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Chandan M. THOMAS  
(Bradenton, FL)

Michihisa TOHDA  
(Tokyo)

Li TONG  
(Xining, Qinghai)
CONTENTS Volume 16, Number 1, 2022

Original Article

1-7 Stability of extemporaneously compounded 5-fluorouracil utilizing high performance liquid chromatography. 
Caitlin Komm, Ankit Rochani, Timothy Fox, Gagan Kaushal

8-13 Association between serum vitamin D level and uterine fibroid in premenopausal women in Indian population. 
Ruchi Kumari, Banashree Nath, Kashika, Harsha S. Gaikwad, Manjula Sharma


23-29 Relationship between low back pain and stress urinary incontinence at 3 months postpartum. 
Megumi Mutaguchi, Ryoko Murayama, Yoko Takeishi, Maiko Kawajiri, Akari Yoshida, Yasuka Nakamura, Toyoko Yoshizawa, Mikako Yoshida

Brief Report

30-36 Waning COVID-19 vaccine effectiveness in Japan. 
Junko Kurita, Tamie Sugawara, Yasushi Ohkusa

37-42 Effectiveness of ultrasound-guided pelvic floor muscle training in improving prolonged urinary incontinence after robot-assisted radical prostatectomy. 
Akiko Matsunaga, Mikako Yoshida, Yusuke Shinoda, Yusuke Sato, Jun Kamei, Aya Niimi, Tetsuya Fujimura, Haruki Kume, Yasuhiko Igawa

Communication

43-46 Probiotic microbes: Are their anti-melanogenicity and longevity promoting activities closely linked through the major "pathogenic" kinase PAK1? 
Hiroshi Maruta, Mok-Ryeon Ahn

Letter to the Editor

Anabel Franco-Moreno, María Soledad Acedo-Gutiérrez, Rodolfo Romero-Pareja, Juan Torres-Macho

49-51 Arsenic intoxication with renal failure managed with hemodialysis alone: A case report. 
Ayush Agarwal, Santhosh Kumar KN, Pankaj Jorwal, Javed Ahsan Quadri, Gaurav Gupta, Ashutosh Biswas
Stability of extemporaneously compounded 5-fluorouracil utilizing high performance liquid chromatography

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SUMMARY The goal of the current study is to determine stability of compounded 5-fluorouracil (5-FU) in Intravia™ bags and CADD™ cassettes stored up to 15 days under refrigeration (2-8°C) and room temperature (25°C with 60% relative humidity), with four different concentrations (20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL) and two diluents (0.9% sodium chloride and 5% dextrose). A stability-indicating high-performance liquid chromatography (HPLC) method was developed to analyze the 5-FU concentrations. The stability of compounded 5-FU infusions was investigated using this method. Two samples from each storage condition were assessed for stability on days 0, 4, 7, 10, and 15 as per United States Pharmacopeia (USP) guidelines. The assay of 5-FU was done utilizing a calibrated stability-indicating HPLC method. The stability-indicating HPLC assay showed 5-FU completely degraded within 1 hour in basic conditions. No cloudiness or color change was observed during the stability study. Precipitation was observed in the CADD™ cassettes at day 15 in both storage conditions and at day 10 in a single room-temperature CADD™ cassette for 40 mg/mL in 5% dextrose (D5W). HPLC assay revealed the infusions in CADD™ cassettes retained greater than 90% of the initial concentrations of 5-FU for 15 days stored at room temperature (25°C and 60% relative humidity) and for 10 days at refrigeration (2-8°C). Intravia™ bags retained stability through 15 days for all the compounded 5-FU concentrations and both the storage conditions. 5-FU infusions in both CADD™ cassettes and Intravia™ bags were stable for extendable periods in multiple concentrations compared to recommended guidelines for hospital use.

Keywords 5-Fluorouracil, compounding, stability, high performance liquid chromatography

1. Introduction

5-Fluorouracil (5-FU), a nucleotide analog and antimetabolite drug, has been approved in the treatment of head and neck cancer, breast cancer, and gastrointestinal cancer, and is one of the oldest known oncologic agent. It works by competing with uracil in RNA with one active metabolite, 5-fluorouridine monophosphate (F-UMP), and inhibiting the synthesis of thymidine-phosphate products via inhibition of thymidylate synthase with another active metabolite, fluorodeoxyuridine monophosphate (F-dUMP). Together, these processes stop growth of cancer cells by inhibiting new DNA and RNA formation, thus preventing the cancer cells from replicating. Dosing varies by type of cancer, but ranges from 200 mg/m² to 3,000 mg/m² per the patient’s body surface area (BSA) (1). 5-FU is commonly compounded in various concentrations at up to 50 mg/mL, either in CADD™ cassettes or Intravia™ bags, as well as other chemotherapy devices such as the Braun Easypump® (2). In order to ensure stability and quality of the compounded product and appropriate assignment of beyond-use dating (BUD), stability studies are essential. Health system pharmacies have often struggled to confidently extrapolate stability data from widely varied drug concentrations, differing diluents, and studies specific to unique elastomeric devices, which may or may not be composed of materials similar to the bags or cassettes utilized by the particular pharmacy. While manufacturers often do these studies for their commercial pre-made products in house, extemporaneously compounded 5-FU requires its own studies for BUD assignment as required by the joint commission and United States Pharmacopeia (USP) guidelines (3).

In this study, we present validated stability studies utilizing high performance liquid chromatography (HPLC) of extemporaneously compounded 5-FU in both 5% dextrose (D5W) and 0.9% sodium chloride (NS), respectively, over the course of 15 days at room
temperature and under refrigeration, as they would be used in the hospital setting. Validation of the method used was achieved using International Council for Harmonisation (ICH) guidelines for stability studies for publication and the studies by Sinha et al. (4) and Galanti et al. (5) demonstrated long term stability of 90-95% of 5-FU in polyvinyl chloride (PVC) and ethylene-vinyl acetate (EVA) bags and for use during freeze thaw cycles, by use of HPLC methodologies. Both PVC and EVA containers produced no precipitation when the drug was frozen for 79 days and then thawed in the microwave and stored for 28 days under refrigeration. This resulted in little to no loss of the efficacious form of the drug in 0.9% sodium chloride. Roberts et al. (2) also demonstrated the stability of 5-FU for up to 21 days in both sodium chloride and dextrose in the Braun Easypump6.

Additionally, the stability of 5-FU admixtures with folic acid was studied by Milano et al. (6) and remained within 10% of the initial concentrations. While the previous studies were consistent regarding stability in EVA bags and PVC bags, it is worth noting that 5-FU precipitated in PVC reservoirs when stored under refrigerated conditions (as demonstrated by Martel et al. (7)), and in irradiated plastic bags (as demonstrated by Dine et al. (8)). Barberi-Heyob et al. (9) studied 5-FU in cassettes and found them to be stable for 7 days at refrigeration and 14 days at room temperature. Previous studies often utilized only one or two concentrations of 5-FU, typically in one device and diluent. However, in our study we utilized three different concentrations and undiluted 5-FU, in two different reservoirs, and in two different diluents. This allows for the analysis of the stability in each device, and at each concentration and diluent as it would be compounded and provides a more comprehensive understanding of 5-FU stability. As 5-FU is dosed by weight (1), institutions will often utilize multiple concentrations for compounding to optimize dosing regimens.

5-FU, as an analytical molecule, is particularly susceptible to alkaline conditions when initially put into solution, as demonstrated by Sinha et al. (4). Formulations with proven stability past manufacturer recommendations would reduce drug waste, potentially mitigate drug shortages, and improve the efficiencies of hospitals and health systems. Hence, in the present study we developed a simple and robust stability-indicating HPLC method to determine the stability of the intravenous 5-FU formulations compounded at the Jefferson Home Infusion Services (JHIS). The objective of this study was to determine whether injectable 5-FU prepared at 50 mg/mL, 40 mg/mL, 30 mg/mL, and 20 mg/mL concentrations in 5% dextrose (D5W) and normal saline (NS) and stored in CADD™ cassettes and Intravia™ bags were stable for up to 15 days when stored at refrigeration (2-8°C) and room temperature conditions (25°C and 60% relative humidity).

2. Materials and Methods

2.1. Materials

All chemicals were of analytical grade. 5-FU (Lot# A0305173, Acros Organics), potassium dihydrogen phosphate (Lot#Q18D028), sodium hydroxide (Lot# 995312), hydrochloric acid (Lot#167712), methanol (HPLC grade, Lot#197075), and all other chemicals were procured from Fisher Chemicals (Fair Lawn, NJ, USA). Fluorouracil for Injection USP, 5,000 mg/100 mL (Lot# P2002967 and expiration: 05/22) per pharmacy bulk package, was purchased from Fresenius Kabi (Lake Zurich, IL, USA). Intravia™ bags, dextrose 5% (Lot# Y343428, and expiration: 12/21), and 0.9% sodium chloride (Lot# Y344029, and expiration: 12/21) were procured Baxter Healthcare Corporation (Deerfield, IL, USA). CADD™ cassettes were purchased through Smiths Medical (Minneapolis, MN, USA). Intravia™ bags are composed of a polyolefin-based material. The solution contact layer is a blend of polypropylene, polyethylene, polyamide, and SEBS (Styrene Ethylene Butylene Styrene). The fluid path of CADD™ cassettes are composed of polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), and polypropylene.

2.2. Compounding of the 5-FU formulations

All compounding was performed in a certified, Class II, Type B2 biological safety cabinet by Jefferson Home Infusion Services (JHIS) pharmacy personnel. All study bags and cassettes were filled to 100 mL total volume with the appropriate volumes of drug (for 50 mg/mL) and drug/diluent (for 20, 30, and 40 mg/mL concentrations), packaged in chemotherapy transport bags, and immediately brought to the study site for proper storage and initiation of analysis.

2.3. Test groups and sampling timeline

Two temperature conditions (room temperature (25°C and 60% relative humidity) and refrigeration (2-8°C)) were used for all compounded preparations of 5-FU. Samples included 5-FU preparations as follows: 50 mg/mL (undiluted), 40 mg/mL, 30 mg/mL, and 20 mg/mL, one for each reservoir, diluent (either D5W or NS), and temperature, with a total number of 140 samples. To most closely reproduce the true compounding procedures and likely volumes, all reservoirs (cassettes and bags) were filled to 100 mL of all of the above concentrations. Due to the exorbitant cost that would have been incurred for additional drug and reservoirs, the study was limited to the above-mentioned 140 samples, deeming that number more than adequate to determine stability across multiple concentrations and storage conditions.
2.4. HPLC conditions

All chromatographic studies were performed on Waters® HPLC system (Milford, MA, USA), Alliance 2695 separations module, attached to the Waters® 2998 photodiode array detector. 5-FU concentration in all the samples was assessed by HPLC. The separations were performed on a Phenomenex Luna 5 μm 100Å (H16-221298) 100 × 4.6 mm column. The mobile phase was a 70:30 ratio of 5 mM potassium phosphate buffer (pH 6) and methanol, with an absorbance wavelength of 254 nm for a run time of 4.0 minutes at 25°C. The mobile phase was filtered and degassed before use. The flow rate was 0.5 mL/min, and the injection volume was 5 μL.

2.5. Development of standard curve

Standard curve was created using a 5-FU analytical standard (Acros Organics, LOT A0305173, and CAS: 51-21-8) diluted to 100, 200, 400, 600, 800, and 1,000 μg/mL in HPLC-grade water. Stock solution for dilution was made via creating a 1,000 μg/mL solution by the addition of 10 mg (10,000 μg) of the standard into 10 mL of HPLC grade water to ensure enough stock could be utilized for dilutions. This solution was then mixed until the 5-FU had been dissolved. Later the dilutions were made in HPLC grade water, for creating calibration curve in the range of 100 to 1,000 μg/mL. Triplicate data was utilized to calculate both intraday variation and interday variations. HPLC was run as described above. The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%. The limit of quantitation (LOQ) of the method was defined as the lowest concentration that could be quantitatively determined with acceptable precision and accuracy. Acceptance limits were defined as accuracy of 80-120% and % coefficient of variance (%CV) of ≤ 10%.

2.6. Physical evaluation

The pH is an important parameter that governs product stability, as changes in pH can affect the solubility of the product and cause precipitation. Thus, pH was measured at each sampling point, beginning at day 0 (initial). The SevenEasy™ pH meter (Columbus, OH, USA) attached to Routine Pro-ISM pH electrode (Mettler Toledo, Columbus, OH, USA) was used after three-point standardization with standard buffer solutions (pH 4.0, 7.0, and 10.0).

2.7. Stability under oxidative, alkaline, and acidic environments

The suitability of the present HPLC conditions to be used as a stability-indicating method was tested by accelerating the degradation of 100 μg/mL 5-FU in 1 N HCl, 1 N NaOH, and hydrogen peroxide solutions. Samples were withdrawn before and after heating each of the solutions at 80°C for 1 hour, 2 hours, and 5 hours. Each sample was analyzed by HPLC using the conditions explained above. Due to rapid hydrolysis in basic conditions, percent of initial values calculated for base was based on the amount in standard concentrations.

2.8. Data analysis

Stability of 5-FU was determined by calculating the percentage of the initial amounts. Wherever possible, the data are presented as mean ± standard deviation.

3. Results

3.1. Standard curve

The chromatogram of 5-FU standards shows a peak at the retention time (Rt) of 2.5 minutes (Figure 1a (blank) and 1b (sample)). A blank sample was also injected into the HPLC system and no peak was observed (Figure 1a). As shown in Figure 1c, a good linearity was exhibited (using analytical pipette) then q.s. to 100 mL using HPLC-grade water. The percentage of 5-FU remaining in each of the injectable formulations after Day 0 was calculated as a percentage based on the expected 5-FU concentration of the sample. The concentration was calculated from the area under the curve in the chromatogram utilizing the equation derived in the standard curve. The drug concentration was considered stable if the concentration of 5-FU was more than 90% of the initial concentration. Chromatogram peak areas were used to determine 5-FU concentrations in the CADD™ cassettes and Intravia™ bags. Samples were run as per the sampling timeline listed above and were run in duplicate per the HPLC protocol listed below.

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Acceptable precision and accuracy were demonstrated by this method for all the standards and quality controls based on the recommended criteria (10). The percentage recovery of 5-FU using the HPLC method was also calculated from the peak areas obtained.

3.2. Stability-indicating HPLC method

The current HPLC method met all acceptance criteria and was reproducible for the study of 5-FU in unknown samples. Failure to recognize degradation profiles of drug or degradation products, or lack thereof, is a most common point leading to erroneous data reporting in stability studies (11). The established HPLC method was able to determine the degradation peaks of 5-FU under various stress conditions (Figure 2a). The blanks were found to be non-interfering in the retention time range of 1.4 to 2.3 min. The data suggest that basic conditions (Figure 2b) cause significant degradation of 5-FU almost immediately. Less oxidative degradation occurred when treated with hydrogen peroxide (Figure 2d), and even less degradation occurred under acidic conditions (Figure 2c). Degradation was confirmed by a reduction in concentration over time, and no degradation peaks could be identified likely due to lack of chromophores, which has been reported in previous studies (4). Hence, this HPLC assay was used to determine the stability of 5-FU in an injectable formulation prepared in JHIS.

3.3. Drug content

5-FU is a treatment for many types of cancer and must be compounded; it has no pre-packaged product available due to BSA dosing. Hospitals compound injectable 5-FU into many types of sterile containers. At JHIS, 5-FU injectable formulations are routinely compounded in Intravia™ bags and CADD™ cassettes.

