014. All the participants that were included in the study provided their informed consent. The participants consisted of three groups of parents: parents of children with ASD as cases, and parents of ID and TD children as controls.

To qualify for inclusion in this study, the parents of children with ASD must have a child with a confirmed diagnosis of ASD. Ascertainment of ASD diagnosis was made according to the Gold-Standard criteria based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)[22]. Accordingly, all participants must be family caregivers of a child with ASD who fulfilled the eligibility for diagnosis of ASD's exhibiting symptoms within the triad of typical autistic traits: communication impairment, social deficits, and ritualistic interests as classified in the DSM-IV-TR. The family caregivers of children with ASD were recruited from two places that were deemed to be acceptable catchment area for the present study. The majority of parents of children with ASD were from Sultan Qaboos University Hospital (SQUH) - Behavioral Medicine Clinic. SQUH is a tertiary care hospital that receives children with ASD from different secondary care hospitals. The second recruitment center is from schools that cater for the needs with special demands and talents (e.g. Al-Wafa Rehabilitation Centers, Muscat Autism Center, Early Intervention Association, and the Association of the Welfare of the Handicapped Children).

Parents of children with ID were recruited from Al-Wafa Rehabilitation Centers. The inclusion criteria included a psychometric assessment that confirmed the presence of intellectual disability, that is, intellectual quotient is defined as falling below 25 percentiles, with marked impairment in adaptive behaviors that have direct bearing on daily living and educational competency. If the child has other comorbid conditions, the family caregivers were excluded from the study.

The third group was parents of TD children, who were recruited from public and private schools. These schools appeared to constitute mainstream education which do not cater to the need for children with failure to thrive. Protracted psychosocial history of the children was solicited from participating parents. If the psychosocial history suggested any form of failure to thrive in cognitive or emotional development, the family caregivers were excluded from the present study.

Pittsburgh Sleep Quality Index (PSQI) was employed to assess various dimensions of quality and quantity of sleep[23]. PSQI is a 19-item self-reported questionnaire that solicits seven indices of sleep (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications, and daytime dysfunction).

Score range is evaluated between 0 (no difficulty) to 3 (severe difficulty). In addition, there are special arrangements for some questions -- from zero (not during the past month) to three (three or more times a week). By adding component scores, a total sleep quality score that ranges from 0 (good sleep quality) to 21 (poor sleep quality) is obtained. The sum of scores of the seven subscales gives the global score that is used to differentiate 'good sleepers' from 'poor sleepers'[23]. The cut-off of >5 of the global PSQI score is used to distinguish those parents who were 'good sleepers' from 'poor sleepers'. PSQI has been validated into more than 52 different languages with adequate psychometric property[24]. The present study utilized the Arabic version of PSQI and its psychometric performance was found to be acceptable for Arabic-speaking population[25,26]. In Oman, it has been employed in different clinical populations[27].

Chi-square analyses were used to evaluate the statistical significance of differences among proportions of categorical data. ANOVA has been used to assess the statistical significance of differences among continuous data. The non-parametric Fisher's

Table 1: Socio-demographic characteristics of parents with children with Autism Spectrum Disorder (ASD), Intellectual Disabilities (ID) and Typical Development (TD), Oman, 2014

Characteristics	Total (N = 454) n (%)	ASD (n = 220) n (%)	Control (n = 234)			p-value*
			Total (n = 234) n (%)	ID (n = 109) n (%)	TD (n = 125) n (%)	
Parent						0.6
Father	215 (47.4)	107 (48.6)	108 (46.2)	50 (45.9)	58 (46.4)	
Mother	239 (52.6)	113 (51.4)	126 (53.8)	59 (54.1)	67 (53.6)	
Age						0.8
Less than 40	254 (56)	124 (56.4)	130 (55.6)	39 (35.5)	91 (72.7)	
40 or above	200 (44)	96 (43.6)	104 (44.4)	70 (64.5)	34 (27.3)	
Education						0.4
Illiterate	45 (9.9)	17 (7.8)	28 (12)	22 (20)	6 (4.1)	
High school	241 (53.1)	116 (52.7)	125 (53.4)	74 (67.6)	51 (41.1)	
University	168 (37.0)	87 (39.5)	81 (34.6)	13 (12.4)	68 (54.8)	
Occupation						0.5
Employed	261 (57.5)	132 (60.0)	129 (55.1)	41 (37.6)	88 (70.4)	
Unemployed	193 (42.5)	88 (40.0)	105 (44.9)	68 (62.4)	37 (29.6)	
Monthly income (OMR)						0.06
Less than 500	140 (30.8)	59 (26.8)	81 (34.6)	61 (56.2)	20 (16.1)	
500 to 1000	123 (27.1)	57 (25.9)	66 (28.2)	24 (21.9)	42 (33.9)	
More than 1000	191 (42.1)	104 (47.3)	87 (37.2)	24 (21.9)	63 (50)	

*p-value has been calculated to compare between total cases and total controls.

exact test (two-tailed) replaced the Chi-square test in case of small sample size, where the expected frequency is less than 5 in any of the cells in 2 x 2 tables. For assessment of difference in means of continuous variables, independent Student's t-test and Mann-Whitney test were performed as parametric and non-parametric tests, respectively. The odds ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression models were taken as the measures of association between 'poor sleep' as indicated by global PSQI and status of children (ASD, ID, and TD). Important confounders were adjusted from using multivariate logistic regression modeling.

All statistical analyses were performed using the Statistical Package for Social Sciences software (Version 20.0, IBM, Chicago, Illinois, USA). A significant association is considered if the 95% CI does not include the value 1.0, and a cut-off p-value of less than 0.05 is used for all tests of statistical significance in this study.

The study was approved by the Medical Research Ethics Committee in the College of Medicine and Health Sciences, Sultan Qaboos University (MREC#786). Consent form was attached with the questionnaire to get the signature from parents who participated in the study.

RESULTS

Table 1 shows the socio-demographic data of parents of ASD children, ID children, and TD children. The control group was composed of ID and TD children. All socio-demographic characteristics were not significantly different in between parents in the two groups: cases and control. Fathers constituted 47.4%, which was less than that of mothers (52.6%). The mean age of all parents was 39.7 years. The majority (56%) of total parents were below 40 years.

Parents with ASD and TD children were more educated than parents with ID children (90% vs. 80%). Illiteracy was more prevalent among parents of ID (20%) than ASD or TD children. Parents with ID children who graduated from university were only 12.4%, while parents of ASD and TD children were 39.5% and 54.4% respectively.

The majority of parents with ASD and TD children had an occupation with 60% and 70.4% respectively. However, parents with ID children who were employed were only 37.6%. Therefore, the family monthly income for parents with ID children was less compared to the other two groups. Parents with ID children who had monthly income more than 500 Omani rials (1 OMR = 2.6 US$) were only 43.8%, whereas parents with ASD and TD were 73.2% and 83.9%, respectively.

Table 2 reveals that parents in the ASD group have, on average, higher scores of sleep problems on five of the seven measures, in comparison to parents in the control group (TD and ID), and the differences were statistically significant ($p < 0.05$). The total PSQI score is higher among parents of ASD children with mean 6.6 (SD = 3.3), compared to parents with TD or ID children with mean 5.5 (SD = 2.4). This result indicated

Table 2: The performance on indices of quality of sleep of Pittsburgh Sleep Quality Index (PSQI) among parents with children with ASD compared to control, Oman, 2014

Variable	ASD (n = 220) mean (SD)	Control (ID +TD) (n = 234) mean (SD)	p-value
Subjective sleep quality	1.2 (0.8)	0.7 (0.7)	0.0001
Sleep latency	1.4 (1.1)	1.2 (0.8)	0.09
Sleep duration	1.7 (0.9)	1.3 (0.8)	0.0001
Sleep efficiency	0.06 (0.2)	0.04 (0.2)	0.5
Sleep disturbances	1.1 (0.6)	1.1 (0.6)	0.5
Sleep medications	0.1 (0.4)	0.1 (0.4)	0.8
Daytime functioning	0.9(0.9)	0.8 (0.8)	0.1
Total score (Global PSQI)	6.6 (3.3)	5.5 (2.4)	0.001

that parents with ASD children have more sleeping problems compared to parents with ID or TD children.

Table 2 shows the performance on indices of quality of sleep of PSQI among parents of children with ASD compared to control. Overall, parameters of sleep quality of parents with ASD children were significantly poorer than parents with ID and TD children (p-value = 0.0001). Most parents of children with ASD needed more than 20 minutes to fall asleep. The total number of sleeping hours with parents of ASD children were less than with parents of ID or TD children (p-value = 0.0001). Parents with ASD children spend more time in bed before falling asleep compared to parents with ID and TD children. There are some sleeping troubles that may disturb sleep quality with parents at night such as feeling hot or cold, cannot breathe comfortably, having bad dreams or pain, cough or snore loudly and waking up in the middle of the night or early morning. These sleep disturbances were similar with parents of ASD children and parents with ID or TD children. Parents of ID and TD children took sleep medications, same as parents with ASD group. Parents with ASD children feel tired in the morning due to less sleep hours in the night.

Table 3 shows the subjective endorsement of sleep quality among parents of children with ASD compared to control group. Sleep quality was assessed as "bad sleep" by 23.6% of the parents who have children with ASD compared to only 8.2% of parents in the control group. Parents of children with ASD who required more than 30 minutes to fall asleep constitute 31.4%, which is more than parents in the control group. About 61% of parents of children with ASD sleep less than 6 hours per day, compared to only 41.5% among the control group. Global score for PSQI indicates that parents of ASD children who had poor sleep were 74%, whereas parents with ID children were 66%.

Table 3: Subjective endorsement of sleep quality among parents with children with ASD and Control, Oman, 2014

Characteristics	Total (N = 454) n (%)	ASD (n = 220) n (%)	Control (n = 234) n (%)	p-value
Subjective sleep Quality				0.0001
Good	383 (84.4)	168 (76.4)	215 (91.8)	
Bad	71 (15.6)	52 (23.6)	19 (8.2)	
Sleep latency				0.03
< 30 min	334 (73.6)	151 (68.6)	183 (78.2)	
> 30 min	120 (26.4)	69 (31.4)	51 (21.8)	
Sleep duration				0.0001
>6 hrs	222 (48.9)	85 (38.6)	137 (58.5)	
<6 hrs	232 (51.1)	135 (61.4)	97 (41.5)	
Global PSQI				
Good	137 (30)	57 (26)	80 (34)	
Poor	317 (70)	163 (74)	154 (66)	

Table 4 shows the association between the status of groups (TD, ASD, ID) and have poor sleep quality as indicated by the global PSQI parameter. Both crude and adjusted regression models indicated that the risk of having 'poor sleep' was significantly higher among parents of children with ASD (p = 0.01) and ID (p = 0.02) compared to those of TD children. Overall and compared to parents with TD children, the risk of having 'poor sleep' in parents of ID children was 2.2 times higher (OR = 2.2; 95% CI = 1.3, 3.8). The risk was even higher among parents of children with ASD (OR = 2.4; 95% CI = 1.3, 3.8).

Table 4: Association between the status of groups (TD, ASD, ID) and have poor sleep quality as indicated by the global PSQI parameter, Oman, 2014

Characteristics	n (%)	Crude Analysis		Adjusteda Logistic Regression	
		OR (C.I)	p-value	OR (C.I)	p-value
Types of Children					
TD	125 (27.5%)	1.0		1.0	
ASD	220 (48.5%)	1.9 (1.2, 3.0)	0.01	2.4 (1.3, 4.8)	0.01
ID	109 (24.0%)	1.7 (1.1, 3.0)	0.04	2.2 (1.3, 3.8)	0.02

DISCUSSION

This study has embarked to compare the quality and quantity of sleep among parents of children with ASD, ID and TD. Out of the seven PSQI parameters, the ASD parents scored significantly higher in the

pathological range compared to the control group (ID and TD). As a whole, this data suggests that parents with ASD children have more compromised quality and quantity of sleep. Risk of having 'poor sleep' was significantly higher among parents of children of ASD compared to control groups.

This study came from an Arab/Islamic population that collaborates the view of parents caring for a child with ASD, who are prone to poor quality and quantity of sleep. This result was consistent with the previous research that reported poor sleep quality in parents of children with ASD[28,29]. It has also been found that parents of children with ASD likely meet the 'poor sleepers' criteria in the PSQI instruments compared to children with TD[30]. The finding was also consistent with previous studies that have confirmed poor sleep quality in parents of children with intellectual disabilities including ASD[31-34]. In addition, Gallagher *et al* found that 72% of parents with ID children were categorized as poor sleepers according to PSQI[35]. Furthermore, Meltzer found that 60% of parents with ASD children were classified as poor sleepers[29].

Parents of children with ASD have shorter duration of sleep compared to parents in the control group. This result was consistent with Meltzer study that showed a shorter total sleep time with parents of ASD children compared to parents with TD children[29].

We found that parents of children with ASD have sleep disturbances at night. This can be explained by the observation that parents of children with ASD who wake up more than once in the night due to their children's sleep problems, which are common among children with Adman confirmed by several studies[8,33].

Longitudinal studies are needed to quantify the reasons why parents of children with ASD have poor quality of sleep. Some studies have clearly indicated that parents of children with ASD are marked with high levels of stress and distress[36-38]. It is possible that unremitting afflictive emotions among family caregivers with developmental disabilities would disturb their sleep behaviors and quality of sleep. It has been shown that one of the fallout of stress and distress is the resultant of poor quality of sleep; as it has been reported in both general and psychiatric population that emotional disorders such as depression or anxiety tend to negatively impact the quality of sleep[39]. It is also possible that sleep disorders and their aftermath are common in children with ASD, which would also affect the rhythm and mood of the family caregivers invariably[8]. Polimeni has reported that over two-thirds of parents of children with ASD suffered from sleep disruptions because of their children's poor sleep pattern[33]. Therefore, the combination of adverse reaction having disabled children and disrupted sleep among children with ASD could act in tandem to exacerbate the observed adverse sleep behaviors and sleep quality in family caregivers.

There are some indications that mechanisms inherent in development of ASD tend to compromise various neuronal systems and functions that are critically involved in sleep-wake cycles and by default, this also impacts the caregivers[40-42]. Both children with ASD and their caregivers are deprived of much needed restorative function of sleep. For children with ASD, they will be rendered with social deficit and probably further exacerbate their behavioral, cognitive and emotional disorders. For the parents, such situation has implications on their competency as caregivers and their ability to have a meaningful existence[35].

Some issues arising from socio-demographic background are worth considering. In terms of education, the family caregivers of children with ASD appear to be more educated than the caregivers of the control group (ID and TD). Previously, it was noted that people who come from lower educational background tend to have more propensity to failure to thrive cognitively and physically[43]. However, this study suggests that ASD is common among people of higher educational levels. By virtue of being educated, it is also possible that these were the parents who are likely to present their children to healthcare and educational systems where the present cohort were drawn upon. In terms of occupation, although previous studies in Oman suggested that family caregivers of children with ASD seem to have more economic problems, this study suggests that majority of them are in fact, employed[21]. It is worthwhile to note that parents of children with ASD have higher education and command higher salaries. Despite this, it has been noted that they perceive themselves to have economic problems. The educational level of parents of children with ASD in Oman has been also linked to several observations such as malnutrition, sub-optimal breastfeeding practices, and the decreased awareness of society about ASD, especially among teachers[44-46].

Obviously, various limitations of this study ought to be highlighted. First, the sample was based on one region of the country, Muscat, the capital city of Oman, and its satellite towns. Therefore, in order to generalize this study to the rest of the country, future studies ought to explore similar phenomenon among parents with challenged children from other parts of the country. Second, Oman's remedial and educational services for children with special needs and talents appear to be rudimental and often cater to the need for those who are higher functioning[21]. Therefore, children with severe form of ASD are likely to be "hidden" in the household since stereotypical

behavior, aggression and impulsivity are likely to be perceived by the family that others may not tolerate them[47]. Third, quality and quantity of sleep was assessed using a subjective measure, PSQI. It is well known that subjective measures are prone to poor reliability[44]. Future studies should employ objective actigraphic recording. Last but not the least, this study has lumped TD and ID as one cohort. This obviously has its strength since the entrance of ID is likely to skew the performance towards more pathological sleep. Despite such combination, the difference between the two cohorts was not statistically significant, albeit, indirectly indicate the veracity of the predicament endured by parents of children with ASD.

CONCLUSION

In conclusion, parents caring for children with ASD compared to parents of TD and ID children reported to have poorer sleep quality. Further research is needed to find out the effect of sleep problems of children on parents' sleep problems with children's behavior problems and family functioning. Future researchers should circumvent some of the limitations of this study.

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Authors' contributions: OF formulated the study concept and collected data. He contributed to data analysis, literature review, and write-up of the manuscript. YF and MS conceptualized the methods and contributed in reviewing results and write-up of the manuscript. YF and OF conceptualized the regression modelling techniques, reviewed the results and contributed to the write-up. MW and SA revised the scientific background of the study and contributed to the literature review and write-up of manuscript, especially the Discussion. All authors read and approved the final manuscript.

There has been no change in affiliation of any of the authors subsequent to the time of the study.

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Original Article

Expressions of p53, KAI1, PTEN and their use as prognostic markers in urothelial carcinoma of the bladder

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ABSTRACT

Objectives: To determine the expression levels of p53, KAI1, PTEN and their relations with the clinicopathologic parameters in patients with urothelial carcinoma of the bladder
Design: Retrospective study
Setting: Department of Pathology, Ankara Diskapi Yildirim Beyazit Education and Research Hospital, Ankara, Turkey
Subjects: Seventy-eight patients with a diagnosis of urothelial carcinoma of the bladder at the Pathology Department of Ankara Diskapi Yildirim Beyazit Education and Research Hospital and who were followed up for two years
Intervention: Archived pathology materials of all patients were reviewed and clinical data were collected from medical database records retrospectively.
Main outcome measures: We considered the following parameters: age, gender, survivals and disease-free survivals of the patients, tumor-stage, lymphovascular-angiovascular invasions, expressions of KAI1, PTEN and p53 in tumorous tissues.
Results: In non-metastatic-patients, 55.2% were p53 negative; however, 45.8% of the metastatic-patients had diffuse-strong p53-expressions. Hence, p53 positivity indicates that the tumor will metastase.
In transurethral resection (TUR) materials, patients with positive KAI1-expression had better survival (p=0.0285). The tumor seemed to be more aggressive and invasive in patients with decreased KAI1-expressions because there was no KAI1 expression in 50% of the metastatic-patients, although it wasn't statistically significant (p=0.550).
For PTEN expression, no statistically-significant relation was found with the tumor-stage (p=0.34) and survival/ disease-free-survival (TUR:p=0.9599/0.7576, radical cystectomy :p=0.8219/0.5790).
Conclusions: The presence of metastasis dramatically decreases the overall survival in patients with urothelial carcinoma. Identification of these patients as early as possible would effect their therapies, follow-up intervals and surveys. p53 and KAI1 seem to be especially better indicators of the prognosis than PTEN.

KEYWORDS: KAI1, PTEN, p53, urinary bladder, urothelial carcinoma

INTRODUCTION

Urothelial malignancy of the bladder is the fifth most common malignancy occurring worldwide. In the genitourinary tract, it is the second most common malignancy. Approximately 90% of bladder tumors are of epithelial origin and the majority of them are the urothelial carcinomas of the bladder (UCB)[1,2]. The ratio of men to women is approximately 3:1[3,4].

About 25% of patients with UCB present with advanced (metastatic or muscle-invasive) disease[3]. The gold standard of treatment for patients with advanced and refractory non-muscle-invasive UCB is radical cystectomy (RC) with bilateral pelvic lymphadenectomy (BPL) with or without perioperative chemotherapy and radiotherapy. In approximately 50% of patients with muscle-invasive UCB, the recurrence of disease and even death from the disease can be seen, in spite of advances in surgical techniques and perioperative chemotherapy[3,5,6].

The standard prognostic, predictive factors and the risk factors for recurrence and progression of UCB can be listed as; multifocality, tumor size of >3 cm,

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Table 1: The sex, mean age and pathological stage (pTstage) distribution of the patients without lymph node/distant metastasis and with lymph node/distant metastasis of the urothelial carcinoma of the bladder.[Number of the patients-(% distribution)].

Parameter	Non-metastatic patients	Metastatic patients	Total
Sex (p = 0.614)			
Male	39 (56.5)	30 (43.5)	69 (100)
Female	5 (55.6)	4 (44.4)	9 (100)
Total number (%)	44 (56.4)	34 (43.6)	78 (100)
Mean age (p = 0.031)	60.04 ± 9.1	55.02 ± 9.06	
pT stage in Transurethral Resection materials			
T1	10 (66.7)	5 (33.3)	15 (100)
T2	19 (55.9)	15 (44.1)	34 (100)
Total number (%)	29 (59.2)	20 (40.8)	49 (100)
pT stage in Radical Cystectomy materials			
T0	2 (100)	-	2 (100)
Ta	1 (100)	-	1 (100)
T1	2 (100)	-	2 (100)
T2a	5 (50.0)	5 (50.0)	10 (100)
T2b	1 (100)	-	1 (100)
T3a	12 (50.0)	12 (50.0)	24 (100)
T3b	1 (3.4)	1 (4.0)	2 (100)
T4a	5 (45.5)	6 (54.5)	11 (100)
Total number (%)	29 (54.7)	24 (45.3)	53 (100)

concurrent carcinoma in situ, tumor extension beyond the bladder on bimanual examination, infiltration of the ureteral orifice, lymph node metastases, presence of systemic dissemination, histological grade, stage, lymphatic and/or vascular invasion, specific subtypes or histological variants of urothelial carcinomas (such as small cell carcinoma, sarcomatoid carcinoma, nested variant, micropapillary carcinoma and lymphoepithelioma-like carcinoma have worse prognosis), margin status after cystectomy and the pattern of tumor growth (pushing or infiltrative)[7].

However, all these factors always do not adequately show the individual biological potential and clinical behaviour of the tumor.

Therefore, it is important to find out the molecular events to explain the clinical heterogeneity of these tumors in order to help clinicians apply individualized adjuvant therapy according to individualized prognostic and predictive factors[8-10]. Some of these controversial molecular events are antibodies like PTEN, KAI1 and p53.

PTEN

PTEN (MMACI-The phosphatase and tensin homolog mutated on chromosome ten) is a tumor suppressor gene located on chromosome 10 that inhibits cell proliferation and tumorogenesis by inducing apoptosis and by arresting G1 phase of cell cycle[4]. PTEN is known to be mutated or deleted in many human cancers, including gliomas, breast, prostate, lung, thyroid and endometrial carcinomas. This gene is also reported to show loss of heterozygosity in bladder cancer and more frequently in muscle-invasive urothelial carcinomas[11-14].

KAI1

KAI1 protein (also named as R2, C33, IA4, 4F9, CD82), located on chromosome 11, is a cell surface glycoprotein initially detected as a specific tumor metastasis suppressor gene in prostate cancer[14]. KAI1 inhibits cell migration and attenuates epidermal growth factor signaling[15-16]. Many experimental evidences have shown that expression of KAI1 is down-regulated or lost in the advanced stages of many different human cancers (breast, stomach, colon, lung cancers, melanoma), indicating that a loss of KAI1 function may be an important step in disease progression, invasiveness and metastasis[17-20].

p53

p53 is a tumor suppressor gene located on chromosome 17 that plays a key role in regulating cell cycle progression and apoptosis. It blocks the damaged cell entrance to the S phase of cell cycle until its DNA is repaired and it sends specific damaged cells to apoptosis. p53 is the most commonly mutated gene in human cancers[3,21,22].

In the present study, we analyzed the expressions of PTEN, KAI1 and p53 in materials of the patients with UCB and compared with pathological parameters to determine the prognostic significance of these proteins in the UCB.

SUBJECTS AND METHODS

Patients and tissue samples

We analyzed 78 patients (69 males and 9 females) retrospectively who underwent RC with BPL (53 patients) and/or transurethral resection (TUR) (49 patients) who were diagnosed as urothelial carcinoma

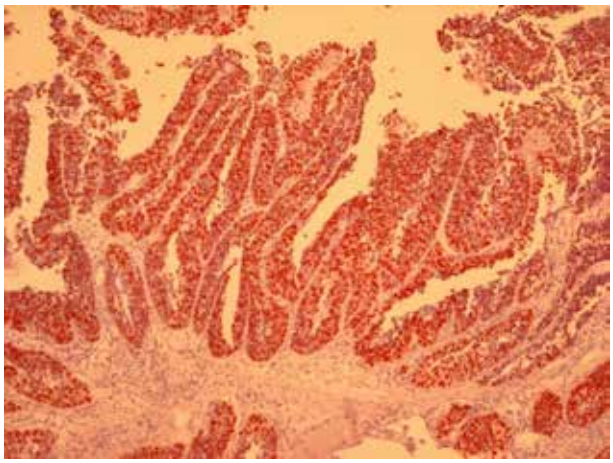

Fig 1: Nuclear p53 positive staining (p53 x 100)

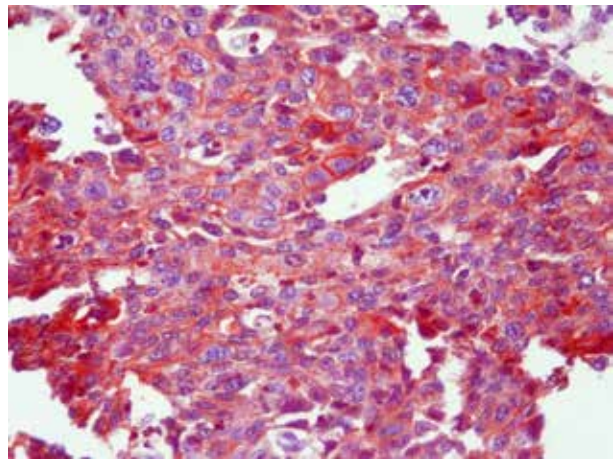

Fig 2: Cytoplasmic KAI1 positive staining (KAI1 x 200)

between 2007 and 2008 and followed up for 2 years (Table 1). We obtained informed consents from all patients and certification from Ankara Dışkapı Yıldırım Beyazıt Education and Research Hospital Ethics Committee about the relevance of the study (Ethics Committee no: 17/19-03.11). We followed up all the patients after the 3rd month control with intervals of 4 months for 2 years.

The pathology slides of RC and TUR materials of the patients were reviewed and representative sections and paraffin blocks of each tumor were selected.

The histological grade of the bladder tumors were determined according to the 2004 World Health Organization classification[7]. Tumor staging was done using Tumor Node Metastasis system according to the American Joint Committee on Cancer[23].

The patients were seperated into two groups as;

Group 1 (non-metastatic group) : Composed of the patients (n = 44) with no lymph node metastasis present at the time of cystectomy and no distant organ metastasis determined on the follow-up.

Group 2 (metastatic group): Composed of the patients (n = 23) who had lymph node metastasis present at the time of cystectomy and the patients (n = 11) who did not have any lymph node metastasis present at the time of cystectomy, but distant organ metastasis occured on the follow-up.

The age, gender, survivals and disease free survivals of the patients, stage of the tumors, lymphovascular-angiovascular invasions, expressions of KAI1, PTEN and p53 in TUR and RC materials were statistically evaluated in two groups (non-metastatic and metastatic).

Immunohistochemistry

For immunohistochemical staining, 4µm sections of selected tumor blocks were cut and mounted on poly-L-lysine–coated slides. Following deparaffinization and rehydration, antigen retrieval was performed using citrate buffer (pH 6.0) at 121 ˚C for 10 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for five minutes. Later, the sections were incubated with antibodies against KAI1 (Santa Cruz, sc-17752, G-2), p53 (Novocastra, NCL-L-p53-D-07) and PTEN (Neomarker, ser370, RB-10620-P1). 3,3'-diaminobenzidine and 3-amino-9-ethylcarbazole were performed as chromogenes and finally slides were counterstained with hematoxylin. Breast carcinoma tissue was used as positive controls for p53 and PTEN. Prostate carcinoma tissue was used as a positive control for KAI1. Cases not treated with primary antibodies served as negative controls.

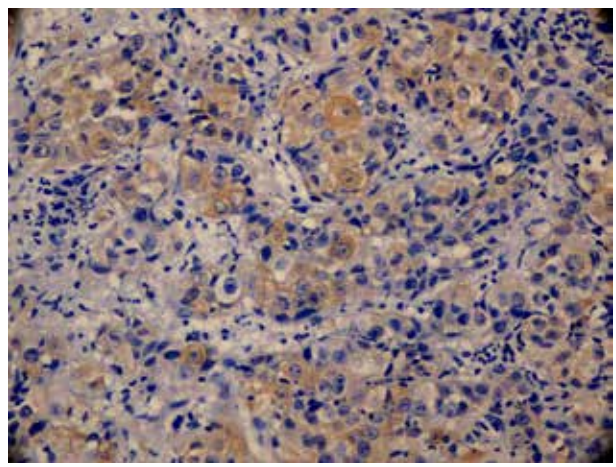

Fig 3: Cytoplasmic PTEN positive staining (PTEN x 400)

Immunostaining was evaluated by two pathologists. Nuclear staining for p53 (Figure 1) and cytoplasmic staining for KAI1 (Figure 2) and PTEN (Figure 3) was accepted as positive. The 'expression rate', the extent of staining for each antibody, was scored according to the number of cytoplasmic or nuclear stained carcinoma cells in 100 tumor cells. Less

Table 2: p53, KAI1 and PTEN expressions of the urothelial carcinoma of the bladder in transurethral resection materials.

Antibody expressions	Number of non-metastatic patients-(%)	Number of metastatic patients-(%)	p-value
P53 expression			0.755
0	9 (31.0)	5 (25.0)	
1	10 (34.5)	6 (30.0)	
2	10 (34.5)	9 (45.0)	
KAI1 expression			0.550
0	16 (55.2)	10 (50.0)	
1	5 (17.2)	6 (30.0)	
2	8 (27.6)	4 (20.0)	
PTEN expression			0.611
0	12 (41.4)	12 (60.0)	
1	3 (10.4)	2 (10.0)	
2	9 (31.0)	4 (20.0)	
3	5 (17.2)	2 (10.0)	

Table 3: p53, KAI1 and PTEN expressions of the urothelial carcinoma of the bladder in radical cystectomy materials.

Antibody expressions	Number of non-metastatic patients-(%)	Number of metastatic patients-(%)	p-value
P53 expression			0.036
0	16 (55.2)	8 (33.4)	
1	9 (31.0)	5 (20.8)	
2	4 (13.8)	11 (45.8)	
KAI1 expression			0.832
0	15 (51.7)	13 (54.2)	
1	6 (20.7)	6 (25.0)	
2	8 (27.6)	5 (20.8)	
PTEN expression			0.514
0	18 (64.3)	19 (79.2)	
1	2 (7.1)	2 (8.3)	
2	1 (3.6)	1 (4.2)	
3	8 (25.0)	2 (8.3)	

than 5% positive cells were accepted as negative, while ≥5% positive cells were being accepted as positive.

Scoring for p53 and KAI1:
0 (negative): <5% positive cells;
1+ (positive): 5-50% positive cells;
2+ (positive): >50% positive cells.

Scoring for PTEN:
0 (negative): <5% positive cells;
1+ (positive): 5-25% positive cells;
2+ (positive): 26-50% positive cells;
3+ (positive): >50% positive cells.

Statistical analysis

Statistical evaluation was performed by using Chi-square test, Mann-Whitney test or Fisher's Exact test. The survival and the factors that have effects on disease free survival were evaluated with Log Rank survival analysis. The patients who died within the first month after the operation were excluded during the survival analysis. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Age and gender of the patients

Patients included 69 males and 9 females. The relation of sex with the status of metastasis was not statistically significant. The mean age was lower in metastatic group and this was statistically significant (Table 1).

TUR materials

In TUR materials, there was no statistically significant relation between p53, KAI1, PTEN expressions and the metastatic, non-metastatic groups (Table 2). Also, there were no statistically significant relations between p53, KAI1, PTEN expressions and pT stages of the tumors in metastatic and non-metastatic groups (p = 0.248, p = 0.755, p = 0.059 respectively).

Radical cystectomy materials

In RC materials, there was a statistically significant relation between p53 expressions and the groups (non-metastatic and metastatic, Table 3). However, this relation was not significant with KAI1, PTEN. Also, there was no statistically significant relation between

Table 4: Survivals-disease free survivals of the patients with the urothelial carcinoma of the bladder and p53, KAI1 and PTEN expressions in transurethral resection and radical cystectomy materials.

Antibody expressions	Transurethral Resection Materials				Radical Cystectomy materials			
	Survival	p-value	Disease free survival	p-value	Survival	p-value	Disease free survival	p-value
P53		0.9378		0.6581		0.7771		0.7923
- (<5%)	50.00		78.57		40.00		71.43	
+(≥ 5%)	44.12		70.59		38.77		69.23	
KAI1		0.0285		0.7461		0.9808		0.5081
-(<5%)	27.27		69.23		37.50		65.22	
+(≥ 5%)	53.33		77.27		36.66		75.00	
PTEN		0.9599		0.7576		0.8219		0.5790
- (<5%)	45.79		69.13		41.89		66.67	
+(≥ 5%)	54.48		75.34		42.75		73.91	

p53, KAI1, PTEN expressions and pT stages of the tumors in metastatic and non-metastatic groups (p = 0.185, p = 0.723, p = 0.34 respectively).

However, when the lymphovascular vessel invasion was evaluated, it was seen positive only in 2 patients (6.8%) in non-metastatic group and in 11 patients (45.8%) in metastatic group. These rates were statistically quite significant (p <0.001). As the angiovascular invasion was evaluated, it was seen positive in 2 patients (6.8%) in non-metastatic group and in 12 patients (50.0%) in metastatic group. These rates were also statistically quite significant (p <0.001).

When the survival, the disease free survival and p53, KAI1 and PTEN expressions were evaluated in TUR and RC materials; according to TUR materials, there was a statistically significant relation in survival only between the patients with positive and negative KAI1 expressions (Table 4).

DISCUSSION

Bladder carcinoma is the second most common cancer of the genitourinary tract and the incidence of it has been increasing in the last 20 years. UCB is a progressive disease typically seen in older ages. The depth of invasion, grade of the tumor and lymph node metastasis are the major factors used in determining clinical treatment. About 70% of bladder tumors are superficial, 25% are invasive and 5% are metastatic. Also, 30% of the superficial tumors become invasive in the follow-up. Long term survivals are better in patients with organ-confined disease[24-25].

BPL helps to understand the local spread of the disease. The risk of pelvic lymph node metastasis increases with the stage of the disease. The risk of pelvic lymph node metastasis in patients with stage pT3 and pT4 is 30 – 60% respectively, while the risk of lymph node positivity at the time of surgery in patients with stage pT2 is about 10 - 30%. Generally, the patients with positive lymph node metastasis determined postoperatively show simultaneous distant organ micrometastasis. Hence, it is important to predict the tumors that tend to metastise before the surgery in order to determine the apropriate clinical treatment and aproach. Still, there is no reliable indicator to use in predicting the metastatic disease[26-27].

The first notable finding in our study was that, the average age of the metastatic group was lower than the non-metastatic group. This shows that the tumors seen in younger ages are more aggressive.

p53 located in the nucleus blocks the cell cycle in G1 phase and prevents the cell passing to the S phase. Then it activates the genes that repair DNA and sends the damaged and unrepaired cells to apoptosis. p53 mutations can be seen in many tumors like lung, colon and breast[26]. It was shown that p53 can be used in diagnosing urothelial carcinoma in situ of the urinary bladder and in predicting the progression of the non-invasive pT1 lesions[28]. In a study, Uygur *et al* evaluated p53 expressions in TUR materials of 31 patients with RC. In 17 patients with p53 positivity (nuclear staining 20% and higher was accepted as positive), 11 had lymph node metastasis. Therefore, patients who had pT2 and pT3a disease with p53 positivity were accepted as high risk group and concluded that early aggressive therapy had to be started as soon as possible[29]. In another study, Esrig *et al* reported that there was a positive relation between p53 staining and the pathological stage. In that study, they also reported that in p53 positive patients, recurrence of the disease was higher and the survival was lower. Hence, p53 was determined as a prognostic factor independent from the stage and the grade[22]. On the other hand, there are studies reporting that there is no relation between p53 expression and the stage[30]. Also Puzio-Kuter *et al* reported similar results; that p53 overexpression was seen in invasive bladder carcinoma compared to non-invasive papillary tumors[1]. Goebell *et al* showed in their international study that p53 positivity was significantly correlated with tumor progression in pT1 disease and advanced bladder cancer and p53 appeared to be predictive in high grade bladder cancer[21].

In our study, we found out that in RC materials; there was a statistically significant relation between p53 expressions and the groups (non-metastatic and metastatic). p53 positivity in metastatic group was higher. We thought that the patients with p53 expression of 50% or higher had more risk to develop metastasis. So, p53 positivity may be a bad prognostic factor and the patients expressing 50% or more p53 may be treated more radically and followed up carefully. However, we could not find this relation in TUR materials and we thought that p53 is not sufficient in predicting lymph node metastasis of the tumor in TUR materials. The reason for this is thought to be that the material obtained by TUR is inadequate and also cautery artefact can limit adequate examination of all material. We also showed that the lymphovascular vessel and the angiovascular invasions were seen more frequently in metastatic group than the non-metastatic group as known before[7]. When we looked at the survivals and disease-free survivals, the increase of p53 expression had negative effects on the survival and disease-free survival. However, this effect was not statistically significant. We think that this effect would be statistically significant in new studies with more patients.

KAI1 was detected in tumors like prostate, stomach, colon, breast carcinomas and the invasiveness and the metastatic potentials of the tumors were shown to increase with the loss of KAI1 expressions[18-20]. In a

study, Su *et al* reported that decreased KAI1 expression was associated with the degree of invasiveness and progression of cancer and was an independent prognostic factor for tumor recurrence in primary pTa and pT1 UCB[16]. We found that according to TUR materials, patients with positive KAI1 expression had better survival than patients with negative KAI1 expression. The tumor was more aggressive and invasive in TUR materials with lower KAI1 expressions. We thought that this was not statistically significant because of the low number of patients.

PTEN inhibites cell proliferation and tumorogenesis by inducing apoptosis and by arresting G1 phase of cell cycle. PTEN was detected in many human cancers, like gliomas, breast, prostate, lung, thyroid and endometrial carcinomas. This gene is also reported to show loss of heterozygosity in bladder cancer, more frequently in muscle-invasive urothelial carcinomas[11-15]. In their study, Lee *et al* reported that loss of PTEN expression was associated with non-papillary histology, high grade and invasive urothelial carcinomas[13].

We only found that the loss of PTEN expression in metastatic group was more than the loss of PTEN expression in non-metastatic group. However, this correlation was not statistically significant.

In UCB, the lymphovascular vessel and the angiovascular invasions are very important as they are indicators of the lymph node or distant metastasis. The presence of lymph node or distant metastasis shortens the surveys. We need more reliable indicators to use at the beginning of the disease before the radical operation to predict the tumors which will be more aggressive, in order to decide the correct therapy, neoadjuvant or early adjuvant therapy. p53, KAI1 and PTEN seem to be good indicators, although we could not reach perfect results. We think that the reason is the limited number of our patients as indicated before. New studies with large series seem to support the usefulness of p53, KAI1 and PTEN.

CONCLUSION

In UCB, it is very important to predict the progression of the disease before the surgery as it frequently relaps and shortens the lives of the patients. It is very important to find new indicators showing the aggressiveness of the disease. The results of this study give the impressions that p53 positivity, KAI1 negativity and PTEN negativity in UCB are good indicators of worse prognosis and aggressiveness.

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Original Article

The effect of acute kidney injury on the success of non-invasive ventilation in COPD patients with hypercapnic respiratory failure

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ABSTRACT

Objective: To investigate the effect of acute kidney injury (AKI) on the success of noninvasive ventilation in chronic obstructive pulmonary disease (COPD) patients with hypercapnic respiratory failure
Design: Retrospective observational cohort study
Setting: Faculty of Meram Medicine, Necmettin Erbakan University, Konya, Turkey
Subjects: This clinical study included 55 patients with decompensated COPD: 29 patients with AKI and 26 patients without AKI.
Intervention: Demographical data, APACHE II scores, urea and creatinine values before non-invasive mechanical ventilation (NIMV) administration were recorded.
Main outcome measures: The effect of AKI on the success of NIMV
Results: There was no significant difference between sex, age and baseline respiratory rate of groups. There were statistically significant differences in baseline mean APACHE II score (21.4±5.3 vs 18.3±4.6, p=0.028), baseline mean pH (7.23±0.1 vs 7.30±0.1, p=0.001), urea (113.7±43.6 vs 45.2±13.0, p<0.001) and creatinine values (3.1±2.0 vs 0.9±0.3, p<0.001) of Group 1 and Group 2. Logistic regression analysis showed that none of these variable values tested have any effect on NIMV outcomes. Of the baseline variables tested, age (OR: 0.85; 95% CI: 0.69 to 1.07), sex (OR: 1.29; 95% CI: 0.89 to 1.89), baseline respiratory rate (OR: 1.02; 95% CI: 0.92 to 1.14), APACHE II score (OR: 1.03; 95% CI: 0.87 to 1.23), and AKI (OR: 0.79; 95% CI: 0.15 to 4.18) were not related to the outcome of NIMV in the logistic regression.
Conclusions: We determined that AKI did not affect the outcome of NIMV in decompensated COPD patients.

KEYWORDS: acute kidney injury, COPD, hypercapnic respiratory failure, noninvasive ventilation, success

INTRODUCTION

Non-invasive mechanical ventilation (NIMV) is an effective treatment for acute hypercapnic respiratory failure complicating the exacerbation of chronic obstructive pulmonary disease (COPD)[1]. However, in some cases, patients do not benefit from NIMV and thus, intubation is required. Several factors have been found to be associated with the risk of NIMV failure. In a randomized controlled study, baseline pH <7.3, or $PaCO_2$ or respiration rate not recovering after 4 hours following treatment was associated with an increase in mortality[2]. Other factors defined as poor prognostic markers were high APACHE II (Physiology and Chronic Health Evaluation II) score, radiologically documented consolidation, hemodynamic instability, impairment of consciousness, presence of comorbidities and metabolic dysfunction. On the other hand, the potential of NIMV success was also associated with the appropriate selection of ventilator modality and interface, experience level of the team, mental state of the patient and advanced age[3-5]. NIMV is currently recommended in acute exacerbations of COPD and seems to improve clinical outcomes[6].

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The most frequent comorbidities in COPD patients include chronic renal disorders. It is well known that renal injury in surgical and internal critical care patients is associated with poor prognosis[7]. As the studies evaluating the impact of a single clinical condition on NIMV are limited, the effect of acute kidney injury (AKI) on prognosis in respiratory failure requiring NIMV administration is not clear enough. In this study, we aimed to investigate whether the presence of AKI on admission in COPD patients with hypercapnic respiratory failure is a determining factor for NIMV outcomes.

MATERIALS AND METHODS

Study design and participants

A retrospective single-center observational cohort study was performed including 55 COPD patients who had been admitted for acute hypercapnic respiratory failure and treated with NIMV between October 2015 and August 2017 in 10-bed third level intensive care unit (ICU) of a university hospital. The study was approved by the Necmettin Erbakan University Medical School Human Research Ethics Committee.

The definition of acute hypercapnic respiratory failure as an exacerbation of COPD and the decision to implement NIMV were made according to the clinical status and arterial blood gas (ABG) levels of the subject at admission, using the following criteria: moderate or severe dyspnea, tachypnea, accessory muscle use, abdominal paradoxical respiration, ABG pH <7.35, and partial arterial carbon dioxide pressure ($PaCO_2$) >45 mmHg[8].

NIMV was performed using a dedicated ventilator (BiPAP Vision; Philips Respironics, Murrysville, PA, USA, or its new version the V60; Philips Respironics) with its single branch circuit, its calibrated intentional leak, and an interface strategy as previously described in detail[9]. Bi-level positive pressure ventilation in spontaneous timed mode was used, and the settings were left at the discretion of the attending physician. NIMV was continuously applied until a significant clinical improvement of the patient ensued. Then, the duration of NIMV was gradually reduced.

The patients were divided into two groups: 29 patients with AKI were categorized as Group 1 and the remaining 26 patients without an AKI were categorized as Group 2. AKI was defined by the Kidney Disease Improving Global Outcomes definition according to creatinine change and urine output criteria, including any of: 1), increase in serum creatinine by ≥0.3 mg/dl within 48 hours; or 2) increase in serum creatinine to ≥1.5 times baseline within the prior 7 days; or 3) urine volume <0.5 ml/kg/h for 6 hours[10].

Patients with one or more of the following conditions were excluded: 1) stable chronic patients receiving NIMV as continuation of their home treatment; 2) previously treated in this ICU; and 3) admitted with chronic kidney disease.

Data collection

The demographic data, personal history, admission and discharge dates (referred to both the hospital and ICU), in-hospital mortality, nighttime, daytime, daily and total NIMV durations, APACHE II score (for the severity of illness), different physiological variables (systolic blood pressure, diastolic blood pressure, respiratory frequency, and heart rate), and laboratory test parameters (ABG values, blood count and biochemical analysis) were collected. The primary outcome was the effect of AKI on the success of

Table 1: Clinical and demographic characteristics of patients with and without AKI

Characteristics	AKI group (n = 29)	Non-AKI group (n = 26)	p-value
Age (mean ± SD)	69.1 ± 10.2	65.9±11.2	0.26
Sex			0.15
Male n (%)	17 (58.6)	20 (76.9)	
Female n (%)	12 (41.4)	6 (23.1)	
Respiration rate (mean ± SD)	27 ± 6	28 ± 8	0.40
Pulse (mean ± SD)	100 ± 20	103 ± 24	0.71
Systolic BP (mean ± SD)	135 ± 31	129 ± 24	0.42
Diastolic BP (mean ± SD)	78 ± 19	77 ± 14	0.83
Arterial blood gas			
pH (mean ± SD)	7.23 ± 0.09	7.30 ± 0.07	0.001
$paCO_2$ (mean ± SD) mmHg	67.8 ± 19.0	67.7 ± 18.5	0.98
paO_2 (mean ± SD) mmHg	57.9 ± 19.6	52.2 ± 16.9	0.48
HCO_3 act. (mean ± SD) mmol/L	28.7 ± 10.4	32.6 ± 8.8	0.14
Urea (mean ± SD) mg/dL	113.7 ± 43.6	45.2 ± 13.0	0.000
Creatinine (mean ± SD) mg/dL	3.1 ± 2.0	0.9 ± 0.3	0.000
APACHE II Score (mean ± SD)	2 1± 5	18 ± 5	0.03

AKI: acute kidney injury; BP: blood pressure; APACHE: Acute Physiology and Chronic Health Evaluation

Table 2: The impact of clinical and demographic characteristics on NIMV outcomes

Characteristics	NIMV success (n = 38) Mean (SD)	NIMV failure (n = 17) Mean (SD)	p-value
Age	67.4 (11.4)	68.1 (9.3)	0.81
Sex			0.79
Male n (%)	26 (68.4)	11 (64.7)	
Female n (%)	12 (31.6)	6 (35.3)	
Respiration rate	27 (6)	28 (7)	0.60
Pulse	99 (19)	106 (27)	0.20
Systolic BP	128 (27)	141 (27)	0.11
Diastolic BP	76 (16)	82 (17)	0.30
APACHE II score	19 (5)	22 (6)	0.09
Renal failure (+), n (%)	18 (47.4)	11 (64.7)	0.23
Durations of NIMV use			
Night time, hours	7.9 (3.0)	8.1 (3.4)	0.82
Day time, hours	5.0 (2.2)	5.3 (3.1)	0.74
Daily, hours	12.7 (4.5)	12.6 (5.3)	0.96
Total, hours	75.7 (49.7)	69.8 (56.5)	0.70
Arterial blood gas			
pH at entry	7.28 (0.08)	7.25 (0.09)	0.28
$paCO_2$ at entry, mmHg	68.6 (19.1)	65.7 (18.0)	0.59
paO_2 at entry, mmHg	54.6 (19.5)	56.5 (16.3)	0.73
HCO_3 act. at entry, mmol/L	31.5 (10.1)	28.3 (8.8)	0.25
1st hour pH	7.34 (0.09)	6.98 (1.29)	0.09
1st hour $paCO_2$, mmHg	61.0 (14.7)	60.2 (17.4)	0.87
1st hour paO_2, mmHg	60.7 (24.4)	60.1 (17.8)	0.92
1st hour HCO_3 act. mmol/L	31.7 (7.8)	27.9 (7.2)	0.11

NIMV: non-invasive mechanical ventilation; BP: blood pressure; APACHE: Acute Physiology and Chronic Health Evaluation

NIMV, and the secondary outcomes included hospital mortality, length of ICU stay, length of hospital stay, *etc*.

Failure of NIMV was defined as the need for intubation and mechanical ventilation following its suspension. Regarding the causes of NIMV failure, labored breathing was defined as the persistence of tachypnea and use of the accessory muscles.

Statistical analysis

SPSS software (SPSS for Windows; Chicago, IL, USA) was used for statistical analysis. Data were recorded as frequency and mean ± SD. In order to compare the differences between the groups, independent sample t-test was used for continuous variables and chi-square test for the categorical variables. Our primary analysis of the independent effects of each variable on the success of NIMV was analyzed using logistic regression. Age, sex, baseline respiratory rate, APACHE II score and AKI were included in logistic regression analysis. A p-value of <0.05 was considered as statistically significant.

Table 3: The impact of AKI on the success of NIMV

Characteristics	Odds Ratio	95% CI
Age	0.85	0.69-1.07
Gender	1.29	0.89-1.89
Baseline respiratory rate	1.02	0.92-1.14
APACHE II score	1.03	0.87-1.23
Acute kidney injury	0.79	0.15-4.18

AKI: acute kidney injury; NIMV: non-invasive mechanical ventilation; APACHE: Acute Physiology and Chronic Health Evaluation; CI: confidence interval

RESULTS

When demographical data were evaluated, the two groups with and without AKI overlapped with each other in terms of age, sex distribution and vital signs, and there was no statistically significant difference between the two groups (p >0.05). However, there were statistically significant differences between Group 1 and Group 2 with respect to APACHE II scores, (p = 0.03), pH at entry (p = 0.001), urea (p <0.001), creatinine (p <0.001) and potassium values (p = 0.04) (Table 1).

The success rate of NIMV in the patient population without an AKI was 76.9% compared with 62.1% in the group with AKI. However, it was determined that the presence of AKI had no effect on the success of NIMV and that none of the parameters assessed had an effect on the successful outcomes of NIMV (Table 2). With logistic regression analysis, it was observed that none of these variables had an effect on NIMV outcomes (Table 3).

Table 4: Clinical outcomes of patients with and without AKI

Clinical Outcomes	AKI group (n = 29)	Non-AKI group (n = 26)	p-value
Duration of ICU stay, mean days (SD)	10.2 (6.5)	10.4 (3.6)	0.88
Duration of hospital stay, mean days (SD)	11.8 (6.8)	11.1 (4.3)	0.68
Mortality rate n(%)	10 (34.5)	3 (11.5)	0.04

AKI: acute kidney injury; ICU: intensive care unit

There were no differences between the population with AKI and the population without in terms of length of hospital stay and length of ICU stay. However, it was determined that the mortality rate in patients with AKI was 3 times higher (p = 0.04) (Table 4).

DISCUSSION

Prospective randomized controlled studies have showed that the addition of NIMV to the standard therapy in COPD patients with acute hypercapnic respiratory failure reduced mortality and the requirement of intubation, and shortened the duration of hospitalization. In addition, there is a consensus that NIMV reduces the respiratory workload and ventilator-related pneumonia arising from endotracheal intubation, and improves gas change

and dyspnea. Therefore, NIMV is a very important development for the management of these patients. Chandra *et al* showed in data covering a period of over 10 years and including over 7.5 million applications in 1000 hospitals in the USA that NIMV usage increased 4-fold. In the same study, they observed a decrease in mortality from 6.5% to 3.5% that corresponded to a 42% decrease in patients administrated with NIMV therapy[11-13].

On the other hand, it reduced mortality to make a prior risk evaluation and to define NIMV failure early in order to determine whether these patients would benefit from NIMV administration in early and late periods[14]. Many studies evaluated the reasons for potential NIMV failure in COPD patients and reported that the severity of blood gas disorders (particularly $PaCO_2$ and pH) on admission and the changes occurring during the 1st and 2nd hours of ventilation had an effect on the outcomes, and suggested that a risk chart be created including much larger clinical parameters[3,5]. These parameters are of high importance in deciding to continue the therapy with NIMV or to directly switch to intubation. However, it was declared that COPD was not an independent disease due to a recent study where comorbidities were high in number and most of them adversely affected 4-year survival[15]. In a large cross-sectional study conducted in the USA, it was emphasized that chronically comorbid conditions were more predictive for in-hospital mortality for these patients compared to other variables[16]. While there are many studies emphasizing the importance of comorbidity in COPD, which is known as a systemic disease, the studies assessing the effect on the success of NIMV used for the management of acute hypercapnic respiratory failure which complicates the disease are quite few.

In our study, success rate of NIMV was approximately 70% and was not significantly different from the rates reported in many other studies[17-21]. Early definition of the group where NIMV administration fails is of crucial importance in terms of preventing unnecessary delay to switch to invasive ventilation and thus, reducing mortality[14]. Although AKI is a well-known marker of prognosis in intensive care patients, its effect on the success of NIMV is not clear. In our opinion, the fact that the demographic data did not differ significantly between the groups with and without AKI in our study was an important finding with regards to the evaluation of the single effect of AKI on NIMV failure.

ABG changes not affecting the success of NIMV was among the results that was clearly inconsistent with the literature. ABG changes occurring after NIMV administration have been shown in studies to affect the success[3,5,22]. Due to the fact that the mean age was high, the acidosis pathogenesis of these patients was multifactorial and because of the buffer effect of the high levels of bicarbonate arising from the relatively low presence of acidosis at baseline and the pre-existing chronic respiratory failure in both groups, we could assume that the changes in pH were homogenous. One of the most physiologically clear pulmonary-renal interactions was the response of renal acidification, and worse outcomes in terms of lower pH at baseline and APACHE II in the presence of AKI could be evaluated as a finding that reflected the importance of renal acidification mechanism[23].

Consistent with our findings, Nicolini *et al*[21] have reported that the presence of comorbid conditions are closely associated with in-hospital mortality. Inconsistent with our findings, Carratù *et al*[7] have reported that the presence of comorbid conditions affect mortality as well as the success of NIMV. However, they emphasized the absence of comorbidity typing as a shortcoming of their studies. Although Miller *et al*[22] did not specifically emphasize renal injury, they stated that patients with lower urea levels showed a significant improvement in pH in one hour and were more likely to benefit from NIMV administration. Our findings demonstrate that improvement in ABG values, the presence of AKI and the parameters involved in the assessment had no effect on the success of NIMV. On the other hand, Pacilli *et al*[17] investigated the effect of comorbidities on the outcomes of COPD admitted to the ICU. Consistent with our findings, they observed that the presence of chronic renal disease was higher in the NIMV failure group, but there was no statistically significant difference when compared with the patients without renal injury. Also, in the same study, it was determined that the most important determinants of NIMV success were the presence of pneumonia, which was an underlying cause of respiratory failure rather than comorbidities.

The limitations of our study include that it was not a multi-center study; there was no detection of comorbidities; and there was a lack of data about the underlying cause of hypercapnic respiratory failure in COPD patients.

CONCLUSION

In this study, we observed that baseline renal injury of hypercapnic COPD patients reduced the success of NIMV but it showed no significant difference, and the ABG parameters including the bicarbonate levels, NIMV durations and all the other factors assessed had no effect on the successful outcomes of NIMV. However, we determined that, while it did not affect the successful outcomes of NIMV, AKI increased mortality rates by 3-fold and AKI was an important marker of mortality in COPD patients with

hypercapnic respiratory failure. While the presence of AKI is an important parameter to be considered by clinicians before initiating NIMV, it should not be a good predictor of the contraindication or failure of NIMV.

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Original Article

Near-infrared spectroscopy monitoring during unilateral antegrade cerebral perfusion: Does time matter?

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ABSTRACT

Objectives: Currently, antegrade cerebral perfusion (ACP) for the protection of brain in thoracic aorta surgery has been considered superior to other methods. Adequate perfusion of the opposite hemisphere may sometimes be challenging in the unilateral application of this method. The present study aims to evaluate the opposite hemisphere perfusion via regional oxygen saturation (rSO_2) values.
Design: Observational and descriptive
Setting: Department of Cardiovascular Surgery and Department of Anesthesiology and Reanimation, Turkey Yuksek Ihtisas Training and Research Hospital, Ankara, Turkey
Subjects: Adult patients undergoing elective ascending and aortic arch surgery with ACP performed by the same team
Intervention: Two groups according to the unilateral ACP duration, Group 1 (n = 22) was composed of 15 minutes or less unilateral ACP duration, Group 2 (n = 17) was more than 15 minutes.
Main outcome measure(s): Effects of unilateral ACP duration in aortic surgery and aortic arch surgery on bilateral cerebral rSO_2 as measured by near-infrared spectroscopy (NIRS) were prospectively investigated. Bihemispheric regional rSO_2 values were measured at nine time points during surgery. Any p-value less than 0.05 was considered statistically significant.
Results: While no difference was observed between the right and left hemisphere rSO_2 values in Group 1, the difference between the right and left hemisphere values in Group 2 statistically increased towards the end of the ACP period ($p < 0.05$).
Conclusions: We concluded that 15 minutes or less unilateral ACP may be performed at moderate hypothermia without any asymmetry between hemispheres with NIRS monitoring.

KEYWORDS: cardiac surgery, cerebral blood flow, thoracic aortic aneurysm

INTRODUCTION

Anesthesia management in aortic surgery is closely related with maintaining the brain perfusion pressure at appropriate levels, decreasing the metabolic needs of the brain by anesthetic drugs and hypothermia, and close monitoring of this demand/supply balance. Thanks to the novel surgical strategies, and cerebral perfusion and intraoperative monitoring techniques developed in recent years, maximal cerebral protection is attained and neurologic events occurring post-aortic surgery are reduced[1].

In thoracic aortic surgery, to reduce the neurologic events, following the deep hypothermic circulatory arrest (DHCA) technique, which is used to protect the brain, other techniques, such as antegrade cerebral perfusion (ACP) and retrograde cerebral perfusion (RCP), have gained popularity. Issues accompanying prolonged DHCA application[2], the association of RCP with cerebral edema, and the concerns about its possible inadequacy in maintaining sufficient perfusion[3,4] resulted in wider use of the ACP technique[5]. The dilemma of unilateral or bilateral application of ACP has been a topic of discussion in the literature[6,7]. The most important drawback of unilateral use of ACP is

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the uncertainty associated with providing sufficient perfusion to the opposite hemisphere[1].

With the use of modern perfusion techniques, aortic surgery is performed at higher body temperatures, sufficiency of perfusion is accurately monitored by real-time monitoring techniques, and thereby malperfusion can be detected at early periods, so sufficient perfusion is provided immediately before the occurrence of an irreversible brain damage[8]. For example, near-infrared spectroscopy (NIRS) is a real-time, useful, easy-to-use, and non-invasive method for cerebral oxygen monitoring.

In the present study, effects of unilateral ACP duration in aortic surgery and aortic arch surgery on bilateral cerebral regional oxygen saturation (rSO_2) as measured by NIRS were prospectively investigated. Our aim was to evaluate the contralateral hemisphere perfusion via regional rSO_2 values in terms of asymmetry.

SUBJECTS AND METHODS

Patient population

The study included patients undergoing elective ascending and aortic arch surgery by the same team after obtaining the ethics committee permission and written consents of the patients. Reporting guideline STROBE has been implemented for this paper. Patients undergoing emergency surgery, those with a history of cerebrovascular event, those with a cerebrovascular disease (any degree of carotid stenosis and any known pathological vascular disease of the brain), and those who were not performed by the unilateral ACP technique were not included in the study. The study was completed after recruiting 39 patients between January and December 2014. Demographic data, intraoperative surgery data and intensive care-hospital stay durations were recorded.

Anesthetic management

Orally 0.15 mg kg^{-1} diazepam was administered as night-time premedication and 0.1 mg kg^{-1} morphine was administered i.m. 30 minutes before the surgery. Cannulation of the two peripheral veins and the left radial artery was made in the OR. Pulse oximetry, electrocardiography, and invasive arterial blood pressure were monitored. Before anesthesia induction of the patient, NIRS optodes (Equanox, Nonin Medical Inc., Minnesota, USA) were placed on the bilateral left and right forehead regions 1 cm above the brow curve line. Following preoxygenation, patients were induced with 10 μg kg^{-1} fentanyl, 0.1 mg kg^{-1} midazolam, 0.6 mg kg^{-1} rocuronium, and 1 mg kg^{-1} lidocaine. Maintenance was attained by the total intravenous anesthesia technique using fentanyl, rocuronium, and midazolam. After intubation, the patient was ventilated with FiO_2 50%, tidal volume 6 mL kg^{-1} and $PaCO_2$ 35-45 mmHg was targeted. Central venous cannulation was attained via the left internal jugular vein. Nasopharyngeal temperature monitoring was performed. Blood gas management during cardiopulmonary bypass was performed with the alpha-stat strategy.

Surgical technique and cerebral protection

Following the right axillary artery and right atrial two-stage venous cannulation, cardiopulmonary bypass was started as described previously[9]. Cardiopulmonary bypass was initiated via roller-pump, open reservoir, and Nipro® oxygenator. Target flow rate was 2.4 L min^{-1} m^{-2} at 36 °C. Composition of the prime volume was Ringer Lactate and adjunctives. The patient was cooled down to nasopharyngeal 28 °C. Cardiac arrest was initiated by crystalloid antegrade and selective coronary ostial cardioplegia (Plegisol®), and thereafter maintained by 1:4 mixed blood retrograde cardioplegia at 20-minute intervals. Surgical field CO_2 flood was not used routinely. Antegrade cerebral perfusion was initiated with flow through the RCA (8-10 mL kg^{-1} min^{-1}), via axillary artery. All arch vessels were clamped during the ACP period. The primary method that has been used to lower cerebral oxygen and metabolic demand and reduce ischemic injury is the establishment of cerebral hypothermia. Additional pharmacologic adjuncts (barbiturates or propofol) that decrease neuronal activity may play a role, so five minutes before low flow, 5 mg kg^{-1} propofol was administered[10,11]. During ACP, all distal anastomoses were performed as an open distal anastomosis in all patients, including the patients undergoing isolated ascending aortic surgery.

Neuromonitoring

NIRS data were monitored throughout the operation, and recorded at the following time points: baseline values before anesthesia induction (Phase 1), at the beginning of cardiopulmonary bypass (Phase 2), during cooling down (32 °C) (Phase 3), at the beginning of ACP application (Phase 4), at the beginning of distal anastomosis with graft during ACP (at about the 5^{th} min of ACP)(Phase 5), at the end of ACP (Phase 6), during warming up (32 °C) (Phase 7), after the end of cardiopulmonary bypass (Phase 8), and end of operation (Phase 9). The control of the perfusion and taking preventive measures, such as checking the oximeter-equipment-cannula position, optimizing the hematocrit, arterial oxygen and carbon dioxide pressure, and increasing the anesthetic depth were planned for cases where there is more than 30% change compared to baseline values, more than 30% difference between two hemispheres, or any value lower than 40%[12].