The drug content analysis of 5-FU from the Intravia™ bags stored at 2-8°C (Figure 3a) found contents to be in the range of 90-110% of the labeled drug amount by the end of 15 days, with no significant difference in stability between formulations. This indicates that formulation was stable in nature. The Intravia™ bags kept at 25°C with 60% humidity contained 5-FU in the range of 90-110% at the end of 15 days (Figure 4a). This indicates that 5-FU is stable through 15 days in either refrigeration or room temperature in Intravia™ bags in concentrations from 20 mg/mL to 50 mg/mL.

![Figure 1. (a) Representative HPLC chromatogram of blank sample shows background peaks and absence of 5-fluorouracil at ~2.5 min; (b) Representative HPLC chromatogram of 5-fluorouracil showing peak at 2.5 minutes; (c) Standard calibration curve of 5-fluorouracil assay.](image1)

![Figure 2. (a) Degradation of 5-FU over time under various stress conditions. It was observed that 5-FU gets completely degraded by the end of 1 h in base. (b-d) Degradation spectra or peaks for the 5-FU in presence of acid, base, and peroxide.](image2)
The drug content analysis of 5-FU from the CADD™ cassettes stored at 2-8°C (Figure 3b) found contents to be in the range of 90-110% by day 15. This indicates that formulation was stable in nature up until the 15-day mark; however, precipitation in the CADD™ cassettes at day 15 suggests 10 days as the maximum stability for refrigerated samples. The CADD™ cassettes kept at 25°C with 60% humidity contained 5-FU in the range of 90-110% (Figure 4b) for 15 days. No further sampling was carried out at controlled room temperature conditions or refrigeration conditions after 15 days. The drug content analysis suggests that the Intravia™ bag and CADD™ cassette formulations of 5-FU with concentrations from 20 mg/mL to 50 mg/mL may be stable for 15 days at room temperature. However, previous reports have suggested presence of precipitates at high concentration around 50 mg/mL, hence is important to perform physical evaluations to verify this (12,13). In addition, refrigeration, CADD™ cassettes are stable for 10 days, and Intravia™ bags are stable for 15 days.

3.4. Physical evaluation

The Intravia™ bags remained clear throughout the study when stored under refrigeration or at room temperature and no precipitation was seen. In the CADD™ cassettes, at day 15 precipitation was observed for all the concentrations at room temperature and refrigeration. At day 10, precipitates were observed in the 40 mg/mL concentration stored at room temperature sample only.

The initial pH of the 5-FU infusion solutions ranged from 9.15-9.26. As shown in Figures 5 and 6, there was no significant change \( p < 0.05 \) in the pH values under the two storage conditions.

4. Discussion

A robust and highly sensitive HPLC method was developed for the quantitation of 5-FU from the extemporaneously formulated injectable solutions in Intravia™ bags and CADD™ Cassettes. The method was able to efficiently quantitate 5-FU concentrations with % CV < 10% and 100 ± 10% accuracy for inter- and intra-day accuracies as per FDA guidelines.

In the present study, 5-FU was exposed to acid, base, and hydrogen peroxide at high temperature conditions. The assay clearly showed that 5-FU degrades in basic and to a lesser extent in peroxide conditions and the HPLC method was able to detect the degradation by a decrease in concentration. The drug completely degraded within 1 hour of exposure to basic conditions in the presence of heating, and even at the time zero point, registered at around 30% of the other initial concentrations on average, suggesting a nearly immediate degradative process when exposed to basic conditions. Previous reports suggested that treatment with base causes complete degradation of 5-FU and peroxide may cause
incomplete degradation of 5-FU (4). Moreover, exposure of 5-FU to thermal heating alone does not cause any degradation. As a result, the previous study may provide limited information about the stability of 5-FU. Hence, in our study we performed heat-induced treatment of 5-FU with acid, base, and peroxide. This extensive evaluation shows that 5-FU gets degraded completely, probably due to base hydrolysis, whereas hydrogen peroxide treatment with heating provides relatively lesser degradation profiles. The parent peak does not interfere with peaks of the peroxide, acid, or base, which indicates that HPLC assay is stability indicating.

Unlike previous evaluations, this stability-indicating method was later employed in studying the stability of compounded injectable formulations in Intravia™ bags and CADD™ Cassettes. Galanti et al. (5) evaluated 8 mg/mL formulations frozen and then thawed at 4°C for up to 28 days, and found that these preparations were stable for 28 days. Martel et al. (7) also evaluated 10 mg/mL at 4°C, 21°C, and 33°C in ambulatory pump reservoirs (both PVC and EVA) and EVA bags. They found these to be stable for 14 days at 21°C and 33°C for the reservoirs but only 3 days at 4°C for the PVC reservoirs and 5 days for the EVA reservoirs due to precipitation. In the EVA bags, they found all formulations were stable up to 14 days. Finally, Dine et al. (8) found 5-FU to be stable in PVC bags after irradiation of the bags at 50 mg/mL and 25 mg/mL through 14 days.

In the present study, we evaluated drug content from seven formulations utilizing two different reservoirs, two different diluents, and four different concentrations for up to 15 days for 5-FU preparations. Intravia™ bags compounded with concentrations from 20 mg/mL to 50 mg/mL may be stable up to 15 days under both refrigeration and room temperature conditions. Overall, our findings were consistent with the previous literature, although different reservoirs were used in previous studies and our study. Differences from the findings of Galanti et al. (5) for beyond 15 days may be attributed to the freezing and microwaving protocols of the preparations in Galanti’s study, which is not commonly done in practice with hazardous preparations.

Precipitation was observed in the CADD™ cassettes at day 15 in the tubing and in the body of the CADD™ cassette in 50 mg/mL concentration at both temperature conditions. The fluid is less mobile in the tubing and can sit for prolonged periods; this may account for the higher likelihood of precipitation at lower temperatures. Precipitation seen in the body of the CADD™ cassette can be attributed to the high concentration in the 50 mg/mL CADD™ cassette. This is consistent with the findings of Martel et al. (7) and Dine et al. (8) also observed precipitation in the 50 mg/mL concentration, although they attributed it to HCl formation due to radiation from the irradiated PVC bags.

Based on the present study we can conclude that injectable 5-FU in CADD™ cassettes are stable for up to 10 days at both, room temperature and under refrigeration. In Intravia™ bags, all the 5-FU
compounded preparations were stable at both the storage conditions for 15 days. These recommendations are longer than the present recommendation by the manufacturers and provide a more comprehensive picture than previous literature has given with the diversity of reservoirs, diluents, and concentrations studied. For instance, our study result of 10-day stability (at 2-8°C) for CADD™ cassettes and 15-day stability for Intravia™ bags at higher concentrations goes beyond the FDA-recommended manufacturer guidelines of 4 hours, the previous research reports of 120 hours for cassettes in admixtures, 7 days for cassettes, or 14 days in bags at room temperature (9). Using our study, efficiencies within the pharmacy can be enhanced. All stability findings and utilization of those findings are subject to BUD limits set forth in the USP Chapter 797.

5. Conclusion

A sensitive HPLC method was developed for the detection and quantitation of 5-FU. This method is stability indicating in nature as it can detect decreases in concentration with sensitivity up to 1,000 µg/mL. The 5-FU injectable Intravia™ bags are stable for 15 days when stored at room temperature (25°C and 60% relative humidity) and refrigeration (2-8°C) for concentrations ranging from 20 mg/mL to 50 mg/mL. The 5-FU injectable CADD™ cassettes are stable for 10 days at refrigeration (2-8°C) for concentrations ranging from 20 to 50 mg/mL. These cassettes are stable for 10 days for concentrations ranging from 20 to 30 mg/mL and 7 days for concentrations ranging 20 to 50 mg/mL at room temperature (25°C and 60% relative humidity). Overall, present study suggests the extended stability of the 5-FU preparations (beyond usual guidelines) in PVC bags and cassettes.

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Association between serum vitamin D level and uterine fibroid in premenopausal women in Indian population

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SUMMARY
We aim to evaluate the association between serum 25-hydroxyvitamin D3 levels and total number, volume and location of uterine fibroids (UFs) in premenopausal women in North Indian population. This case control study was undertaken in 310 women between 18 years and 45 years of age. Cases comprised of 102 women with fibroid lesion and the control group included 208 women with normal uterine morphology on ultrasonography. Blood samples were taken for measuring 25-hydroxyvitamin D3 levels. The mean serum 25-hydroxyvitamin D3 level in the study and control group was 14.52 ± 7.89 ng/mL and 26.6 ± 14.36 ng/mL respectively (p < 0.05). There was significant inverse correlation between serum 25-hydroxyvitamin D3 levels and total volume of fibroids (p = 0.000) while none between 25-hydroxyvitamin D3 levels with location, number of fibroids. 25-hydroxyvitamin D3 deficiency was more common in the study group (54.90%) compared to healthy controls (6.7%) while sufficiency was more common among controls (67.8% vs. 27.45) (p < 0.05). Women with deficient 25-hydroxyvitamin D3 levels have an odds of 18.36 for developing uterine fibroid. Women with low parity, those belonging to higher socioeconomic status and having less than 1-hour sun exposure per day were independently found to have high risk for development of UFs. Vitamin D may have a role in growth of UFs. Women not able to get adequate sun exposure due to indoor working conditions may need evaluation and supplementation as prophylaxis for development of fibroid.

Keywords
Serum 25-hydroxyvitamin D3 levels, uterine leiomyomas, pelvic imaging

1. Introduction
Uterine fibroids (UFs) are the most common benign pathology of female genital tract with 5-70% of women developing the tumor. Uterine fibroids may be associated with abnormal uterine bleeding, abdominal and pelvic pain, infertility and obstetric complications like miscarriage and premature labor, anemia, gastric disorders like bloating, constipation, and voiding symptoms (1). Conventional medical therapies provide only a short-term relief (2), and surgical intervention still remains the mainstay of treatment. Vitamin D, which is shown to have an antitumor activity, may play one of the major roles in UF biology and prophylaxis (1).

Vitamin D is a prohormone produced in the skin via a sunlight-initiated reaction and converted to the active metabolite 1, 25-dihydroxyvitamin D3 mainly in the liver and kidneys (3). It exerts its effects via activation of its cellular receptor (vitamin D receptor), which in turn alters the transcription rates of target genes responsible for various biological responses (4). Vitamin D receptors are present in various human tissues like skin, colon, brain, monocytes, macrophages, and UF tissues of human uterus throughout the menstrual cycle. The active vitamin D has the ability to inhibit growth and promote differentiation of a variety of cell types (5). Besides, studies have shown that vitamin D is an antifibrotic factor that inhibits the growth of human fibroid cells in a dose-dependent fashion by reducing many of immediate effects of transforming growth factor beta 3 (TGF-β3) significantly. It has also been demonstrated to shrink UF legions in Eker rats and authentic preclinical animal model for UFs (4). Besides vitamin D has good safety profile, and only chronic overdosage in the presence of markedly impaired renal function may lead to toxicity (6).

Presently, there is paucity of Indian data on the association between vitamin D insufficiency and the development of UFs. Keeping this in view, this study was conducted to compare serum 25-hydroxyvitamin D3 levels in women with and without UFs. It was also aimed to evaluate the association between serum...
25-hydroxyvitamin D, levels and total number, volume and location of UFs.

2. Materials and Methods

2.1. Design

The cross-control study was conducted in the Department of Obstetrics and Gynaecology in a tertiary care centre in the northern India for a period of 1 year from 2014 to 2015 in collaboration with the Department of Biochemistry. Clearance was taken from ethical committee of the institution prior to starting the study.

2.2. Study population

Premenopausal women 18-45 years of age who attended gynaecological outpatient department (GOPD) with different complaints for the period of 1 year and matched for age and BMI were recruited to the study. Those patients specially coming with complaints of abnormal uterine bleeding and infertility were approached to participate. There were a few patients who were diagnosed with UFs elsewhere and had attended GOPD with ultrasound scan report were too included. All of them were posted for an ultrasound scan. Those patients with at least one fibroid lesion with a mean volume of ≥ 2 cm³ were taken as cases. The rest of the patients who had normal uterine morphology on ultrasonography were approached to participate as controls. Following women were excluded from our study: current pregnancy or pregnancy within the last 6 months, currently lactating or lactating within the last 6 months, history of abortion within 6 months prior to start of study, history of myomectomy, women currently using vitamin D supplements or hormonal treatment (including oral contraceptives) or H/O use within the last 6 months.

2.3. Study procedure

Blood samples from patients consenting to participate as cases and controls were taken for measuring 25-hydroxyvitamin D₃ levels. Written informed consent was taken from all the selected women in a language understood by them. A detailed history regarding their diet and history of calcium intake was taken. The selected women were specifically asked for hours of exposure to sunlight between 10:00 AM and 3:00 PM. A thorough general and gynaecological examination was carried out. Age and BMI (Body Mass Index) were matched for all cases and controls. Ultrasonography was performed through both transabdominal and transvaginal routes on all consenting subjects.

The ultrasonography assessment was performed by the same ultrasonologist using ultrasound machine fitted with endovaginal probe 4-8 MHz for transvaginal sonography and 2-5 MHz convex probe for transabdominal sonography. The number of fibroid lesions, volume, and location of all fibroid lesions were accurately noted. The volume of each fibroid was determined according to the ellipsoid formula \( V = \frac{4}{3} \pi abc \), where \( a \) is the height, \( b \) is the width, and \( c \) is the depth (in cm). The total volume of fibroid in a patient was calculated by adding the volumes of all lesions detected. The location of each fibroid lesion within the uterus was classified as submucosal, intramural, or subserosal. A myoma with > 50% of its diameter bulging out of the uterine contour line was defined as subserous. Intramural fibroids were those mostly within the uterine shape. Myomas distorting the cavity line were defined as submucosal.

The estimation of serum 25-hydroxy vitamin D levels in both the study and control groups was carried out at Biochemistry Department of the same hospital by ELISA technique. The intra-assay coefficient of variation was based on 40 measurements for each serum, and the interassay coefficient of variation on the four measurements was performed in six different test runs. Vitamin D₃ status of the study and control population was categorized into 3 groups: deficient (levels < 10 ng/mL), insufficient (levels between 10 ng/mL and 19.9 ng/mL), and sufficient (≥ 20 ng/mL).

2.4. Statistical analysis

The association between 25-hydroxy vitamin D levels and the presence of fibroid and total fibroid volume (TFV) was analysed using means, frequencies, standard deviations, and percentages. The independent \( t \) test was used to compare serum 25-hydroxy vitamin D levels across groups. Correlations were assessed by Spearmen's rank correlation test. \( p \) value of < 0.05 was considered significant.

2.5. Sample size calculation

Paffoni A. et al. (7) found in controls a mean serum concentration of 25-hydroxyvitamin D₃ to be 20.8 ± 11.1 ng/mL. Presuming a 20% difference in 25-hydroxyvitamin D₃ level as clinically significant at 5% type 1 error with 80% power and assuming 10 % drop out rate when taking controls twice as cases, sample size was estimated to be 95 cases and 190 controls.

3. Results

3.1. Recruitment of participants

A total of 392 women with complaints of abnormal uterine bleeding or coming with ultrasound diagnosis of UF elsewhere were recruited and posted for ultrasonography. Uterine fibroid was diagnosed in 166...
patients, and other uterine/ovarian pathology was found in 7 patients. A total of 123 patients with UF consented to participate in the study. In total, 102 patients were ultimately recruited who fulfilled eligibility criterion. A total of 219 patients who had normal uterine morphology on ultrasonography were approached to participate as controls. Ultimately, 208 patients were found to be eligible to participate in the study as controls. Recruitment of patients in study and control groups is shown in Figure 1.