Statistical analysis

Continuous variables with normal distribution were expressed as mean ± standard deviation, and categorical variables were expressed as number and percentage. Demographic features and perioperative variables were compared by Mann-Whitney U test and chi-square test. Right and left rSO_2 values recorded at nine time points during the operation were compared by Mann-Whitney U test for both all patients and individually for each group. rSO_2 change compared to the baseline was calculated in terms of percentage and analyzed. The standard deviation values of the NIRS group were found to be varying in the range of 10 - 12%. In this case, considering that 1 standard deviation between groups is significant, and that 5% type 1 error and 0.80 type 2 error are assumed to occur, with 14 patients in each group, the power of the study was calculated as 82%. Any p-value less than 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA).

Table 1: Preoperative patient characteristics

Features	Group 1 ACP ≤ 15 min (n = 22)	Group 2 ACP > 15 min (n = 17)	p-value
Male gender	13 (59.1%)	8 (47.1%)	0.455
Age (years)	60.18 ± 10.03	56.47 ± 13.83	0.510
Creatinine (mg/dl)	0.94 ± 0.23	0.92 ± 0.21	0.812
Hematocrit (%)	40.72 ± 4.34	43.05 ± 4.57	0.138

Values are means ± SD or number (%) where shown.

RESULTS

Patient data

Mean ACP duration for the entire study population was found to be 16.4 ± 5.9 minutes. The patient population was divided into 2 groups, namely Group 1 (22 patients with ACP duration 15 minutes and less) and Group 2 (17 patients with ACP duration more than 15 minutes). No difference was observed between groups in terms of individual differences, such as age, gender, and creatinine and hematocrit values (Table 1). Cross-clamping and total perfusion durations, the lowest intraoperative hematocrit value, blood transfusions performed, neurological event (permanent stroke or transient ischemic attack), and ICU-hospital stay were similar in both groups (Table 2).

Table 2: Intraoperative and postoperative variables

Variables	Group 1 ACP ≤ 15 min (n = 22)	Group 2 ACP > 15 min (n = 17)	p-value
CPB time (min)	124.95 ± 42.25	123.00 ± 31.10	0.967
Cross-clamp time (min)	79.77 ± 37.53	71.65 ± 26.69	0.812
ACP time (min)	13.77 ± 1.48	19.71 ± 7.46	<0.001
Intraoperative transfusion (units)	1.45 ± 1.14	1.06 ± 1.09	0.305
Lowest hematocrit (%)	21.60 ± 3.12	23.91 ± 3.68	0.081
Neurological dysfunction	1 (4.5%)	1 (6.3%)	1.000
ICU stay (days)	2.45 ± 6.16	2.00 ± 2.71	0.942
Hospital stay (days)	9.05 ± 5.92	8.69 ± 7.58	0.529

Values are means ± SD or number (%) where shown.
ACP: Antegrade cerebral perfusion; CPB: Cardiopulmonary bypass; ICU: Intensive care unit

NIRS measurements

In terms of the nine rSO_2 values recorded, right and left rSO_2 values for Group 1 (ACP duration ≤ 15 minutes) were similar at all measurement phases; however, a statistically significant difference was observed in Group 2 (ACP duration > 15 minutes) between the right and left rSO_2 values at Phases 5 and 6 (p = 0.024 and 0.023, respectively)(Figure 1). The left rSO_2 value in Group 2 was lower than the right rSO_2 value at Phase 5 (at the beginning of distal arch anastomosis with graft) and Phase 6 (at the end of ACP). In this group, the right and left rSO_2 values were recorded as 58.6 ± 11.9% (95% CI: 52.5 - 64.7) and 49.5 ± 10.2% (95% CI: 44.2 - 54.7), respectively, at Phase 5. At Phase 6, the right and left rSO_2 values were observed as 58.8 ± 11.1% (95% CI: 53.1 - 64.5) and 49.8 ± 8.8% (95% CI: 45.2 - 54.3), respectively (Table 3). When the two groups were compared, no statistically

Table 3: Right and left rSO_2 values of each group

NIRS Phases	Group 1 - ACP ≤ 15 min			Group 2 - ACP > 15 min		
	Right	Left	p-value	Right	Left	p-value
Phase 1	62.3 ± 7.3	62.2 ± 8.8	0.805	66.9 ± 12.9	65 ± 9.2	0.986
Phase 2	55.0 ± 6.9	55.3 ± 5.8	0.733	61.4 ± 11.1	57.8 ± 10.6	0.334
Phase 3	54.6 ± 9.2	53.2 ± 6.5	0.760	55.1 ± 8.3	52.7 ± 11	0.407
Phase 4	52.3 ± 6.8	51.1 ± 6.9	0.733	56.5 ± 13.1	50.1 ± 9.7	0.163
Phase 5	52.9 ± 5.9	50.4 ± 7.6	0.323	58.6 ± 11.9	49.5 ± 10.2	0.024
Phase 6	53.2 ± 6.9	52.4 ± 6.5	0.869	58.8 ± 11	49.8 ± 8.8	0.023
Phase 7	54.9 ± 9.5	53.4 ± 9.7	0.638	57.8 ± 11.2	53.3 ± 12.3	0.301
Phase 8	60.9 ± 10.1	59.8± 8.6	0.753	59.9 ± 9.6	57.6 ± 10	0.448
Phase 9	59.5 ± 10.3	58.1 ± 7.4	1.000	63.5 ± 9.9	61.2 ± 9.5	0.361

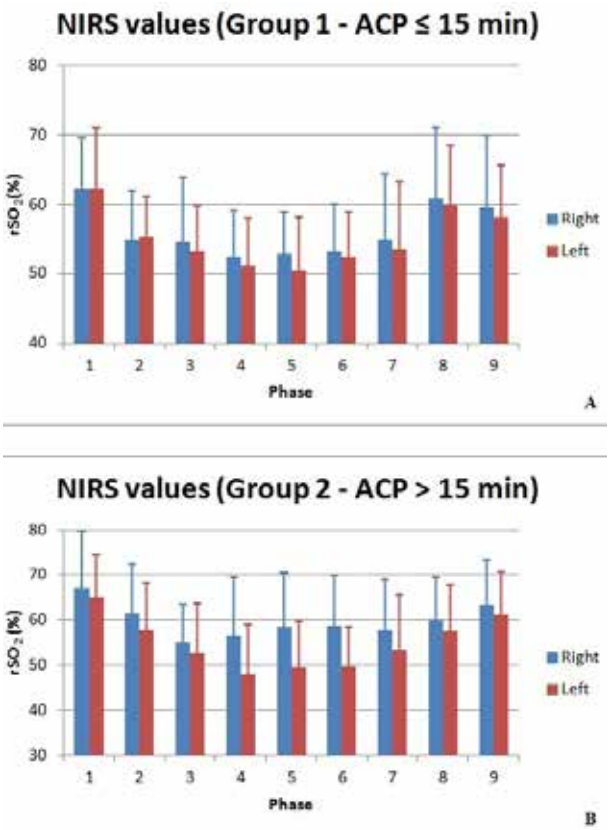

Fig 1. Figure shows the trend monitoring of rSO_2 for each group (A: Group 1 and B: Group 2). At the upper part of the figure (A), it can be seen that the trend was similar along the time period for the Group 1 patients, whereas for Group 2, there is a gap between the trends at the ACP period corresponding to the period of midway to the end of ACP. (ACP: antegrade cerebral perfusion, NIRS: near-infrared spectroscopy, rSO_2: regional oxygen saturation)

significant difference was observed in both right and left hemisphere in terms of rSO_2 values recorded at Phases 5 and 6.

No statistically significant difference was observed between the groups in terms of right and left rSO_2 value percentage differences between baseline and each time interval ($p > 0.05$).

Follow-up

No statistically significant difference was observed between the groups in terms of postoperative ventilation duration, low cardiac output syndrome, duration of intensive care and hospital stay, neurologic complications, nor in the need for inotropes. In one patient in each group, temporary postoperative neurological dysfunction ($p = 1.000$) was observed. Mean ICU stay, and mean hospital stay for Groups 1 and 2 were found to be 2.45 ± 6.16 and 2.00 ± 2.71 days, and 9.05 ± 5.92 and 8.69 ± 7.58 days, respectively (Table 2).

DISCUSSION

In the present study, in patients with 15 minutes or less ACP duration, no statistically significant difference was observed between right and left hemisphere rSO_2 values, and they were within normal limits. However, as the ACP duration prolonged, a difference between hemispheres in terms of rSO_2 values, as measured by NIRS, occurred, and the left rSO_2 values started to decrease. While the perfusion of the left hemisphere was similar to that of the right hemisphere following an average ACP of 13.8 minutes (Group 1), it decreased after an average ACP of 19.7 minutes (Group 2).

NIRS is a non-invasive continuous trend monitor used in brain oxygenation observation. Any change over 30% compared to baseline, a difference of over 30% between two hemispheres, and any value falling below 40% is considered as a risk for brain hypoperfusion and an intervention is recommended. The advantage of NIRS monitoring is that it can be used during hypothermia, low flow perfusion, and even circulatory arrest because its application does not depend on pulse, pressure, or temperature[13-15].

In aortic aneurysm surgery, compared to unilateral perfusion, bilateral cerebral perfusion allows longer time for circulatory arrest. However, the manipulation of arch vessels by direct cannulation may increase the stroke incidence[15]. The unilateral ACP method involves the unilateral brain perfusion by right axillary, or proximal brachial or subclavian artery, and assumes that the circle of Willis is intact, and thus contra lateral hemisphere is perfused. Previous anatomical and angiographic studies reported that variations of Willis polygon occur in more than 50% of the cases[1,16-18]. Therefore, bilateral monitoring of cerebral oxygenation by NIRS is quite crucial for unilateral ACP. Between patients who experienced a neurological event following aortic surgery with ACP and those who did not, there was a statistically significant difference in terms of operation duration and low rSO_2 duration[13,14]. Since metabolic demands are suppressed by mild hypothermia and anesthesia, low-flow and low-pressure perfusion during ACP are considered adequate[19]. However, despite this suppression, when the Willis polygon is incomplete or the ACP duration takes longer, cross-perfusion may not be sufficient and neurologic sequelae secondary to the hypoperfusion may occur[13,14]. This condition warrants the need for the determination of a reliable period for ACP. In the present study, with increasing ACP duration, and in about the middle and towards the end of ACP, the difference between right and left hemisphere rSO_2 values increased. NIRS monitoring is a trend analysis method, and it showed no difference was observed in percentage changes

compared to baseline values, which shows that, even though a difference was observed in the over 15 minutes group at phases 5 and 6, this difference was not a pathological change. However, when each rSO_2 value is considered individually, rSO_2 values were not found to be lower than the previously set cut-off values in any of the patients. Therefore, no alternative strategies, such as switching to bilateral perfusion or cooling down to lower temperatures, were needed in the present study group. However, even though there was no difference in relative changes in the trend analysis, the differences occurring in Group 2 (ACP duration > 15 minutes) at these phases, albeit within normal limits, may indicate that both surgeons and anesthetists should be alert regarding longer-lasting complex aortic arch interventions. Angeloni *et al* reported recently in their updated meta-analysis that longer circulatory arrest times during unilateral ACP were significantly associated with increased mortality[20]. They strongly recommended bilateral ACP for prolonged circulatory arrests over 30 minutes. In case of prolonged ACP, deeper hypothermia or bilateral perfusion technique may be more advantageous.

In all patients undergoing ascending aortic replacement in our clinic, the open distal anastomosis technique is used. Even in patients with isolated ascending aortic aneurysm, distal anastomosis is carried out via the open technique during ACP[21]. In cases where only hemiarch replacement is adequate, this anastomosis takes about 10 minutes. The results of the present study showed that the unilateral ACP method can even be used in isolated ascending aortic replacement cases because it allows open distal anastomosis.

Limitations

The present study has three major limitations. First, the number of patients included in the study is relatively low. Second, the duration of ACP is within the normal limits when all the study population is considered. The reason for this is that isolated ascending aortic surgery was performed in most patients and thus distal anastomosis was carried out in shorter terms in these patients. Besides, the number of patients needing a complex aortic arch intervention is low. Third, this is an observational study and the results may be biased by our protocol of surgical technique which forecloses randomization (unilateral vs. bilateral). Through studies involving cases with longer ACP duration, precise time intervals may be set. Nevertheless, the present prospective observational study highlights the effectiveness of a surgical and brain protection technique that is clearly beneficial in patients with a short ACP duration.

CONCLUSION

In aortic surgery, 15 minutes or less unilateral ACP may be performed at moderate hypothermia without any asymmetry between hemispheres with NIRS monitoring. As the ACP duration prolonged, a difference between hemispheres in terms of rSO_2 values occurred, and the left rSO_2 values started to decrease, and they were within normal limits after all.

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Original Article

BRAF^{V600E} gene mutation can predict central lymph node metastasis in thyroid papillary microcarcinoma

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ABSTRACT

Objective: To explore the relationship between v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene mutation and central lymph node metastasis in papillary thyroid microcarcinoma (PTMC)
Design: Retrospective
Setting: Taizhou Hospital of Zhejiang Province, Linhai, China
Subjects: Paraffin-embedded specimens from a total of 200 PTMC patients
Intervention: Intraoperative rapid pathology and postoperative pathology; real time polymerase chain reaction
Main outcome measure(s): BRAF mutation with clinicopathological features
Results: The incidence of BRAFV600E mutation in patients with PTMC was 84.5% (169/200). Both univariate and multivariate analyses indicated a correlation between BRAFV600E mutation and central lymph node metastasis ($p = 0.030$). For patients with tumors >5 and to ≤10 mm in diameter, BRAFV600E mutation had a significant effect on central lymph node metastasis ($p = 0.043$). BRAFV600E mutation had no effect on central lymph node metastasis with tumors ≤5 mm in diameter ($p = 0.581$).
Conclusions: BRAFV600E gene mutation is helpful to predict the central lymph node metastasis in patients with PTMC. It could be an easy predictor for central lymph node dissection in preoperatively detected BRAFV600E mutation positive PTMC patients and that lymphatic and adipose tissues should be routinely removed.

KEYWORDS: BRAF^{V600E} gene, central lymph node metastasis, mutation, papillary thyroid microcarcinoma

INTRODUCTION

Thyroid carcinoma is the most common endocrine system malignant tumor[1]. The incidence of thyroid cancer has increased dramatically over the past decades world wide[2,3], especially in the Chinese coastal city, such as the Taizhou area, where a lot of new cases have been found every year. Most of these new cases are papillary thyroid microcarcinoma (PTMC)[4,5]. PTMC refers to papillary thyroid carcinoma (PTC) with a diameter ≤10 mm[6]. Although the biological behavior of PTMC tends to be benign and the mortality rate is low, in clinical practice, it is found that a small part of PTMC will recur and even affect the patient's life[7]. The v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene mutation is the most common genetic event in thyroid carcinoma[8], with the highest prevalence in PTC (29 - 88%)[9]. Some studies[7,10-12] have found that the mutation of BRAF^{V600E} is related to the invasiveness of PTMC, such as extrathyroidal invasion, lymph node metastasis, tumor recurrence and prognosis. With the increasing incidence of PTMC and highest prevalence BRAF^{V600E} mutation in PTMC, it is important to identify the subgroup of high-risk PTMC of patients who may be traditionally thought to be a more indolent disease[13]. This study retrospectively analyzed the BRAF^{V600E} mutation status and the clinical data in samples from a total of 200 PTMC patients.

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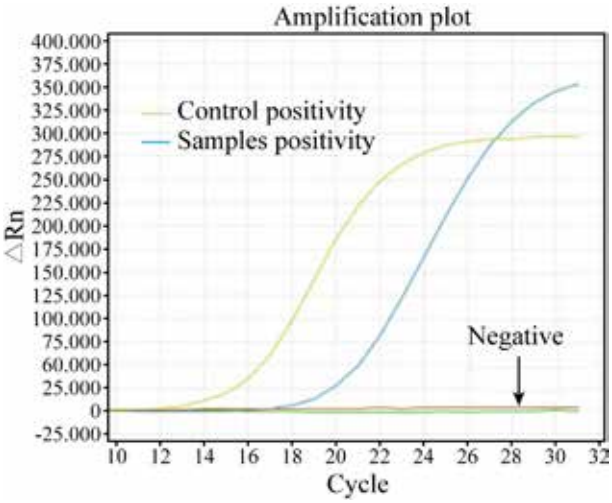

Fig 1. Gene mutation test results (real time-polymerase chain reaction signal curves).

SUBJECTS AND METHODS

Patients and samples

Paraffin-embedded specimens from a total of 200 PTMC patients at our hospital collected between October 2014 and March 2016 were used in this study. All patients included in this study met the following requirements: 1. Unilateral tumor was diagnosed by ultrasound before surgery; 2. PTMC diagnosis by both intraoperative rapid pathology and postoperative pathology; 3. Treatment via ipsilateral lobectomy, isthmectomy, and prophylactic ipsilateral central lymph node dissection (CLND); and 4. the BRAF^{V600E} gene was detected in all samples routinely. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Wenzhou Medical College. Written informed consent was obtained from all participants.

Real time polymerase chain reaction (PCR)

Eluent containing DNA was obtained from 5 paraffin-embedded tissue sections (3-5ìm in thickness) per sample using a DNA kit (Aide Biotechnology Inc, Xiamen, China) according to the manufacturer's instructions. The BRAF^{V600E} mutation was detected by analyzing real time PCR signals using a human BRAF^{V600E} mutation detection kit (Fluorescence PCR' Aide Biotechnology Inc, Xiamen, China), to determine whether the sample carried a mutation-positive gene, as shown in Figure 1.

Table 1: Clinicopathological characteristics of 200 patients with PTMC

Clinicopathologic characteristics	Patients (n)	Percentage (%)
Gender		
Male	38	19.0
Female	162	81.0
Age (year)	44.5 ± 10.0	
≥45	100	50.0
<45	100	67.0
Tumor size (mm)	6.3 ± 2.0	
>5 and to ≤10 vs ≤5	134	33.0
Multifocality		
Multiple (≥2)	66	21.5
Single	43	78.5
Extrathyroid invasion		
Yes	157	29.0
No	58	71.0
Hashimoto's disease		
Yes	142	16.5
No	33	83.5
Lymph node metastases		
Yes	167	44.5
No	89	55.5
BRAFV600E mutation	111	
Yes	169	84.5
No	31	15.5

Statistical analysis

The SPSS13.0 statistical software package (SPSS, Inc., Chicago, IL, USA) was used for all analyses. Normally distributed measurement data were expressed as means ± standard deviations, and non-normally distributed data were expressed as medians (quartiles). Enumeration data were expressed as frequencies and percentages. The independent

Table 2: Univariate and multivariate analyses of the BRAF^{V600E} mutation and clinicopathological features in PTMC

Variable (n = 200)	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender (male/female)	1.027 (0.389-2.72)	0.956	1.377 (0.494-3.835)	0.540
Age, yr (≥45 vs <45)	0.795 (0.368-1.715)	0.558	0.787 (0.354-1.750)	0.557
Tumor size, mm (>5 and to ≤10 mm vs ≤5)	0.633 (0.289-1.385)	0.252	0.749 (0.326-1.721)	0.496
Multifocality (multiple/single)	1.332 (0.549-3.231)	0.526	1.355 (0.527-3.479)	0.528
Extrathyroidal invasion (yes/no)	0.675 (0.273-1.666)	0.394	0.993 (0.377-2.611)	0.988
Hashimoto's disease (yes/no)	2.472 (1.017-6.005)	0.046	2.552 (0.981-6.642)	0.055
LNM (yes/no)	0.378 (0.160-0.892)	0.026	0.371 (0.151-0.907)	0.030

Note: A significant correlation between BRAFV600E mutation and central lymph node metastasis in both univariate (p = 0.026) and multivariate analysis (p = 0.030).

samples-test (normally distributed data), rank-sum test (non-normally distributed data) or χ^2-test (enumeration data) was used to compare two variables. Univariate and multivariate logistic regression analyses were used to evaluate the influences of clinicopathological factors on $BRAF^{V600E}$ mutation and central lymph node metastasis (central lymph node metastasis as the dependent variable). The level of significance was defined as $p < 0.05$.

RESULTS

Clinicopathological data

The clinical and pathological features of patients are shown in Table 1. A total of 200 cases of unilateral PTMC in 38 male and 162 female patients were included in this study (average age: 44.5 ± 10.0 years).

Table 3: Correlation of central lymph node metastasis with $BRAF^{V600E}$ mutation

Central lymph node metastasis	$BRAF^{V600E}$ Mutation Positive	Negative	p-value
*LNM (n/%)			0.023
Yes	81 (91.0)	8 (9.0)	
No	88 (79.3)	23 (20.7)	
ΔLymph node dissection (n, mean ± SD)	4.90±3.33	5.48±4.64	0.402
ΔLNM (n, mean ± SD)	0.97±1.35	0.71±1.67	0.342

Note: *: X^2-test; Δ: t-test. $BRAF^{V600E}$ mutation was not significantly associated with the number of lymph nodes dissected as well as the number of central metastatic lymph nodes ($p > 0.05$).
LNM : lymph node metastatics

Association of BRAF mutation with clinicopathological features

The relationships between $BRAF^{V600E}$ mutation and clinicopathological features of PTMC are shown in Table 2. The univariate and multivariate analysis indicated a significant correlation between $BRAF^{V600E}$ mutation and central lymph node metastasis ($p = 0.030$).

The relationship between BRAF mutation and central lymph node metastasis

The correlation between $BRAF^{V600E}$ mutation and features of central lymph node metastasis is shown in Table 3. Compared with $BRAF^{V600E}$ negative patients, a significant correlation between BRAF mutation and central lymph node metastases was observed in $BRAF^{V600E}$ mutation positive patients ($p = 0.023$). However, the mutation status was not significantly associated with the number of lymph nodes dissected from the patient as well as the number of central metastatic lymph nodes ($p = 0.402$; $p = 0.342$).

Correlations between tumor size, $BRAF^{V600E}$ mutation, and central lymph node metastasis are shown in Table 4. In patients with tumors ≤5 mm in diameter, the BRAF mutation status was not associated with lymph node metastasis ($p = 0.581$). but patients with tumors >5 and to ≤10 mm in diameter, the $BRAF^{V600E}$ mutation status had a significant correlation with lymph node metastasis ($p = 0.043$).

DISCUSSION

The $BRAF^{V600E}$ mutation is the most common genetic event in PTC, with a reported incidence from 29 to 88%[8,9]. In PTMC patients, we found a $BRAF^{V600E}$ mutation prevalence of 84.5%. $BRAF^{V600E}$ aberrantly activates the MAPK pathway, a central regulator of cell growth and proliferation. Through the univariate and multivariate analysis conducted in our study, $BRAF^{V600E}$ mutation was found to be associated with central lymph node metastasis ($p = 0.030$). Fraser *et al*[14] found that $BRAF^{V600E}$ mutation in PTC predicts an increased risk of lymph node metastasis, extra-thyroidal extension and reduced disease-free survival. Zheng *et al* [15] found that $BRAF^{V600E}$ mutation was closely related to a poor clinicopathological outcome and could lead to an increase in PTMC recurrence. Chen *et al*[11] found that BRAF mutational status correlated with recurrence of PTMCs. It is believed that $BRAF^{V600E}$ mutation is an additional useful predictor of central lymph node metastasis. This result is consistent with the study results published by Lin *et al*[16] and Sun *et al*[9].

Our study also analyzed the correlation of central lymph node metastasis with $BRAF^{V600E}$ mutation and tumor size. Furthermore, a significant correlation was found between central lymph node metastasis and $BRAF^{V600E}$ mutation ($p = 0.023$). It can indicate the effect and predictive value of $BRAF^{V600E}$ mutation with respect to central lymph node metastasis. Moreover,

Table 4: Correlations of tumor size and $BRAF^{V600E}$ mutation with lymph node metastasis

Tumor size (mm)	Mutation	Lymph node metastases Yes (n)	Percentage (%)	No (n)	Percentage (%)	p-value
≤5	Positive	19	35.8	34	64.2	0.581
	Negative	3	23.1	10	76.9	
>5 and to ≤10	Positive	62	53.4	54	46.6	0.043
	Negative	5	27.8	13	72.2	

this study also found that patients with tumors >5 and ≤10 mm in diameter, the BRAF^{V600E} mutation status had a significant correlation with lymph node metastasis (p = 0.043). This result is consistent with the study results published by Wang *et al* and Vorasubin *et al*[17,18]. Furthermore, patients with tumors ≤5 mm in diameter, the BRAF^{V600E} mutation status was not associated with lymph node metastasis (p = 0.581). This may be due to the limited number of patients in the group; the final conclusion is yet to be further clarified.

The above results showed that the PTMC with BRAF^{V600E} mutation and in diameter >5 mm is more aggressive than those with BRAF^{V600E} negative and in diameter ≤5 mm. It suggested that the tumors with diameter >5 mm and BRAF^{V600E} mutation positive patients may be more susceptible to recurrence and metastasis after surgery. That was a group of high-risk patient population, who needed to perform CLND with preoperative BRAF^{V600E} mutation positivity and that lymphatic and adipose tissues should be routinely removed[19,20].

CONCLUSIONS

This study found a BRAF^{V600E} mutation prevalence of 84.5% in PTMC patients and determined that BRAF^{V600E} mutation is an independent predictor of central lymph node metastasis in PTMC. Primary PTMC positive for the BRAF^{V600E} mutation and in diameter >5 mm may represent a group of tumors that require more prompt surgical management and that lymphatic and adipose tissues should be routinely removed. Patients without these high-risk features may be suitable candidates for observation, after sufficient counseling.

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Conflict of interest: The authors declare no conflict of interests.

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Original Article

Maternal and neonatal outcomes based on the amniotic fluid index in cases of preterm premature rupture of membranes

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ABSTRACT

Objectives: The management of cases of preterm premature rupture of membranes (PPROM), which is known to cause significant levels of maternal-neonatal morbidity and mortality, is based on induction of birth or monitoring with a conservative approach. PPROM is associated with some complications such as preterm delivery, low APGAR scores, perinatal infections, respiratory distress syndrome and neonatal sepsis.

Design: Retrospective clinical study

Setting: Erciyes University Obstetric Clinic Perinatology Unit, Turkey

Subjects: Ninety-seven patients with a singleton pregnancy complicated by PPROM between 26 and 34 gestational weeks

Intervention(s): Patients were divided into two groups based on their amniotic fluid index (AFI), <5 cm (n: 54, Group 1) and >5 cm (n: 43, Group 2).

Main outcome measure(s): To assess maternal and neonatal outcomes in cases of PPROM in cases with AFI values <5 cm or >5 cm.

Results: Low birth weight and premature birth were determined in the AFI <5 cm group ($p < 0.05$). Levels of neonatal mechanical ventilator requirement, necrotizing enterocolitis, intracranial hemorrhage and early neonatal sepsis were similar between the groups. Surfactant requirement and mortality rates were significantly high in Group 1 ($p < 0.05$).

Conclusion(s): Although morbidity rates in cases with AFI <5 cm and AFI >5 cm were similar, severe oligohydroamnios is associated with high neonatal mortality.

KEYWORDS: fetal morbidity-mortality, oligohydramniosis, preterm premature rupture of membranes

INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as the rupture of membranes without labor before the 37th week of pregnancy. Seen in approximately 3% of pregnant women, PPROM is responsible for approximately one premature birth in three[1]. Increased morbidity and mortality are observed in cases of PPROM secondary to complications with a high possibility of occurring during the period from rupture of membranes to birth, such as perinatal infections, entrapment of the umbilical cord between the fetal head or extremities and the uterus in association with a decrease in amniotic fluid, or in some cases, prolapse of umbilical cord[2].

Pathogen micro-organisms deriving from the subgenital system are thought to lead to intrauterine infections through the ascending route in the period between PPROM and birth[3]. The incidence of serious complications such as intracranial hemorrhage (ICH), necrotizing enterocolitis (NEC) and respiratory distress syndrome (RDS) increases in cases of PPROM together with infections responsible for neonatal morbidity and mortality, such as early-onset neonatal sepsis[4].

Close patient monitoring by clinicians in cases of PPROM, prophylactic antibiotic therapy aimed at preventing infections, the planning of steroids for lung maturation and birth timing are very important in terms of maternal-neonatal morbidity and mortality.

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The general approach in these cases is to reach the 24th week of pregnancy with antibiotic prophylaxis and steroid therapy for lung maturation.

Some studies have shown that, although a decrease in prematurity-related morbidity secondary to the conservative approach approved in cases of PPROM has been achieved, perinatal infections have increased[5,6].

The purpose of this study was to determine the effect of amount of residual amniotic fluid on maternal and neonatal morbidity and mortality in cases of PPROM.

SUBJECTS AND METHODS

Ninety-seven patients with a singleton pregnancy between gestational weeks 26 and 34 complicated with PPROM at the Erciyes University Obstetric Clinic Perinatology Unit, Turkey, in 2012 - 2015, together with the newborns, were included in this retrospective study. Patients were divided into two groups depending on their amniotic fluid indices, amniotic fluid index (AFI) <5 cm (n:54, Group 1) and AFI >5 cm (n:43, Group 2). The postnatal, perinatal and maternal outcomes of patients monitored up to birth were then compared. Patients with multiple pregnancies, with identified delayed intrauterine development, or with diseases such as maternal diabetes and pre-eclampsia were excluded. Patients presenting to our clinic were first examined with a sterile speculum. PPROM was diagnosed through observation of amniotic fluid discharge from the cervical passage at sterile speculum examination or pooling of amniotic fluid in the posterior vaginal fornix. Diagnosis was confirmed using the nitrazine test in uncertain cases.

Gestational age was determined on the basis of the last menstrual period. In patients uncertain of their last menstrual periods, gestational age was determined on the basis of ultrasonographic measurement before the 20th week of pregnancy. Ultrasonographic criteria were also used for patients uncertain of their last menstrual periods and who had not received previous ultrasonography.

Ultrasonography was applied for confirmation of gestational age, to observe fetal heartbeat and to determine amounts of amniotic fluid. AFI measurement was performed by measuring the deepest fluid pocket in four quadrants using B-mode ultrasound[3-11].

Patients were hospitalized for observation up to the 34th week of pregnancy. The pregnancies of patients who did not give birth for fetal and maternal reasons during that time were concluded on the 34th week of pregnancy by normal spontaneous vaginal or cesarean delivery in the light of obstetric history.

Twelve milligrams of betamethasone were administered for the purpose of lung maturation, and the same dose was repeated 24 hours later. All patients received a single course of steroid, with no repeat course. A daily non-stress test was applied to assess fetal status. Biophysical profile measurement was added to this in cases requiring it. Patients received ampicillin-azithromycin antibiotic prophylaxis for 7 days, the first 2 days intravenously. Body temperature was monitored every 4 hours, and patients were closely monitored in terms of chorioamnionitis findings, such as uterine sensitivity and maternal and fetal tachycardia. White cell count, sedimentation and C - reactive protein (CRP) were measured daily.

Clinical chorioamnionitis was based on the presence of two or more maternal body temperature values measured at hourly intervals of 37.8 °C or more, tachycardia in the mother (120/min), tachycardia in the fetus (160/min), uterine sensitivity, purulent discharge, maternal leukocytosis (20,000/mm^3) and positive CRP (reference interval 0-5 mg/L) (1-13). Diagnosis of neonatal sepsis was based on a clinical picture of infection together with infection being shown in blood or cerebrospinal fluid culture (1-15), while RDS was diagnosed based on a frosted glass appearance at pulmonary radiology in addition to clinical picture.

The association between post premature rupture of membranes-residual AFI level and complications such as grade 2 or above intraventricular hemorrhage, NEC stage 2 or above, early natal sepsis within 72 hours and RDS was investigated in the patients in this study. We also sought to determine the effect on development of these complications of CRP levels, length of EMR, birth weight and gestational age in the mothers of babies with these complications.

Statistical analysis

Normality of data distribution was assessed using the Shapiro-Wilk normality test. Normally distributed variables between groups were compared using the independent samples test and non-normally distributed variables were compared using the Mann-Whitney U test. The chi-square test was used to determine the relations between categorical variables. Statistical significance was set at $p < 0.05$.