3.2. Serum vitamin D level

The mean serum 25-hydroxy vitamin D level among cases was 14.52 ± 7.89 ng/mL, while in the control group, it was 26.6 ± 14.36 ng/mL, and the difference was statistically significant (p < 0.05). On further categorical analysis, it was observed that vitamin D deficiency was more common in the study group (54.90%) as compared to healthy controls (6.7%) while sufficiency was more common among controls (67.8% vs. 27.45%), the difference being statistically significant (p < 0.05).

3.3. Association between serum vitamin D level and uterine fibroid

Women with deficient vitamin D levels have an odds of 18.36 for the development of UF (Table 1). There was an association found between multiple fibroids with deficient vitamin D levels. The majority of women with two (18/27, 66.67%) and three fibroid lesions (6/7, 85.71%) were vitamin D deficient. However, no statistically significant association was observed between vitamin D levels and number of UF (p = 0.105). The most common fibroid lesion found in our study was intramural (60/102, 58.82%) followed by subserosal (42/102, 41.17%). No association was found between vitamin D levels and location of the fibroid lesion (p = 0.760). The total fibroid volume (TFV) in the study group ranged from a minimum of 2 cm³ to a maximum of 248.6 cm³. Among the 56 women with deficient vitamin D levels (< 10 ng/mL), 44 women (44/56, 78.57%) had TFV between 50 cm³ and 100 cm³, and 12 women (12/56, 21.42%) had TFV above 100 cm³. Among the women with insufficient levels, the majority (14/18, 77.8%) had TFV between 10 cm³ and 50 cm³, whereas among women with sufficient vitamin D levels, most (13/28, 46.42%) had TFV < 10 cm³. There was a significant inverse correlation between serum vitamin D levels and total volume of UF (p < 0.001) (Table 2).

3.4. Secondary analysis of demographic factors

Demographic factors of recruited women as cases and controls are listed in Table 3. The age and BMI of the women were matched among cases and controls. The mean age of women in cases and controls were 34.50 ± 5.38 and 34.45 ± 5.37, respectively. The control group had a higher proportion of women who were housewives (90.9%) and belonging to lower socioeconomic status (86.53%) associating lower odds of UF with these demographic variables. Parity was also found to offer protection for development of UF with higher proportion (85.1%) of multiparous women in control group.

![Figure 1. Flowchart showing recruitment of patients in study and control group. AUB: Abnormal uterine bleeding, UF: Uterine fibroid.](#)

Table 1. Distribution of women among cases and controls according to serum vitamin D levels

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Study, n (%)</th>
<th>Control, n (%)</th>
<th>OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>56 (54.90)</td>
<td>14 (6.7)</td>
<td>18.36</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Insufficient</td>
<td>18 (17.64)</td>
<td>53 (25.5)</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>28 (27.45)</td>
<td>141 (67.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean vitamin D ± SD (ng/mL)</td>
<td>14.52 ± 7.89</td>
<td>26.6 ± 14.36</td>
<td></td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

*Chi-square test. *Independent t test.
3.5. Sun exposure and uterine fibroid

Of the women in control group, 64% had more than 1-hour sun exposure/day in contrast to 32.35% in cases, the difference being statistically different ($p < 0.05$). The cases with sun exposure less than 1 hour/day have an odds of 9.08 (95% CI 4.53-18.21) for developing UF, while the risk reduces to 1.43 (95% CI 0.744-2.77) when exposure is 1 hour (Table 4).

4. Discussion

The present cross-sectional study showed that vitamin D levels were significantly low in patients with UF when compared with those having normal uterine morphology similar to other studies (7-10). The total volume of fibroids had a significant inverse correlation with vitamin D levels identical to a recent study (9). There was a positive correlation of the number with serum vitamin D levels similar to most other studies (9,10) with no statistically significant difference. Our study observed that sun exposure ≥ 1 hour/day (weather permitting) provided protection against UFs. This was in tune with two other studies (7,10).

Oskovi Kaplan et al. (10) reported no relation of fibroid volume with vitamin D level. However, they found...
very low vitamin D levels in both cases and controls (6.54 ± 4.66 ng/mL vs. 8.18 ± 5.16 ng/mL, respectively). The apparent effect of vitamin D on the growth of UFs in the study without a vast margin of difference may have missed a causal relationship between fibroid volume with vitamin D level.

Paffoni et al. (7) however discovered a relationship between lower vitamin D levels and higher number of leiomyomas (≥ 3) with no statistical significance (p = 0.08) but none with volume and location of lesions. Hence, they suggested the role of vitamin D in the development and none on growth. But we observed a significant relation between deficiency and dimension of lesions like Ciavattini et al. (9). Volume of fibroid (< 50 mL, > 50 mL) and vitamin D level (deficient < 20, sufficient > 20) were assumed as dichotomous variables for regression analysis. Univariate analysis showed that the odds of developing a fibroid with > 50 mL volume is 2.51 times higher in women with deficient vitamin D levels (< 20 ng/mL).

Baird et al. (8) observed population with medium and high sun exposure have the same reduction in adjusted odds of fibroids relative to low sun exposure (40% reduction in adjusted odds of fibroids relative to low sun exposure). Oskovi Kaplan et al. (10) reported significantly higher prevalence of severe 25-hydroxy vitamin D deficiency (< 10 ng/mL) in women with covered clothing style (p = 0.002). Our findings are in tune with these studies as we observed that the patients with less than 1 hour of sun exposure/day were having an odds of 9.08 of developing UFs after adjusting for parity, socioeconomic status and sun exposure (Table 4). It is however pertinent to note that there is significant difference of parity among working women and housewives in our study. 81.8% of working women had low parity (parity 0 or 1) while only 17% women among housewives had parity 0 or 1. The overlap of optimum reproductive age and fulfilment of higher education and career ambitions among women in the past few decades has deferred the events of marriage and conception to late thirties. A systematic review to evaluate the relative strengths of various risk factors in UF epidemiology opined parity as the factor exerting greatest protective effect for the development of UFs (12). A monocentric analysis in Japan discovered fivefold reduction in the risk of UFs in women giving births for three or more times when compared to nulliparous women (13). The hypothesis for such finding is due to effects of pregnancy hormones along with cessation of menstruation and extensive remodelling in the immediate puerperium which can induce elimination of early as well as large leiomyoma lesions (12,14). We however discovered a twofold protective effect of parity on UFs presumably failing to consider the genetic and racial influence on its occurrence. The discovery of an increased incidence of UF in working women in our study is probably explained by the finding of lower parity in high numbers among them.

With only 40% of fibroids exhibiting genomic instability (15), a comprehensive analysis of multifactorial influence on UF development and growth is necessitated. However, the cross-sectional design of our study limits the precise understanding of the role of vitamin D in the causation of UFs. More robust prospective cohort studies with monitoring of vitamin D status and serial ultrasound imaging for uterine fibroids at predetermined time spaced intervals can shed light on the role of vitamin D in development vs growth of UFs or both. Being a hospital based study, it may not be representative of the true burden of the problem with respect to demographic variables in the community.

Hence, we suggest vitamin D deficiency may have a role in the growth of UFs. Women working indoor for maximum hours of the day may undergo serum vitamin D estimation and subsequently opt for supplementation, which could be simple, non-invasive, and cheap means of preventing the most common benign pathology in women.
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The effects of curcumin as dietary supplement for patients with COVID-19: A systematic review of randomized clinical trials

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SUMMARY Accumulating evidence has been reported regarding the effect of curcumin as a dietary antiviral on patients with COVID-19; however, findings are controversial. Our systematic review aimed to evaluate the effects of curcumin in patients with COVID-19. Electronic databases (PubMed, EMBASE, Scopus, Web of Science, Cochrane Central, and Google Scholar) were systematically searched to identify only randomized clinical trials (RCTs) that assessed curcumin in patients with COVID-19 from inception to September 23, 2021 relevant keywords. The Cochrane risk-of-bias tool for randomized trials was used to evaluate the risk of bias. After a critical review of 1,098 search hits, only six RCTs were selected for discussion. A total of 480 patients were included, with 240 amongst the curcumin groups and 240 in the control group. The lymphocyte count was significantly higher in the curcumin group compared to the placebo group. Curcumin was found to decrease the number of T-helper 17 cells, downregulate T-helper-17 cell‐related factors, reduce levels of T-helper-17 cell‐related cytokines, yet increase the gene expression of Treg transcription factor forkhead box P3 (FOXP3), and decrease T-Box transcription factor 21 (TBX21). Our review revealed that curcumin might have a positive effect on relieving COVID-19 related inflammatory response due to its powerful immune‐modulatory effects on cytokines production, T-cell responses, and gene expression. These findings suggest that curcumin confers clinical benefits in patients with COVID-19. However, due to the limited number of the included studies, further high-quality studies are needed to establish the clinical efficacy of the curcumin.

Keywords curcumin, COVID-19, SARS-CoV-2, cytokines, gene expression, systematic review

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first reported in December 2019 in China, then the virus spread expeditiously worldwide, causing a pandemic that led to health, economic and social disruption. The clinical presentation is variable, ranging from mild symptoms such as cough, myalgias, and headache to serious illnesses like acute respiratory distress syndrome (ARDS), multisystem failure, and death (1). As of October 17, 2021, 232 million infections, including 4.87 million deaths, have been recorded globally (2).

SARS-CoV-2 causes overactivation of the immune system, which leads to excessive cytokine production, including interleukin (IL) -6, IL-7, IL-8, IL-18, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and granulocyte-macrophage colony-stimulating factor (GM-CF) (1). Excessive cytokine production may cause peripheral lymphopenia, neutrophilia, and cytokine storm (3). Subsequently, ARDS, multisystem organ failure, and death may develop consequently. Medications targeting the suppression of cytokine storms are vital to prevent...
permanent parenchymal damage and ARDS caused by COVID-19. Among herbal medicines, curcumin has documented anti-inflammatory, antiviral, and antioxidant effects (4), which could have potential application when used against COVID-19. Curcumin, scientifically known as diferuloylmethane, is derived from the *Curcuma longa* plant and has been traditionally used in many countries as a spice and food coloring due to its yellow color. Interestingly, curcumin has been documented to improve glycemic control (5), have anticancer activity (6,7), and multiple antimicrobial effects, including antifungal, antibacterial, and antiviral (8). The prominent anti-inflammatory and immunomodulatory effects of curcumin make it a promising candidate for the treatment and prevention of COVID-19. Few randomized clinical trials (RCTs) have proposed mechanisms explaining the effect of curcumin on patients with COVID-19. However, no review has been conducted to critically and systematically evaluate these mechanisms.

Thus, this systematic review aims to evaluate the effects of curcumin among patients with COVID-19 to deliver reliable information for clinical decision-making in such cases. Furthermore, the pathophysiological mechanism of COVID-19 induced inflammation is discussed with relevance to the mechanism of action of curcumin in reducing that.

2. Methods

2.1. Protocol and registration

Our systematic review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and Cochrane Handbook for Systematic Reviews of Interventions (9,10). We registered our review at OSF Registries with DOI: 10.17605/OSF.IO/RKEMY

2.2. Eligibility criteria

Type of study: RCTs. Type of subject: Patients with COVID-19, no age criterion. Type of intervention: Studies that evaluated the effect of curcumin consumption versus placebo in patients with COVID-19. Primary results: change in the lymphocyte level, cytokines level, and gene expression.

2.3. Data sources and search strategy

We identified the studies by searching the electronic databases PubMed, EMBASE, Scopus, Web of Science, Cochrane Central, and Google Scholar. We used the following search terms ((COVID-19 OR covid-19 OR COVID OR coronavirus OR SARS-CoV-2 OR SARS virus) AND (Curcumin OR Turmeric Yellow OR yellow turmeric OR Curcuma OR Turmeric)), and the search terms were modified according to each database (Table S1, Supplementary Material, http://www.ddtjournal.com/action/getSupplementalData.php?ID=91). No language restrictions were carried out. The literature search was conducted on September 23, 2021, and included all studies from inception to that data. We also evaluated the studies mentioned in the bibliographies or suggested by co-authors for eligibility.

2.4. Study selection and data extraction

Two authors independently evaluated the eligibility of studies (AKA and MAE). Any conflict was resolved by a third author (BA). All search results were transferred to Covidence Software (11). In the first phase of the selection, the title and the summary of the search results were evaluated based on the inclusion and exclusion criteria. Then, the pre-selected studies were reviewed in full to determine eligibility. From the studies that met our inclusion criteria, two authors (AKA and MAE) extracted the following information: authors, publication year, study design, study duration, subject baseline characteristics (sample size, mean age, gender, lymphocyte count, platelet counts, C-reactive protein), intervention design (type of curcumin, dose, and control group), and outcome measures of interest (interleukins, genes). Tahmasebi et al. conducted two RCTs on the same patient sample, but they published two articles discussing different outcomes measured, and they divided the patient into mild and severe COVID-19 (12,13). We reported each patient group separately (mild vs. severe) in our tables as each had unique baseline characteristics.

2.5. Risk of bias assessment

We used the Cochrane Collaboration's tool for assessing the risk of bias in randomized trials for risk of bias assessment (14). Two authors (AKA and MAE) separately classified the included RCTs as having a low, high, or unclear risk of bias. Each RCT was evaluated for the following biases: selection, performance, detection, attrition, and reporting bias. BA resolved discrepancies between the reviewers. We used Review Manager V. 5.3 to create the graphs for the risk of bias assessment (15).

2.6. Outcomes of interest

Our outcome of interest was the effect of curcumin on lymphocyte count, change in cytokine levels, and gene expression of transcription factors and cytokines in patients with COVID-19.

3. Results

3.1. Study identification and selection

We identified a total of 1,098 studies from our literature
search. We removed 584 studies as duplicate and 499 studies based on the title and abstract screening. The remaining fifteen studies were read in full for eligibility. Nine studies were excluded; five had wrong study designs (16-20), one had a wrong population (21), and three were protocols. As a result, six RCTs met our inclusion criteria for the systematic review (12,13,22-25) (Figure 1).

3.2. Characteristics of included studies

RCTs evaluating the effect of curcumin on patients with COVID-19 were included in this systematic review. Two RCTs were triple blinded (22,23), and four were double-blinded (12,13,24,25). One RCT was done in India (24), and the rest in Iran (12,13,22,23,25). The RCTs included in our systematic review differ in the type and dose of Curcumin dosage, which ranged from 80 mg to 160 mg for 2-3 weeks in five RCTs (12,13,22,23,25), and 950 mg for two weeks in the last RCT (24). The total number of patients included was 480 patients (240 patients in each group), with a mean age of 51 years. All RCTs involved both genders with a male predominance (58% total patients). Table 1 and Table 2 show the characteristics of the included studies, patient demographics, and baseline characteristics as reported by the authors.