RESULTS

Ninety-seven patients were included in the study, 54 (55.67%) were classified in Group 1 (AFI <5 cm) and 43 (44.33%) in Group 2 (AFI >5 cm). The general demographic and clinical characteristics of the patients according to the groups are shown in Table 1.

No significant difference was observed between the groups in terms of maternal age, gravida, parity, prenatal maternal CRP and white cell count, pregnancy developing chorioamnionitis, length of EMR (time to birth) or newborn gender and white cell count.

Table 1: Demographic and clinical characteristics of the groups

Demographic parameters	Group 1 (n = 54)	Group 2 (n = 43)	p-value
Maternal age (years)	29.41 ± 5.80	29.72 ± 6.63	0.80
Gravidity	2.50 (1-8)	2.00 (1-8)	0.55
Parity	1.00 (0-7)	1.00 (0-6)	0.25
Prenatal maternal WBC	12104 ± 3532	12269 ± 3748	0.82
Prenatal maternal CRP	7.21 (3-102)	6.20 (3-80)	0.43
CA pregnancies (n,%)	4 (4.1%)	2 (2.1%)	0.69
PROM week	27.15 (161-232)	30.85 (173-235)	<0.001
Delivery week	30.42 (173-253)	32.71 (187-239)	0.003
PROM duration (day)	11.00 (2-62)	8.00 (2-40)	0.14
Birth weight (g)	1504 ± 586	1775 ± 494	0.02
Female gender (n,%)	22 (22.7%)	19 (19.6%)	0.83
First day neonatal WBC	12480(4170-78110)	12410(6220-38060)	0.64
Neonatal CRP	3.45 (3.17-68)	3.34 (3.08-55)	0.01

CRP: C-reactive protein; CA: Chorioamnionitis; WBC: white blood cell; PROM: premature rupture of membranes; SD: standard deviation
Group 1 : AFI < 5cm ; Group 2: AFI > 5cm

Week of PPROM was significantly lower in the AFI <5 cm group compared to the AFI >5 cm group (27.15 vs 30.85, p <0.001). Birth in Group 1 also occurred significantly earlier than in Group 2 (30.42 vs 32.71, p: 0.003). In terms of birth indications, birth was induced in 37 patients in Group 1 due to preterm movements, compared to 21 patients in Group 2. Fourteen patients in Group 2 reached the 34th week of pregnancy, compared to 4 in Group 1. Levels of births induced due to fetal distress and chorioamnionitis were similar between the groups. The mean birth weight in Group 1 was 1504 ± 586 gr and 1775 ± 494 gr in Group 2 (p <0.05). Newborn CRP levels were significantly lower in patients with AFI >5 cm compared to those in Group 1 (p <0.05).

General neonatal mortality was 12.3% (12/97). Mortality among all newborns was higher in the AFI <5 cm group. A statistically significant difference was observed between the mean gestational age of the exitus babies, (28w+6d) ± 22d, and that of the surviving babies, (32w+3d) ± 17d (p = 0.002). A statistically significant difference was also observed between the mean birth weight of the exitus babies and that of the surviving babies (1154 ± 413 gr vs. 1828 ± 526 gr; p = 0.014). In terms of neonatal morbidity, surfactant requirement was significantly higher in Group 1, while levels of early neonatal sepsis, retinopathy of prematurity, ICH, bronchopulmonary dysplasia, NEC and mechanical ventilation requirements were similar between the groups. While these complications were all greater in Group 1, the differences were not statistically significant (Table 2).

Analysis of the effect of maternal CRP level, length of EMR, week of PPROM, birth weight and gestational age on newborn results identified low birth weight, low EMR and birth week as significant factors in newborn mortality and morbidity (p <0.05) (Table 3).

Table 2: Neonatal outcomes in the PPRM study groups

Neonatal complications	Group 1 (n = 54)	Group 2 (n = 43)	p-value
Length of stay (days)	13 (1-149)	12 (4-98)	0.98
MV requirement	26	14	0.14
CPAP requirement	13	7	0.45
Surfactant requirement	24	10	0.03
Grade 2 and > ICH	6	3	0.72
Grade 2 and > NEC	4	5	0.50
BPD	10	6	0.59
BPD postnatal steroid	44	38	0.40
Grade 2 and > ROP	8	4	0.54
PDA	11	3	0.08
APGAR score < 7	14	8	0.46
Early neonatal sepsis	14	11	0.57
Neonatal mortality	12	0	0.001

AFI: amniotic fluid index; MV: mechanical ventilator; CPAP: continuous positive airway pressure; ICH: intracranial hemorrhage; NEC: necrotizing enterocolitis; BPD: bronchopulmonary dysplasia; ROP: retinopathy of prematurity; PDA: patent ductus arteriosus
Group 1 : AFI < 5cm ; Group 2: AFI > 5cm

DISCUSSION

The management of cases of PPROM, which is known to cause significant levels of maternal-neonatal morbidity and mortality, is based on induction of birth or monitoring with a conservative approach. These decisions are closely associated with the determination of several risk factors involving the mother and newborn and the establishment of these factors' cause and effect relations.

In this study, an AFI <5 cm was not identified as a significant factor in maternal and neonatal morbidity, although neonatal mortality was significantly higher in group 1 than group 2. One multicenter study of 290 singleton pregnancy patients with PPROM between weeks 24 and 32 investigated the relation between amount of amniotic fluid and chorioamnionitis. Similar

Table 3: Impact on neonatal morbidity of C-reactive protein, gestational age, birth weight, PPROM week and duration

Maternal parameters	Group without neonatal morbidity (n:64) mean±SD	Group with neonatal morbidity (n:33) mean±SD	p-value
Birth weight (g)	1880 ± 456	1113 ± 355	<0.0001
Maternal CRP levels (mg/L)	5.95 (3-78)	7.3 (3-102)	0.34
PPROM weeks	(30w+1d) ± 20d	(26w+2d) ± 13d	<0.0001
PPROM duration /day	9 (2-62)	13 (2-59)	0.96
Birth weeks	(32w+5d) ± 14d	(28w+3d) ± 13d	<0.0001

SD: standard deviation; CRP: C-reactive protein; PPROM: preterm premature rupture of membranes

levels of clinical and subclinical chorioamnionitis were determined in the group with less amniotic fluid and the group with more[7]. Vermillion *et al* and Park *et al* emphasized that a decrease in the amount of amniotic fluid led to an increase in levels of chorioamnionitis[8,9]. Another study of 95 patients reported significantly high levels of chorioamnionitis in cases of PPROM with amniotic fluid levels below 5 cm[5]. In contrast to those studies, Tavvasolli *et al* and Mercer *et al* determined no relation between level of residual amniotic fluid after PPROM and chorioamnionitis[4,7].

Prophylactic antibiotic use in cases of PPROM is known to reduce neonatal morbidity and mortality by lowering the risk of chorioamnionitis[10] and also that of premature birth[11]. The polymicrobial etiology of chorioamnionitis in these cases means that prophylaxis with broad spectrum antibiotics is essential. The American College of Obstetricians and Gynecologists recommends amoxicillin and erythromycin therapy for 5 days following 48 hours intravenous ampicillin and erythromycin therapy in cases of PPROM[12]. It has also been reported that azithromycin therapy can be administered in addition to ampicillin in some cases. However, no clear superiority among these antibiotic regimens has been demonstrated. Pierson *et al* compared an ampicillin-erythromycin combination with ampicillin-azithromycin in cases of PPROM and reported levels of chorioamnionitis of 8.6% in patients using ampicillin-azithromycin and 12% in those receiving ampicillin-erythromycin[13].

Examination of patients' neonatal sepsis results showed that amount of amniotic fluid did not significantly affect sepsis rates. Vintzileous *et al* demonstrated greater neonatal sepsis and chorioamnionitis in cases with less amniotic fluid[14]. In contrast, in their multicenter study, Mercer *et al* reported that amount of amniotic fluid did not affect neonatal sepsis levels in 290 cases of PPROM with singleton pregnancy[7]. While Vintzelieous *et al*[14] did not administer antibiotics to all newborns with PPROM, in our study we gave antibiotic prophylaxis to all babies born to mothers with PPROM in our hospital's obstetric clinic and neonatal unit. We encountered no sepsis. We attribute this to the absence of any significant relation between a low level of amniotic fluid and neonatal sepsis in this study. Neonatal inflammatory response syndrome may bear a clinical resemblance to sepsis. Azithromycin is known to reduce fetal inflammatory response syndrome due to its anti-inflammatory effectiveness. The decreased prevalence of neonatal infection-sepsis may therefore be related to the use of azithromycin in our clinic.

All newborns were compared in terms of APGAR scores. The number of newborns with Apgar scores <7 in Group 1 (AFI <5 cm) was higher than that in Group 2 (AFI >5 cm). However, in contrast to Piazze *et al*'s study[6], the difference was not statistically significant. Although surfactant requirements were higher in the AFI <5 cm group than in the AFI >5 cm group, RDS levels in the two groups were similar. Sims *et al* reported that RDS developed in 17% of cases of PPROM[15]. Borna *et al* and Mercer *et al* reported that RDS was less common in patients with AFI >5 cm[5,7]. Similar to our own research, several studies have reported no significant correlation between amount of amniotic fluid and development of RDS[4,5].

Analysis of the effect on newborn findings of maternal CRP level, duration of EMR, week of PPROM, birth weight and gestational age identified, in agreement with the literature, low birth weight, low EMR and birth week as significant factors in neonatal morbidity.

No neonatal mortality occurred in the AFI >5 cm group in our study. Eleven of the 12 newborns in the AFI <5 cm group died from prematurity-related complications (NEC, ICH and sepsis) and one baby died due to pneumothorax. Mortality levels being high while neonatal morbidity levels were similar in the groups might appear to represent an inconsistency. When the two groups were examined, however, all complications such as ICH, NEC, RDS and neonatal sepsis were greater in the AFI <5 cm group, albeit not to a statistically significant extent. In addition, PPROM week was considerably lower in the AFI <5 cm group

compared to the AFI >5 cm group (27.15 - 30.85 w, p <0.05). Although times until birth were similar, babies in the AFI <5 cm group with early EMR were born at earlier weeks than babies in the AFI >5 cm group. The mean gestational age of the exitus babies, (28w+6d) ± 22d, was significantly lower than that of the surviving babies, (32w+3d) ± 17d (p = 0.002). Similarly, the mean birth weight in the exitus babies (1154 ± 413 gr) was significantly lower than the birth weight in the surviving babies (1828 ± 526 gr, p = 0.014). This variation in neonatal morbidity may be attributed to secondary factors.

CONCLUSION

The amount of amniotic fluid in cases of PPROM did not affect maternal or neonatal morbidity. However, as previous studies have shown, increased mortality associated with greater premature birth and low birth weight is observed in cases with low levels of amniotic fluid.

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Original Article

Correlation of antiplatelet agent and statin use with degree of vascular calcification in patients on peritoneal dialysis

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ABSTRACT

Objective: Vascular calcification is a major cause of cardiovascular mortality in patients with end-stage renal disease. Therefore, this study was designed to evaluate whether the use of antiplatelet agents and statins improve vascular calcification and other risk factors in patients undergoing peritoneal dialysis.
Design: Retrospective study
Setting: Division of Nephrology, Department of Internal Medicine, Inha University Hospital, Republic of Korea
Subject: This study included patients with end-stage renal disease who were administered peritoneal dialysis center.
Intervention: Medical records of all the patients were reviewed and the data were collected retrospectively.
Main outcome measures: The severity and extent of vascular calcification were scored using a semi-quantitative scale ranging from 0 to 8 (Adragao's method). The patient characteristics were compared according to their vascular calcification score.
Results: The 156 study patients undergoing peritoneal dialysis had an average vascular calcification score (VCS) of 2.9 ± 2.7. The VCS was significantly higher in patients with diabetes and increased age but was significantly lower in those prescribed an antiplatelet agent or statin (for >2 years). Patients who used both statins and antiplatelet agents (1.9 ± 2.4) showed a more significant improvement in vascular calcification than those who used antiplatelet agents alone (3.3 ± 2.6) or none of the agents (4.2 ± 2.6) did. In the logistic regression analysis, the statin and antiplatelet agent group showed 0.65 times lower VCS than the control group.
Conclusions: Concurrent use of antiplatelet agents and statins decreased vascular calcification in patients undergoing peritoneal dialysis.

KEYWORDS: antiplatelet agent, continuous ambulatory peritoneal dialysis, diabetes mellitus, statins, vascular calcification

INTRODUCTION

Patients with end-stage renal disease are at a greater risk of cardiovascular mortality than the general population[1]. Several recent studies have reported that vascular calcification is an important risk factor for cardiovascular disease in patients on dialysis[2,3]. Vascular calcification reflects the severity of a vascular disease and is a simple and powerful predictor of the morbidity and mortality of cardiovascular diseases in patients with end-stage renal disease[3,4].

The severity of vascular calcification is scored using simple X-ray chest films, ultrasonography, electron beam computed tomography, and multi slice computed tomography[2,5-7]. Among these methods, scoring using plain radiographic films of the pelvis and both hands is not only inexpensive but also effective in predicting the incidence and mortality of vascular diseases including coronary heart disease, according to the degree of the vascular calcification score[2,8]. Several risk factors associated with vascular calcification have been studied recently, and the traditional risk factors include aging, hypertension, diabetes, smoking, and dyslipidemia. The non-traditional risk factors include mineral metabolism disorders such as hyperphosphatemia and elevated serum levels of intact parathyroid hormone, anemia, and chronic inflammation[9]. However, the effects of medications, particularly antiplatelet agents and statins that are frequently administered to patients with end-stage renal disease on vascular calcification have not been well investigated. Therefore, this study was performed to evaluate the effects of risk factors on vascular

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Table 1: Comparison of clinical baseline parameters according to medication

Parameter	None	Antiplatelet agent	Antiplatelet plus statin	p-value
Patient no.	32	61	63	
Age	55.66 ± 10.72	55.79 ± 11.04	53.81 ± 11.73	0.576
Diabetes mellitus (+)	15 (46.8)	32 (52.4)	34 (53.9)	0.102
Hypertension (+)	3 (15.6)	29 (47.5)	31 (49.2)	0.315
Hemoglobin	10.1 ± 4.6	10.0 ± 4.5	10.3 ± 4.4	0.173
Albumin	3.5 ± 1.5	3.5 ± 1.5	3.5 ± 1.5	0.661
Cacium	8.15 ± 1.22	8.40 ± 1.14	8.05 ± 1.33	0.294
Phosphate	5.51 ± 1.14	4.95 ± 1.45	4.74 ± 1.43	0.038
Caciumxhosphate	47.33 ± 9.32	44.13 ± 12.44	42.46 ± 14.32	0.213
ALP	152.82 ± 117.89	120.91 ± 103.14	203.57 ± 219.06	0.032
iPTH	167.46 ± 150.60	177.24 ± 179.24	176.59 ± 114.27	0.950
FGF23	2557.15 ± 1240.73	2663.65 ± 1293.41	2166.24 ± 1526.08	0.212
Vitamin D	16.68 ± 2.53	17.08 ± 4.08	14.60 ± 8.89	0.447
Cholesterol	169.6 ± 46.5	169.2 ± 44.2	167.4 ± 41.5	0.114
BUN	55.12 ± 18.15	51.69 ± 17.10	52.31 ± 17.34	0.653
Creatinine	9.92 ± 3.58	8.06 ± 3.35	8.73 ± 2.92	0.034
RRF	2.95 ± 7.44	0.10 ± 0.58	1.70 ± 2.25	0.002
Pre-VC score	4.34 ± 2.30	4.69 ± 2.18	4.33 ± 2.48	0.657
Post-VC score	4.59 ± 2.61	3.74 ± 2.72	2.10 ± 2.57	0.001

Values for continuous variables are means ± standard deviation and variables not normally distributed are median and interquartile range; values for categorical variables are percentages.
ALP: alkaline phosphatase; iPTH: intact parathyroid hormone; FGF23: fibroblast growth factor 23; BUN: blood urea nitrogen; RRF: residual renal function; VC: vascular calcification

calcification, and the association of antiplatelet agents and statins with the degree of vascular calcification in patients with end-stage renal disease.

SUBJECTS AND METHODS

Study population

Patients with end-stage renal disease (aged ≥ 18 years) who underwent dialysis treatment at the Inha University Dialysis Center between 2008 and 2012 were recruited for this study. According to the inclusion criteria, the registered patients had stage 5 chronic kidney disease prior to peritoneal dialysis. Some patients with end-stage renal disease were excluded owing to incomplete, unclear, or missing information, and covariate outlier data. Finally, 156 patients were enrolled. This study was approved by the Medical Ethics Committees of the participating hospitals, and informed consent was obtained from all patients before inclusion in the study.

Data collection

Baseline demographic and clinical data including age, gender, height, weight, body mass index, comorbidities, laboratory parameters, and therapeutic characteristics were recorded. Cardiovascular disease was defined as the presence of coronary artery disease, congestive heart failure, peripheral vascular disease, or cerebrovascular disease. Serum hemoglobin, albumin, aspartate aminotransferase, alanine aminotransferase, calcium, phosphorus, intact parathyroid hormone, total cholesterol, triglyceride, uric acid, and high-sensitivity C-reactive protein levels were determined from the blood samples of patients. The residual renal function was calculated using the chronic kidney disease epidemiology collaboration creatinine equation[10]. For the vascular calcification evaluation, patients who received antiplatelet and statin medications for more than 2 years were enrolled[11].

Vascular calcification scoring

Vascular calcification scores were calculated from plain radiographic films of the pelvis and both hands by using Adragao's method[2]. The pelvis images were separated into four sections by two imaginary lines, horizontal and vertical, and were used to assess the calcification of the iliac and femoral arteries. Similarly, the hand images were also separated into four sections and used to assess the calcification of the radial and digital arteries. The existence and nonexistence of vascular calcification in each section were scored as 1 and 0, respectively. The total vascular calcification score was the sum of vascular calcification scores from all the sections, ranging from 0 to 8.

Outcomes

The direct relationship between vascular calcification and the antiplatelet agents, evaluation of vascular calcification scores according to each risk factor, and whether an antiplatelet agent and a statin were used were investigated.

Statistical analyses

The data of the continuous variables with normal distributions are presented as the mean ± standard

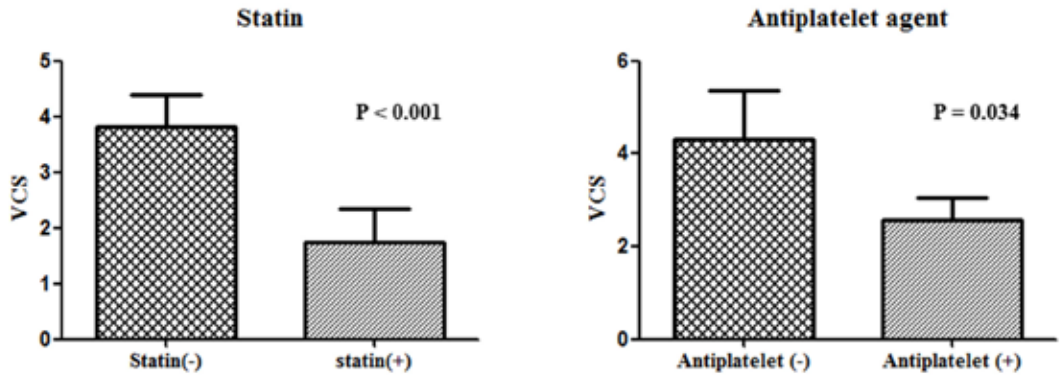

Fig 1: Vascular calcification scores according to use of antiplatelet agents or statins

deviation, while those without normal distributions are presented as the median with ranges as appropriate for the type of variable. The Student's *t*-test, Mann-Whitney U test, one-way analysis of variance, or Kruskal-Wallis test were used, as appropriate, to determine the statistical differences between the continuous variables. Categorical variables are presented as percentages. The Pearson's chi-square or Fisher's exact test was used to determine the differences in categorical variables. Absolute mortality rates were calculated per 100 person/years of follow-up while a simple linear regression analysis was used for variables with relevant vascular calcification levels. Then, all the significant variables assessed using simple linear regression were entered into a backward stepwise procedure. A p-value <0.05 was considered statistically significant, and all statistical analyses were performed using the statistical package for the social sciences 16.0 software (Chicago, IL, USA).

RESULTS

The clinical characteristics of the patients are shown in Table 1. Of the 156 patients studied, 50.7% had diabetes, and their mean age was 55.2 ± 9.7 years. The mean baseline vascular calcification score was 4.47 ± 2.32, and it ranged from 0 to 8. The patients were classified into three groups based on their use of medication, and the comparisons of clinical parameters between the three groups are presented. No differences were observed in age, the presence of diabetes and hypertension, intact parathyroid hormone levels, calcium, fibroblast growth factor 23, vitamin D level, and phosphate among the groups. However, the group without medication showed higher phosphate levels than the other groups did (p = 0.038).The antiplatelet plus statin group showed higher alkaline phosphatase levels than the other groups.

The vascular calcification score of patients who used an antiplatelet agent for more than 2 years (n = 61) was 3.74 ± 2.72, whereas that of patients who had never used an antiplatelet agent (n = 32) was 4.59 ± 2.61 (p = 0.034, Figure 1). A similar outcome was observed in patients administered statins. The vascular calcification score for statin users was lower (n = 72; 2.3 ± 2.54) compared to that of the non-drug users (n = 32, p <0.001). Patients who used both statins and antiplatelet agents showed a more significant improvement in the vascular calcification (2.10 ± 2.57) than those who used only antiplatelet agents (3.74 ± 2.72) or neither agent (4.59 ± 2.61) did (Figure 2).

In the multivariate analysis, the factors that affected vascular calcification independently were age, diabetes, and use of both antiplatelet agents and statins. The highest vascular calcification was observed in patients with diabetes who did not use either of the drugs (6.3 ± 2.9). Furthermore, the degree of vascular calcification was similar between the patients without diabetes who did not use either of the drugs and patients with diabetes who used both medications concurrently (Figure 3).

The logistic regression analysis was performed to compare patients using antiplatelet agents and statins concurrently with those who did not use any

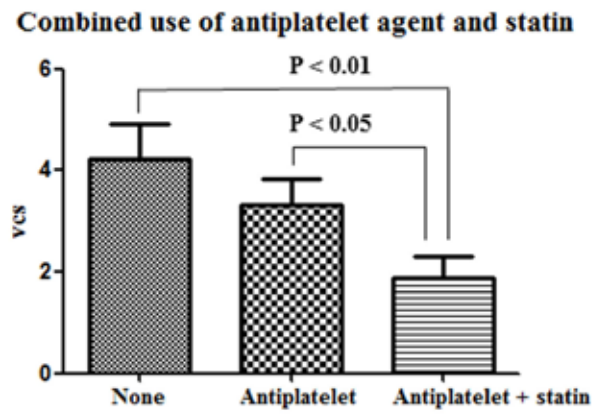

Fig 2: Vascular calcification scores according to combined use of antiplatelet agents and statins

Table 2: Univariate and multivariate logistic regression analyses of clinical outcomes between two groups

Group	Crude model			Model 1			Model 2		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
None		1 (reference)			1 (reference)			1 (reference)	
Antiplatelet agent (AP)	0.899	0.578 – 1.397	0.635	0.847	0.537 – 1.337	0.477	0.852	0.533 – 1.363	0.505
AP + Statin	0.691	0.655 – 0.730	0.009	0.666	0.625 – 0.709	0.016	0.650	0.598 – 0.707	0.021

AP: antiplatelet agent; OR: odds ratio; CI: confidence interval
Model 1: multivariate model including age and sex.
Model 2: multivariate model including model 1 +, diabetes mellitus, hypertension (HTN), serum albumin level, serum Ca_2+ level, intact parathyroid hormone (iPTH), serum phosphate level, total cholesterol, and warfarin.

of them (Table 2). Notably, calcification in the group using both antiplatelet agents and statins decreased to 0.691 times its original value. In addition, even after adjusting numerous factors, calcification was found to have decreased to 0.65 times its original value. However, the results of the antiplatelet agent group were not significant.

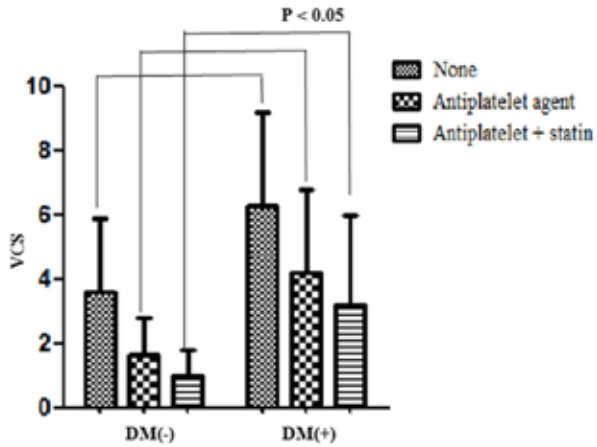

Fig 3: Vascular calcification scores according to presence of diabetes and use of antiplatelet agents and statins

DISCUSSION

This study demonstrated the effects of traditional risk factors as well as antiplatelet agent and statin use for more than 2 years in patients with end-stage renal disease. The results revealed that the vascular calcification scores of patients with diabetes were higher than those of patients without diabetes were. Antiplatelet agents were used more often in patients with diabetes than they were in those without diabetes. Patients who used both statins and antiplatelet agents showed significantly lower vascular calcification scores than those who used only antiplatelet agents.

Various imaging modalities are available for screening for the presence of vascular calcification[12], and the analysis of plain radiological films was used in this study using Adragao's method[2]. Adragao's method, which scores the degree of vascular calcification in the hands and pelvic region, provides relatively accurate prognostic information at a lower cost than that of other radiologic diagnostic tools. For example, a vascular calcification score of the hands and pelvic region using Adragao's method was an independent predictor of cardiovascular mortality and fatal or non-fatal cardiovascular events[2,13].

Plain radiological films are used in evaluating calcification in various European countries including Portugal, indicating that it is used in countries with diverse cultural environments, medical insurance, and economic differences under similar conditions to those used in our study[14,15]. Vascular calcification score determination using this method provides a simple, non-invasive, and inexpensive tool for evaluating the cardiovascular risks associated with vascular calcification in patients with end-stage renal disease. Therefore, this method was selected for our study.

The direct relationship between vascular calcification and the use of antiplatelet agents has not been well established. Cilostazol is a phosphodiesterase III inhibitor that suppresses platelet aggregation and acts as an arterial vasodilator. It is currently used for the treatment of peripheral arterial occlusive disease, which is highly related to vascular calcification clinically[16]. Among the different antiplatelet agents, cilostazol and pentoxifylline improved vascular calcification more effectively than aspirin did in patients with end-stage renal disease. In addition, the concurrent use of antiplatelet agents and statins showed better outcomes than the use of antiplatelet agents alone.

Statins, which are potent lipid-lowering agents, have been shown to exhibit antioxidant effects, improve endothelial function in uremic condition, and inhibit the calcification of human vascular smooth muscle cells induced by inflammatory mediators[17]. The findings of this study will enhance the understanding of the mechanism underlying the statin-induced prevention of the progression of calcification in inflammatory vascular diseases such as atherosclerosis[18]. However, clinical side effects associated with the use of statins for vascular calcification and cardiovascular diseases have been observed in patients with end-stage renal

disease. No current prospective clinical studies have shown the direct effects of statins on the progression of vascular calcification. However, several studies on the effects of statins have shown conflicting results for the relationship between statins, vascular calcification, and cardiovascular diseases. For example, Nigwekar *et al* recently reported that statin use is negatively associated with the odds ratio (0.20, 95% confidence interval: 0.05 - 0.88) of calcific uremic arteriolopathy, which is characterized by vascular calcification, thrombosis, and intense inflammation in patients undergoing hemodialysis[19]. Callister *et al* demonstrated that statins reduced the volume of calcified plaque in coronary arteries[20]. In contrast, Saremi *et al* reported that the frequent use of statins is associated with accelerated coronary artery calcification in patients with type II diabetes with advanced atherosclerosis[21]. Similarly, Terry *et al* showed that simvastatin treatment did not reduce the progression of coronary artery calcium or abdominal aortic calcium compared to the placebo[22].

The majority of patients with end-stage renal disease receiving statin treatment did not show any improvement. However, the post hoc subgroup analyses of data from the SHARP trial showed a significant reduction in the major adverse events and mortality due to cardiovascular diseases in the following three groups: patients with significant hypercholesterolemia, those with mild to moderate chronic kidney disease, and those undergoing peritoneal dialysis[23]. There are several reasons why patients in these groups showed a relatively better response to statin therapy than those undergoing hemodialysis did.

The primary causes of cardiovascular disease in patients with end-stage renal disease are chronic inflammation, oxidative stress[24], high-density lipoprotein deficiency[25], and the presence of small, dense, low-density lipoproteins[26]. The inhibition of cholesterol synthesis by statins cannot correct these disorders. Disorders of calcium and phosphate metabolism, uremic toxins, anemia, and diabetes, which are unrelated to cholesterol metabolism, are unresponsive to statin treatment[27]. Patients undergoing peritoneal dialysis have a greater risk of developing metabolic syndromes than those undergoing hemodialysis do because glucose metabolism is altered in patients with chronic kidney disease undergoing peritoneal dialysis and manifests as diabetes and dyslipidemia[28]. Thus, these differences may explain the divergent responses of patients with hypercholesterolemia, mild to moderate chronic kidney disease, and peritoneal dialysis to statins.

Treatment with statins did not significantly decrease cardiovascular events or mortality in randomized controlled trials conducted in a population of patients on hemodialysis[29,30]. The anti-vascular calcification properties of statins are yet to be confirmed in this population. However, various mechanisms of statins may be important in preventing vascular calcification, especially in patients with end-stage renal disease.

This study has some limitations such as the small number of patients in each group, which may have influenced the statistical significance of the results. In addition, several important data such as the low-density lipoprotein and high-density lipoprotein cholesterol levels, nutritional status, and inflammatory markers were not obtained. This was an observational and retrospective study, and therefore, the results should be carefully interpreted. Furthermore, additional prospective and randomized studies are necessary to confirm the positive effect of antiplatelet agents and statins in patients with end-stage renal disease.

CONCLUSIONS

This study showed that the use of antiplatelet agents and statins is negatively associated with vascular calcification development. The well-known traditional risk factors of vascular calcification such as age and diabetes are positively associated with vascular calcification in patients with end-stage renal disease. The confirmation of this relationship using large, prospective studies in the future will offer potential strategies for the prevention and treatment of vascular calcification, which is highly associated with major vascular complications and mortality. Furthermore, this study revealed significant treatment implications for patients with end-stage renal disease using the investigated substances.

ACKNOWLEDGMENT

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

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Ethical approval: All procedures involving human participants were performed in accordance with the ethical standards of the Institutional and National Research Committee and the 1964 Helsinki declaration as well as its subsequent amendments or comparable ethical standards. This study was approved by the medical ethics committees of the participating hospitals.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Case Report

Small cell carcinoma of the urinary bladder: Radical cystectomy opportunity should not be missed

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ABSTRACT

Small cell carcinoma of the bladder (SCCB) is rare, but often has distant metastasis when diagnosed. SCCB has poorer prognosis than urothelial bladder carcinoma. The optimal treatment is not well established and usually multimodal approaches are needed. Presented here are two cases of SCCB; the first case had mixed histology and the second case had pure SCCB.

KEYWORDS: bladder cancer, neuroendocrine tumor, small cell carcinoma

INTRODUCTION

Neuroendocrine tumors (NET) can develop epithelium of all kinds, but more frequently occur in the epithelium rich in enterochromaffin cells. NET of the urinary bladder consists of carcinoid tumors, large cell carcinomas and small cell carcinomas. Small cell carcinoma of the bladder (SCCB) is the most frequent of extrapulmonary SCC[1], but accounting for only 0.35-0.70% of all bladder tumors[2]. SCCB is histologically similar with extrapulmonary SCC and has a poorer prognosis than urothelial bladder carcinomas[3,4]. Median survival of all those with SCCB is 20 - 23 months[5]. SCCB is mostly diagnosed in advanced stages and multimodal therapy is recommended due to its aggressive nature[6,7].

In this article, we report on two cases of the SCCB.

CASE REPORT 1

A 68-year-old man presented for painless gross hematuria. Computed tomography (CT) showed a 7 x 3 cm polypoid mass in the right lateral bladder wall (Figure 1). CT scan showed liver metastasis. Transurethral resection bladder tumor (TUR-BT) was performed. A diagnosis of small cell neuroendocrine carcinoma invading the mucosa and muscularis propria (pT2G3) was made (Figure 2,3). There was high-grade urothelial carcinoma concomitant with primary bladder small cell neuroendocrine carcinoma. The patient refused further radical cystectomy. The patient received six cycles of platinum-based chemotherapy. The chemotherapy consisted of intravenous etoposide 100 mg/m^2 dose and intravenous cisplatin 70-100 mg/m^2, repeated every 3 weeks. TUR-BT was performed 2 more times (1st month and 1st year respectively after the first treatment.) He died 26 months after the initial diagnosis.

CASE REPORT 2

Our patient was a 57-year-old man with a major complaint of macroscopic hematuria. CT scan of the abdomen and pelvis showed a well-defined, polypoid mass (4 x 3 cm) involving the wall of the urinary bladder (Figure 4). He underwent transurethral resection of this bladder mass for pathologic diagnosis. A diagnosis of pure small cell carcinoma invading the mucosa and muscularis propria (pT2) was made (Figure 5, 6A-

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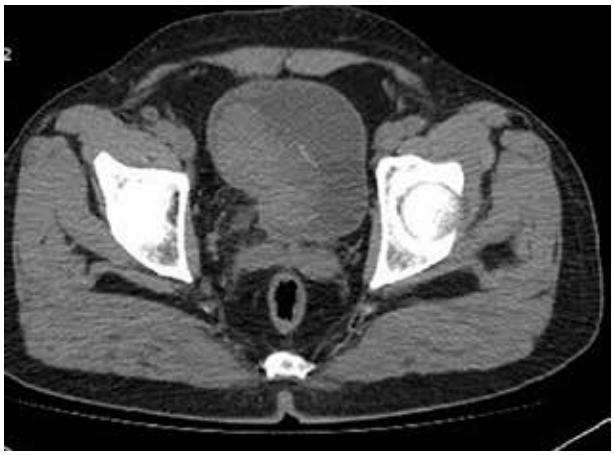


Fig 1. Computed tomography showed 7 x 3 cm polypoid mass at the right bladder wall.

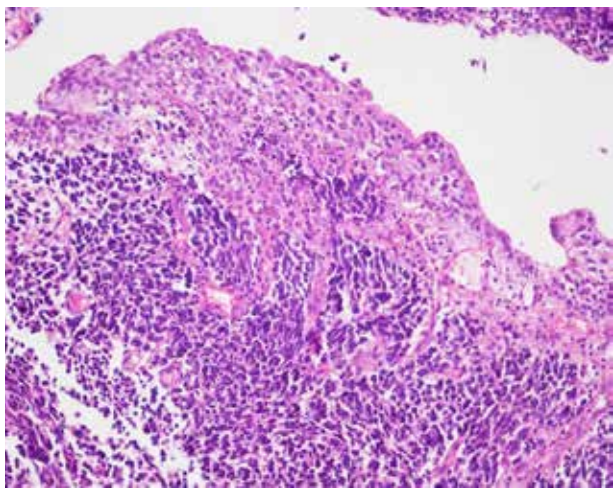


Fig 2. Proliferation comprised small to intermediate sized cells with minimal cytoplasm, hyperchromatic nuclei, indistinct nucleoli infiltrating the subepithelial region (HEX200)

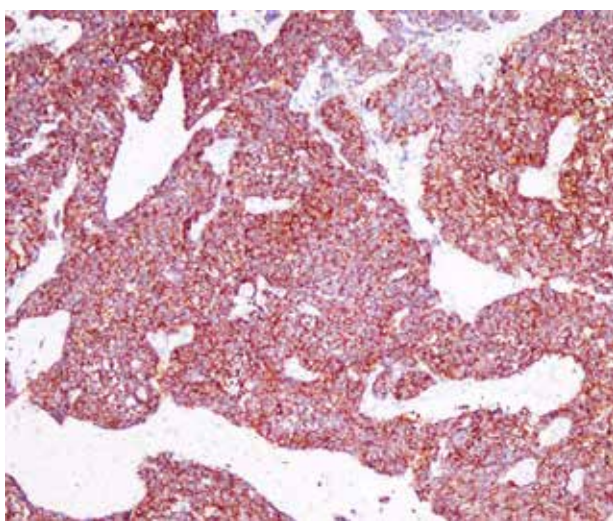


Fig 3. Diffuse cytoplasmic positivity in tumor cells with Synaptoptophisin immunohistochemically (X100)

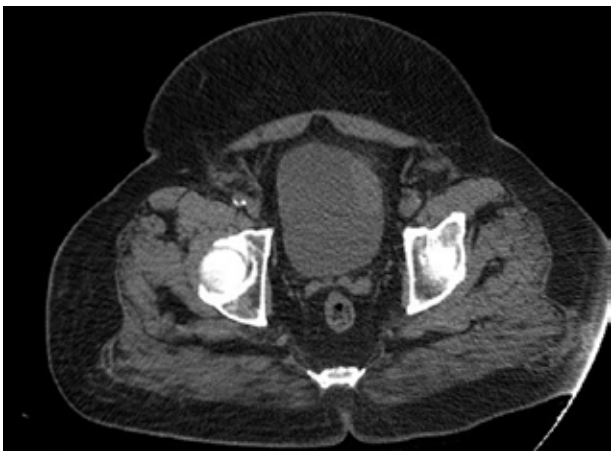


Fig 4. Computed tomography showed 4 x 3 cm polypoid mass at the left bladder wall.

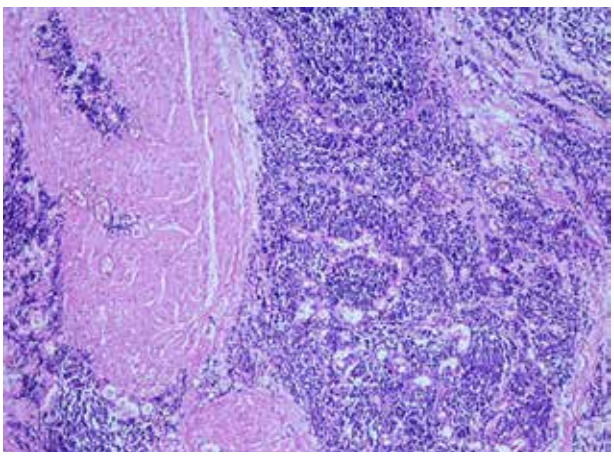
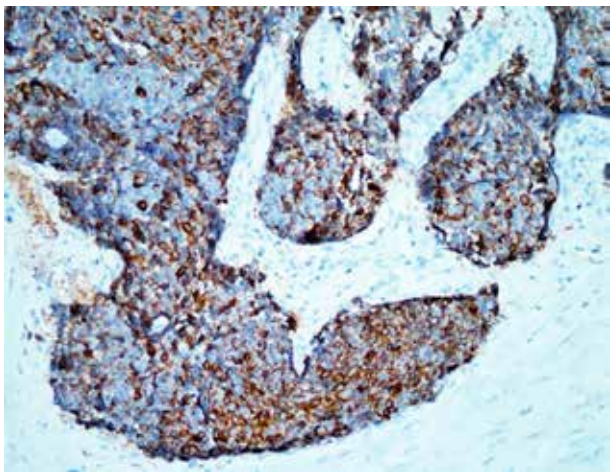


Fig 5. Hematoxylin-eosin staining, small to intermediate sized cells with hyperchromatic nuclei infiltrating the muscle (HEX100)

B). The patient received six cycles of platinum-based chemotherapy. TUR-BT was performed 1st month after the first treatment. He died twelve months after surgery.

DISCUSSION

Small cell bladder cancer is rare, but often has distant metastasis when diagnosed[2,7]. Primary small cell carcinoma of the bladder was firstly described by Cramer *et al* in 1981[8]. SCCB has characteristics similar to urothelial carcinoma of the bladder in terms of epidemiology, risk factor and symptom[3,9]. SCCB is by a majority mixed histology and is often accompanied by urothelial carcinoma[7]. Our present study, in the first case had mixed histology and in the second case had pure SCCB. Pure SCCB has an overall survival 2 to 3 times shorter compared to mixed type, as in our case[10,11].



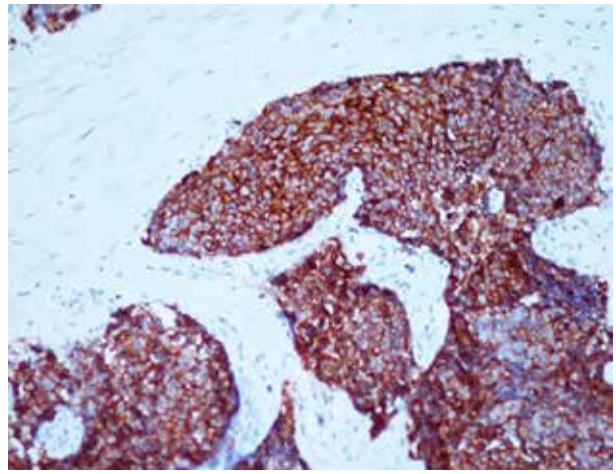


Fig 6A-6B. Diffuse cytoplasmic immunoreactivity in tumor cells with Chromogranin and Synaptoptophisin immunohistochemically (X100)

The initial diagnosis of SCCB is usually made by cystoscopy and transurethral biopsy. The most important point in diagnosis is tumor pathology. It is important to use immunohistochemical staining to confirm the diagnosis. Immunohistochemical neuroendocrine differentiation should be demonstrated to exclude other possible tumors[4]. When SCCB is diagnosed, computerized tomography, magnetic resonance, chest radiograph and bone scan is recommended for staging and treatment planning[3,4,6].

Because SCCB is rare, it is difficult to determine optimal treatment algorithms. Principal surgery alternatives are radical cystectomy and TUR-BT. TUR-BT alone is usually not curative and is associated with survival rates of 3 to 6 months[12]. Radical cystectomy in patients suitable for surgery is the gold standard treatment[3]. Mackey *et al* published that the platinum-based chemotherapy is the only statistical significant factor for improved survival in SCCB patients[13]. Lynch *et al* reported median overall survival of up to 159.5 months in patients with neoadjuvant chemotherapy and cystectomy[14]. Therefore, radical cystectomy and neoadjuvant chemotherapy were offered to both patients. Both patients refused radical cystectomy.

CONCLUSION

SCCB is an uncommon and aggressive disease. Immunohistochemical stains provide an important confirmation for diagnosis. Best treatment for this tumor cannot be established for certain. Radical cystectomy and neoadjuvant chemotherapy are the primary treatments in patients suitable with surgery. Unfortunately, both of our patients refused radical cystectomy.

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Case Report

Cytomegalovirus papillitis and cholangiopathy in renal transplant patients

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ABSTRACT

Clinically significant cytomegalovirus cholangitis is frequently observed in patients with AIDS, but is less common in other groups of immunocompromised patients. The present article reports a case involving a 65-year-old man, seven years post-renal transplant, who presented with abdominal pain, weight loss and loss of appetite with elevated levels of alkaline phosphatase and gamma glutamyl transferase. Endoscopic retrograde cholangiopancreatography identified a papillary mass and a distal common bile duct stricture. Histopathological analysis of papillary mass revealed a cytomegalovirus infection. The patient improved clinically following treatment with valganciclovir.

KEYWORDS: ampulla of vater mass, cholangiopathy, cytomegalovirus, papillitis, renal transplant

INTRODUCTION

Human cytomegalovirus (CMV) is an enveloped, double-stranded DNA virus belonging to the human herpes virus family. CMV remains the single most important pathogen affecting the outcome of solid organ transplantation (SOT)[1]. CMV infection has direct effects on morbidity and mortality[2]. These include acute and chronic allograft rejection, increased susceptibility to the development of other opportunistic infections, allograft dysfunction or failure, and death[1,3]. CMV has also been associated with the development of new-onset diabetes mellitus in SOT recipients[4].

CASE REPORT

The patient was a 65-year-old man with diabetes mellitus who had undergone renal transplantation seven years previously and was on treatment with mycophenolate mofitel, prednisolone and tacrolimus. The patient complained of epigastric pain associated with heartburn, nausea, vomiting, diarrhea, anorexia and weight loss (10 kg) over a three-month period. A scar from the previous transplant and mild tenderness of the upper abdomen were noted on examination. Investigation revealed a white blood cell count of 2.3×109 cells/L. Total bilirubin was normal at 7 μmol/L. Alkaline phosphatase (ALK, 1108 U/L) and gamma glutamyl transferase (GGT, 574 U/L) levels were elevated. Creatinine was 113 μmol/L and albumin was 28 g/L and were within normal range, and hepatitis B surface antigen was positive; the workup, including tumor markers, was otherwise normal. A polymerase chain reaction (PCR) test for CMV (blood sample) was negative. A computed tomography scan showed dilated intra- and extrahepatic biliary radicals with a soft tissue mass at the ampulla of Vater; no lymphadenopathy was observed. Endoscopic retrograde cholangiopancreatography showed a prominent ampulla of Vater with an inflamed orifice (Figure 1) and a dilated common bile duct with a

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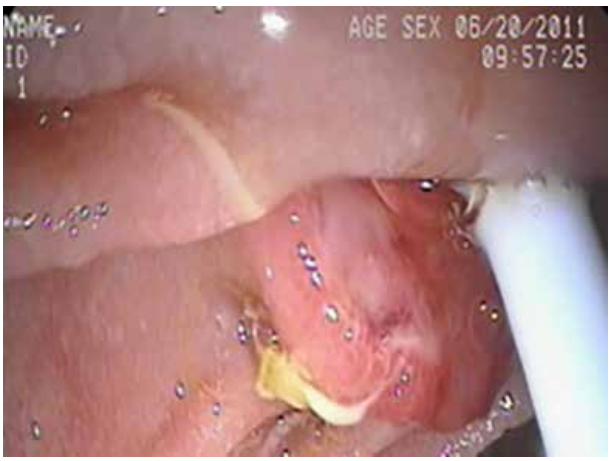


Fig 1: Endoscopic retrograde cholangiopancreatography showing a prominent ampulla of Vater with an inflamed orifice

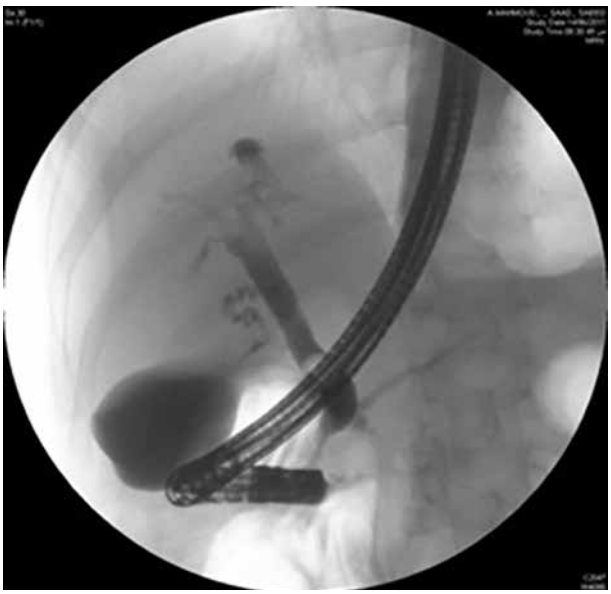
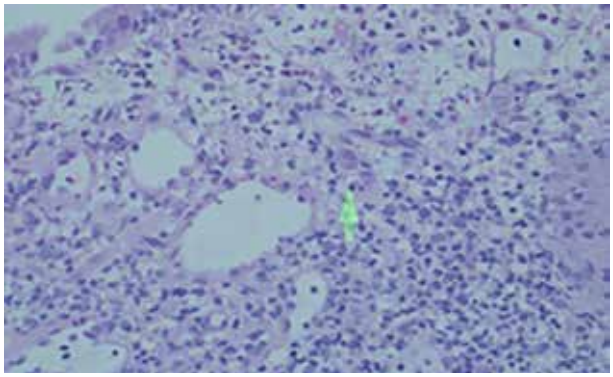


Fig 2: Endoscopic retrograde cholangiopancreatography showing a dilated common bile duct with a short distal common bile duct stricture

short distal common bile duct stricture (Figure 2). Histopathological analysis revealed mild chronic inflammation with an inclusion body as evidence of CMV infection (Figure 3,4). Following nephrological review, immunosuppressive medication was decreased and treatment with valganciclovir (900 mg orally twice daily) was initiated. The patient improved clinically and was discharged.

DISCUSSION

CMV infections are among the most common infections that occur following SOT. The symptoms of a CMV infection range from asymptomatic viremia to CMV syndrome to tissue-invasive disease. The most common manifestation of tissue-invasive CMV disease



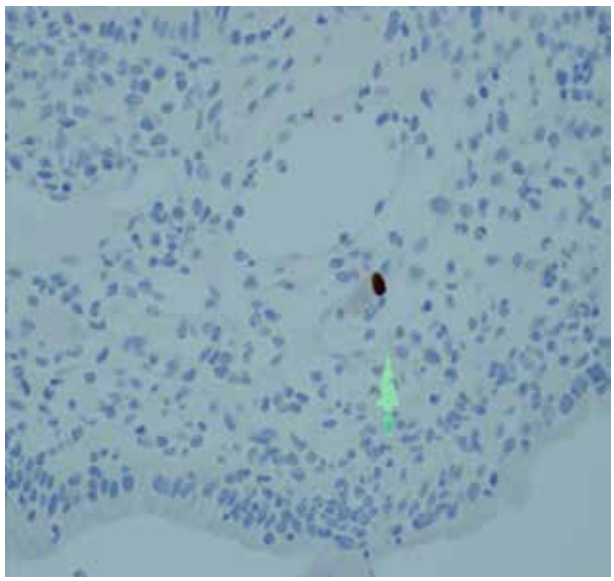


Fig 3, 4: Histopathological analysis revealing mild chronic inflammation with an inclusion body as evidence of CMV infection

is CMV infection of the gastrointestinal tract, which is a significant cause of morbidity and mortality in the SOT recipient[5]. The most common clinical syndromes are esophagitis, colitis and hepatitis; however, infection can occur anywhere in the gastrointestinal tract[6]. CMV cholangitis is a rare manifestation of CMV infection that has been reported mostly in patients with AIDS[7]. There are a small number of cases reported in the literature of CMV cholangitis in renal transplant patients[8-10] and patients on corticosteroid therapy[11]. Transplant recipients may develop CMV disease via primary infection (transmission through the donor allograft to a CMV seronegative recipient), reactivation infection (latent endogenous CMV reactivation in the recipient after transplantation) or reinfection (donor-transmitted infection superimposed on endogenous viral reactivation)[1]. In the gastrointestinal tract, CMV leads to inflammation and mucosal ulcerations and may affect the vascular endothelium.

The main factor determining the occurrence of CMV infection after SOT is the viral status of

both the donor and recipient. Infection will occur in approximately 60 - 80% of seronegative patients who receive a kidney from a positive donor, and 60 - 80% will develop CMV disease[12]. Additional risk factors for CMV in transplant recipients include more potent immunosuppression, acute rejection, advanced age and poor kidney transplant graft function[13]. In the present case, the CMV status of both the patient and his donor were unknown.

All of the previously described patients underwent endoscopic retrograde cholangiopancreatography with multiple biopsies to confirm the diagnosis, as in the present case, with the exception of one diagnosis that was based on magnetic resonance cholangiopancreatography and a positive CMV PCR test[9], and another case in which diagnosis was based on histopathology of a specimen obtained from a Whipple procedure. For tissue-invasive gastrointestinal tract disease, viral culture and histopathology of tissue specimens provide the highest diagnostic yield. Identification of viral inclusion bodies and viral antigens by immunohistochemistry and/or in situ DNA hybridization in biopsy specimens maximizes sensitivity and may improve the predictive value of a positive culture[14]. Noninvasive molecular diagnostic testing for CMV infection of the blood is also available, including the CMV PCR and CMV pp65 antigenemia assays. However, a number of studies have shown these tests to have variable sensitivity and specificity for the diagnosis of gastrointestinal CMV disease in SOT recipients, and a negative test should not be used to eliminate CMV as the cause of gastrointestinal symptoms in the SOT population[15]. Indeed, "CMV gastrointestinal disease" is defined by identification of a combination of clinical symptoms from the upper or lower gastrointestinal tract, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV infection in a gastrointestinal tract biopsy specimen[16]. Although, the PCR test for CMV in our patient was negative, the tissue biopsy was positive for CMV inclusion body. CMV cholangitis may present with abdominal pain, fever, loss of weight and appetite, hemobilia or obstructive jaundice with elevated ALK and GGT levels[8-11]. Our patient was unique in his presentation with abdominal pain, loss of weight and appetite, and elevated ALK and GGT with normal bilirubin. There have also been reported cases of pancreatitis due to concomitant papillitis or immunosuppressive medication, and choledocholithiasis due to cyclosporine treatment or CMV pancreatitis. Eight cases of CMV cholecystitis[17] and CMV hepatitis, observed more frequently in liver transplant patients, have also been reported[18].

Intravenous administration of ganciclovir remains the route of choice when the patient is seriously unwell, or when oral drug absorption is uncertain or poorly tolerated. However, oral valganciclovir was demonstrated to be of equal efficacy to intravenous ganciclovir for treating CMV disease in a mixed group of SOT recipients, three-quarters of whom were renal transplant recipients[19,20]. In recent years, substantial clinical experience has reinforced that this is an appropriate treatment strategy, which has the advantage of being able to offer outpatient management for some patients. In general, the duration of treatment for gastrointestinal CMV disease should be patient specific and guided by virological and clinical improvement. If CMV viremia is present with gastrointestinal infection, the viremia should be cleared before discontinuation of therapy. Patients should demonstrate at least two consecutive negative CMV PCR or antigenemia assays one week apart to ensure viral clearance[14]. Renal function and white blood cell count should be monitored frequently during therapy. Renal adjustment of antiviral therapy may be necessary; however, dose reduction for side effects such as leukopenia should be avoided as much as possible due to the potential for the development of resistance or breakthrough infection. Consideration should be made for a reduction in immunosuppressive therapy to the lowest possible safe dose, particularly in patients with severe CMV disease, nonresponse to therapy, high viral loads or leucopenia[14]. Primary gastrointestinal CMV disease has a relatively high rate of relapse following treatment. A recent study by Eid *et al*[21] involving SOT recipients with CMV donor positive/recipient negative serostatus and CMV gastrointestinal disease demonstrated a relapse rate of 27%, which was significantly associated with extensive upper and lower gastrointestinal tract disease. Interestingly, CMV seroconversion, viral load, treatment duration, maintenance therapy and endoscopic findings at the end of therapy were not associated with relapse. Given these findings, routine end-of-treatment upper endoscopy or colonoscopy should not regularly be performed before the discontinuation of antiviral therapy[1]. Secondary prophylaxis with valganciclovir (900 mg once daily) for one to three months after the completion of treatment may be considered in patients at high risk for recurrence. Periodic CMV PCR or antigenemia assays should be performed during the secondary prophylaxis to screen for breakthrough infection, although the appropriate monitoring interval is not known[14].

CONCLUSION

In conclusion, cytomegalovirus cholangitis should be considered in the differential diagnoses of ampullary mass and bile duct stricture in solid organ

transplant patients, especially in patients in whom the lesion is limited to the ampulla and common bile duct.

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Case Report

Nasopharyngeal branchial cleft cyst with a presenting symptom of bilateral otitis media with effusion

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ABSTRACT

Branchial cleft cysts are uncommon malformations that result from various degrees of incomplete closure of branchial apparatus during embryologic development. Clinical presentation of these lesions can be cystic and/or fistula formation on the head and neck region. Nasopharyngeal region is one of the unexpected anatomical sites of these malformations, and definite diagnosis was only disclosed by histopathologic examination of the specimen. The common presenting symptoms are one sided or bilateral nasal obstruction and one-sided otitis media with effusion. In this paper, we presented a nasopharyngeal branchial cleft cyst with a presenting symptom of bilateral otitis media with effusion. According to the best of our knowledge, this is the first case of bilateral otitis media with effusion due to a nasopharyngeal branchial cleft cyst, which is a very rare malformation.

KEYWORDS: branchial cleft cyst, microdebrider, nasopharyngeal cyst, otitis media with effusion

INTRODUCTION

Otitis media with effusion (OME) is a disease of the middle ear that is an inflammatory response due to various factors without an infection and is presented with sterile fluid partially or fully filled middle ear and mastoid space with complaints of hearing loss, aural fullness, and tinnitus in varying degree. The factors that mostly lead to OME are bacterial and/or viral acute upper airway and ear infections and allergy. Nevertheless, lesions that disturb eustachian tube function by obstructing also cause OME. Nasopharyngeal cysts and malignancies could cause OME unilaterally or bilaterally, as in our case.

Branchial cyst as a congenital malformation mostly detected at the neck region resulted from incomplete closure of branchial apparatus in embryogenic period of life, classically anterior to sternocleidomastoid (SCM) muscle. Although it is a congenital malformation, the diagnosis of the disease is mostly in the second and fourth decades of life[1,2].

The four kinds of anomalies are presently labeled as first, second, third and fourth branchial cleft cysts[3]. The most common type is the second type[4]. Although they are generally located in the lateral neck, anterior to the SCM; these cysts may present anywhere along the course of the entire embryologic tract, proceeding from the anterior part and deep to the SCM muscle into tonsillar fossa[1,5].

Although rare, this anomaly can be clinically present in anatomic locations other than the usual. These unusual presentations cause confusion in diagnosis of the lesion. In this study, we present a nasopharyngeal branchial cleft cyst with bilateral otitis media with effusion as presenting symptom. According to the best of our knowledge, this is the first nasopharyngeal branchial cleft cyst which presented with bilateral OME as a first sign.

CASE REPORT

A 65-year-old female patient applied to our clinic with complaints of hearing loss, tinnitus and a slight nasal obstruction for two months. Otomicroscopic examination revealed bilateral dullness and retraction of ear drums. In pure tone audiometric examination,

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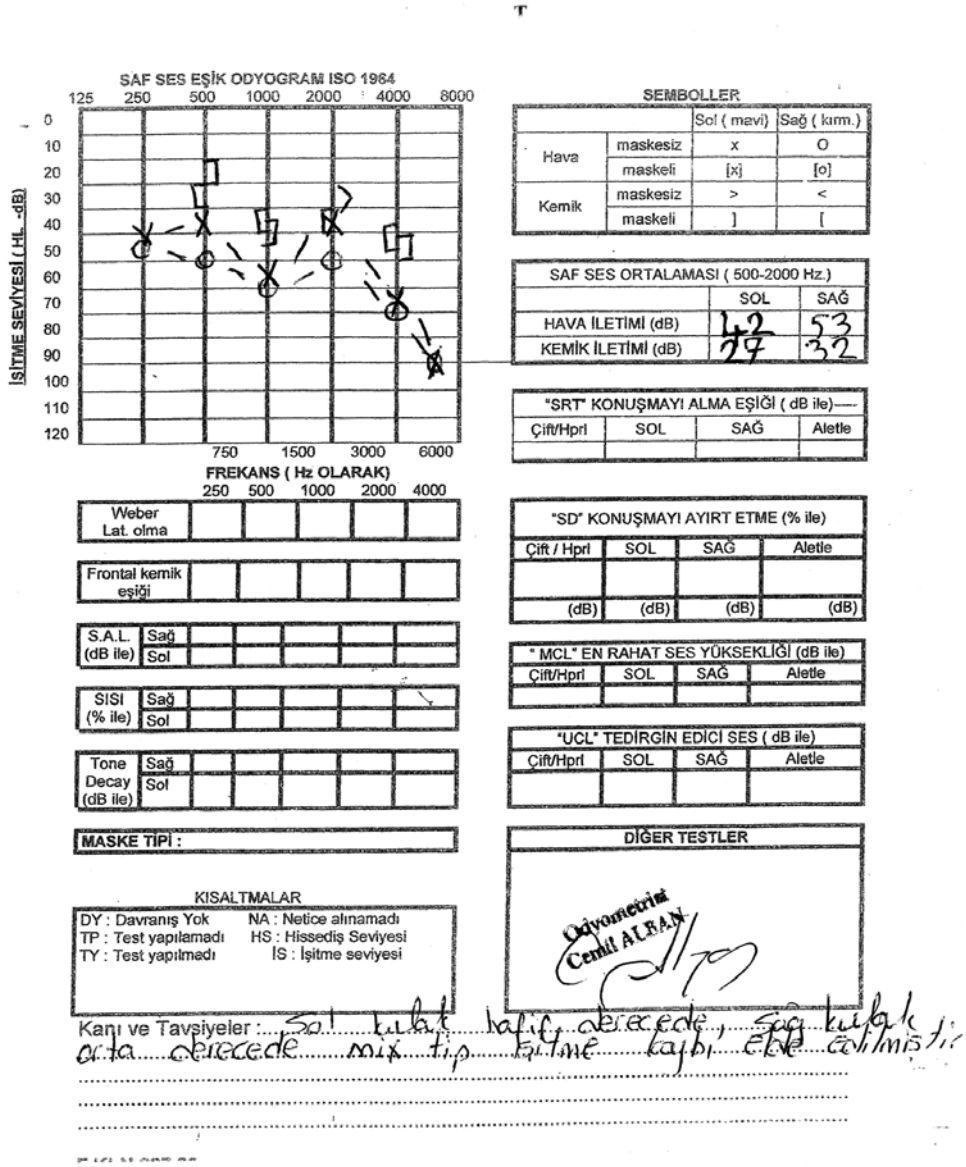

Fig 1. Preoperative audiometric examination of the patient.

there was a mild to moderate mixed type hearing loss in both ears (Figure 1). Air-conduction and bone-conduction hearing threshold averages of 0,5 KHz, 1 KHz and 2 KHz for the right ear and the left ear were 53/32 dB and 42/27 dB, respectively and air-bone gaps were 21 dB for the right ear and 15 dB for the left ear. Tympanometry was bilateral type B (Figure 2). A smooth-cystic lesion that nearly totally occluded both choana and the nasopharynx was detected on flexible fiberoptic nasopharyngoscopy. A polypoid, cystic soft tissue projected from the posterior nasopharynx into the lumen with obstruction of pharyngeal recesses and Eustachian tube openings on CT. Additionally, MRI showed a 2 cm diameter cystic homogenous lesion with minimal contrast enhancement in the same region (Figures 3, 4). An intranasal endoscopic surgery with microdebrider for cyst excision and bilateral myringotomy for glue ears were planned under general anesthesia. Patient declined grommet ventilation tube insertion.