3.3. Risk of bias of the included studies

Figure 2 shows the risk of bias summary and graph. All RCTs were classified as having a low risk of bias for random sequence generation except Valizadeh et al., who used the intervention method to divide the patients (25). Five RCTs had an unclear risk of bias for allocation concealment. One RCT was at low risk of bias, as they used a randomization application to create the allocation sequence (24). Performance bias was deemed low risk in all included RCTs as they were double or triple blinded. Detection bias was low risk in Hassaniazad et al. since they used coded capsule containers to achieve the triple blindness of the participants, physicians, nurses, and

![Identification of studies via databases and registers](image)

Figure 1. PRISMA 2020 flow diagram for updated systematic reviews, which included searches of databases, registers, and other sources.
<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Study design</th>
<th>Year of Research</th>
<th>Hospital, Country</th>
<th>Duration (weeks)</th>
<th>Sample size (n)</th>
<th>Treatment group (n)</th>
<th>Control (n)</th>
<th>Curcumin Dose and form</th>
<th>Outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmadi et al., 2021 (22)</td>
<td>Triple-blinded RCT</td>
<td>2020</td>
<td>Imam Reza Hospital, Iran</td>
<td>16</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>Two soft gels (40 mg of nano-curcumin capsule) twice a day for 14 days.</td>
<td>CRP, lymphocyte count.</td>
</tr>
<tr>
<td>Hassaniazad et al., 2021 (23)</td>
<td>Triple-blinded RCT</td>
<td>2020</td>
<td>Shahid Mohammadi hospital, Iran</td>
<td>2</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>40 mg of the nano-curcumin capsule, four times per day for 14 days.</td>
<td>INF γ, IL-4, IL-17, TGF-β, CRP, lymphocyte count, lactate dehydrogenase, gene expression of TBX21, GATA3, RORC, and FOXP3 genes.</td>
</tr>
<tr>
<td>Valizadeh et al., 2020 (25)</td>
<td>Double-blinded RCT</td>
<td>2020</td>
<td>Imam Reza hospital, Iran</td>
<td>2</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>160 mg of nano-curcumin (four 40 mg capsules) daily for 14 days</td>
<td>Lymphocyte count, platelet count, creatinine, lactate dehydrogenase, gene expression of IL-1β and IL-6.</td>
</tr>
<tr>
<td>Tahmasebi, Saeed, et al., 2021 (12)</td>
<td>Double-blinded RCT</td>
<td>2020</td>
<td>Imam Reza hospital, Iran</td>
<td>3</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>Two doses of nano-curcumin (Sina Curcumin) in an 80 mg capsule, two times for 21 days.</td>
<td>TGF-β, CRP, lymphocyte count, platelet count, creatinine, lactate dehydrogenase, gene expression of Treg transcription factor forkhead box P3 (FoxP3), and cytokines (IL-10, IL-35, and TGF-β).</td>
</tr>
<tr>
<td>Tahmasebi, El-Esawi, et al., 2021 (13)</td>
<td>Double-blinded RCT</td>
<td>2020</td>
<td>Imam Reza hospital, Iran</td>
<td>3</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>Two doses of nano-curcumin (Sina Curcumin) in 80 mg capsule, two times for 21 days.</td>
<td>In mild and severe COVID: Th-17 frequency, serum level of Th17, mRNA expression levels of Th17, cytokines secretion levels of Th-17, gene expression levels of Th17 cells, serum levels of IL-23, 21, GM-CSF.</td>
</tr>
<tr>
<td>Pawar et al., 2021 (24)</td>
<td>Double-blinded RCT</td>
<td>2021</td>
<td>India</td>
<td>2</td>
<td>140</td>
<td>70</td>
<td>70</td>
<td>Curcumin (diferuloylmethane; 2.5 mg) with 2.5 mg Bioperine twice a day for 14 days</td>
<td>Absolute neutrophil to absolute lymphocyte ratio &gt; 3.5; PaO2/FiO2 ratio &lt; 300; rising CRP, ferritin, D-dimer, LDH, and triglycerides; troponin I positive and positive CK-MB.</td>
</tr>
</tbody>
</table>

RCT: randomized control trial; n: Number; y: year; SD: standard deviation; CRP: C-reactive protein; IL: interleukin; INF: Interferon; TGF: tumor growth factor; CK-MB: Creatine kinase-MB.
### Table 2. Baseline characteristics of the included patients

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Population number</th>
<th>Age (mean ± SD)</th>
<th>Sex (M: F)</th>
<th>Lymphocyte count (number/μL)</th>
<th>creatinine (μmol/L)</th>
<th>lactate dehydrogenase (U/L)</th>
<th>C-reactive protein (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUR</td>
<td>CO</td>
<td>CUR</td>
<td>CO</td>
<td>CUR</td>
<td>CO</td>
<td>CUR</td>
</tr>
<tr>
<td>Ahmadi et al., 2021 (22)</td>
<td>30</td>
<td>30</td>
<td>41.33 ± 12.04</td>
<td>44.97 ± 11</td>
<td>20:10</td>
<td>15:15</td>
<td>2.016 ± 1.294</td>
</tr>
<tr>
<td>Hassaniazad et al., 2021 (23)</td>
<td>20</td>
<td>20</td>
<td>48.7 ± 10.8</td>
<td>48.3 ± 11</td>
<td>10:10</td>
<td>12:8</td>
<td>* &gt; 0.99 20.6%</td>
</tr>
<tr>
<td>Tahmasebi et al., 2020 (25)</td>
<td>20</td>
<td>20</td>
<td>53.3 ± 8.4</td>
<td>51.4 ± 7.9</td>
<td>15:5</td>
<td>16:4</td>
<td>* &lt; 1.0 × 10^7/L, 13 ≥ 1.0, 7</td>
</tr>
<tr>
<td>Tahmasebi et al., 2021 (12,13) (for mild COVID-19 patients)</td>
<td>20</td>
<td>20</td>
<td>54.2 ± 9.1</td>
<td>52.4 ± 8.5</td>
<td>24:16</td>
<td>24:16</td>
<td>* &lt; 1.0 × 10^7/L, 14, (70%)</td>
</tr>
<tr>
<td>Tahmasebi et al., 2011 (12,13) (for severe COVID-19 patients)</td>
<td>20</td>
<td>20</td>
<td>54.2 ± 9.1</td>
<td>52.4 ± 8.5</td>
<td>24:16</td>
<td>24:16</td>
<td>* &lt; 1.0 × 10^7/L, 15, (75%)</td>
</tr>
<tr>
<td>Pawar et al., 2021 (24)</td>
<td>70</td>
<td>70</td>
<td>51.5 ± 14.13</td>
<td>54.25 ± 12.44</td>
<td>45:25</td>
<td>54:16</td>
<td>NA</td>
</tr>
</tbody>
</table>

M: male; F: female; SD: standard deviation; CUR: curcumin; CO: control; L: liter. *Data are presented as the variable’s value, patient number, (patient percentage compared to total patients included). **Data of C-reactive protein is reported using the qualitative method rather than the quantitative method used in the rest of the studies.
3.4. Outcomes of interest

All RCTs evaluated the effects of curcumin on the lymphocyte count, reporting that lymphocyte count was significantly higher in the curcumin group than in the placebo group (12,13,22-25).

Four RCTs reported outcomes evaluating the effect of curcumin on gene expression of transcription factors and cytokines (12,13,23,25). Tahmasebi, El-Esawi et al. reported a significant decrease in the number of T-helper 17 cells, downregulation of T-helper 17 cell-related factors (RAR-related orphan receptor γt, IL-17, IL-21, IL-23, and GM-CF), and decreased levels of T-helper 17 cell-related cytokines were found in both mild and severe COVID-19 patients treated by curcumin compared to the placebo group (13). In addition, Tahmasebi, Saeed et al. reported that curcumin upregulates the frequency of T regulatory cells leading to an increase in the gene expression transcription factor forkhead box P3 (FOXP3) and cytokines (IL-10, IL-35, and TGF-β) (12). Those findings were consistent with Hassaniazad et al., who...
also reported that curcumin significantly increased gene expressions of FOXP3 and decreased T-Box transcription factor 21 (TBX21) genes (23). Finally, Valizadeh et al. assessed the mRNA expression and cytokine secretion levels of IL-1β, IL-6, TNF-α, and IL-18, and curcumin decreased IL-6 and IL-1β expression and secretion but did not affect TNF-α and IL-18 (25).

4. Discussion

Due to the lack of strict regulation, the need to demonstrate the efficacy, safety, and quality of a marketed product is less reinforced by manufacturers of dietary supplements than in the pharmaceutical sector. As a result, many of the available products can be ineffective (26,27). However, systematic reviews and meta-analyses are at the top of the clinical evidence hierarchy and can decide whether nutraceutical agents should be used in the clinical setting. Fortunately, there were recent advances in the management of COVID-19, including antiviral agents, monoclonal antibodies along with supportive care measures, oxygen therapy, and usage of corticosteroids (28-30). The purpose of this systematic review is to highlight the multi-therapeutic effects of curcumin as a dietary supplement in reducing inflammation, relieving the symptoms of COVID-19, and accelerating the recovery process. To the best of our knowledge, the present review is the first to gather and present the currently available evidence on the effects of curcumin on COVID-19 patients.

Regulatory T-cells are involved in the downregulation of immune responses by producing inhibitory cytokines like TGF-β, IL-10, and IL-35. On the other hand, T helper 17 cells enhance the inflammatory response by producing IL-17, IL-21, IL-22, and GM-CS. An imbalanced ratio of T regulatory/T helper 17 cells is one of the underlying mechanisms of immune system dysregulation and hyper inflammation in COVID-19 that is associated with high mortality rates (31). According to Valizadeh 2020 et al. (25), cytokine storm might be the cause of the higher mortality rates observed in the placebo group 40% (8 out of 20) in comparison to the curcumin group that had a mortality rate of 20% (4 out of 20); moreover, Tahmasebi 2021 et al. (12) stated mortality rates in the curcumin group of 0% (0 out of 20) and 5% (1 out 20), compared to the placebo group with 5% (1 out 20) and 25% (5 out 20) in the mild and severe group, respectively. Furthermore, studies (12,23) indicate that curcumin has a significant effect on gene expression, which can subsequently modulate the immune response in COVID-19 patients compared to placebo. Curcumin can attenuate the T helper 1 inflammatory response by downregulating the TBX21 gene (the transcription factor involved in developing T helper cells to the T helper lineage). Curcumin also downregulates T helper 17 and the expression of the RAR Related Orphan Receptor (ROR) C gene, which is the transcription factor RORγt for T helper 17 cell differentiation (23). Moreover, curcumin acts as a modulator of T regulatory cells by upregulating FOXP3 and GATA binding protein 3 genes. With the effects of curcumin detailed above, it is reasonable to infer that curcumin may restore normal homeostasis in COVID-19 patients by suppressing T helper 1 and T helper 17 cell responses and augmenting the T regulatory cell responses.

COVID-19 patients had a significantly higher neutrophil-to-lymphocyte ratio compared to their healthy counterparts. Neutrophil to lymphocyte ratio also correlates with disease severity and could potentially be used to predict disease outcomes in COVID-19 (32). Surprisingly, curcumin has been shown to increase lymphocyte percentage, decrease the neutrophil percentage, and decrease neutrophil to lymphocyte ratio in COVID-19 patients (23). This can be explained by curcumin’s ability to inhibit the nuclear factor-κB (NF-κB) pathway in addition to other proinflammatory pathways (i.e., mitogen-activated protein kinase (MAPK) and the Janus kinase (JAK) (33)). These pathways promote neutrophil apoptosis and suppression of sustained inflammatory response.

Additionally, curcumin significantly suppresses mRNA expressions of proinflammatory markers IL-1β and IL-6 markers in COVID-19 patients, as was experimentally measured by (25). But, it did not decrease the level of IL-18 and TNF-α. Interestingly, IL-6 was significantly elevated in critically ill COVID-19 patients with ARDS than patients without ARDS and was strongly associated with death (34). Thus, curcumin may improve mortality by reducing inflammatory cytokines (mainly IL-6). Curcumin has also demonstrated molecular effects by adjusting several inflammatory molecules like histone acetylase, histone deacetylase, protein kinases, protein reductases, glyoxalase I, proteasome, and carrier proteins (35). The antioxidative effect of curcumin occurs by increasing the activity of superoxide dismutase and enhancing the expression of glutamate-cysteine ligase, both of which control the production of the cellular antioxidant glutathione (36). Glutathione is essential for the regulation of cellular proliferation, apoptosis, and immune response (37).

Curcumin is generally considered safe in its use as a food additive by the Food and Drug Administration. The allowable daily intake of curcumin is 0-3 mg/kg body weight (38). Potential adverse effects have been documented and include diarrhea, headache, rash, and yellow stool (39), but no serious adverse effects have been reported to date. One limitation of using curcumin as a therapeutic agent is its poor bioavailability. It is poorly absorbed, has a rapid metabolism, and is subsequently eliminated rapidly from the body. Several formulations have been prepared to enhance its bioavailability. For example, The combination of curcumin with piperine, the main active ingredient of black pepper, was associated with a 2,000% increase.
in curcumin bioavailability (19, 40). Nano-formulations of curcumin improve curcumin's solubility in aqueous solutions (41) and have improved concentration in the blood (42).

Curcumin effectively improves myalgia, cough, taste, and olfactory disturbances in COVID-19 patients (22). In addition, another open-label nonrandomized clinical trial has documented that Curcumin alleviated COVID-19 related symptoms like fever, chills, tachypnea, myalgia, and cough (20). These characteristics add to the curcumin activity in reducing inflammation, accelerating the healing process, and decreasing the mortality in COVID-19 patients through the above-noted mechanisms. Therefore, curcumin is considered a potential therapeutic agent for COVID-19. However, further RCTs are required to evaluate the bioavailability, efficacy, and safety of the use of Nanocurcumin in COVID-19 patients.

The main limitation of this systematic review was that we could not perform a meta-analysis. Statistical analysis was not applicable due to the insufficient number of the RCTs in each outcome and lack of homogeneity between the RCTs. Other limitations are related to the included RCTs, such as a short course of follow-up, a small sample size, and different dosages of curcumin used among the trials. This may lead to heterogeneity in clinical effects and further affect the results. Finally, the external validity and applicability of the results to other populations are questionable as most of the RCTs were conducted in Iran. Therefore, more RCTs should be conducted to study curcumin's application as a potential treatment of COVID-19.

5. Conclusion

In conclusion, the results of the present review suggest that curcumin is a promising herbal medicine that may be effective in treating COVID-19 due to its powerful immune-modulatory effects on cytokines production, T-cell responses, and gene expression. However, it is noteworthy that further high-quality studies are required to confirm our results based on the limited available data.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

References

20. Saber-Moghaddam N, Salari S, Hejazi S, Amini M,


Relationship between low back pain and stress urinary incontinence at 3 months postpartum

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SUMMARY

Previous studies have proposed that pelvic misalignment may be associated with stress urinary incontinence through a decrease in the contractile function of the pelvic floor muscles; however, this relationship remains unclear. This study aimed to clarify the relationship between low back pain, an indication of pelvic misalignment, and stress urinary incontinence at 3 months postpartum. We conducted a cross-sectional study of women who gave birth to full-term babies between July 2008 and July 2009. Stress urinary incontinence was defined as urinary leakage when coughing, sneezing, or exercising. Low back pain was defined as pain between the ribs and gluteal sulcus in the preceding 2 months. Of the 228 subjects included in the study, the prevalence of stress urinary incontinence was 22.8% (n = 52). The prevalence of low back pain in the stress urinary incontinence group was significantly higher than that in the non-stress urinary incontinence group (78.8% [n = 41] vs. 57.4% [n = 101]; p = 0.005). Stress urinary incontinence was associated with older age, primiparity, vaginal delivery, and low back pain at 3 months. In conclusion, low back pain was associated with stress urinary incontinence after adjusting for pregnancy and delivery factors, suggesting pelvic misalignment contributes to the development of stress urinary incontinence. We propose that including care for pelvic misalignment in pelvic floor muscle training, the treatment of choice for stress urinary incontinence, could be beneficial.