A myringotomy and aspiration of glue in both of the ears was performed under general anesthesia. An incision to the cystic lesion under the view of a rigid 0º telescope through the nasal cavity was made. A gelous mucoid discharge was aspirated, and all the cystic space cleaned out. Multiple punch biopsies including full thickness of the cystic wall were taken for histopathologic examination. The entire remaining lesion cleaned out with the assistance of a microdebrider. Nasal obstruction improved dramatically just after the operation. Approximately 4 months after the surgery, hearing loss in both ears improved. Tympanometry

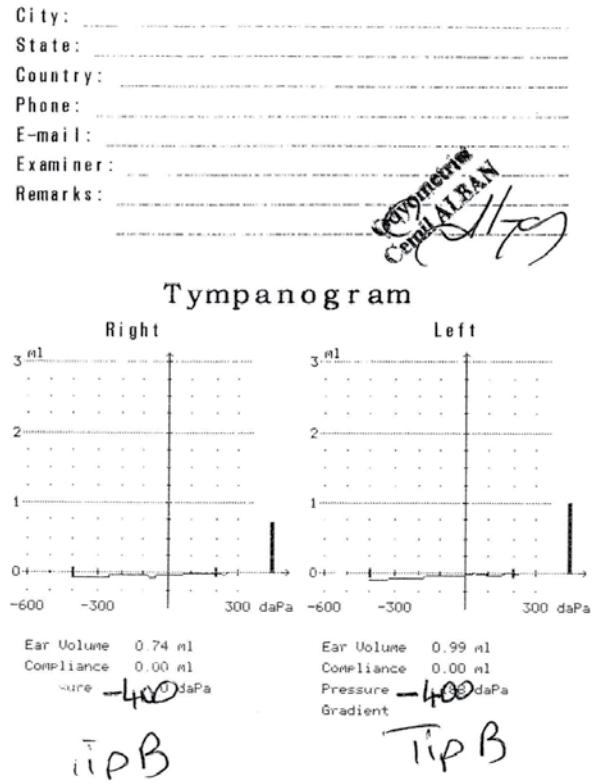

Fig 2. Preoperative tympanometric examination of the patient.

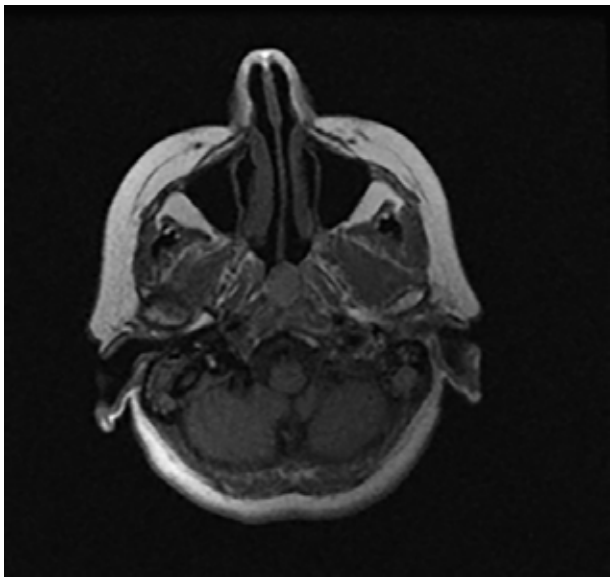

Fig 3. Contrast-enhanced T1 & T2 weighted magnetic resonance imaging (MRI) of nasopharyngeal branchial cyst is seen. The cyst is hypo-dense in T1 weighted images

was type A for the right ear and type C for the left ear. According to histopathologic examination of the specimen, the lesion was diagnosed as a branchial cleft cyst (Figure 5). The patient had not shown any recurrence in the follow-up period of more than 3 years, and her

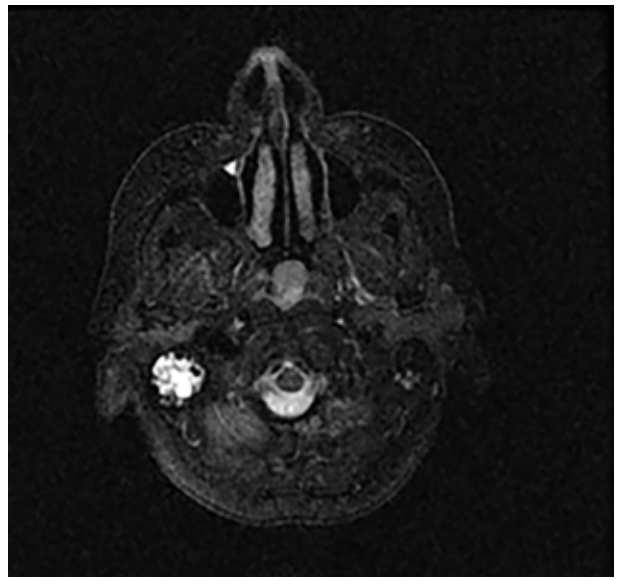

Fig 4. Contrast-enhanced T1 & T2 weighted magnetic resonance imaging (MRI) of nasopharyngeal branchial cyst is seen. The cyst is hyper-intense in T2 weighted images

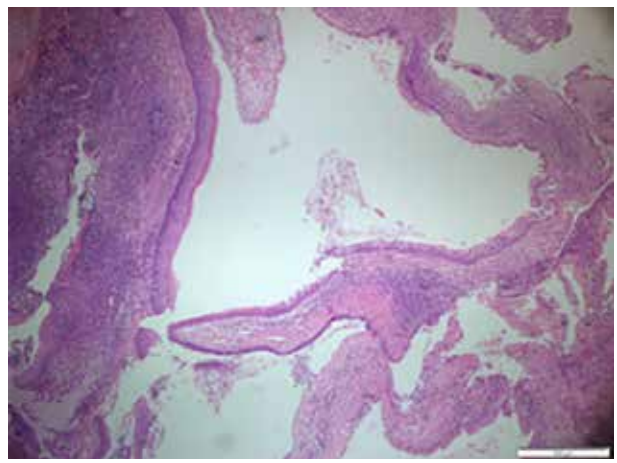

Fig 5. It is seen a cystic mass that is lined with stratified squamous epithelium with a neighborhood of a ciliated stratified epithelium. There is stroma that contains reactive lymphoid aggregates under the epithelium (H&E-40X).

otitis media with effusion was completely recovered in both ears, with no recurrence in this follow-up period.

DISCUSSION

Clinical presentation of a branchial anomaly can be as a cyst, fistula and sinus formation with a varying degree. The most common form is second branchial cleft cyst, which consists of nearly 95% of all malformations[6]. The other types are first branchial (8 - 10%), third branchial (2 - 8%) and fourth branchial (1 - 2%) malformations, respectively. Nevertheless, this malformation can be clinically detected very rarely in anatomic locations other than the usual; such as the nasopharyngeal location in our case.

Nasopharyngeal branchial anomalies exist in the literature only as case reports due to rarity of the disease[3,7-9]. However, very rarely, oropharyngeal location of this malformation is also presented as a few cases[2,5]. Differential diagnosis for nasopharyngeal cysts are Tornwald's cyst, branchial cyst, retention cyst, mucocele, angiofibroma[8].

This malformation is seen on MRI T2 weighted imaging as a hyper-intense cystic lesion, and MRI is a superior imaging technique to CT[10]. Detection of this malformation is either by being symptomatic, as in our case, or by chance during routine otorhinolaryngologic examination and/or radiologic imaging. In our case, bilateral hearing loss due to OME was the presenting symptom. The enlargement of these cysts may be due to repeated mechanical stimulation such as intense swallowing, sneezing, coughing or blowing of nose.

Nasopharyngeal locations are very rare variants of branchial anomalies with no tract[2,5,7,8]. *Kim et al* managed two nasopharyngeal branchial cleft cysts with marsupialization via endoscopic route by using powered instruments[7]. They concluded that there was no recurrence in a follow-up of 2 years. *Chen et al* managed one case of nasopharyngeal branchial cleft cyst by marsupialization with diode laser via endoscopic endonasal surgery, in which patient had been free of disease for six months[3]. In our case, we preferred excising the entire remaining cystic lesion with a microdebrider after aspirating the cystic contents and biopsies taken from the cyst's wall. No recurrence occurred after more than 3 years. Nevertheless, marsupialization seems an adequate surgical procedure for these lesions. On the other hand, it is difficult to decide definitely because of the rarity of the malformation.

CONCLUSION

Nasopharyngeal branchial cleft cysts, although very rare, should be kept in mind in the differential diagnosis of nasopharyngeal cysts. Definitive diagnosis can be achieved only by histopathologic examination of the specimen. However, hyper-intense cystic lesion imaging on T2-weighted MRI would arouse suspicion of an initial diagnosis of a branchial cleft cyst. The striking points with these unusual located lesions are the choice of instrumentation for the surgery (cold steel, laser, radiofrequency energy) is not critical, and marsupialization of the lesion seems adequate surgical manipulation. Additionally, myringotomy and aspiration of glue/fluid in middle ear without insertion of a ventilation tube should be presented as a management option because it would be sufficient therapeutic management in these patients as in our case.

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Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

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Kuwait bone marrow transplantation activities

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Kuwait is located in the Arabian Gulf and has a population of 3.5 million. The stem cell transplantation program started in 2000. Autologous peripheral blood stem cell transplantation started first, as it was easier technically to establish. In 2011, the allogeneic program started with focus on acute leukemia and hemoglobinopathies. The success of both programs required teamwork and support of health planners. The Kuwait National Bone Marrow Registry was established in 2012. The issue of donor availability and drug shortage remain the two main obstacles for expanding the bone marrow transplantation program.

Development and validation of Attitude Toward Nutrition Counselling Questionnaire for use among Kuwaiti healthcare professionals

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OBJECTIVE
This study aims to report the developmental processes and validation of Attitude Toward Nutrition Questionnaire in Arabic language.

RESULTS
A total of 173 (response rate = 86.93%) participants responded to the survey. There were a total of 92 (53.2%) nutritionists and 81(46.8%) doctors/surgeons. Principal component analyses revealed followed by visualization of Cattell's scree plot, suggested a four-factor solution for the 36-item Attitude Toward Nutrition Counselling Questionnaire. It was found to have an acceptable validity. These four factors cumulatively explained 37.9% of the variance in the factor structure of the ATNQ. Cronbach's alpha revealed an acceptable level of reliability for each subscale of the ATNQ. The first subscale named "Factual knowledge about nutrition" comprised of nine items. It yielded a Cronbach's alpha value of 0.78. The second subscale "knowledge about nutrition in morbidities" comprised of seven items and yielded a Cronbach's alpha value of 0.71. The third subscale "counselling of patients" comprised of 11 items and yielded a Cronbach's alpha of 0.68. The fourth subscale comprising nine items yielded a Cronbach's alpha value of 0.64 and was named, "Dietary programs and supplementation".

Normal reference ranges for the left ventricular mass and left ventricular mass index in preterm infants

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OBJECTIVE
The objective of this study is to establish normal reference ranges for the left ventricular mass (LVM) and LVM index (LVMI) in preterm infants according to the body surface area (BSA) and assess their correlation with body weight and gestational age.

SUBJECTS AND METHODS
In a prospective study, 268 preterm babies who fulfilled the criteria for inclusion were examined. Echocardiograms were performed to measure the LVM and LVMI on 0-6 day (s) of life and at weekly intervals until the babies reached 36 weeks. The preterm infants were divided into six groups according to their BSA: 0.07-0.08 m2, 0.09-0.10 m2, 0.11-0.12 m2, 0.13-0.14 m2, 0.15-0.16 m2, and 0.17-0.19 m2.

RESULTS
The mean gestational age was 29.8 (±2.38 standard deviation [SD]) weeks, ranging from 24 to 35 weeks. The mean body weight was 1479 (±413 SD) g, ranging from 588 to 3380 g, and the mean BSA was 0.13 m2, ranging from 0.07 to 0.19 m2. The LVM correlated well with the gestational age, body weight, and BSA. The LVMI correlated well with body weight and BSA. Reference ranges with the mean ± SD, range, and interquartile range were calculated for the LVM and LVMI according to the BSA. A significant gradual increase was observed in a LVM with increasing BSA. Overall, a progressive and significant increase in the LVM was observed during the first 9 weeks of life.

CONCLUSION
The LVM and LVMI exhibited a significant correlation with the BSA and body weight. This study provides reference data that can be used as a normal reference tool for the LVM and LVMI for preterm infants based on the BSA.

The effect of adjuvants and delivery systems on Th1, Th2, Th17 and Treg cytokine responses in mice immunized with Mycobacterium tuberculosis-specific proteins

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Tuberculosis (TB) is a major health problem of global concern. The control of this disease requires appropriate preventive measures, including vaccines. In TB, T helper (Th)1 cytokines provide protection whereas Th2 and T regulatory (Treg) cytokines contribute to the pathogenesis and Th17 cytokines play

a role in both protection and pathogenesis. Previous studies with Mycobacterium tuberculosis-specific proteins have identified seven low molecular weight proteins, PE35, ESXA, ESXB, Rv2346c, Rv2347c, Rv3619c, and Rv3620c, as immunodominant antigens inducing Th1-cell responses in humans following natural infection with M. tuberculosis. The aim of this study was to characterize the cytokine responses induced in mice immunized with these proteins, using various adjuvants and delivery systems, i.e. chemical adjuvants (Alum and IFA), non-pathogenic mycobacteria (M. smegmatis and M. vaccae) and a DNA vaccine plasmid (pUMVC6). The immune responses were monitored by quantifying the marker cytokines secreted by Th1 (IFN-◎), Th2 (IL-5), Treg (IL-10), and Th17 (IL-17A) cells. DNA corresponding to pe35, esxa, esxb, rv2346c, rv2347c, rv3619c, and rv3620c genes were cloned into the expression vectors pGES-TH-1, pDE22 and pUMVC6 for expression in Escherichia coli, mycobacteria and eukaryotic cells, respectively. Mice were immunized with the recombinants using different adjuvants and delivery systems, and spleen cells were stimulated in vitro with peptides of immunizing proteins to investigate antigen-specific secretion of Th1 (IFN-◎), Th2 (IL-5), Treg (IL-10), and Th17 (IL-17A) cytokines. The results showed that spleen cells, from mice immunized with all antigens, secreted the protective Th1 cytokine IFN-◎, except ESXB, with one or more adjuvants and delivery systems. However, only Rv3619c consistently induced Th1-biased responses, without the secretion of significant concentrations of Th2, Th17 and Treg cytokines, with all adjuvants and delivery systems. Rv3619c also induced antigen-specific IgG antibodies in immunized mice.

Livestock-Associated Methicillin-Resistant Staphylococcus aureus in Patients Admitted to Kuwait Hospitals in 2016-2017

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Front Microbiol. 2020 Jan 8;10:2912. doi: 10.3389/fmicb.2019.02912. eCollection 2019.

Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) has been reported to colonize and cause infections in animals as well as in humans. LA-MRSA isolates have only recently been identified in patients admitted to Kuwait hospitals. This study was conducted to characterize LA-MRSA isolates obtained from patients admitted to Kuwait hospitals. A total of 202 (7.1%) of 2,823 MRSA isolates obtained from clinical samples in 2016 and 2017 in 11 public Kuwait hospitals were assigned to lineages previously known to be associated with livestock. They were characterized using antibiogram, spa typing, and DNA microarray for the assignment of clonal complexes (CCs) and detection of antibiotic resistance and virulence determinants. Identification as putative LA-MRSA clones was based on the molecular definition inferred from DNA microarray. The LA-MRSA isolates consisted of CC96 (N = 31), CC97 (N = 169), and CC398 (N = 2). Isolates belonging to CC96 and CC398 were resistant to erythromycin and clindamycin mediated by erm(A) and erm(C). CC97 isolates were multiresistant to gentamicin, kanamycin, erythromycin, clindamycin, tetracycline, chloramphenicol, fusidic acid, trimethoprim, and ciprofloxacin and harbored aacA-aphD, erm(A), erm(C), msr(A), tet(K), cat, fusC, and dfrS1. In total, 35 spa types were identified among the isolates. CC398 isolates consisted of t899 and t034. Ten spa types were identified among CC96 with t11822 (N = 13) as the most prevalent. CC97 consisted of 26 spa types with most belonging to t267 (N = 73) followed by t359 (N = 39). CC398 was composed of CC398-MRSA-IV and CC398-MRSA-V (PVL+). CC96 belonged to CC96-MRSA-IV and CC96-MRSA-IV (PVL+) Central Asian caMRSA/WA MRSA-119. CC97 consisted of six strains including CC97-MRSA-V (fusC +), CC97-MRSA-IV WA MRSA-54/63, CC97-MRSA-V, CC97-MRSA-(V+fus), CC97-MRSA-(mec VI+fus), and CC97-MRSA (mecV/VT+fus+ccrAB2). Whereas CC96 and CC97 isolates were identified in 2016 and 2017, CC398 isolates were detected only in 2016. This study identified four LA-MRSA clones among MRSA isolated from patients in Kuwait hospitals in 2016-2017 with CC97-MRSA-V (fusC +) as the dominant clone. The presence of LA-MRSA with different genetic backgrounds suggests its independent acquisition from different sources.

Neuromyelitis optica spectrum disorders in the Arabian Gulf: challenges and growing experience

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[3]Department of Neurology, Rashed Hospital, Dubai, United Arab Emirates
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Mult Scler J Exp Transl Clin. 2020 Jan 10;6(1):2055217319850195. doi: 10.1177/2055217319850195. eCollection 2020 Jan-Mar.

Neuromyelitis optica spectrum disorders (NMOSD) have been studied in different ethnic groups, including Asians, African-Americans, and Caucasians. Demonstrating the clinical features among diverse communities is important to understand the variable disease phenotypes, which will lead to further classification and better clinical management. Testing for antibody against aquaporin-4 (AQP4), the most common target antigen in NMOSD, is not available in many countries and tests use different methods, with variable sensitivity. With negative antibody results, the diagnosis of NMOSD becomes challenging and may affect the outcomes of patients with NMOSD. There are no adequate studies that assess NMOSD cohorts in the Arabian Gulf region, despite the increasing number of diagnosed cases. It is worth assessing NMOSD cohorts in the Arabian Gulf population to study the natural history of disease and to establish an epidemiological background for future perspectives. Various challenges to implement such a mission are outlined, including disease rarity, overlapping presenting symptoms and signs, which posed the issue of mimickers in the differential diagnosis, lack of specialized clinics, absence of highly sensitive testing methods for diagnosis, and the indefinite agreement on the negative AQP4 NMOSD criteria. Collaborative efforts started to take a place among many experts in the region to establish a registry of NMOSD patients for better perception of the disease pattern.

Childhood leukaemia incidence and trends in a Middle Eastern country during 1980-2014: a population-based study

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[2]Department of Surgery, Farwaniya Hospital, PO Box 33978, 7346, Al Rawdha, Kuwait
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Cancer Causes Control. 2020 Jan 20. doi: 10.1007/s10552-020-01267-3. [Epub ahead of print]

BACKGROUND
This retrospective cohort study examines the trends in childhood leukaemia age-standardized incidence rates (ASIRs) (per million person-years) using cases which were diagnosed at age 0-19 years from 1980 to 2014 and recorded in the Kuwait Cancer Control Center (KCCC) registry.

METHODS
Childhood leukaemia age-specific incidence rates overall and by sub-cohorts defined by age (0-4, 5-9, 10-14, and 15-19 years), sex (male, female) and nationality (Kuwaiti, non-Kuwaiti) were computed and age-standardized. Joinpoint regression models were used to evaluate trends in childhood leukaemia ASIRs. Average annual percent change (AAPC) and its 95% confidence interval (CI) were used to interpret the observed trends.

RESULTS
During the study period, 1077 childhood leukaemia cases of 32.3 million person-years were diagnosed. From 1980 to 2014, the average annual childhood leukaemia ASIR was 53.1 (95% CI 20.9, 85.2). Overall childhood leukaemia ASIRs significantly decreased on average by 6.8% per year (AAPC = -6.8; 95% CI -12.1, -1.1; p = 0.02) from 1980 to 1993, but a marginally significant increase in ASIRs from 1993 to 2014 was recorded (AAPC = 2.5; 95% CI -0.5, 5.5; p = 0.10). During the entire period, childhood leukaemia ASIRs trends significantly ($p < 0.05$) increased among 6 of 16 sub-cohorts, which was more pronounced among females and 10-14-year-old children.

CONCLUSIONS
Overall, ASIRs significantly increased from 1993 to 2014, which specifically seems to be driven by an increase in ASIRs among females and 10-14 -year-old children. These increasing trends underscore the potential involvement of a range of exposures. Future studies on unravelling such factors may help develop preventive measures to minimize childhood leukaemia risk in this and similar settings in the region.

Forthcoming Conferences and Meetings

Compiled and edited by
Vineetha Elizabeth Mammen

Kuwait Medical Journal 2020; 52 (1): 94 - 101

Alcohol and the **Nervous System** by Gordon Research Conferences
Mar 01 - 06, 2020
United States / Galveston, Texas
Contact: Gordon Research Conferences (GRC)
Phone: 401-360-1521
Email: ngray@grc.org

AO Trauma - Introduction Seminar
Mar 01, 2020
India / New Delhi, Delhi
Contact: AOTrauma
Phone: +91 8587899960
Email: bhavnesh.mittal@aocourses.org

T Cell Memory (X2) Conference
Mar 01 - 05, 2020
Canada / Banff, Alberta
Contact: Keystone Symposia on Molecular and Cellular Biology
Phone: +1 800-253-0685
Email: info@keystonesymposia.org

Multi-Specialty Conference in **Medicine**
Mar 01 - 03, 2020
Philippines / Makati, Metro Manila
Contact: American Academy of Family Medicine (AAFM)
Phone: 011 63 908 451 3700
Email: aafm123@gmail.com

British **FRCR** 2B Rapid Reporting, Long cases and Database Review
Mar 01 - 05, 2020
United Arab Emirates / Dubai
Contact: Radicon Radiology Courses (RRC)
Phone: +971 445147475
Email: info@radiologycourses.org

ESMO Targeted **Anticancer Therapies** Congress 2020
Mar 02 - 04, 2020
France / Paris, Ile-de-France
Contact: European Society for Medical Oncology (ESMO)
Email: meetings@esmo.org

Alzheimer's and **Dementia** Care Seminar
Mar 02, 2020
United States / Fort Myers, Florida
Contact: Wilson Shepard (WS) Education Associates
Email: jdangelo@wshep.com

5th **Ostomy Care** Management Course
Mar 04 - 06, 2020
United Arab Emirates / Abu Dhabi
Contact: Stars Medical Assistance Center (SMAC)
Phone: +971 2 6227887
Email: info@smacuae.com

Faculty of **Forensic Psychiatry** Annual Conference 2020
Mar 04 - 06, 2020
United Kingdom / Liverpool, England
Contact: Royal College of Psychiatrists (RCPSYCH)
Phone: 020 7235 2351
Email: reception@rcpsych.ac.uk

16th Annual **Women's Health** Update 2020
Mar 05 - 07, 2020
United States / Scottsdale, Arizona
Contact: Mayo Clinic
Phone: 4803014580
Email: mca.cme@mayo.edu

Radiology for Non-radiologists Course 2020
Mar 05, 2020
United Arab Emirates / Dubai
Contact: Radicon Radiology Courses (RRC)
Phone: +971 45147475
Email: info@radiologycourses.org

10th SEHA International **Neonatology** Conference
Mar 05 - 07, 2020
United Arab Emirates / Abu Dhabi
Contact: MENA Conference
Phone: +971 2 4919888
Email: afsal@menaconference.com

Bedside Ultrasound in the Acute Care Setting 2020
Mar 06 - 07, 2020
United States / St Louis, Missouri
Contact: Washington University School of Medicine in St. Louis
Phone: 314-362-6891
Email: cme@wustl.edu

Coalition of State **Rheumatology** Organizations (CSRO) 2020 Fellows Conference
Mar 06 - 07, 2020
United States / San Francisco, California
Contact: Coalition of State Rheumatology Organizations (CSRO)
Phone: (847) 517-7225
Email: info@csro.info

First **Pediatric** Advance Conference 2020
Mar 06, 2020
United Arab Emirates / Dubai
Contact: First Professional (FP)
Phone: 0097126544136
Email: info@firstprofessional.co

Dubai **FRCR** 2B Course 2020
Mar 06 - 07, 2020
United Arab Emirates / Dubai
Contact: Radicon Radiology Courses (RRC)
Phone: +971 445147475
Email: info@radiologycourses.org

8th Annual UCLA-Mellinkoff **Gastroenterology** and **Hepatology** Symposium
Mar 06 - 07, 2020
United States / Beverly Hills, California
Contact: David Geffen School of Medicine (DGSOM) at UCLA, Office of Continuing Medical Education
Phone: (310) 794-2620
Email: UCLACME@mednet.ucla.edu

AOCMF Course - Management of **Facial Trauma**
Mar 06 - 07, 2020
Kingdom of Saudi Arabia / Jeddah, Makkah
Contact: AOCMF
Phone: +9714297200
Email: edina.buzas@aocmf.org

Neurological Rehabilitation for Physiotherapy Assistants Programme – Course 3
Mar 07, 2020
United Kingdom / Salford, England
Contact: Manchester Neurotherapy Centre (MNC)
Phone: 0161 7930003
Email: admin@mncweb.co.uk

Evidence Based **Rehabilitation** of Traumatic Elbow Injuries
Mar 07, 2020
United States / Detroit, Michigan
Contact: Gold Standard Seminars (GSS), LLC
Phone: 516-455-6433
Email: info@goldstandardseminars.com

Colorado **Rheumatology** Summit 2020
Mar 07, 2020
United States / Denver, Colorado
Contact: ARTHROS
Phone: 973-588-8176
Email: amichaels@focusmeded.com

AOCMF Advanced Seminar - **Orthognathic Surgery**
Mar 08, 2020
Kingdom of Saudi Arabia / Jeddah, Makkah
Contact: AOCMF
Phone: +41 81 414 25 54
Email: edina.buzas@aocmf.org

Tumor Metabolism (C2) 2020
Mar 08 - 12, 2020
Canada / Banff, Alberta
Contact: Keystone Symposia on Molecular and Cellular Biology
Phone: +1 800-253-0685
Email: info@keystonesymposia.org

300 **CT and MRI** cases review
Mar 08 - 12, 2020
United Arab Emirates / Dubai
Contact: Radicon Radiology Courses (RRC)
Phone: +971 582047475
Email: info@radiologycourses.org

11th Global **Drug Delivery & Formulation** (DDF) Summit
Mar 09 - 11, 2020
Germany / Berlin
Contact: Mark Allen Group (MAG)
Phone: +44 (0)20 7738 5454
Email: maxconferences@markallengroup.com

Rewire the Anxious Brain: Neuroscience-Informed Treatment of **Anxiety, Panic and Worry**
Mar 09, 2020
United States / Billings, Montana
Contact: PESI HealthCare
Phone: (800) 844-8260
Email: info@pesi.com

High Risk Obstetrics: Current Trends, Treatments & Issues
Mar 09, 2020
United States / Portland, Maine
Contact: PESI HealthCare
Phone: (800) 844-8260
Email: info@pesi.com

Asia-Pacific **Healthcare Compliance Certificate** Program
Mar 09 - 12, 2020
Singapore / Singapore
Contact: Seton Hall University School of Law
Phone: 973-642-8871
Email: healthlaw@shu.edu

Gulf Thoracic 2020
Mar 11 - 14, 2020
United Arab Emirates / Dubai
Contact: Saudi Thoracic Society (STS)
Phone: +966-11-2488966
Email: sts.exo@gmail.com

IOC World Conference on Prevention of **Injury & Illness in sport**
Mar 12 - 14, 2020
France / Monaco
Contact: Publi Creations SAM
Phone: + 377 97 97 35 55
Email: info@ioc-preventionconference.org

IBD for Health Care Providers: Advances in **Patient Care** - CME
Mar 12, 2020
United States / Nashville, Tennessee
Contact: Vanderbilt University Medical Center (VUMC)
Phone: 615-322-4030
Email: cme@vanderbilt.edu

5th International **Dermatology and Cosmetology** (INDERCOS) Congress
Mar 12 - 15, 2020
Turkey / Istanbul
Contact: Figur Congress & Organizations
Phone: + 90 212 381 46 00
Email: indercos@figur.net

Emirates **Thyroid** Congress (ETC) 2020
Mar 13 - 14, 2020
United Arab Emirates / Abu Dhabi
Contact: InfoPlus Events LLC (IPE)
Phone: +971 4 4218996
Email: Plus@InfoPlusEvents.com

Dubai World **Dermatology and Laser** Conference & Exhibition 2020
Mar 16 - 18, 2020
United Arab Emirates / Dubai
Contact: INDEX Conferences & Exhibitions
Phone: 00971 4 520 8888
Email: index@emirates.net.ae

7th World Conference on **Pharmaceutical Science and Drug Manufacturing**
Mar 18 - 19, 2020
United Arab Emirates / Dubai
Contact: Bioleagues Worldwide
Phone: +91-9884076645
Email: info@pharma-dubai.com

7th International **GGSD** Conference 2020
Mar 19 - 21, 2020
Oman / Muscat, Muscat
Contact: MCI Middle East
Phone: 971 431 16 300
Email: neha.choudhary@mci-group.com

8th International **Maternal & Fetal Nutrition** in the first 1000 days
Mar 19 - 21, 2020
Turkey / Cankaya, Ankara
Contact: FTS Tourism Congress Organization / FTS Turizm Kongre Organizasyon, Halit Riza Ozturk
Phone: +90 312 439 68 04
Email: first1000days2020@ftskongre.org

7th Evolving Practice of **Ophthalmology** Middle East Conference (EPOMEC) 2020
Mar 19 - 21, 2020
United Arab Emirates / Dubai
Contact: iMeet International Business & Professional Organizations
Phone: +971544404202
Email: admin@epomec.ae

3rd UAE International Conference on **Antimicrobial Resistance** (ICAMR)
Mar 19 - 20, 2020
United Arab Emirates / Dubai
Contact: InfoPlus Events LLC (IPE)
Phone: +971 4 4218996
Email: Icamr@InfoPlusEvents.com

The 10th Biennial Johns Hopkins **Breast Cancer** Conference: A Multidisciplinary Perspective
Mar 20 - 21, 2020
United States / Hanover, Maryland
Contact: Johns Hopkins Medicine (JHM)
Phone: 410-955-5000
Email: cmenet@jhmi.edu

Diabetes in 2020: Understanding Evidence and Interventions
Mar 21, 2020
United Kingdom / Sheffield, England
Contact: MediConf UK Ltd
Phone: 01253 712894
Email: janet@mediconf.co.uk

Advances in **Cancer Immunotherapy** (C4) 2020
Mar 22 - 26, 2020
Canada / Whistler, British Columbia
Contact: Keystone Symposia on Molecular and Cellular Biology
Phone: +1 800-253-0685
Email: info@keystonesymposia.org

The UK **Transoral Robotic Surgery** Conference and **Cadaveric Hands** on Dissection Course 2020
Mar 23 - 24, 2020
United Kingdom / Newcastle upon Tyne, England
Contact: British Association of Plastic Reconstructive and Aesthetic Surgeons (BAPRAS)
Phone: +44 (0)20 7831 5161
Email: louise.sore@nuth.nhs.uk

World Congress on **Dementia** & Dementia Care
Mar 24 - 25, 2020
United Kingdom / London, England
Contact: Conference Mind
Phone: +91 7735912022
Email: dementia@conferencemind.com

1st **Kuwait Medical Microbiology** Conference
Mar 24 – 26, 2020
Kuwait / Marina Hotel
Contact: www.kmmconference.com

Obstetrics (OB) Emergencies
Mar 24, 2020
United States / Tacoma, Washington
Contact: PESI HealthCare
Phone: (800) 844-8260
Email: info@pesi.com

International Society of **Nephrology** (ISN) World Congress of Nephrology 2020
Mar 26 - 29, 2020
United Arab Emirates / Abu Dhabi
Contact: International Society of Nephrology (ISN)
Phone: +32 2 808 04 20
Email: info@theisn.org

2nd **Kuwait Gastroenterology Association** Conference
Mar 27 – 28, 2020
Kuwait / The Regency
Contact: www.kgaconf.com

24th Pan Arab Conference on **Diabetes** (PACD24)
Mar 27 - 29, 2020
Morocco / Casablanca
Contact: Pure Spot Events Management
Phone: +20 2 267 21 944
Email: registration@arab-diabetes.com

Society of Interventional **Radiology** (SIR) 2020 Annual Scientific Meeting
Mar 28 - Apr 02, 2020
United States / Seattle, Washington
Contact: Society of Interventional Radiology (SIR)
Phone: (703) 691-1805
Email: AnnualMeeting@SIRweb.org

Endo **Micro Surgical Retreatment** (Management of Endodontic Failure)
Mar 28 - 29, 2020
United Arab Emirates / Dubai
Contact: Centre for Advanced Professional Practices (CAPP)
Phone: +971 4 347 6747
Email: events@cappmea.com