Keywords Low back pain, pelvic misalignment, postpartum period, urinary incontinence, vaginal delivery

1. Introduction

The prevalence of urinary incontinence (UI) in women older than 20 years is as high as approximately 25%, and approximately 80% of these women have stress urinary incontinence (SUI) (1,2), defined as any involuntary loss of urine on effort or physical exertion (e.g., sporting activities), sneezing, or coughing (3). SUI impairs the affected person’s quality of life (QOL) both psychologically and socially. Those affected may experience the fear of smelling like urine and of contamination of their clothes and they may restrict social activities, quit work, and experience depression (4,5) because SUI can occur suddenly in daily life.

The main cause of SUI is urethral closure dysfunction due to stretching of the pelvic floor fascia, ligaments, and muscles by the increasing weight of the fetus, amniotic fluid, and uterus during pregnancy (6-8), and defects of the pelvic floor that may occur during vaginal delivery (9-11). Additionally, recovery of pelvic floor function following delivery is delayed in women who have delivered a baby for the first time at ≥ 35 years old (12). Thus, it is essential that patients receive appropriate treatment to promote the recovery of pelvic floor function and reduce SUI symptoms in the postpartum period.

The pelvic floor includes the muscles, fascia, and ligaments that support the pelvic organs (bladder, uterus, and intestines). It is responsible for closing the urethra and anus to prevent involuntary leakage of urine and stool, respectively. Pelvic floor muscle training is recommended to improve its supportive and urethral closure functions by strengthening the pelvic floor muscles (13-15), which are the main muscles of the pelvic floor. In fact, 3 months of pelvic floor muscle training in the postpartum period can restore pelvic floor function and improve SUI (16). However, approximately 30% of women with SUI remained symptomatic even after undergoing pelvic floor muscle training (16). To improve the effectiveness of treatment...
in the postpartum period, an additional approach is needed to reduce SUI symptoms.

Pelvic misalignment may be a cause of decreased pelvic floor function because the pelvic floor muscles adhere to the pelvis. Increased secretion of relaxin during pregnancy alters the properties of the cartilage and tendons around the pelvis, resulting in loosening of the pubic symphysis and sacroiliac joints (17). In addition, the lax pelvis is exposed to the chronic load of the fetus, amniotic fluid, and uterus during late pregnancy. When this load is applied to one side of the pelvis or to an area that differs from the usual placement, the pelvis is distorted (18,19), and pelvic girdle pain and low back pain (LBP) occur during pregnancy and after childbirth (20). Considering the increased activity level of the pelvic floor muscles at rest and loss of motor control in patients with LBP (21-23), pelvic misalignment causes symptoms due to pelvic floor dysfunction, including SUI.

The evaluation methods for pelvic misalignment include pelvic radiography, assessment of the postural alignment when standing, and manual examination (18-22,24). However, these methods have the following shortcomings: radiography results in radiation exposure (24), and postural alignment evaluation and manual examination require special skills and a considerable amount of time (18-22). Thus, previous studies (21,22) that investigated the relationship between pelvic misalignment and SUI struggled with small sample sizes and/or no control of other pregnancy and delivery factors.

LBP mainly results from pelvic misalignment because pelvic misalignment is associated with LBP among adults (24) and sacroiliac joint pain among pregnant women (25). Thus, we propose that LBP may be useful as an alternative indication of pelvic misalignment in a large sample questionnaire study. Although a study found that many women have LBP and SUI simultaneously 12 months after childbirth (26), the study did not evaluate the relationship between LBP and SUI after adjusting for pregnancy and delivery factors. It remains unclear whether LBP in postpartum causes SUI independently from pelvic floor damage caused by vaginal childbirth. Therefore, this study aimed to clarify the relationship between LBP and SUI at 3 months postpartum, when SUI and LBP were likely to persist for a long period of time (27,28), and adjust for factors relating to pregnancy and delivery.

2. Materials and Methods

2.1. Study design, participants, and procedure

We conducted a cross-sectional study among postpartum women at an obstetrics facility in Tokyo between July 2008 and July 2009. Women who gave birth to full-term babies were recruited while they were hospitalized immediately after delivery, regardless of their SUI history. The exclusion criteria were as follows: (1) stillbirth/neonatal death, (2) neurogenic bladder dysfunction, (3) mental illness, (4) difficulty in understanding Japanese, and (5) age < 20 years old. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of the institution. After obtaining consent for participating in the study, a researcher obtained the patients’ demographic and obstetric data from medical records. The women answered a questionnaire regarding SUI and LBP that was delivered by mail at 3 months postpartum.

2.2. Measurements

The Japanese version of the scored International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) (29) was used to evaluate the presence, degree, and type of UI. The ICIQ-SF consists of four items: frequency of UI (0-5 points), amount of urinary leakage (0-6 points), effects of UI on daily life (0-10 points), and subjective evaluation of UI symptoms (not included in the score). Higher scores indicated severe UI and a poor QOL. Women who answered that they had experienced "leakage when coughing or sneezing" or "leakage when moving or exercising" were diagnosed with SUI.

The incontinence QOL questionnaire (I-QOL) (30) was used to examine disease-specific QOL. The I-QOL consists of a 22-item, 5-point scale (1-5 points) that represents the effects of UI on the patient’s life. The score is converted to a maximum of 100 points (range, 20-100 points). A lower score indicated that UI had a greater impact on daily living.

We also included two of our own questions that asked about whether the women experienced LBP (yes or no) and the location of the LBP in the past 2 months. Based on the definition of the LBP as published in the Japanese Orthopaedic Association guidelines (31), LBP was defined as pain between the lowermost rib and gluteal sulcus.

Demographic data (age, smoking history, height, weight before the current pregnancy, medical history, pregnancy history, and delivery) and information about the current pregnancy (weight gain during the pregnancy, complications, mode and duration of delivery, treatment during delivery, gestational age at delivery, birth weight, and baby's head circumference) were collected from the women's medical records.

2.3. Analysis

The Mann-Whitney U test, Chi-square test, or Fisher's exact test was used to compare the results of the women with and without SUI. Variables with a p-value of < 0.05 in the univariate analysis and the number of deliveries
considered to be theoretically essential were included in the binary logistic regression analysis. The adjusted odds ratios (AORs) and 95% confidence intervals (95% CIs) were calculated for the presence of SUI. IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY) was used for the analysis. A p-value of < 0.05 was considered statistically significant.

3. Results

Of the 286 women who participated in the study during their puerperal hospitalization, 234 responded to the questionnaire at 3 months postpartum (response rate: 81.8%). Of these women, 228 were included in the analysis after 6 women were excluded because they did not answer the questions on the subjective evaluation of their UI symptoms on the ICIQ-SF. Fifty-two women (22.8%) answered that they had SUI at 3 months postpartum. Among the 52 women with SUI, 31 experienced UI "2 to 3 times a week," followed by "about once a day" in 11 (59.6% and 21.2%, respectively; Figure 1). Forty-nine women (94.2%) had moderate urinary leakage. The I-QOL score was significantly lower in the SUI group than in the non-SUI group (90.0 ± 10.7 vs. 97.3 ± 8.0, p < 0.001).

The mean age (in years) and proportion of patients who delivered vaginally in the current pregnancy in the SUI group were significantly higher than those in the non-SUI group (p < 0.01, Table 1). Among the women who delivered vaginally, the duration of the second stage of labor was significantly longer in the SUI group than in the non-SUI group (98.6 ± 102.9 vs. 66.9 ± 95.6 minutes, p = 0.012). Infants born to women in the SUI group had significantly heavier birth weights than those born to women in the non-SUI group (3063 ± 403.7 vs. 2944 ± 338.4 g, p = 0.028).

The prevalence of LBP at 3 months postpartum was 142 (62.3%) in all the participants, and it was significantly higher in the SUI group than in the non-SUI group (41 [78.8%] vs. 101 [57.4%], p = 0.005; Figure 2). Logistic regression analysis showed that SUI was associated with LBP at 3 months postpartum, age, multiparity, and vaginal delivery (AOR [95% CI]: 3.60 [1.55-8.34], 1.14 [1.04-1.24], 2.39 [1.05-5.43], and 8.63 [1.07-69.68], respectively) (Table 2).

4. Discussion

This study found that the prevalence of SUI and LBP at 3 months postpartum was 22.8% and 62.3%, respectively. In addition, the study showed that LBP was associated with SUI even after adjusting for pregnancy and delivery factors.

The mean age in this study (35.1 and 33.7 years...
Table 1. Comparison of the differences in the demographic and obstetric data between the two groups

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>SUI group (n = 52)</th>
<th>non-SUI group (n = 176)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.7 ± 4.2</td>
<td>33.7 ± 4.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td>0.691</td>
</tr>
<tr>
<td>Never</td>
<td>47 (90.4%)</td>
<td>163 (92.6%)</td>
<td></td>
</tr>
<tr>
<td>Past smoker</td>
<td>5 (9.6%)</td>
<td>12 (6.8%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>0 (0.0%)</td>
<td>1 (0.6%)</td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (kg/m²)</td>
<td>20.6 ± 4.1</td>
<td>20.7 ± 2.7</td>
<td>0.739</td>
</tr>
<tr>
<td>Medical history (yes)</td>
<td>27 (51.9%)</td>
<td>82 (46.6%)</td>
<td>0.511</td>
</tr>
<tr>
<td>Gynecological diseases</td>
<td>8 (15.4%)</td>
<td>37 (21.0%)</td>
<td></td>
</tr>
<tr>
<td>Respiratory disorders</td>
<td>3 (5.8%)</td>
<td>9 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>5 (9.6%)</td>
<td>14 (8.0%)</td>
<td></td>
</tr>
<tr>
<td>Orthopedic disease</td>
<td>6 (11.5%)</td>
<td>6 (3.4%)</td>
<td></td>
</tr>
<tr>
<td>Urological disease</td>
<td>3 (5.8%)</td>
<td>5 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Current pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravid women</td>
<td>29 (55.8%)</td>
<td>117 (66.5%)</td>
<td>0.157</td>
</tr>
<tr>
<td>Gestational weight gain (kg)</td>
<td>8.6 ± 3.4</td>
<td>9.2 ± 3.2</td>
<td>0.588</td>
</tr>
<tr>
<td>Complications (multiple answers)</td>
<td></td>
<td></td>
<td>0.219</td>
</tr>
<tr>
<td>Imminent preterm birth</td>
<td>4 (66.7%)</td>
<td>10 (43.5%)</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>0 (0.0%)</td>
<td>5 (21.7%)</td>
<td></td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>1 (16.7%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>1 (16.7%)</td>
<td>3 (13.0%)</td>
<td></td>
</tr>
<tr>
<td>Imminent uterine rupture</td>
<td>0 (0.0%)</td>
<td>3 (13.0%)</td>
<td></td>
</tr>
<tr>
<td>Placenta previa</td>
<td>0 (0.0%)</td>
<td>2 (8.7%)</td>
<td></td>
</tr>
<tr>
<td>Delivery mode (vaginal delivery)</td>
<td>50 (96.2%)</td>
<td>124 (70.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total duration of labor (minutes)</td>
<td>388.3 ± 357.1</td>
<td>323.2 ± 284.1</td>
<td>0.294</td>
</tr>
<tr>
<td>First stage</td>
<td>288.4 ± 322.6</td>
<td>251.4 ± 241.2</td>
<td>0.812</td>
</tr>
<tr>
<td>Second stage</td>
<td>98.56 ± 102.9</td>
<td>66.93 ± 95.6</td>
<td>0.012</td>
</tr>
<tr>
<td>Treatment at delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>20 (39.2%)</td>
<td>47 (33.3%)</td>
<td>0.396</td>
</tr>
<tr>
<td>Epidural birth</td>
<td>5 (9.8%)</td>
<td>5 (3.5%)</td>
<td>0.078</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>30 (57.7%)</td>
<td>76 (43.2%)</td>
<td>0.785</td>
</tr>
<tr>
<td>Perineal tear</td>
<td>22 (42.3%)</td>
<td>50 (40.7%)</td>
<td>0.762</td>
</tr>
<tr>
<td>Gestational age at delivery (days)</td>
<td>274.4 ± 8.0</td>
<td>274.1 ± 8.6</td>
<td>0.757</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3063.3 ± 403.7</td>
<td>2943.9 ± 338.4</td>
<td>0.028</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.5 ± 1.3</td>
<td>33.1 ± 2.9</td>
<td>0.227</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± standard deviation or n (%), Mann-Whitney U test, or Fisher's exact test. Abbreviation: SUI, stress urinary incontinence.

Figure 2. Prevalence of LBP.

Table 2. SUI-related factors

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>AOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>1.10 (1.03-1.19)</td>
<td>0.009</td>
<td>1.14 (1.04-1.24)</td>
<td>0.004</td>
</tr>
<tr>
<td>LBP at 3 months</td>
<td>1.00 (1.00-1.00)</td>
<td>0.036</td>
<td>1.00 (0.10-1.00)</td>
<td>0.345</td>
</tr>
<tr>
<td>Multiparas</td>
<td>2.77 (1.33-5.74)</td>
<td>0.006</td>
<td>3.60 (1.55-8.34)</td>
<td>0.003</td>
</tr>
<tr>
<td>Second stage (minutes)</td>
<td>1.57 (0.84-2.95)</td>
<td>0.159</td>
<td>2.39 (1.05-5.43)</td>
<td>0.038</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>1.00 (1.00-1.01)</td>
<td>0.055</td>
<td>1.00 (0.10-1.01)</td>
<td>0.129</td>
</tr>
</tbody>
</table>

Abbreviations: LBP: low back pain, SUI: stress urinary incontinence, OR: odds ratio, AOR: adjusted odds ratio, 95% CI: 95% confidence interval. Logistic regression analysis. LBP at 3 months: 0 = none, 1 = yes; Multiparas: 0 = primipara, 1 = multiparas vaginal delivery: 0 = cesarean section, 1 = vaginal delivery.
in the SUI and non-SUI groups, respectively) was relatively higher than that of the average pregnant Japanese women at the time of delivery in 2018 (1st, 2nd, and 3rd child: 30.7, 32.7, and 33.7 years old, respectively) (32). Although the prevalence of SUI and LBP generally increases with age (4), in this study, the prevalence of SUI and LBP (22.8% and 62.3%, respectively) was consistent with that found among postpartum Japanese women in previous studies (SUI: 26.2% and LBP: 47.8%-71.8%) (33-35). Our results can be considered representative of the prevalence of SUI and LBP among general postpartum Japanese women.

This study used LBP rather than postural alignment or manual examination as an indicator of pelvic misalignment because we prioritized the conduction of a survey in a group with a large sample size. In general, the origins of LBP are divided into five categories (31): the spinal cord and surrounding locomotor disease, neurofibromata in the spinal cord or cauda equina, visceral organ disease (e.g., renal or urinary tract stones and gynecological disease), vascular origin (aortic dissection etc.), and mental illness. Considering the following, we propose that most of the subjects in this study had LBP that derived from the spine: LBP of vascular origin only occurs in serious situations; the exclusion criteria for this study included neurofibromata and psychiatric disorders; all the subjects with a history of gynecological disease were undergoing treatment or had been treated; and pain related to gynecological illnesses was controlled. LBP derived from the spine is roughly divided into nervous system diseases, such as lumbar disc herniation and lumbar spinal canal stenosis, and myofascial LBP due to postural changes and pelvic misalignment. The postpartum pelvic alignment remains wider than that at 12 weeks of gestation, while postural changes during pregnancy disappear (25). Moreover, in this study, none of the subjects had a history of nervous system diseases such as lumbar disc herniation; thus, we concluded that the subjects' chief complaint of LBP appropriately represented pelvic misalignment.

After adjusting for pregnancy and delivery factors, we found that LBP was associated with SUI. Considering that LBP represented pelvic misalignment, our results suggest that the decrease in pelvic floor function due to pelvic misalignment is a risk factor for the development of SUI. Pool-Goudzwaard et al. (21) speculated that the insufficient contraction of the pelvic floor muscles to ensure urethral closure when the intra-abdominal pressure increases is a cause of SUI in women with LBP. This is because the pelvic floor muscles are constantly activated to eliminate the pelvic instability caused by pelvic misalignment. Complex combinations of anterior-posterior, left-right, and twisting pelvic misalignments and pelvic instability during pregnancy and delivery may be triggers for SUI through decreased pelvic floor function.

Multiparity and vaginal delivery were associated with postpartum SUI. Considering that pelvic floor muscle abnormalities occur after vaginal delivery (36) and the risk of SUI increases with parity (37), this result confirmed that pelvic floor dysfunction due to defects in the pelvic floor muscles causes postpartum SUI.

Our findings from the I-QOL score that showed that SUI impairs the QOL of women were consistent with those of previous studies (5,38). Although many healthcare professionals have attempted to improve the effectiveness of pelvic floor muscle training by adding biofeedback tools, increasing the frequency and duration of training, and providing support to keep patients motivated in their training (e.g., group sessions) (15,16), there are still some limitations to pelvic floor muscle training including a high dropout rate and moderate cure rate. As LBP coexists with SUI in many postpartum women and SUI and LBP have a strong effect on postpartum daily life (38), our finding of the possibility of a relationship between pelvic misalignment and SUI would provide an alternative approach to the treatment of SUI in terms of correcting pelvic misalignment.

This study had some limitations. First, it did not investigate whether subjects had any episodes of UI before the current pregnancy because we did not focus on the causal relationship between SUI and pelvic misalignment caused by the current pregnancy or delivery. Although this study included women who had pelvic misalignment or SUI regardless of their pregnancy or delivery information, this did not have an impact on the finding of a relationship between pelvic misalignment and SUI. Second, pelvic misalignment was not directly evaluated. The relationship between pelvic misalignment and decreased pelvic floor function/SUI is still under scrutiny. Nonetheless, this study revealed that LBP (pelvic misalignment) was related to postpartum SUI independent of the pelvic floor damage caused by pregnancy and delivery. Thus, we propose that further studies are required to reveal the mechanisms underlying SUI caused by pelvic misalignment. Finally, data were obtained more than 10 years ago. Although it has been a long time since the data were collected, the method that was used to determine SUI using the ICQF-SF and to evaluate LBP based on the guidelines remain the standard methods to date. In addition, in Japan, there were no differences in the delivery circumstances between the present and 10 years ago, such as the cesarean section rate (17.4% in 2005 vs. 20.4% in 2017) (39) and average maternal age at birth of the first child (29.1 years old in 2005 vs. 30.7 years old in 2019) (40). Therefore, it can be said that the results of this study fully reflect the current situation for postpartum women in Japan.

In conclusion, our results showed that the prevalence of SUI and LBP at 3 months postpartum was 22.8% and 62.3%, respectively, and the prevalence of LBP in women with SUI was as high as 78.8%. Even after
adjusting for delivery factors, it became clear that postpartum SUI was associated with LBP. This suggests that the decrease in pelvic floor function due to pelvic misalignment, which is a potential cause of LBP, is associated with the development of SUI. In future, we propose that SUI can be improved more efficiently by including correction of pelvic misalignment in conventional pelvic floor muscle training.

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References


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Waning COVID-19 vaccine effectiveness in Japan

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As of the end of November, 2021, the rate of completion for second-dose COVID-19 vaccine administration was almost 80% in Japan. We evaluated waning COVID-19 vaccine effectiveness in Japan, controlling for mutated strains, the Olympic Games, and countermeasures. The effective reproduction number $R(t)$ was regressed on current vaccine coverage and data of a certain number of days prior, as well as shares of mutated strains, and an Olympic Games dummy variable along with data of temperature, humidity, mobility, and countermeasures. The study period was February, 2020 through November 4, as of November 25, 2021. Estimation results indicate that vaccine coverage of more than 90 days prior raises $R(t)$ significantly. Especially, vaccine coverage with 90 or 120 days prior cancelled vaccine effectiveness completely. Results indicate significant waning of vaccine effectiveness from 90 days after the second dose.

1. Introduction

Wide coverage of COVID-19 vaccination has altered outbreak situations in European countries and in the US. Unfortunately, vaccination in Japan started only in February, 2021 using BNT162b2 mRNA (Pfizer Inc., BioNTech) and mRNA-1273 (Moderna, Inc.) vaccines: among the latest of starting dates of vaccination programs in economically developed countries. Later, ChAdOx1 adenoviral vector (Oxford, AstraZeneca) also became available. By the end of November, 2021, the rate of completion for second dose vaccine administration had reached almost 80% in Japan (Figure 1) (1,2). The next challenge posed by vaccine issues in Japan might be waning of vaccine effectiveness.

In fact, waning vaccine effectiveness has been reported (3,4). One study revealed that the log of IgG antibody titer decreased by a factor of 18.3 when measured six months after second-dose vaccination. Another study revealed vaccine effectiveness as 77.5% at one month after the second vaccination, but it had decreased to about 20% when measured 5-7 months later. In the real world, vaccines of several types have been used. Moreover, vaccinated persons might change their behaviors. Therefore, this study assessed vaccine effectiveness and its waning capabilities against infectiousness in the real world, particularly in Japan.

By the time vaccinations started in Japan, the alpha variant strain had emerged and had expanded to dominate the recorded infections. Subsequently, a new mutant alpha variant strain appeared in May. Based mainly on data reported by the UK, its infectiousness and pathogenicity were estimated as 35-90% higher than those of the original strain circulating before the emerging variant strain (5-8). Therefore, we consider the prevalence of these mutated strains together when evaluating vaccine effects.

The object of this study was to estimate waning of vaccine effectiveness against SARS-CoV-2 infectiousness for the outbreak in Japan as a result of the vaccine effectiveness itself, the mutated strain, the Olympic Games, countermeasures, and other factors that might affect infectiousness.

2. Methods

2.1. Calculation procedure for $R(t)$

This study examined the numbers of symptomatic patients reported by the Ministry of Health, Labour and Welfare (MHLW) for February 1, 2020 – August 29, 2021 published (9) as of October 18, 2021. Some patients were excluded from data for Japan: patients presumed to be persons infected abroad or infected as Diamond Princess passengers. Those patients were presumed not to represent community-acquired infection in Japan. For some symptomatic patients with unknown onset dates, we estimated the onset dates from an empirical
distribution with duration extending from onset to the report date among patients for whom the onset date had been reported.

Onset dates among patients who did not report this information and a reporting delay were adjusted using the same procedures as those used for earlier studies (10,11). As described hereinafter, we estimated the onset dates of patients for whom onset dates had not been reported. Letting \( f(k) \) represent this empirical distribution of the incubation period and letting \( N_k \) denote the number of patients for whom onset dates were not published and available at date \( t \), then the number of patients for whom the onset date was known is \( t-1 \). The number of patients with onset date \( t-1 \) for whom onset dates were not available was estimated as \( f(1)N_k \). Similarly, patients with onset date \( t-2 \) and for whom onset dates were not available were estimated as \( f(2)N_k \). Therefore, the total number of patients for whom the onset date was not available, given an onset date of \( s \), was estimated as \( \Sigma_{s\leq t} f(k)N_k + k \) for the long duration extending from \( s \).

Moreover, the reporting delay for published data from MHLW might be considerable. In other words, if \( s+k \) is larger than in the current period \( t \), then \( s+k \) represents the future for period \( t \). For that reason, \( N_{s+k} \) is not observable. Such a reporting delay engenders underestimation of the number of patients. For that reason, it must be adjusted as \( \Sigma_{s\leq t} f(k)N_k + k / \Sigma_{s\leq t} f(k) \). Similarly, patients for whom the onset dates were available are expected to be affected by the reporting delay. Therefore, we have \( M_{s+k} / \Sigma_{s\leq t} f(k) \), where \( M_{s+k} \) represents the reported number of patients for whom onset dates were period \( s \) as of the current period \( t \).

We defined \( R(t) \) as the number of infected patients on day \( t \) divided by the number of patients who were presumed to be infectious. The number of infected patients was calculated from the epidemic curve by the onset date using an empirical distribution of the incubation period, which is \( \Sigma_{s\leq t} f(k)E_{s\leq t} \), where \( E \) denotes the number of patients for whom the onset date was period \( t \). The distribution of infectiousness in symptomatic and asymptomatic cases \( g(k) \) was assumed to be 30% on the onset day, 20% on the following day, and 10% for the subsequent five days (12). Then the number of infectious patients was \( \Sigma_{s\leq t} g(k)E_{s\leq t} \). Therefore, \( R(t) \) was defined as \( \Sigma_{s\leq t} g(k)E_{s\leq t} / \Sigma_{s\leq t} f(k)E_{s\leq t} \).

2.2. Data of other factors

Data indicating the shares of mutated variants among all cases were published by the Tokyo Metropolitan Government. Unfortunately, detailed information about mutated strains has not been published for the entirety of Japan. We used two measures for the mutant strain shares in Tokyo, Japan: alpha and delta variant strains (13).

We use average temperature and relative humidity data for Tokyo during the day as climate data because national average data are not available. We obtained data from the Japan Meteorological Agency (https://www.data.jma.go.jp/gmd/risk/obsdl/index.php). Additionally, we identified several remarkable countermeasures in Japan: four state-of-emergency declarations, a travel campaign, and school closure and voluntary event cancellation (SCVEC). The latter, SCVEC, extended from February 27 through March in 2020: this countermeasure required school closure and cancellation of voluntary events, and even cancellation of private meetings. The first state of emergency was declared on April 7, 2020. It ceased at the end of May. It required school closures, shutting down of some businesses, and voluntary restriction against going out. To subsidize travel and shopping at tourist destinations, the "Go To Travel Campaign (GTTC)" started on July 22, 2020. It was halted temporarily at the end of December.

The second state of emergency was declared on January 7, 2021 for the 11 most-affected prefectures. This countermeasure required restaurant closure at 8:00 p.m., with voluntary restrictions against going out, but it did not require school closure. It continued until March 21, 2021. The third state of emergency was declared on April 25, 2021 for four prefectures: Tokyo, Osaka, Hyogo, and Kyoto. Later, the application areas were extended gradually. They never covered the entirety of Japan.

2.3. Estimation model for \( R(t) \)

To clarify associations among \( R(t) \) and current and the past vaccine coverage in addition to the mutant strains, climate, mobility, the Olympic Games, and countermeasures, we used ordinary least squares regression to regress the daily \( R(t) \) on daily current vaccine coverage and daily past vaccine coverage as well as dummy variables for the Games, weekly shares of alpha and delta variant strains, daily climate, mobility, and dummy variables for countermeasures. Temperatures were measured in degrees Celsius, with humidity and mobility as percentages in regression, not as standardized. Variables found to be not significant were excluded from explanatory variables. Then the equation was estimated again.

We define vaccine coverage as the completion rate of the second dose without delay. If a vaccine perfectly protects the recipient from infection, then the estimated coefficient of vaccine coverage would be 0.01 if one assumes an average of \( R(t) \) with no vaccination in the study period. That would indicate that vaccine coverage increased by one percentage point could be expected to reduce \( R(t) \) by one percentage point. If the estimated coefficient of vaccine coverage were smaller than -0.01, then it might reflect imperfect personal prevention. Conversely, if the estimated coefficients of vaccine coverage were smaller than -0.01, then herd immunity can be inferred to have contributed to prevention of infection among non-recipients.

Waning of vaccine effectiveness was measured by the estimated coefficient of vaccine coverage in the past. Particularly, we examined every 30 days prior until
180 days prior. We expected the estimated coefficient to be positive if waning was occurring. If its estimated coefficient was positive but smaller than the absolute value of the estimated coefficient of current vaccine coverage, then waning was presumed to be partially occurring. Vaccination was presumed to be effective even if a part of effectiveness was waning. If the estimated coefficient of vaccine coverage in the past was positive and almost equal to the absolute value of the estimated coefficient of current vaccine coverage, then waning was presumed to be complete. We might not expect vaccine effectiveness until that time. Conversely, if the estimated coefficient of vaccine coverage in the past was positive and larger than the absolute value of the estimated coefficient of current vaccine coverage, then the vaccine might raise infectiousness eventually. We adopted 5% as the level at which we inferred significance of the results.

3. Results and Discussion

3.1. Data

Figure 1 depicts vaccine coverage for the first dose with a 12-day delay and depicts the second dose as scatter diagrams. It also shows the shares of alpha and delta variant strains as bars. These are increasing almost monotonically during the period. Adjustments were made for double counting for the number of vaccine recipients. Therefore, the vaccine coverage was sometimes less than it was earlier. Figure 2 depicts $R(t)$ during the study period.

Figure 3 presents an empirical distribution of the duration of onset to reporting in Japan. The maximum delay was 31 days. Figure 4 presents an empirical distribution of incubation periods among 91 cases for

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**Figure 1. Vaccine coverage and shares of alpha and delta variant strains in 2021.** The black line represents shares of the alpha variant strain in Tokyo, as measured at the left-hand side. The gray line represents shares of the delta variant strain. Black scattered points denote vaccine coverage as defined by the first dose with a 12-day delay. Gray scattered points denote vaccine coverage defined by the second dose. The vaccine coverage data are measured at the right-hand side scale. Because the daily vaccine coverage was not reported on weekends or national holidays, data of vaccine coverage are missing for these days. Moreover, there were adjustments for double counting for the number of vaccine recipients. Therefore, the vaccine coverage was sometimes less than it was earlier.

**Figure 2. Effective reproduction number from February, 2020 through November 4, 2021.** The line represents the effective reproduction number in Japan from February, 2020 through November 4, 2021, as of November 25, 2021. Calculation procedures are explained in the main text.

**Figure 3. Empirical distribution of duration from onset to report by MHLW, Japan.** Bars represent the probability of duration from onset to report based on 657 patients in Japan for whom the onset date was available. Data were obtained from MHLW, Japan.

**Figure 4. Empirical distribution of the incubation period published by MHLW, Japan.** Bars show the distribution of incubation periods for 91 cases for which the exposure date and onset date were published by MHLW, Japan. Patients for whom incubation was longer than 14 days are included in the bar shown for day 14.
which the exposed date and onset date were published by MHLW in Japan. The mode was six days; the average was 6.6 days.

3.2. Estimation results

Table 1 presents estimation results. The Olympic Games, climate conditions, and the fourth state of emergency were not found to have a significant effect. Therefore, we excluded these variables from the explanatory variables. All remaining variables in the specification were found to be significant in the final specification.

Current vaccine coverage reduced infectiousness significantly when vaccine coverage in the past was defined as 90 days prior. The estimated coefficients increased along with their duration of time into the past: from -0.0549 for vaccination coverage of 90 days prior to -0.0241 for vaccination coverage of 180 days prior.

The estimated coefficients of vaccine coverage in the past were significant and positive when vaccine coverage in the past was defined as 90 days prior. The estimated coefficients also increased with time into the past: from 0.0550 to 18.49.

The sums of the estimated coefficients of current and past vaccine coverage were not significantly different from zero for 90 or 120 days prior assumed for the past vaccine coverage. When assuming coverage as 150 or 180 days prior, the estimated coefficients of the past vaccine coverage were significantly larger in absolute terms than the current vaccine coverage.