SGO 2020 Annual Meeting on **Women's Cancer**
Mar 28 - 31, 2020
Canada / Toronto, Ontario
Contact: Society of Gynecologic Oncology (SGO)
Phone: 312-235-4060
Email: sgo@sgo.org

Microbiology Society Annual Conference 2020
Mar 30 - Apr 03, 2020
Scotland / Edinburgh, Scotland
Contact: Microbiology Society
Phone: +44 (0)20 7685 2400
Email: info@microbiologysociety.org

Fourth Annual HITEC: Hopkins International **Therapeutic Endoscopy** Course
Apr 01 - 03, 2020
United States / Baltimore, Maryland
Contact: Johns Hopkins Medicine (JHM)
Phone: (410) 955-2959
Email: cmenet@jhmi.edu

2020 **Radiosurgery** Society (RSS) Annual Scientific Meeting
Apr 02 - 04, 2020
United States / Washington, District of Columbia
Contact: The Radiosurgery Society (RSS)
Phone: (408) 385-9411
Email: rssevents@therss.org

AOTrauma Symposium - Management of **Fractures** of the Hand and Wrist
Apr 02, 2020
Egypt / Cairo
Contact: AOTrauma
Phone: +41 81 414 27 00 ext. 700
Email: EGutierrez@aotrauma.org

Bahrain **Pediatric** Summit Conference 2020
Apr 02 - 04, 2020
Bahrain / Manama, Bahrain
Contact: Smart Management Consultancy
Phone: +973 13112750
Email: info@smartmcbh.com

2020 IEEE 17th International Symposium on **Biomedical Imaging** (ISBI)
Apr 04 - 07, 2020
United States / Iowa City, Iowa
Contact: Institute of Electrical and Electronics Engineers (IEEE)
Phone: +1 732 562 3878
Email: ieee-mce@ieee.org

12th Global Hands-on **Musculoskeletal Ultrasound** with Basic MRI Correlation Congress for Physicians & General Practitioners
Apr 05 - 06, 2020
United States / Penang, Pulau Pinang
Contact: Global Futuristic Management Services
Phone: +6012-2773799
Email: globalfuturistic@gmail.com

4th Annual Dubai International **Paediatric Neurology** Congress
Apr 09 - 11, 2020
United Arab Emirates / Dubai, Dubai
Contact: Maarefah Management
Phone: +971 4 361 9616
Email: info@ipncongress.com

5th Annual Dubai International Conference on **Infectious Diseases & Vaccination** (DICID)
Apr 09 - 11, 2020
United Arab Emirates / Dubai, Dubai
Contact: Maarefah Management
Phone: +971 4 361 9616
Email: info@dic-id.com

Hands-On **Echocardiography**
Apr 13 - 18, 2020
United States / Irving, Texas
Contact: Keith Mauney & Associates (KMA) Ultrasound Training Institutes
Phone: (972) 353-3200
Email: info@kmaultrasound.com

International Interprofessional **Wound Care** Course (IIWCC-U.A.E)
Apr 14 - 17, 2020
United Arab Emirates / Abu Dhabi, Abu Dhabi
Contact: International Inter-Professional Wound Care Group (IIWCG)
Phone: +971 581776415
Email: info@iiwcg.com

Kuwait's 10th Critical Care Conference
Apr 16 – 18, 2020
Kuwait / Sheikh Jaber Al-Ahmad Cultural Centre
Contact: www.criticalcarekuwait.com

Pediatric Neurology Symposium 2020
Apr 17 - 18, 2020
United States / Memphis, Tennessee
Contact: Methodist MD
Phone: (901) 516-8933
Email: Rexann.Pickering@mlh.org

2020 - 5th International Conference on **Research in Life-Sciences & Healthcare** (ICRLSH)
Apr 17 - 18, 2020
United Kingdom / London, England
Contact: Eurasia Research
Phone: +91 7290808650
Email: convener@eurasiaresearch.info

International Society for **Pharmacoepidemiology** (ISPE) 2020 Mid-Year Meeting
Apr 18 - 21, 2020
United States / Orlando, Florida
Contact: International Society for Pharmacoepidemiology (ISPE)
Phone: 301-718-6500
Email: info@pharmacoepi.org

Biennial **Cardio-Oncology** Symposium 2020
Apr 18, 2020
United States / Chicago, Illinois
Contact: Meeting Achievements, LLC
Phone: 2194651115
Email: Lisa@MeetingAchievements.com

Cancer Survivorship Summit: Improving Outcomes for People Living with and Beyond Cancer 2020
Apr 20, 2020
United Kingdom / London, England
Contact: Healthcare (HC) - UK Conferences
Phone: 01932 429933
Email: jayne@hc-uk.org.uk

7th Annual **GCC Healthcare 5.0 Congress**
Apr 20 - 21, 2020
United Arab Emirates / Dubai, Dubai
Contact: Maarefah Management
Phone: +971 4 361 9616
Email: info@gcchealthcareinnovation.com

Obesity Medicine 2021 Conference
Apr 21 - 25, 2020
United States / Denver, Colorado
Contact: Obesity Medicine Association (OMA)
Phone: 303-770-2526
Email: info@obesitymedicine.org

2020 International Conference on **Pharmaceutics & Advanced Pharmacy**
Apr 22 - 23, 2020
United Arab Emirates / Dubai, Dubai
Contact: Research Lake International Inc.
Phone: +1 (647) 551-8989
Email: connect@researchlake.com

2nd Annual Meeting on **Neuroscience & Neurology**
Apr 23 - 24, 2020
Malaysia / Kuala Lumpur,
Contact: BioLEAGUES Worldwide
Phone: 8925457124
Email: neuroscience@bioleagues.com

Cardiac Disease and Anaesthesia Symposium 2020
Apr 23 - 24, 2020
United Kingdom / London, England
Contact: The Royal College of Anaesthetists (RCoA)
Phone: 020 7092 1673
Email: events@rcoa.ac.uk

Nitrous Oxide/Oxygen Sedation for the Registered Dental Hygienist
Apr 27, 2020
United States / Ann Arbor, Michigan
Contact: University of Michigan School of Dentistry
Phone: 734-763-5070
Email: cde.umich@umich.edu

Challenging Geriatric Issues: **Dementia** Behaviors
Apr 29, 2020
United States / Phoenix, Arizona
Contact: PESI HealthCare
Phone: (800) 844-8260
Email: info@pesi.com

5th North American Echocardiography Course on **Congenital Heart Disease**
Apr 30 - May 02, 2020
United States / Stanford, California
Contact: Stanford Center for Continuing Medical Education
Phone: 650-724-7166
Email: msisney@stanford.edu

Update on **Chronic Kidney Disease** 2020
May 05, 2020
United States / Hershey, Pennsylvania
Contact: Penn State University (PSU) College of Medicine Continuing Education
Phone: 717-531-6483
Email: comweb@pennstatehealth.psu.edu

Cardiology Updates Anaheim 2020
May 05, 2020
United States / Anaheim, California
Contact: Pri-Med
Phone: (877) 477-4633
Email: support@pri-med.com

International Society for **Autism Research** (INSAR) 2020 Annual Meeting
May 06 - 09, 2020
United States / Seattle, Washington
Contact: International Society for Autism Research (INSAR)
Phone: 816.595.4852
Email: info@autism-insar.org

2nd International Conference on **Heart & Diabetes**
May 11 - 12, 2020
United Arab Emirates / Dubai
Contact: In Conference Ltd
Phone: 0131 336 4203
Email: diabetesconferenceglobal@gmail.com

The Leading Edge in **Diagnostic Ultrasound** Annual Conference 2020
May 12 - 14, 2020
United States / Atlantic City, New Jersey
Contact: Thomas Jefferson University (TJU)
Phone: 215-955-8533
Email: jurei@jefferson.edu

AOTrauma Course - Basic Principles of **Fracture Management**
May 14 - 16, 2020
India / Dehradun, Uttarakhand
Contact: AOTrauma
Phone: +91 8587899960
Email: bhavnesh.mittal@aocourses.org

International Team for **Implantology** (ITI) World Symposium 2020
May 14 - 16, 2020
Singapore / Singapore
Contact: International Team for Implantology (ITI)
Phone: +41 61 270 83 83
Email: headquarters@iti.org

The 6th World Congresses on Controversies in **Multiple Myeloma** (COMy)
May 14 - 16, 2020
France / Paris, Ile-de-France
Contact: CME Congresses
Phone: +44-20-32899552
Email: info@cme-congresses.com

4th **Probiotics** Congress: Europe
May 18 - 20, 2020
Netherland / Rotterdam, South Holland
Contact: Global Engage Ltd
Phone: +44 (0)1865 849841
Email: info@globalengage.co.uk

Cell Symposia: **Infection Biology** in the Age of the Microbiome
May 18 - 20, 2020
France / Paris, Ile-de-France
Contact: Elsevier
Phone: +1 212 989 5800
Email: a.cascio@elsevier.com

2020 **Eating Disorders** Conference
May 18, 2020
United States / Baltimore, Maryland
Contact: Johns Hopkins Medicine (JHM)
Phone: 410-955-5000
Email: cmenet@jhmi.edu

BAS **Sclerotherapy** Conference 2020
May 19, 2020
United Kingdom / Windsor, England
Contact: British Association of Sclerotherapists (BAS)
Phone: + 01264736500
Email: enq@bassclerotherapy.com

High Risk Postpartum Patients: Conquer the Mother-Baby Warning Signs
May 20, 2020
United States / Bellevue, Washington
Contact: PESI HealthCare
Phone: (800) 844-8260
Email: info@pesi.com

Cell Therapy at the Limits 2020
May 21 - 22, 2020
United Kingdom / London, England
Contact: At the Limits
Phone: +44 1491 419800
Email: info@atthelimits.org

International Congress on **Pharmaceutical Sciences** 2020
May 21 - 22, 2020
Czech Republic / Prague
Contact: Medical Conferences
Phone: +31 208 080 653
Email: angela@medical-conferences.com

Human Anatomy Physiology Society (HAPS) 34th Annual Conference
May 23 - 27, 2020
Canada / Ottawa, Ontario
Contact: Human Anatomy and Physiology Society (HAPS)
Phone: 1-800-448-4277
Email: peter@hapsconnect.org

3rd International Conference on **Gynaecology and Obstetrics**
May 25 - 26, 2020
Indonesia / Bali, Bali
Contact: Colloquium, LLC
Phone: +1 307-222-6372
Email: contact@gynaecologycongress.com

American College of Sports Medicine (ACSM) 67th Annual Meeting
May 26 - 30, 2020
United States / San Francisco, California
Contact: American College of Sports Medicine (ACSM)
Phone: (317) 637-9200
Email: meeting@acsm.org

Consortium of **Multiple Sclerosis** Centers (CMSC) 34th Annual Meeting
May 27 - 30, 2020
United States / Orlando, Florida
Contact: Consortium of Multiple Sclerosis Centers (CMSC)
Phone: 01-487-1050
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18th European Meeting on **HIV & Hepatitis**
May 27 - 29, 2020
France / Paris, Ile-de-France
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Nutrition 2020
May 30 - Jun 02, 2020
United States / Seattle, Washington
Contact: American Society for Nutrition (ASN)
Phone: (240) 428-3650
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Singapore **Hepatology** Conference (SHC) 2020
Jun 05 - 06, 2020
Singapore / Singapore
Contact: Singapore Hepatitis Conference (SHC) Pte Ltd
Phone: 65 8620 3404
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Ultrasound Guided Interventional **Pain Management** Procedures
Jun 07, 2020
United States / New York City, New York
Contact: Empire Medical Training (EMT), Inc
Phone: 866-366-1576
Email: info@empiremedicaltraining.com

International Conference on **Eating Disorders** (ICED) 2020
Jun 11 - 13, 2020
Australia / Sydney, New South Wales
Contact: Academy for Eating Disorders (AED)
Email: info@aedweb.org

12th Singapore International **Physiotherapy** Congress (SIPC) 2020
Jun 19 - 22, 2020
Singapore / Singapore, Singapore
Contact: Singapore Physiotherapy Association (SPA)
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British Association of **Otorhinolaryngology** (BACO) 2020
Jul 08 - 10, 2020
United Kingdom / Birmingham, England
Contact: Ear, Nose and Throat (ENT) UK
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WHO-Facts Sheet

1. Animal Bites
2. Campylobacter
3. Food safety
4. Healthy diet
5. Malaria

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1. ANIMAL BITES

KEY FACTS

- Animal bites are a significant cause of morbidity and mortality worldwide.
- Worldwide, up to five million people are bitten by snakes every year; the majority in Africa and South-East Asia.
- Prompt medical treatment with appropriate antivenom is required for poisonous snake bites.
- Dog bites account for tens of millions of injuries annually; the highest risk is among children.
- Rabies is a significant health concern following dog bites, cat bites and monkey bites.

Animal bites pose a major public health problem in children and adults worldwide. The health impacts of animal bites are dependent on the type and health of the animal species, the size and health of the bitten person, and accessibility to appropriate health care.

Numerous animal species have the potential to bite humans; however, the most important are tabhose arising from snakes, dogs, cats and monkeys.

Snake bites

Scope of the problem

Worldwide, up to five million people are bitten by snakes every year. Of these, poisonous (envenoming) snakes cause considerable morbidity and mortality. There are an estimated 2.4 million envenomations (poisonings from snake bites) and 94 000–125 000 deaths annually, with an additional 400 000 amputations and other severe health consequences, such as infection, tetanus, scarring, contractures, and psychological sequelae. Poor access to health care and scarcity of antivenom increases the severity of the injuries and their outcomes.

Who is most at risk?

The majority of snake bites occur in Africa and South-East Asia. Snake bites are most common among people living in rural, resource-poor settings, who subsist on low-cost, non-mechanical farming and other field occupations. Agricultural workers, women and children are the groups most frequently bitten by snakes. Adding to the burden of these injuries is their socioeconomic impact on families and communities. Adult victims are often the wage earners or care providers of the family unit; and child victims can suffer lifelong disability intensifying demands on families and communities

Treatment

Approximately 600 species of snake are venomous and approximately 50-70% of bites by these cause envenomation. At the time of a bite, the cornerstone of care is complete immobilization of the affected body part and prompt transfer to a medical facility. Tourniquets and cutting wounds can worsen the effects of the venom and should not be used as first aid.

Frequently, victims of snake bites will require treatment with antivenom. It is important that the antivenom is appropriate for snakes endemic to the region. Additional measures include wound cleansing to decrease infection risk, supportive therapy such as airway support, and administration of tetanus vaccine upon discharge if the person has been inadequately vaccinated against tetanus.

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Prevention of snake bites and their serious health consequences

Prevention of snake bites involves informing communities about snake bite risks and prevention techniques, such as to:

- avoid tall grassy areas;
- wear protective shoes/boots;
- keep storage areas clear of rodents;
- remove rubbish, woodpiles and low brush from around the home;
- store food in rodent-proof containers, raise beds above floor level and tuck mosquito nets securely under sleeping mats within the home.

To prevent or limit the serious health consequences of snake bites, health-care providers should be educated on snake-bite management, including the proper use and administration of antivenom. Public health authorities and policy-makers should ensure appropriate supplies of safe and effective antivenoms to communities, countries and regions where they are most needed, and prioritize research initiatives that will further determine the burden of these injuries.

Dog bites

Scope of the problem

There are no global estimates of dog bite incidence, however studies suggest that dog bites account for tens of millions of injuries annually. In the United States of America for example, approximately 4.5 million people are bitten by dogs every year. Of these, nearly 885 000 seek medical care; 30 000 have reconstructive procedures; 3–18% develop infections and between 10 and 20 fatalities occur. Other high-income countries such as Australia, Canada and France have comparable incidence and fatality rates.

Low- and middle-income country data are more fragmented, however some studies reveal that dogs account for 76–94% of animal bite injuries. Dog bite fatality rates are higher in low- and middle-income countries than in high-income countries as rabies is a problem in many of these countries, and there may be a lack of post-exposure treatment and appropriate access to health care. An estimated 59 000 people die annually from rabies, and bites from rabid dogs account for the vast majority of these deaths.

Who is most at risk?

Children make up the largest percentage of people bitten by dogs, with the highest incidence in mid-to-late childhood. The risk of injury to the head and neck is greater in children than in adults, adding to increased severity, necessity for medical treatment and death rates.

In some countries, males have a higher frequency of dog bites than females. Dog bites account for over 50% of animal-related injuries in people who are travelling.

Treatment

Treatment depends on the location of the bite, the overall health condition of the bitten person and whether or not the dog is vaccinated against rabies. The main principles of care include:

- early medical management;
- irrigation and cleansing of the wound;
- primary closure if the wound is low-risk for developing infection;
- prophylactic antibiotics for high-risk wounds or people with immune deficiency;
- rabies post-exposure treatment depending on the dog vaccination status;
- administration of tetanus vaccine if the person has not been adequately vaccinated.

Prevention of dog bites and their serious health consequences

Communities – especially children – should be informed about the risks of dog bites and prevention techniques such as avoiding stray dogs and never leaving a child unattended around any dog.

Health-care providers should be educated on the appropriate management of dog bites. Health authorities and policy-makers should ensure rabies control within dog populations, ensure appropriate supplies of rabies vaccines for potential rabies exposure in people, and develop data collection systems to further document the burden of this problem.

Cat bites

Scope of the problem

Worldwide, cat bites account for 2–50% of injuries related to animal-bites. They are commonly second to dog bites in terms of incidence. In Italy for example, the incidence of cat-related injuries is 18 per 100 000 population, while in the United States of America, there are an estimated 400 000 cat bites and 66 000 visits to hospital emergency departments every year.

Who is most at risk?

Female adults have the highest rate of cat bites.

Treatment

Treatment depends on the location of the bite and the rabies vaccination status of animal species inflicting the bite. The main principles of care include:

- early medical management including wound cleansing;
- prophylactic antibiotics to decrease infection risk;
- rabies post-exposure treatment depending on the

animal vaccination status;
- administration of tetanus vaccine if the person has not been adequately vaccinated.

Prevention of cat bites and their serious health consequences

Communities should be informed about the risks of cat bites and prevention techniques for cat bites including vaccinating cats against rabies.

Health-care providers should be educated on the appropriate management of these injuries. Health authorities and policy-makers should ensure rabies control within animal populations, and appropriate supplies of post-exposure rabies treatment and antibiotic prophylaxis for bitten people. They should also support research initiatives directed at providing more information on the burden of cat bites.

Monkey bites

Scope of the problem

Monkey bites account for 2–21% of animal bite injuries. In India for example, two studies found monkeys to be second to dogs as the most common source of animal bite injuries.

Who is most at risk?

Monkey bites are an important risk among travellers, being the second most common animal bite risk to travellers after dog bites.

Treatment

Treatment depends on the health status of the patient, the location of the bite and whether or not there is a suspicion of rabies in the monkey. The main principles of care include:
- early medical management including wound cleansing;
- prophylactic antibiotics to decrease infection risk;
- rabies post-exposure treatment depending on the animal vaccination status;
- administration of tetanus vaccine if the person has not been adequately vaccinated.

Prevention of monkey bites and their serious health consequences

Communities and travellers should be informed about risks of monkey bites and prevention techniques.

Health-care providers should be educated on the appropriate management of these injuries. Health authorities and policy-makers should ensure rabies control within monkey populations, and appropriate supplies of post-exposure rabies treatment and antibiotic prophylaxis for bitten people. They should also support research initiatives directed at providing more information on the burden of monkey bites.

WHO response

WHO is working to address the public health problem of animal bite injuries.

For snake bites, WHO has launched several tools to help guide the appropriate development, distribution and administration of antivenom.

For rabies, WHO advocates greater access to post-exposure treatment through promoting increased production of rabies biologicals, continuing education in rabies prevention and control, and widespread immunization of dog populations.

For all animal-bite injuries, WHO:
- prioritizes data collection initiatives to help determine the burden and risk factors of these injuries;
- advocates the strengthening of emergency response services for people that are injured;
- promotes research initiatives that focus on effective prevention interventions and populations most affected.

2. CAMPYLOBACTER

KEY FACTS
- Campylobacter is 1 of 4 key global causes of diarrhoeal diseases. It is considered to be the most common bacterial cause of human gastroenteritis in the world.
- Campylobacter infections are generally mild, but can be fatal among very young children, elderly, and immunosuppressed individuals.
- Campylobacter species can be killed by heat and thoroughly cooking food.
- To prevent Campylobacter infections, make sure to follow basic food hygiene practices when preparing food.

The burden of foodborne diseases, including Campylobacteriosis, is substantial: every year almost 1 in 10 people fall ill and 33 million of healthy life years are lost. Foodborne diseases can be severe, especially for young children. Diarrhoeal diseases are the most common illnesses resulting from unsafe food, with 550 million people falling ill yearly (including 220 million children under the age of 5 years). *Campylobacter* is 1 of the 4 key global causes of diarrhoeal diseases.

The high incidence of *Campylobacter* diarrhoea, as well as its duration and possible complications, makes it highly important from a socio-economic perspective. In developing countries, *Campylobacter* infections in children under the age of 2 years are especially frequent, sometimes resulting in death.

Campylobacter are mainly spiral-shaped, "S"-shaped, or curved, rod-shaped bacteria. Currently,

there are 17 species and 6 subspecies assigned to the genus *Campylobacter*, of which the most frequently reported in human diseases are *C. jejuni* (subspecies *jejuni*) and *C. coli*. Other species such as *C. lari* and *C. upsaliensis* have also been isolated from patients with diarrhoeal disease, but are reported less frequently.

The disease

Campylobacteriosis is the disease caused by the infection with *Campylobacter*:

- The onset of disease symptoms usually occurs 2 to 5 days after infection with the bacteria, but can range from 1 to 10 days.
- The most common clinical symptoms of *Campylobacter* infections include diarrhoea (frequently bloody), abdominal pain, fever, headache, nausea, and/or vomiting. The symptoms typically last 3 to 6 days.
- Death from campylobacteriosis is rare and is usually confined to very young children or elderly patients, or to those already suffering from another serious disease such as AIDS.
- Complications such as bacteraemia (presence of bacteria in the blood), hepatitis, pancreatitis (infections of liver and pancreas, respectively), and miscarriage have been reported with various degrees of frequency. Post-infection complications may include reactive arthritis (painful inflammation of the joints which can last for several months) and neurological disorders such as Guillain-Barré syndrome, a polio-like form of paralysis that can result in respiratory and severe neurological dysfunction in a small number of cases.

Sources and transmission

Campylobacter species are widely distributed in most warm-blooded animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep and ostriches; and in pets, including cats and dogs. The bacteria have also been found in shellfish.

The main route of transmission is generally believed to be foodborne, via undercooked meat and meat products, as well as raw or contaminated milk. Contaminated water or ice is also a source of infection. A proportion of cases occur following contact with contaminated water during recreational activities.

Campylobacteriosis is a zoonosis, a disease transmitted to humans from animals or animal products. Most often, carcasses or meat are contaminated by *Campylobacter* from faeces during slaughtering. In animals, *Campylobacter* seldom causes disease.

The relative contribution of each of the above sources to the overall burden of disease is unclear but consumption of undercooked contaminated poultry is believed to be a major contributor. Since common-source outbreaks account for a rather small proportion of cases, the vast majority of reports refer to sporadic cases, with no easily discernible pattern.

Estimating the importance of all known sources is therefore extremely difficult. In addition, the wide occurrence of *Campylobacter* also hinders the development of control strategies throughout the food chain. However, in countries where specific strategies have been put in place to reduce the prevalence of *Campylobacter* in live poultry, a similar reduction in human cases is observed.

Treatment

Treatment is not generally required, except electrolyte replacement and rehydration. Antimicrobial treatment is recommended in invasive cases (when bacteria invade the intestinal mucosa cells and damage the tissues) or to eliminate the carrier state (the condition of people who harbour *Campylobacter* in their bodies and keep shedding the bacteria while remaining asymptomatic).

Prevention methods

There are a number of strategies that can be used to prevent disease from *Campylobacter*:

- Prevention is based on control measures at all stages of the food chain, from agricultural production on a farm, to processing, manufacturing and preparation of foods both commercially and domestically.
- In countries without adequate sewage disposal systems, faeces and articles soiled with faeces may need to be disinfected before disposal.
- Measures to reduce the prevalence of *Campylobacter* in poultry include enhanced biosecurity to avoid transmission of *Campylobacter* from the environment to the flock of birds on the farm. This control option is feasible only where birds are kept in closed housing conditions.
- Good hygienic slaughtering practices reduce the contamination of carcasses by faeces, but will not guarantee the absence of *Campylobacter* from meat and meat products. Training in hygienic food handling for abattoir workers and raw meat producers is essential to keep contamination to a minimum.
- Prevention methods against infection in domestic kitchens are similar to those used against other foodborne bacterial diseases.
- Bactericidal treatment, such as heating (for example, cooking or pasteurization) or irradiation, is the only effective method of eliminating *Campylobacter* from contaminated foods.

WHO response

In partnership with other stakeholders, WHO is strongly advocating the importance of food safety as an essential element in ensuring access to safe and nutritious diets. WHO is providing policies and recommendations that cover the entire food chain from production to consumption, making use of different types of expertise across different sectors.

WHO is working towards the strengthening of food safety systems in an increasingly globalized world. Setting international food safety standards, enhancing disease surveillance, educating consumers and training food handlers in safe food handling are amongst the most critical interventions in the prevention of foodborne illnesses.

In collaboration with the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the WHO Collaborating Centre at the University of Utrecht, WHO published the report *The global view of campylobacteriosis in 2012*.

- The global view of campylobacteriosis

WHO is strengthening the capacities of national and regional laboratories in the surveillance of foodborne pathogens, such as *Campylobacter* and *Salmonella*.

- Global Foodborne Infections Network (GFN)

WHO is also promoting the integrated surveillance of antimicrobial resistance of pathogens in the food chain, collecting samples from humans, food and animals and analysing data across the sectors.

- WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR)

WHO, jointly with FAO, is assisting Member States by coordinating international efforts for early detection and response to foodborne disease outbreaks through the network of national authorities in Member States.

- International Network of Food Safety Authorities (INFOSAN)

WHO also provides scientific assessments as basis for international food standards, guidelines and recommendations developed by the FAO/WHO Codex Alimentarius Commission to prevent foodborne diseases.

- Codex Alimentarius Commission

Recommendations for the public and travellers

The following guidance will help people to stay safe while travelling:

- Ensure food is properly cooked and still hot when served.
- Avoid raw milk and products made from raw milk. Drink only pasteurized or boiled milk.
- Avoid ice unless it is made from safe water.
- When the safety of drinking water is questionable, boil it, or if this is not possible, disinfect it with a reliable, slow-release disinfectant agent (usually available at pharmacies).
- Wash hands thoroughly and frequently using soap, in particular after contact with pets or farm animals, or after having been to the toilet.
- Wash fruits and vegetables carefully, particularly if they are eaten raw. If possible, vegetables and fruits should be peeled.
- A guide on safe food for travellers

Recommendations for food handlers

WHO provides the following guidance for people handling food:

- Both professional and domestic food handlers should be vigilant while preparing food and should observe hygienic rules of food preparation.
- Professional food handlers who suffer from fever, diarrhoea, vomiting, or visible infected skin lesions should report to their employer immediately.
- The WHO *Five keys to safer food* serve as the basis for educational programmes to train food handlers and educate consumers. They are especially important in preventing food poisoning. The Five keys are:
  - keep clean
  - separate raw and cooked
  - cook thoroughly
  - keep food at safe temperatures
  - use safe water and raw materials.
- Five keys to safer food

3. FOOD SAFETY

KEY FACTS

- Access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health.
- Unsafe food containing harmful bacteria, viruses, parasites or chemical substances, causes more than 200 diseases – ranging from diarrhoea to cancers.
- An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (DALYs).
- Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year.
- Diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230 000 deaths every year.
- Food safety, nutrition and food security are

inextricably linked. Unsafe food creates a vicious cycle of disease and malnutrition, particularly affecting infants, young children, elderly and the sick.
- Foodborne diseases impede socioeconomic development by straining health care systems, and harming national economies, tourism and trade.
- Food supply chains now cross multiple national borders. Good collaboration between governments, producers and consumers helps ensure food safety.

Major foodborne illnesses and causes

Foodborne illnesses are usually infectious or toxic in nature and caused by bacteria, viruses, parasites or chemical substances entering the body through contaminated food or water.

Foodborne pathogens can cause severe diarrhoea or debilitating infections including meningitis.

Chemical contamination can lead to acute poisoning or long-term diseases, such as cancer. Foodborne diseases may lead to long-lasting disability and death. Examples of unsafe food include uncooked foods of animal origin, fruits and vegetables contaminated with faeces, and raw shellfish containing marine biotoxins.

Bacteria

- ***Salmonella, Campylobacter, and Enterohaemorrhagic Escherichia coli*** are among the most common foodborne pathogens that affect millions of people annually – sometimes with severe and fatal outcomes. Symptoms are fever, headache, nausea, vomiting, abdominal pain and diarrhoea. Examples of foods involved in outbreaks of salmonellosis are eggs, poultry and other products of animal origin. Foodborne cases with *Campylobacter* are mainly caused by raw milk, raw or undercooked poultry and drinking water. *Enterohaemorrhagic Escherichia coli* is associated with unpasteurized milk, undercooked meat and fresh fruits and vegetables.
- ***Listeria*** infection leads to unplanned abortions in pregnant women or death of newborn babies. Although disease occurrence is relatively low, listeria's severe and sometimes fatal health consequences, particularly among infants, children and the elderly, count them among the most serious foodborne infections. Listeria is found in unpasteurised dairy products and various ready-to-eat foods and can grow at refrigeration temperatures.
- ***Vibrio cholerae*** infects people through contaminated water or food. Symptoms include abdominal pain, vomiting and profuse watery diarrhoea, which may lead to severe dehydration and possibly death. Rice, vegetables, millet gruel and various types of seafood have been implicated in cholera outbreaks.

Antimicrobials, such as antibiotics, are essential to treat infections caused by bacteria. However, their overuse and misuse in veterinary and human medicine has been linked to the emergence and spread of resistant bacteria, rendering the treatment of infectious diseases ineffective in animals and humans. Resistant bacteria enter the food chain through the animals (e.g. *Salmonella* through chickens). Antimicrobial resistance is one of the main threats to modern medicine.

Viruses

Norovirus infections are characterized by nausea, explosive vomiting, watery diarrhoea and abdominal pain. Hepatitis A virus can cause long-lasting liver disease and spreads typically through raw or undercooked seafood or contaminated raw produce. Infected food handlers are often the source of food contamination.

Parasites

Some parasites, such as fish-borne trematodes, are only transmitted through food. Others, for example tapeworms like *Echinococcus spp*, or *Taenia solium*, may infect people through food or direct contact with animals. Other parasites, such as *Ascaris, Cryptosporidium, Entamoeba histolytica* or *Giardia*, enter the food chain via water or soil and can contaminate fresh produce.

Prions

Prions, infectious agents composed of protein, are unique in that they are associated with specific forms of neurodegenerative disease. Bovine spongiform encephalopathy (BSE, or "mad cow disease") is a prion disease in cattle, associated with the variant Creutzfeldt-Jakob Disease (vCJD) in humans. Consuming bovine products containing specified risk material, e.g. brain tissue, is the most likely route of transmission of the prion agent to humans.

Chemicals

Of most concern for health are naturally occurring toxins and environmental pollutants.

- **Naturally occurring toxins** include mycotoxins, marine biotoxins, cyanogenic glycosides and toxins occurring in poisonous mushrooms. Staple foods like corn or cereals can contain high levels of mycotoxins, such as aflatoxin and ochratoxin, produced by mould on grain. A long-term exposure can affect the immune system and normal

development, or cause cancer.

- **Persistent organic pollutants (POPs)** are compounds that accumulate in the environment and human body. Known examples are dioxins and polychlorinated biphenyls (PCBs), which are unwanted by-products of industrial processes and waste incineration. They are found worldwide in the environment and accumulate in animal food chains. Dioxins are highly toxic and can cause reproductive and developmental problems, damage the immune system, interfere with hormones and cause cancer.
- **Heavy metals**such as lead, cadmium and mercury cause neurological and kidney damage. Contamination by heavy metal in food occurs mainly through pollution of air, water and soil.

The burden of foodborne diseases

The burden of foodborne diseases to public health and welfare and to economy has often been underestimated due to underreporting and difficulty to establish causal relationships between food contamination and resulting illness or death.

The 2015 WHO report on the estimates of the global burden of foodborne diseases presented the first-ever estimates of disease burden caused by 31 foodborne agents (bacteria, viruses, parasites, toxins and chemicals) at global and regional level.

The evolving world and food safety

Safe food supplies support national economies, trade and tourism, contribute to food and nutrition security, and underpin sustainable development.

Urbanization and changes in consumer habits, including travel, have increased the number of people buying and eating food prepared in public places. Globalization has triggered growing consumer demand for a wider variety of foods, resulting in an increasingly complex and longer global food chain.

As the world's population grows, the intensification and industrialization of agriculture and animal production to meet increasing demand for food creates both opportunities and challenges for food safety. Climate change is also predicted to impact food safety, where temperature changes modify food safety risks associated with food production, storage and distribution.

These challenges put greater responsibility on food producers and handlers to ensure food safety. Local incidents can quickly evolve into international emergencies due to the speed and range of product distribution. Serious foodborne disease outbreaks have occurred on every continent in the past decade, often amplified by globalized trade.

Examples include the contamination of infant formula with melamine in 2008 (affecting 300 000 infants and young children, 6 of whom died, in China alone), and the 2011 Enterohaemorrhagic *Escherichia coli* outbreak in Germany linked to contaminated fenugreek sprouts, where cases were reported in 8 countries in Europe and North America, leading to 53 deaths and significant economic losses.

Food safety: a public health priority

Unsafe food poses global health threats, endangering everyone. Infants, young children, pregnant women, the elderly and those with an underlying illness are particularly vulnerable. Every year 220 million children contract diarrhoeal diseases and 96 000 die.

Unsafe food creates a vicious cycle of diarrhoea and malnutrition, threatening the nutritional status of the most vulnerable. Where food supplies are insecure, people tend to shift to less healthy diets and consume more "unsafe foods" – in which chemical, microbiological and other hazards pose health risks.

The Second International Conference on Nutrition (ICN2), held in Rome in November 2014, reiterated the importance of food safety in achieving better human nutrition through healthy nutritious diets. Improving food safety is thus a key in achieving Sustainable Development Goals. Governments should make food safety a public health priority, as they play a pivotal role in developing policies and regulatory frameworks, establishing and implementing effective food safety systems that ensure that food producers and suppliers along the whole food chain operate responsibly and supply safe food to consumers.

Food can become contaminated at any point of production and distribution, and the primary responsibility lies with food producers. Yet a large proportion of foodborne disease incidents are caused by foods improperly prepared or mishandled at home, in food service establishments or markets. Not all food handlers and consumers understand the roles they must play, such as adopting basic hygienic practices when buying, selling and preparing food to protect their health and that of the wider community.

Everyone can contribute to making food safe. Here are some examples of effective actions:

Policy-makers can

- build and maintain adequate food systems and infrastructures (e.g. laboratories) to respond to and manage food safety risks along the entire food chain, including during emergencies;
- foster multi-sectoral collaboration among public health, animal health, agriculture and other sectors for better communication and joint action;
- integrate food safety into broader food policies and

programmes (e.g. nutrition and food security);
- think globally and act locally to ensure the food produce domestically be safe internationally.

Food handlers and consumers can:
- know the food they use (read labels on food package, make an informed choice, become familiar with common food hazards);
- handle and prepare food safely, practicing the WHO Five Keys to Safer Food at home, or when selling at restaurants or at local markets;
- grow fruits and vegetables using the WHO Five Keys to Growing Safer Fruits and Vegetables to decrease microbial contamination.

WHO response

WHO aims to facilitate global prevention, detection and response to public health threats associated with unsafe food. Ensuring consumer trust in their authorities, and confidence in the safe food supply, is an outcome that WHO works to achieve.

To do this, WHO helps Member States build capacity to prevent, detect and manage foodborne risks by:
- providing independent scientific assessments on microbiological and chemical hazards that form the basis for international food standards, guidelines and recommendations, known as the Codex Alimentarius, to ensure food is safe wherever it originates;
- assessing the safety of new technologies used in food production, such as genetic modification and nanotechnology;
- helping improve national food systems and legal frameworks, and implement adequate infrastructure to manage food safety risks. The International Food Safety Authorities Network (INFOSAN) was developed by WHO and the UN Food and Agriculture Organization (FAO) to rapidly share information during food safety emergencies;
- promoting safe food handling through systematic disease prevention and awareness programmes, through the WHO Five Keys to Safer Food message and training materials; and
- advocating for food safety as an important component of health security and for integrating food safety into national policies and programmes in line with the International Health Regulations (IHR - 2005).

WHO works closely with FAO, the World Organization for Animal Health (OIE) and other international organizations to ensure food safety along the entire food chain from production to consumption.

4. HEALTHY DIET

KEY FACTS
- A healthy diet helps to protect against malnutrition in all its forms, as well as noncommunicable diseases (NCDs), including such as diabetes, heart disease, stroke and cancer.
- Unhealthy diet and lack of physical activity are leading global risks to health.
- Healthy dietary practices start early in life – breastfeeding fosters healthy growth and improves cognitive development, and may have longer term health benefits such as reducing the risk of becoming overweight or obese and developing NCDs later in life.
- Energy intake (calories) should be in balance with energy expenditure. To avoid unhealthy weight gain, total fat should not exceed 30% of total energy intake (1, 2, 3). Intake of saturated fats should be less than 10% of total energy intake, and intake of trans-fats less than 1% of total energy intake, with a shift in fat consumption away from saturated fats and trans-fats to unsaturated fats (3), and towards the goal of eliminating industrially-produced trans-fats (4, 5, 6).
- Limiting intake of free sugars to less than 10% of total energy intake (2, 7) is part of a healthy diet. A further reduction to less than 5% of total energy intake is suggested for additional health benefits (7).
- Keeping salt intake to less than 5 g per day (equivalent to sodium intake of less than 2 g per day) helps to prevent hypertension, and reduces the risk of heart disease and stroke in the adult population (8).
- WHO Member States have agreed to reduce the global population's intake of salt by 30% by 2025; they have also agreed to halt the rise in diabetes and obesity in adults and adolescents as well as in childhood overweight by 2025 (9, 10).

Overview

Consuming a healthy diet throughout the life-course helps to prevent malnutrition in all its forms as well as a range of noncommunicable diseases (NCDs) and conditions. However, increased production of processed foods, rapid urbanization and changing lifestyles have led to a shift in dietary patterns. People are now consuming more foods high in energy, fats, free sugars and salt/sodium, and many people do not eat enough fruit, vegetables and other dietary fibre such as whole grains.

The exact make-up of a diversified, balanced and healthy diet will vary depending on individual

characteristics (e.g. age, gender, lifestyle and degree of physical activity), cultural context, locally available foods and dietary customs. However, the basic principles of what constitutes a healthy diet remain the same.

For adults

A healthy diet includes the following:

- Fruit, vegetables, legumes (e.g. lentils and beans), nuts and whole grains (e.g. unprocessed maize, millet, oats, wheat and brown rice).
- At least 400 g (i.e. five portions) of fruit and vegetables per day *(2)*, excluding potatoes, sweet potatoes, cassava and other starchy roots.
- Less than 10% of total energy intake from free sugars *(2, 7)*, which is equivalent to 50 g (or about 12 level teaspoons) for a person of healthy body weight consuming about 2000 calories per day, but ideally is less than 5% of total energy intake for additional health benefits *(7)*. Free sugars are all sugars added to foods or drinks by the manufacturer, cook or consumer, as well as sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates.
- Less than 30% of total energy intake from fats *(1, 2, 3)*. Unsaturated fats (found in fish, avocado and nuts, and in sunflower, soybean, canola and olive oils) are preferable to saturated fats (found in fatty meat, butter, palm and coconut oil, cream, cheese, ghee and lard) and *trans*-fats of all kinds, including both industrially-produced *trans*-fats (found in baked and fried foods, and pre-packaged snacks and foods, such as frozen pizza, pies, cookies, biscuits, wafers, and cooking oils and spreads) and ruminant *trans*-fats (found in meat and dairy foods from ruminant animals, such as cows, sheep, goats and camels). It is suggested that the intake of saturated fats be reduced to less than 10% of total energy intake and *trans*-fats to less than 1% of total energy intake *(5)*. In particular, industrially-produced *trans*-fats are not part of a healthy diet and should be avoided *(4, 6)*.
- Less than 5 g of salt (equivalent to about one teaspoon) per day *(8)*. Salt should be iodized.

For infants and young children

In the first 2 years of a child's life, optimal nutrition fosters healthy growth and improves cognitive development. It also reduces the risk of becoming overweight or obese and developing NCDs later in life.

Advice on a healthy diet for infants and children is similar to that for adults, but the following elements are also important:

- Infants should be breastfed exclusively during the first 6 months of life.
- Infants should be breastfed continuously until 2 years of age and beyond.
- From 6 months of age, breast milk should be complemented with a variety of adequate, safe and nutrient-dense foods. Salt and sugars should not be added to complementary foods.

Practical advice on maintaining a healthy diet

Fruit and vegetables

Eating at least 400 g, or five portions, of fruit and vegetables per day reduces the risk of NCDs *(2)* and helps to ensure an adequate daily intake of dietary fibre.

Fruit and vegetable intake can be improved by:

- always including vegetables in meals;
- eating fresh fruit and raw vegetables as snacks;
- eating fresh fruit and vegetables that are in season; and
- eating a variety of fruit and vegetables.

Fats

Reducing the amount of total fat intake to less than 30% of total energy intake helps to prevent unhealthy weight gain in the adult population *(1, 2, 3)*. Also, the risk of developing NCDs is lowered by:

- reducing saturated fats to less than 10% of total energy intake;
- reducing *trans*-fats to less than 1% of total energy intake; and
- replacing both saturated fats and *trans*-fats with unsaturated fats *(2, 3)* – in particular, with polyunsaturated fats.

Fat intake, especially saturated fat and industrially-produced *trans*-fat intake, can be reduced by:

- steaming or boiling instead of frying when cooking;
- replacing butter, lard and ghee with oils rich in polyunsaturated fats, such as soybean, canola (rapeseed), corn, safflower and sunflower oils;
- eating reduced-fat dairy foods and lean meats, or trimming visible fat from meat; and
- limiting the consumption of baked and fried foods, and pre-packaged snacks and foods (e.g. doughnuts, cakes, pies, cookies, biscuits and wafers) that contain industrially-produced *trans*-fats.

Salt, sodium and potassium

Most people consume too much sodium through salt (corresponding to consuming an average of 9–12 g of salt per day) and not enough potassium (less than 3.5 g). High sodium intake and insufficient potassium intake contribute to high blood pressure, which in turn increases the risk of heart disease and stroke *(8, 11)*.

Reducing salt intake to the recommended level of less than 5 g per day could prevent 1.7 million deaths each year *(12)*.

People are often unaware of the amount of salt they consume. In many countries, most salt comes from processed foods (e.g. ready meals; processed meats such as bacon, ham and salami; cheese; and salty snacks) or from foods consumed frequently in large amounts (e.g. bread). Salt is also added to foods during cooking (e.g. bouillon, stock cubes, soy sauce and fish sauce) or at the point of consumption (e.g. table salt).

Salt intake can be reduced by:

- limiting the amount of salt and high-sodium condiments (e.g. soy sauce, fish sauce and bouillon) when cooking and preparing foods;
- not having salt or high-sodium sauces on the table;
- limiting the consumption of salty snacks; and
- choosing products with lower sodium content.

Some food manufacturers are reformulating recipes to reduce the sodium content of their products, and people should be encouraged to check nutrition labels to see how much sodium is in a product before purchasing or consuming it.

Potassium can mitigate the negative effects of elevated sodium consumption on blood pressure. Intake of potassium can be increased by consuming fresh fruit and vegetables.

Sugars

In both adults and children, the intake of free sugars should be reduced to less than 10% of total energy intake *(2, 7)*. A reduction to less than 5% of total energy intake would provide additional health benefits *(7)*.

Consuming free sugars increases the risk of dental caries (tooth decay). Excess calories from foods and drinks high in free sugars also contribute to unhealthy weight gain, which can lead to overweight and obesity. Recent evidence also shows that free sugars influence blood pressure and serum lipids, and suggests that a reduction in free sugars intake reduces risk factors for cardiovascular diseases *(13)*.

Sugars intake can be reduced by:

- limiting the consumption of foods and drinks containing high amounts of sugars, such as sugary snacks, candies and sugar-sweetened beverages (i.e. all types of beverages containing free sugars – these include carbonated or non-carbonated soft drinks, fruit or vegetable juices and drinks, liquid and powder concentrates, flavoured water, energy and sports drinks, ready-to-drink tea, ready-to-drink coffee and flavoured milk drinks); and
- eating fresh fruit and raw vegetables as snacks instead of sugary snacks.

How to promote healthy diets

Diet evolves over time, being influenced by many social and economic factors that interact in a complex manner to shape individual dietary patterns. These factors include income, food prices (which will affect the availability and affordability of healthy foods), individual preferences and beliefs, cultural traditions, and geographical and environmental aspects (including climate change). Therefore, promoting a healthy food environment – including food systems that promote a diversified, balanced and healthy diet – requires the involvement of multiple sectors and stakeholders, including government, and the public and private sectors.

Governments have a central role in creating a healthy food environment that enables people to adopt and maintain healthy dietary practices. Effective actions by policy-makers to create a healthy food environment include the following:

- Creating coherence in national policies and investment plans – including trade, food and agricultural policies – to promote a healthy diet and protect public health through:
    - increasing incentives for producers and retailers to grow, use and sell fresh fruit and vegetables;
    - reducing incentives for the food industry to continue or increase production of processed foods containing high levels of saturated fats, *trans*-fats, free sugars and salt/sodium;
    - encouraging reformulation of food products to reduce the contents of saturated fats, *trans*-fats, free sugars and salt/sodium, with the goal of eliminating industrially-produced *trans*-fats;
    - implementing the WHO recommendations on the marketing of foods and non-alcoholic beverages to children;
    - establishing standards to foster healthy dietary practices through ensuring the availability of healthy, nutritious, safe and affordable foods in pre-schools, schools, other public institutions and the workplace;
    - exploring regulatory and voluntary instruments (e.g. marketing regulations and nutrition labelling policies), and economic incentives or disincentives (e.g. taxation and subsidies) to promote a healthy diet; and
    - encouraging transnational, national and local food services and catering outlets to improve the nutritional quality of their foods – ensuring the availability and affordability of healthy choices – and review portion sizes and pricing.
- Encouraging consumer demand for healthy foods and meals through:

- promoting consumer awareness of a healthy diet;
- developing school policies and programmes that encourage children to adopt and maintain a healthy diet;
- educating children, adolescents and adults about nutrition and healthy dietary practices;
- encouraging culinary skills, including in children through schools;
- supporting point-of-sale information, including through nutrition labelling that ensures accurate, standardized and comprehensible information on nutrient contents in foods (in line with the Codex Alimentarius Commission guidelines), with the addition of front-of-pack labelling to facilitate consumer understanding; and
- providing nutrition and dietary counselling at primary health-care facilities.

- Promoting appropriate infant and young child feeding practices through:

  - implementing the International Code of Marketing of Breast-milk Substitutes and subsequent relevant World Health Assembly resolutions;
  - implementing policies and practices to promote protection of working mothers; and
  - promoting, protecting and supporting breastfeeding in health services and the community, including through the Baby-friendly Hospital Initiative.

WHO response

The "WHO Global Strategy on Diet, Physical Activity and Health" *(14)* was adopted in 2004 by the Health Assembly. The strategy called on governments, WHO, international partners, the private sector and civil society to take action at global, regional and local levels to support healthy diets and physical activity.

In 2010, the Health Assembly endorsed a set of recommendations on the marketing of foods and non-alcoholic beverages to children *(15)*. These recommendations guide countries in designing new policies and improving existing ones to reduce the impact on children of the marketing of foods and non-alcoholic beverages to children. WHO has also developed region-specific tools (such as regional nutrient profile models) that countries can use to implement the marketing recommendations.

In 2012, the Health Assembly adopted a "Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition" and six global nutrition targets to be achieved by 2025, including the reduction of stunting, wasting and overweight in children, the improvement of breastfeeding, and the reduction of anaemia and low birthweight *(9)*.

In 2013, the Health Assembly agreed to nine global voluntary targets for the prevention and control of NCDs. These targets include a halt to the rise in diabetes and obesity, and a 30% relative reduction in the intake of salt by 2025. The "Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–2020" *(10)* provides guidance and policy options for Member States, WHO and other United Nations agencies to achieve the targets.

With many countries now seeing a rapid rise in obesity among infants and children, in May 2014 WHO set up the Commission on Ending Childhood Obesity. In 2016, the Commission proposed a set of recommendations to successfully tackle childhood and adolescent obesity in different contexts around the world *(16)*.

In November 2014, WHO organized, jointly with the Food and Agriculture Organization of the United Nations (FAO), the Second International Conference on Nutrition (ICN2). ICN2 adopted the Rome Declaration on Nutrition *(17)*, and the Framework for Action *(18)* which recommends a set of policy options and strategies to promote diversified, safe and healthy diets at all stages of life. WHO is helping countries to implement the commitments made at ICN2.

In May 2018, the Health Assembly approved the 13th General Programme of Work (GPW13), which will guide the work of WHO in 2019–2023 *(19)*. Reduction of salt/sodium intake and elimination of industrially-produced *trans*-fats from the food supply are identified in GPW13 as part of WHO's priority actions to achieve the aims of ensuring healthy lives and promote well-being for all at all ages. To support Member States in taking necessary actions to eliminate industrially-produced *trans*-fats, WHO has developed a roadmap for countries (the REPLACE action package) to help accelerate actions *(6)*.

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5. MALARIA

KEY FACTS

- **Malaria is a life-threatening disease caused by** parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is preventable and curable.
- In 2018, there were an estimated 228 million cases of malaria worldwide.
- The estimated number of malaria deaths stood at 405 000 in 2018.
- Children aged under 5 years are the most vulnerable group affected by malaria; in 2018, they accounted for 67% (272 000) of all malaria deaths worldwide.
- The WHO African Region carries a disproportionately high share of the global malaria burden. In 2018, the region was home to 93% of malaria cases and 94% of malaria deaths.
- Total funding for malaria control and elimination reached an estimated US$ 2.7 billion in 2018. Contributions from governments of endemic countries amounted to US$ 900 million, representing 30% of total funding.

Malaria is caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected female *Anopheles* mosquitoes, called "malaria vectors." There are 5 parasite species that cause malaria in humans, and 2 of these species – *P. falciparum* and *P. vivax* – pose the greatest threat.

- In 2018, *P. falciparum* accounted for 99.7% of estimated malaria cases in the WHO African Region 50% of cases in the WHO South-East Asia Region, 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific.
- *P. vivax* is the predominant parasite in the WHO Region of the Americas, representing 75% of malaria cases.

Symptoms

Malaria is an acute febrile illness. In a non-immune individual, symptoms usually appear 10–15 days after the infective mosquito bite. The first symptoms – fever, headache, and chills – may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death.

Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ failure is also frequent. In malaria endemic areas, people may develop partial immunity, allowing asymptomatic infections to occur.

Who is at risk?

In 2018, nearly half of the world's population was at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, the WHO regions of South-East Asia, Eastern Mediterranean, Western Pacific, and the Americas are also at risk.

Some population groups are at considerably higher risk of contracting malaria, and developing severe disease, than others. These include infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travellers. National malaria control programmes need to take special measures to protect these population groups from malaria infection, taking into consideration their specific circumstances.

Disease burden

According to the latest World malaria report, released in December 2019, there were 228 million cases of malaria in 2018 compared to 231 million cases in 2017. The estimated number of malaria deaths stood at 405 000 in 2018, compared with 416 000 deaths in 2017.

The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2018, the region was home to 93% of malaria cases and 94% of malaria deaths.

In 2018, 6 countries accounted for more than half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (12%), Uganda (5%), and Côte d'Ivoire, Mozambique and Niger (4% each).

Children under 5 years of age are the most vulnerable group affected by malaria; in 2018, they accounted for 67% (272 000) of all malaria deaths worldwide.

Transmission

In most cases, malaria is transmitted through the bites of female *Anopheles* mosquitoes. There are more than 400 different species of *Anopheles* mosquito; around 30 are malaria vectors of major importance. All of the important vector species bite between dusk and dawn. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment.

Anopheles mosquitoes lay their eggs in water, which hatch into larvae, eventually emerging as adult mosquitoes. The female mosquitoes seek a blood meal to nurture their eggs. Each species of *Anopheles* mosquito has its own preferred aquatic habitat; for example, some prefer small, shallow collections of fresh water, such as puddles and hoof prints, which are abundant during the rainy season in tropical countries.

Transmission is more intense in places where the mosquito lifespan is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. The long lifespan and strong human-biting habit of the African vector species is the main reason why approximately 90% of the world's malaria cases are in Africa.

Transmission also depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees.

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides complete protection, it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in Africa occur in young children, whereas in areas with less transmission and low immunity, all age groups are at risk.

Prevention

Vector control is the main way to prevent and reduce malaria transmission. If coverage of vector control interventions within a specific area is high enough, then a measure of protection will be conferred across the community.

WHO recommends protection for all people at risk of malaria with effective malaria vector control. Two forms of vector control – insecticide-treated mosquito nets and indoor residual spraying – are effective in a wide range of circumstances.

Insecticide-treated mosquito nets

Sleeping under an insecticide-treated net (ITN) can reduce contact between mosquitoes and humans by providing both a physical barrier and an insecticidal effect. Population-wide protection can result from the killing of mosquitoes on a large scale where there is high access and usage of such nets within a community.

In 2018, about half of all people at risk of malaria in Africa were protected by an insecticide-treated net, compared to 29% in 2010. However, ITN coverage has been at a standstill since 2016.

Indoor spraying with residual insecticides

Indoor residual spraying (IRS) with insecticides is another powerful way to rapidly reduce malaria transmission. It involves spraying the inside of housing structures with an insecticide, typically once or twice per year. To confer significant community protection, IRS should be implemented at a high level of coverage.

Globally, IRS protection declined from a peak of 5% in 2010 to 2% in 2018, with decreases seen across all WHO regions, apart from the WHO Eastern Mediterranean Region. The declines in IRS coverage are occurring as countries switch from pyrethroid insecticides to more expensive alternatives to mitigate mosquito resistance to pyrethroids.

Antimalarial drugs

Antimalarial medicines can also be used to prevent malaria. For travellers, malaria can be prevented through chemoprophylaxis, which suppresses

the blood stage of malaria infections, thereby preventing malaria disease. For pregnant women living in moderate-to-high transmission areas, WHO recommends intermittent preventive treatment with sulfadoxine-pyrimethamine, at each scheduled antenatal visit after the first trimester. Similarly, for infants living in high-transmission areas of Africa, 3 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine are recommended, delivered alongside routine vaccinations.

Since 2012, WHO has recommended seasonal malaria chemoprevention as an additional malaria prevention strategy for areas of the Sahel sub-region of Africa. The strategy involves the administration of monthly courses of amodiaquine plus sulfadoxine-pyrimethamine to all children under 5 years of age during the high transmission season.

Insecticide resistance

Since 2000, progress in malaria control has resulted primarily from expanded access to vector control interventions, particularly in sub-Saharan Africa. However, these gains are threatened by emerging resistance to insecticides among *Anopheles* mosquitoes. According to the latest World malaria report, 73 countries reported mosquito resistance to at least 1 of the 4 commonly-used insecticide classes in the period 2010-2018. In 27 countries, mosquito resistance was reported to all of the main insecticide classes.

Despite the emergence and spread of mosquito resistance to pyrethroids, insecticide-treated nets continue to provide a substantial level of protection in most settings. This was evidenced in a large 5-country study coordinated by WHO between 2011 and 2016.

While the findings of this study are encouraging, WHO continues to highlight the urgent need for new and improved tools in the global response to malaria. To prevent an erosion of the impact of core vector control tools, WHO also underscores the critical need for all countries with ongoing malaria transmission to develop and apply effective insecticide resistance management strategies.

Diagnosis and treatment

Early diagnosis and treatment of malaria reduces disease and prevents deaths. It also contributes to reducing malaria transmission. The best available treatment, particularly for *P. falciparum* malaria, is artemisinin-based combination therapy (ACT).

WHO recommends that all cases of suspected malaria be confirmed using parasite-based diagnostic testing (either microscopy or rapid diagnostic test) before administering treatment. Results of parasitological confirmation can be available in 30 minutes or less. Treatment, solely on the basis of symptoms should only be considered when a parasitological diagnosis is not possible. More detailed recommendations are available in the third edition of the "WHO Guidelines for the treatment of malaria", published in April 2015.

Antimalarial drug resistance

Resistance to antimalarial medicines is a recurring problem. Resistance of *P. falciparum* malaria parasites to previous generations of medicines, such as chloroquine and sulfadoxine-pyrimethamine (SP), became widespread in the 1950s and 1960s, undermining malaria control efforts and reversing gains in child survival.

Protecting the efficacy of antimalarial medicines is critical to malaria control and elimination. Regular monitoring of drug efficacy is needed to inform treatment policies in malaria-endemic countries, and to ensure early detection of, and response to, drug resistance.

In 2013, WHO launched the *Emergency response to artemisinin resistance (ERAR)* in the Greater Mekong subregion (GMS), a high-level plan of attack to contain the spread of drug-resistant parasites and to provide life-saving tools for all populations at risk of malaria. But even as this work was under way, additional pockets of resistance emerged independently in new geographic areas of the subregion. In parallel, there were reports of increased resistance to ACT partner drugs in some settings. A new approach was needed to keep pace with the changing malaria landscape.

At the World Health Assembly in May 2015, WHO launched the Strategy for malaria elimination in the Greater Mekong subregion (2015–2030), which was endorsed by all the countries in the subregion. Urging immediate action, the strategy calls for the elimination of all species of human malaria across the region by 2030, with priority action targeted to areas where multidrug resistant malaria has taken root.

With technical guidance from WHO, all countries in the region have developed national malaria elimination plans. Together with partners, WHO is providing ongoing support for country elimination efforts through the Mekong Malaria Elimination programme, an initiative that evolved from the ERAR

Surveillance

Surveillance entails tracking of the disease and programmatic responses, and taking action based on the data received. Currently, many countries with a high burden of malaria have weak surveillance systems and are not in a position to assess disease distribution and trends, making it difficult to optimize responses and respond to outbreaks.

Effective surveillance is required at all points on the path to malaria elimination. Stronger malaria surveillance systems are urgently needed to enable a timely and effective malaria response in endemic regions, to prevent outbreaks and resurgences, to track progress, and to hold governments and the global malaria community accountable.

In March 2018, WHO released a reference manual on malaria surveillance, monitoring and evaluation. The manual provides information on global surveillance standards and guides countries in their efforts to strengthen surveillance systems.

Elimination

Malaria elimination is defined as the interruption of local transmission of a specified malaria parasite species in a defined geographical area as a result of deliberate activities. Continued measures are required to prevent re-establishment of transmission. Malaria eradication is defined as the permanent reduction to zero of the worldwide incidence of malaria infection caused by human malaria parasites as a result of deliberate activities. Interventions are no longer required once eradication has been achieved.

Globally, the elimination net is widening, with more countries moving towards the goal of zero malaria. In 2018, 27 countries reported fewer than 100 indigenous cases of the disease, up from 17 countries in 2010.

Countries that have achieved at least 3 consecutive years of 0 indigenous cases of malaria are eligible to apply for the WHO certification of malaria elimination. Over the last decade, 10 countries have been certified by the WHO Director-General as malaria-free: Morocco (2010), Turkmenistan (2010), Armenia (2011), Maldives (2015), Sri Lanka (2016), Kyrgyzstan (2016), Paraguay (2018), Uzbekistan (2018), Algeria (2019) and Argentina (2018). The WHO *Framework for Malaria Elimination* (2017) provides a detailed set of tools and strategies for achieving and maintaining elimination.

Vaccines against malaria

RTS,S/AS01 (RTS,S) is the first and, to date, the only vaccine to show that it can significantly reduce malaria, and life-threatening severe malaria, in young African children. It acts against *P. falciparum*, the most deadly malaria parasite globally and the most prevalent in Africa. Among children who received 4 doses in large-scale clinical trials, the vaccine prevented approximately 4 in 10 cases of malaria over a 4-year period.

In view of its public health potential, WHO's top advisory bodies for malaria and immunization have jointly recommended phased introduction of the vaccine in selected areas of sub-Saharan Africa. Three countries – Ghana, Kenya and Malawi – began introducing the vaccine in selected areas of moderate and high malaria transmission in 2019. Vaccinations are being provided through each country's routine immunization programme.

The pilot programme will address several outstanding questions related to the public health use of the vaccine. It will be critical for understanding how best to deliver the recommended 4 doses of RTS,S; the vaccine's potential role in reducing childhood deaths; and its safety in the context of routine use.

This WHO-coordinated programme is a collaborative effort with Ministries of Health in Ghana, Kenya and Malawi and a range of in-country and international partners, including PATH, a non-profit organization, and GSK, the vaccine developer and manufacturer.

Financing for the vaccine programme has been mobilized through a collaboration between 3 major global health funding bodies: Gavi, the Vaccine Alliance, the Global Fund to Fight AIDS, Tuberculosis and Malaria, and Unitaid.

WHO response

WHO Global technical strategy for malaria 2016-2030

The WHO *Global technical strategy for malaria 2016-2030* – adopted by the World Health Assembly in May 2015 – provides a technical framework for all malaria-endemic countries. It is intended to guide and support regional and country programmes as they work towards malaria control and elimination.

The Strategy sets ambitious but achievable global targets, including:

- reducing malaria case incidence by at least 90% by 2030;
- reducing malaria mortality rates by at least 90% by 2030;
- eliminating malaria in at least 35 countries by 2030;
- preventing a resurgence of malaria in all countries that are malaria-free.

This Strategy was the result of an extensive consultative process that spanned 2 years and involved the participation of more than 400 technical experts from 70 Member States.

The Global Malaria Programme

The WHO Global Malaria Programme coordinates WHO's global efforts to control and eliminate malaria by:

- setting, communicating and promoting the adoption of evidence-based norms, standards, policies, technical strategies, and guidelines;
- keeping independent score of global progress;
- developing approaches for capacity building, systems strengthening, and surveillance; and

- identifying threats to malaria control and elimination as well as new areas for action.

The Programme is supported and advised by the Malaria Policy Advisory Committee (MPAC), a group of global malaria experts appointed following an open nomination process. The mandate of MPAC is to provide strategic advice and technical input, and extends to all aspects of malaria control and elimination, as part of a transparent, responsive and credible policy-setting process.

"High burden high impact approach"

At the World Health Assembly in May 2018, the WHO Director-General, Dr Tedros Adhanom Ghebreyesus, called for an aggressive new approach to jump-start progress against malaria. A new country-driven response – " High burden to high impact" – was launched in Mozambique in November 2018.

The approach is currently being driven by the 11 countries that carry a high burden of the disease (Burkina Faso, Cameroon, Democratic Republic of the Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda and United Republic of Tanzania). Key elements include:

1. political will to reduce the toll of malaria;
2. strategic information to drive impact;
3. better guidance, policies and strategies; and
4. a coordinated national malaria response.

Catalysed by WHO and the RBM Partnership to End Malaria, "High burden to high impact" builds on the principle that no one should die from a disease that can be prevented and diagnosed, and that is entirely curable with available treatments.