The estimated coefficients of the share of the variant strain were negative. These findings were inconsistent with the expected effects of the variant strain. Especially, shares of the alpha variant strain were significant and negative consistently, although the shares of the delta variant strain were not significant, except when assuming past vaccine coverage as 30 or 60 days prior.

Mobility was found to be positive and significant. The first three state-of-emergency periods and GTTC were found to be negative and significant. However, SCVEC was found to be significant and positive. The fourth state-of-emergency period was significant but positive only when assuming past vaccine coverage as 60 or 180 days prior. Effects of the Olympic Games were not significant.

3.3. Brief history of countermeasures

The Olympic Games and Paralympic Games of 2020 began on July 23, 2021. A subject of great concern for COVID-19 outbreak effects in Japan was whether audiences would be allowed to attend game events, or not. As part of this controversy, some experts asserted that the Games should be abandoned because they would expand the outbreak explosively (14). As a result, the game events were held with no live audience. Under the state of emergency declared in Tokyo, effects of the 2020 Tokyo Games must be included to evaluate vaccine effectiveness.

As countermeasures against the COVID-19 outbreak in Japan, school closure and voluntary event cancellation were adopted from February 27, 2020 through the end of March. Large commercial events were cancelled. Subsequently, a state of emergency was declared for April 7 through 25 May, stipulating voluntary restrictions against leaving home. Consumer businesses such as retail shops and restaurants were shuttered. During this period, the first peak of infection was reached on April 3. Infections subsequently decreased through July 29. The so-called "Go To Travel Campaign" (GTTC) was launched on July 22 as a 50% subsidized travel program aimed at supporting sightseeing and tourism businesses with government-issued coupons for use in shopping at tourist destinations. It was expected that the campaign might expand the outbreak. Thereafter, GTTC continued to the end of December, by which time a third wave of infection had emerged. The third wave in December, which was larger than either of the preceding two waves, reached its highest peak at the end of December. Therefore, GTTC was inferred as the main reason underlying the third wave (15).

To suppress that third wave of infection, a second state of emergency was declared from January 8, 2021 through March 15, 2021. However, a fourth wave emerged at the end of February, probably because of the spread of variant strains. To support hosting of the Olympics and Paralympics games in Tokyo in July, a third state of emergency was declared on April 25, 2021. It had ceased on June 20, 2021 in Tokyo. Nevertheless, the outbreak commenced again before the Tokyo Games 2020 started. Therefore, a fourth state of emergency was declared on July 13, 2021. It continued thereafter until the Tokyo Games 2020 had closed.

Although results have been mixed, some findings from earlier studies suggest that COVID-19 is associated with climate conditions (16-19). If that were true for Japan, then GTTC might not have been the main factor contributing to the third wave. In fact, mobility was inferred as the main cause of the outbreak dynamics for the first wave in Japan (20) and throughout the world (21-24).

3.4. Implications of estimation results

Results showed complete waning by 90 days after the second dose of vaccine was administered. This duration is remarkably shorter than those reported from earlier studies of waning (3,4), which reached their conclusions based on antibody titer or test negative design. Readers must be reminded that waning estimated for the present study might include behavioral changes among the vaccinated persons to adoption of more risky behavior that is prone to exacerbating infectiousness. Such behaviors and the vaccine itself affect waning results, but they are not separately discernible based on results of this study. Weakening of immunoreaction and behavioral change are separate factors, but their mutual effects might be the most
Table 1. Estimation results of R(t) with vaccine coverage, prevalence of the variant strains, and Olympic Games with the climate condition, mobility, and countermeasures

<table>
<thead>
<tr>
<th>Lag for waning</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated coefficient</td>
<td>p-value</td>
<td>Estimated coefficient</td>
<td>p-value</td>
<td>Estimated coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.0053</td>
<td>0.158</td>
<td>-0.0042</td>
<td>0.277</td>
<td>-0.0034</td>
<td>0.373</td>
</tr>
<tr>
<td>Humidity</td>
<td>-0.0007</td>
<td>0.582</td>
<td>0.0004</td>
<td>0.772</td>
<td>0.0006</td>
<td>0.686</td>
</tr>
<tr>
<td>Mobility</td>
<td>0.0085</td>
<td>0.000</td>
<td>0.0082</td>
<td>0.000</td>
<td>0.0080</td>
<td>0.000</td>
</tr>
<tr>
<td>SCVEC</td>
<td>0.7430</td>
<td>0.000</td>
<td>0.7909</td>
<td>0.000</td>
<td>0.8064</td>
<td>0.000</td>
</tr>
<tr>
<td>1st State of emergency</td>
<td>-0.8875</td>
<td>0.000</td>
<td>-0.8692</td>
<td>0.000</td>
<td>-0.8671</td>
<td>0.000</td>
</tr>
<tr>
<td>GTTC</td>
<td>-0.9088</td>
<td>0.000</td>
<td>-0.8801</td>
<td>0.000</td>
<td>-0.8720</td>
<td>0.000</td>
</tr>
<tr>
<td>2nd State of emergency</td>
<td>-1.0461</td>
<td>0.000</td>
<td>-1.0071</td>
<td>0.000</td>
<td>-0.9933</td>
<td>0.000</td>
</tr>
<tr>
<td>3rd State of emergency</td>
<td>-0.3107</td>
<td>0.017</td>
<td>-0.6941</td>
<td>0.000</td>
<td>-0.7406</td>
<td>0.000</td>
</tr>
<tr>
<td>4th State of emergency</td>
<td>0.2832</td>
<td>0.290</td>
<td>0.7525</td>
<td>0.007</td>
<td>0.4292</td>
<td>0.134</td>
</tr>
<tr>
<td>Olympic Games</td>
<td>0.2927</td>
<td>0.049</td>
<td>0.4532</td>
<td>0.022</td>
<td>-0.0487</td>
<td>0.802</td>
</tr>
<tr>
<td>Vaccine coverage (%)</td>
<td>0.1486</td>
<td>0.000</td>
<td>0.0337</td>
<td>0.243</td>
<td>-0.0539</td>
<td>0.001</td>
</tr>
<tr>
<td>Vaccine coverage with lag (%)</td>
<td>-0.1416</td>
<td>0.000</td>
<td>-0.0406</td>
<td>0.095</td>
<td>0.0550</td>
<td>0.012</td>
</tr>
<tr>
<td>Share of alpha variant strain (%)</td>
<td>-0.0107</td>
<td>0.000</td>
<td>-0.0041</td>
<td>0.008</td>
<td>-0.0031</td>
<td>0.033</td>
</tr>
<tr>
<td>Share of delta variant strain (%)</td>
<td>-0.0447</td>
<td>0.000</td>
<td>-0.0313</td>
<td>0.010</td>
<td>0.0074</td>
<td>0.411</td>
</tr>
<tr>
<td>Constant</td>
<td>1.1571</td>
<td>0.000</td>
<td>1.0542</td>
<td>0.000</td>
<td>1.0384</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Adjusted $R^2$ | 0.6042 | 0.5714 | 0.5740 | 0.5737 | 0.5736 | 0.5730 |

Number of observations | 604 |

Notes: The dependent variable was R(t); GTTC stands for "Go To Travel Campaign"; SCVEC denotes school closure and voluntary event cancellation. The sample period was February 1, 2021 through November 4, 2021, as of November 25, 2021.
important for management of public health.

However, when we assumed the past vaccine coverage as greater than 90 days prior, the current vaccine coverage was found to be significant and to have absolute value greater than 0.01.

Vaccine efficacy was estimated as 95% through clinical trials (25). In the real world, it was also estimated as 46-80% for the first dose and 86-90% for the second dose (26-31) through case-control studies or test-negative design. However, even in the real world, such studies specifically examine protection for vaccine recipients only and ignore herd immunity, representing vaccine effects on non-vaccine recipients. The latter was not able to be estimated through clinical trials, case-control studies, or test negative design. In this sense, these earlier studies have been incapable of evaluating the overall effects of vaccination on the community. Instead of those methods, we evaluated vaccine effectiveness on the entire community, of course including herd immunity, through its effects on SARS-CoV-2 infectiousness.

Results indicated no significant result of momentary effects from the current vaccine coverage or waning from past vaccine coverage when the past vaccine coverage was defined as less than 60 days prior. Particularly, these estimated coefficients had unexpected signs but were significant. These results were probably caused by multicollinearity among the current and past vaccine coverage. Because of smaller time differences among these variables, correlation among them can be expected to be higher. Therefore, the smaller time differences distorted estimation results.

Conversely, when the past vaccine coverage was defined as more than 120 days prior, the estimated coefficients of the past vaccine coverages were much larger than the estimated coefficients of the current vaccine coverage in absolute terms. Statistically, the result probably reflected that the past vaccine coverage occurring longer ago should be a very small number, as shown in Figure 1. Therefore, the estimated coefficients should be larger than the correspondence when the past vaccine coverage was defined as 60 or 90 days prior. Expressed semantically, because that waning might not reduce the immunization level to less than before the vaccination was administered, these results imply that behavioral changes to adopt more risky behaviors prone to infection among vaccinated persons raise infectiousness considerably. No evidence exists to indicate that the Tokyo Games 2020 exacerbated the outbreak of COVID-19. Expectations by some experts before the Olympic Games might have been wrong. It seems likely that most Japanese people watched TV at home and rooted for athletes. The no-audience policy might have contributed to reduction in infectiousness during the Games. Even though lower infectiousness prevailed during the Games, if it actually became higher than unity, then the number of newly infected or newly confirmed patients would be expected to grow during the period. Therefore, the number of patients has not represented the outbreak situation accurately. Infectiousness must be specifically examined during that period to evaluate policies adequately.

Alpha variant strain effects were significant and negative. Additionally, the share of delta variant strain was not found to be significant, with some exceptions. These results were not consistent with results reported from earlier studies (5-8).

3.5. Limitations

First, we assumed implicitly that epidemiological characteristics including incubation period or delay in reports were the same among the original strain, alpha and delta variant strains. However, results of one study indicated that the delta variant strain has a shorter incubation period than either original strain (32).

Secondly, readers must be reminded when interpreting the obtained results that they do not indicate causality. Results of this study demonstrated that a negative association exists between the vaccine coverage and infectiousness. That finding does not necessarily mean that the vaccine coverage reduced infectiousness. The lower infectiousness might have caused or might have even simply coincided with higher vaccine coverage.

4. Conclusion

The estimation results are evidence of significant waning in vaccine effectiveness from 90 days after the second dose. (The present study is based on the authors’ opinions: it does not reflect any stance or policy of their professionally affiliated bodies.)

Acknowledgements

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Conflict of Interest: The authors have no conflicts of interest to disclose.

Ethical considerations: All information used for this study was from official data published on the internet. There is therefore no ethical issue related to this study.

References


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Effectiveness of ultrasound-guided pelvic floor muscle training in improving prolonged urinary incontinence after robot-assisted radical prostatectomy

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SUMMARY Persistence of urinary incontinence (UI) after robot-assisted radical prostatectomy (RARP) is a bothersome problem because of its negative effect on the patient’s quality of life (QOL). This study aimed to evaluate the effect of transperineal ultrasound (TPUS)-guided pelvic floor muscle training (PFMT) on prolonged UI after RARP. Thirty men with stress UI persisting for > 1 year after RARP underwent biofeedback PFMT using TPUS once every 2-3 weeks for 3 months. The frequency and duration of sustaining pelvic floor muscle (PFM) contractions were assessed using ultrasound imaging. The severity of UI and UI-related QOL were evaluated using a 24-hour pad test and the incontinence quality of life (I-QOL) questionnaire. Twenty-four men (mean age, 72.2 years) completed the TPUS-guided PFMT. The mean duration from RARP to PFMT was 1,228.9 days. The mean cumulative session and the total duration of TPUS-guided PFMT were 4.6 times and 73.3 days, respectively. Compared with the data before TPUS-guided PFMT, the frequency of PFM contractions and duration of sustaining contraction significantly improved after TPUS-guided PFMT (p < 0.05). Additionally, the total amount of urinary leakage after TPUS-guided PFMT was reduced significantly (248.6 ± 280.6 g vs. 397.0 ± 427.0 g, p = 0.024). The I-QOL score was significantly increased after TPUS-guided PFMT (72.1 ± 16.8 vs. 61.0 ± 19.0, p < 0.001). TPUS-guided PFMT may be effective in improving prolonged UI occurring > 1 year after RARP.

Keywords biofeedback, physiotherapy, urinary leakage, quality of life, transperineal ultrasound

1. Introduction

Urinary incontinence (UI) after robot-assisted radical prostatectomy (RARP) is a common complication and has a negative effect on the patient’s quality of life (QOL) (1,2). Approximately 90% of patients experience UI after RARP (1,2) and approximately 10% experience UI at 1 year after RARP (3,4). Although artificial urethral sphincter placement is recommended as the gold standard of treatment for these patients (5,6), inherent incidences of adverse events, such as device infection and malfunction, urethral erosion, and atrophy, have been reported (7). These risks deter possible placements of the artificial urethral sphincter in patients. However, patients with prolonged UI for > 1 year after RARP are reluctant to accept a lower QOL caused by UI. The long-term negative effects of UI on the QOL of these patients are concerning.

Generally, pelvic floor muscle training (PFMT) is advocated as the first choice of conservative treatment (8) for all patients immediately after RARP (9). This is because the main cause of UI is insufficient urethral closure because of urinary sphincter dysfunction following intraoperative nerve damage (10). PFMT promotes urethral closure by increasing the strength of the pelvic floor muscle (PFM). To reduce the amount of urinary leakage after RARP, the patients should relearn how to contract the PFM to close the urethra sufficiently. Modalities that utilize biofeedback (BF) for self-recognition of PFM contractions, such as digital
palpation, electromyography (11,12), and ultrasound (US) imaging (13), have been recommended additions to PFMT. Our recent study (14) suggests that transperineal ultrasound (TPUS) guided PFMT effectively helps men after RARP relearn PFM contractions to reduce the amount of urinary leakage. US images showed the extent of urethral closure when a patient contracts the PFM. As a BF, TPUS allows visualization of urethral closures and facilitates self-awareness of urethral closure during PFM contractions.

Considering that patients presenting with prolonged UI are likely to have more severe damage to the urinary sphincter, TPUS may be more useful than digital palpation or electromyography in these patients. However, the effectiveness of TPUS-guided PFMT in improving prolonged UI after RARP has not been adequately studied. Therefore, this study aimed to evaluate the effect of PFMT with TPUS on prolonged UI that has lasted for > 1 year after RARP.

2. Materials and Methods

2.1. Study design and setting

This prospective interventional study was conducted between April 2018 and October 2019 at a university hospital in Tokyo. The study protocol was approved by the Research Ethics Committee of the authors' institute (approval no: 10921-(1), 2373-(2)). Each study participant provided written informed consent.

2.2. Patients

The study included male patients who underwent RARP using the peritoneal approach (15) at our institution and complained of stress urinary incontinence (SUI) persisting for > 1 year after RARP. The exclusion criteria were as follows: < 20 g per day of urinary leakage, urgency UI, disability-associated UI, severe mental disease or cognitive impairment, a known neurological disorder affecting the lower urinary tract function, restricted physical activity, and an inability to understand Japanese.

2.3. Procedure

Patients who met the inclusion criteria were introduced to the TPUS-guided PFMT protocol by their physicians. If the patient showed a willingness to take the TPUS-guided PFMT, they underwent TPUS-guided PFMT that was performed by a physiotherapist. The physiotherapist provided the patient with instruction on the anatomy of the pelvic floor and the mechanism of continence using a leaflet and an anatomical model of the pelvis. All participants attended TPUS-guided PFMT once every 2-3 weeks for up to 3 months. The frequency of PFMT depended on the patient's circumstances, such as work schedule and transportation difficulty. After receiving individual BF in every TPUS-guided PFMT session, the patient was instructed on the training load of PFMT at home.

2.4. TPUS-guided PFMT

The procedure of performing TPUS-guided PFMT has been described in our previous study (14). US observation was conducted with the patient in a lateral position, in which the penis and scrotum were not seen or touched when the US transducer was placed on the perineal area. A 1- to 5-MHz two-dimensional curved array ultrasound transducer was used (Noblus; Hitachi, Ltd., Tokyo, Japan) to visualize PFMT contraction. When the US transducer was placed on the perineal skin in the midsagittal plane, the image showed the bladder neck, proximal urethra, paraurethral tissue, and PFM (Figure 1). When a patient contracted the PFM correctly, movement of the anorectal angle toward the posterior aspect of the pubic symphysis was observed, resulting in shortening of the diameter of the membranous urethra and closure of the bladder neck on US images. Therefore, patients could recognize the strength and sensation of optimal PFM contractions without the activity of other muscles, such as the abdominal muscles. When providing BF using US images, the physiotherapist gave verbal instructions to the patients to retract the penis, elevate the penis from the root, and pull the scrotum upward to help them become aware of the contractions of the PFM. If patients could contract the PFM correctly, the physiotherapist instructed the patients to perform the following two types of PFM contractions at maximum strength: the first type was to repeat the contractions up to 10 times, and the second type was to sustain the contraction for up to 10 seconds (16). Based on the patient's performance at each TPUS-guided PFMT, the physiotherapist discussed with the patient the individual PFMT load that should be performed at home.

2.5. Measurements

The function of PFM, the severity of urinary leakage, and QOL related to UI were measured before and after TPUS-guided PFMT. The function of PFM, including the frequency of PFM contractions, and the duration of maintaining the contraction, was evaluated by the physiotherapist. The frequency of PFMT contractions was measured by counting the number of times the patients could repeat the contractions correctly at maximum strength on the US monitor. The duration of sustained contraction was evaluated by observing how long they could hold the maximum contraction on the US monitor.

The two-day data of the 24-hour pad test were used to evaluate the severity of UI. To determine the
The patients’ demographic data and perioperative parameters (nerve-sparing, lymph node dissection, resected prostate volume) were obtained from their medical records.

2.6. Statistical analysis

Means and standard deviations were used for descriptive data. A paired t-test was performed to compare data obtained before and after TPUS-guided PFMT. All p-values were two-sided. A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows version 23.0 software (IBM Corp, Armonk, NY, USA).

3. Results and Discussion

3.1. Patients

Among the 30 patients who met the inclusion criteria, three were excluded because of lack of data on urinary leakage before TPUS-guided PFMT, and three other patients withdrew from the study because of difficulty in visiting the hospital regularly. Thus, 24 patients who completed the TPUS-PFMT regimen were included in the analysis (Figure 2).

The patients had a mean age of 72.2 years (range, 60-79 years) and mean body mass index of 23.8 ± 1.9 kg/m². Of them, 13 patients (54.2%) were employed; 6 patients (25.0%) regularly took medications for lower urinary tract symptoms; and 5 patients (20.8%) had received additional radiation or androgen deprivation therapy after RARP (Table 1). There was no change in

![Figure 1](image1.png)

Figure 1. Transperineal ultrasound images of a patient who underwent RARP shows urethral closure in the midsagittal plane. First TPUS-guided PFMT (A) at rest and (B) during contraction of the pelvic floor muscle. Last TPUS-guided PFMT (C) at rest and (D) during contraction of the pelvic floor muscle. RARP, robot-assisted radical prostatectomy; TPUS, transperineal ultrasound; PFMT, pelvic floor muscle training.

![Figure 2](image2.png)

Figure 2. Flow chart of the participants in the study. UI, urinary incontinence; RARP, robot-assisted radical prostatectomy; DUI, disability-associated UI; UUI, urgency UI; SUI, stress UI; PFMT, pelvic floor muscle training.
medication during the period of TPUS-guided PFMT.

The mean duration from RARP to TPUS-guided PFMT was 1,228.9 ± 610.7 days. The cumulative session of TPUS-PFMT was 4.6 ± 0.9 times. The interval between each session was 20.3 ± 4.8 days. The total duration of TPUS-guided PFMT was 73.3 ± 17.4 days (Table 2).

3.2. Change of the PFM function after the TPUS-guided PFMT

Table 3 summarizes the changes in the function of the PFM, UI amount, and I-QOL score after TPUS-guided PFMT. The frequency of PFM contractions after TPUS-guided PFMT was significantly higher than that before TPUS-guided PFMT (pre-PFMT: 7.5 ± 2.5 vs. post-PFMT: 10.0 ± 0 times, $p < 0.001$); furthermore, all patients could repeat PFM contractions 10 times. The duration of maintaining the contraction was significantly longer after TPUS-guided PFMT than before training (pre-PFMT 2.6 ± 1.9 s, $p = 0.017$). The average amount of urinary leakage for 2 days after TPUS-PFMT was significantly lower than the pre-training amounts (pre-PFMT 397.0 ± 427.0 vs. post-PFMT 248.6 ± 280.6, $p = 0.024$). The I-QOL score after TPUS-guided PFMT was significantly higher than that before TPUS-guided PFMT (pre-PFMT 61.0 ± 19.0 vs. post-PFMT 72.1 ± 16.8, $p < 0.001$).

3.3. Change of urinary leakage after the TPUS-guided PFMT

The amount of urinary leakage decreased significantly after TPUS-guided PFMT with a mean total duration of 73.3 ± 17.4 days (Table 3). The 3-month duration has been recommended for PFMT in both male and female patients (18,19), as it takes approximately 3 months for the PFM, a skeletal muscle, to be strong enough to continuously close the urethra (20,21). Generally, men use quick contraction of the PFM to squeeze urine out of the urethra after urination, and this sensation of muscle contraction is retained even after RARP. These quick contractions can maintain urethral closure during momentary increases in intra-abdominal pressure, such as when coughing or standing up. The sustained contractions keep the urethra closed during activities of daily living (ADL), such as walking and performing housework (22). Enhancement of the ability to sustain these contractions is the target of PFMT to ensure urethral closure during ADL. Thus, the 3-month PFMT

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**Table 1. Demographic data and surgical outcomes of the participants ($n = 24$)**

| Variable                      | Age (years)  | Body mass index (kg/m$^2$) | Having a job (yes) | Leisure activity (yes) | Disease          | Hypertension | Diabetes | Heart disease | Hyperlipidemia | Surgery of inguinal hernia | Drug related to LUTS* | Anticholinergic drugs | β3 adrenoceptor agonists | Initial serum PSA | Nerve sparing (yes) | Unilateral (yes) | Bilateral (yes) | Lymph node dissection (yes) | Resected prostate volume (ml) | Radiation therapy after RARP (yes) | Androgen deprivation therapy after RARP (yes) |
|-------------------------------|--------------|----------------------------|-------------------|------------------------|-----------------|----------------|------------|--------------|----------------|-------------------------|---------------------|----------------------|------------------------|-----------------------|---------------------|------------------|----------------------|-------------------------|------------------------|----------------------|
|                               | 72.2 ± 5.1   | 23.8 ± 1.9                 | 13 (54.2%)        | 12 (50.0%)             | Disease         | 11 (45.8%)    | 4 (16.7%)  | 3 (12.5%)    | 1 (4.2%)       | 6 (25.0%)                | 2 (8.3%)            | 4 (16.7%)            | 1 (4.2%)              | 6 (25.0%)             | 12.0 ± 13.5         | 5 (20.9%)          | 4 (16.7%)           | 1 (4.2%)               | 6 (25.0%)                | 39.3 ± 13.4            | 2 (8.3%)            | 3 (12.5%)           |

Mean ± standard deviation (range), $n$ (%). *LUTS: lower urinary tract symptoms; PSA: prostate-specific antigen; RARP: robot-assisted radical prostatectomy.

**Table 2. Attendance of clinic pelvic floor muscle training**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Starting day of training after RARP* (days)</th>
<th>Cumulative session of PFMTs (times)</th>
<th>Interval between each session (days)</th>
<th>Total duration of PFMTs (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1228.9 ± 610.7 (431-2,618)</td>
<td>4.6 ± 0.9 (3-6)</td>
<td>20.3 ± 4.8 (11-42)</td>
<td>73.3 ± 17.4 (40-101)</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (range). *RARP: robot-assisted radical prostatectomy; PFMT: pelvic floor muscle training.

**Table 3. Changes in PFM strength, amount of urinary incontinence per day, and QOL before and after the US-guided PFMT**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of PFM contraction (times)</td>
<td>7.5 ± 2.5</td>
<td>10.0 ± 0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of keeping contraction (sec)</td>
<td>2.6 ± 1.8</td>
<td>9.0 ± 1.9</td>
<td>0.017</td>
</tr>
<tr>
<td>Total urine leakage (g)</td>
<td>397.0 ± 427.0</td>
<td>248.6 ± 280.6</td>
<td>0.024</td>
</tr>
<tr>
<td>QOL*</td>
<td>61.0 ± 19.0</td>
<td>72.1 ± 16.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

for prolonged UI in our study is useful in improving the function of the PFM to prevent urinary leakage, and our findings are comparable to those of previous studies (23).

A previous study (24) showed that behavioral therapy, including PFMT, bladder control strategies, fluid management, and self-monitoring with bladder diaries improved prolonged UI of > 1 year after RARP. However, their study could not demonstrate the added value of BF using surface electromyography. Given that patients with UI after RARP are presumed to have decreased awareness of the contraction of the PFM around the urethra, BF would be beneficial for patients who are relearning how to perform PFM contractions. Based on our previous study (14) that showed the advantages of visualizing the urethra by TPUS as BF, we hypothesized that TPUS facilitates relearning of PFM contractions as well as controlling optimal contractions to close the urethra even years after RARP. In the present study, patients with prolonged UI could not sustain maximum PFM contractions for more than 3 seconds at the commencement of TPUS-guided PFMT. However, after 3 months of TPUS-guided PFMT, the strength of the PFM improved significantly enough to sustain maximum contractions for up to 9 seconds. TPUS allowed the patients to visualize the correct PFM contraction required to close the urethra. This is an advantage of TPUS, giving patients the ability to strengthen the PFM any time without supervision (e.g., self-training at home). TPUS is speculated as an ideal BF for promoting self-awareness of urethral closure for patients with prolonged UI.

Our data show that the I-QOL score improved after 3 months of TPUS-guided PFMT, despite the mean amount of urinary leakage being 248.6 g per day (Table 3). Changing pads a few times a day for moderate amounts of urinary leakage is still burdensome to patients. The I-QOL score improved possibly because of the reduction in overall urinary leakage, which gave patients the confidence that PFMT will eventually improve their UI, even years after RARP. In a previous study (25), I-QOL scores increased when the amount of urinary leakage decreased immediately after RARP. The patients felt accomplished when they could successfully manage their incontinence even though they only had small improvements in their symptoms. Another explanation could be that being able to control PFM contraction, thereby preventing urinary leakage, helped the patients to regain their self-esteem.

Generally, patients receive PFMT once every 2-4 weeks (18, 23). Owing to the more severe damage of the PFM, patients with prolonged UI should undergo frequent sessions to retrain the PFM. In our TPUS-guided PFMT regimen, patients visited the hospital once every 2-3 weeks. TPUS-guided PFMT allowed patients to look at images and repeatedly match the sensations of PFM contraction to the actual contractions. The awareness of the correct contraction gave patients the confidence to continue PFMT at home and minimize the frequency of hospital visits. Most patients undergo RARP in their early 70s. Half of them still have jobs or engage in leisure activities. Considering that a few participants withdrew from the study because of the difficulty in visiting the hospital regularly, the TPUS-guided PFMT protocol has the advantage of maintaining the patient’s lifestyle.

This study has several limitations. First, this study did not have a control group to confirm the efficacy and feasibility of the TPUS-guided PFMT for prolonged UI. A randomized controlled trial is required to verify the effectiveness of this treatment strategy. Second, this study included a few patients with severe urinary leakage of > 500 g per day. These patients are considered to have more severe damage to the PFM. Thus, the effectiveness of the TPUS-PFMT in these patients with severe UI needs to be interpreted cautiously, which require further investigation.

In conclusion, our results suggest that the TPUS-PFMT may improve UI prolonged for > 1 year after RARP by increasing the strength of PFM.

Acknowledgements

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Probiotic microbes: Are their anti-melanogenicity and longevity promoting activities closely linked through the major "pathogenic" kinase PAK1?

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SUMMARY  
PAK1-deficient mutant of *C. elegans* lives 60% longer than the wild-type. Interestingly, PAK1-deficient mutant of melanocytes produces less melanin (only a half compared with the wild-type) in the presence of either serum (PDGF) or α-MSH (alpha-melanocyte stimulating hormone). These observations indicate that the major "pathogenic" kinase PAK1 is responsible for both shortening the healthy lifespan, and PDGF/α-MSH-dependent melanogenesis. For screening of PAK1-blocking probiotic bacteria or their products, their anti-melanogenic as well as longevity promoting properties were examined. Recently it was found that *C. elegans* fed with *Lactobacillus rhamnosus* in Xinjiang cheese lives 40% longer than the worm fed with the standard *E. coli*. Interestingly, a Chinese traditional medicine called "ChiBai" fermented with the *Lactobacillus rhamnosus* also inhibited the α-MSH-induced melanogenesis, and this bacteria itself produces butyric acid that blocks the oncogenic HDAC (histone deacetylase)-PAK1 signaling pathway. These findings strongly suggest, if not proven, that anti-melanogenic activity of *Lactobacillus* and many other probiotic bacteria might serve as a reliable indicator for their longevity promoting activity. In this context, a popular Japanese *Lactobacillus*-fermented milk drink called "Calpis", developed a century ago, and recently proven to inhibit the melanogenesis by suppressing the PAK1-dependent tyrosinase gene expression, may potentially prolong our healthy lifespan.

Keywords  
PAK1, *Lactobacillus*, melanogenesis, longevity, *C. elegans*, *Bacillus*, COVID

1. Introduction

In 1908, a Russian/Ukrainian physiologist, Ilya Mechnikov (1845-1916), shared a Nobel Prize in Medicine with Paul Ehrlich (1854-1915), a German/Jewish pathologist who developed the first chemotherapeutic called "Salvarsan" (or 606) against Syphilis in 1909. The former was an expert in phagocytes called macrophages, and published a rather sensational book entitled "Prolongation of life: Optimistic Studies" in 1907. In this book he proposed a theory that *Lactobacillus* from stomach from long-living Bulgarians who ingest routinely the Bulgarian local yogurts fermented with *Lactobacillus* could potentially be useful for our longevity. More than a century later, there are increasing biochemical evidences, provided mainly from the Far-East research groups, supporting this theory in principle.

In this century, the majority of scientists studying on the longevity opt for testing the potential longevity-promoting (so-called elixir) effect of given chemicals or organisms on a tiny worm called *C. elegans*, mainly because its lifespan is the shortest among aminal kingdom, around 15 days at 20°C. Not suprisingly among the genes responsible for shortening the life-span of this organism shared with mammals are "oncogenic" genes encoding PI-3 kinase (AGE), PAK1, ILK, AKT and TOR (1-4). PI-3 kinase deficient mutant of this worm lives 100% longer than the wild-type (1), and PAK1-deficient mutant lives 60% longer than the wild-type (2). Interestingly in both cases, these mutants show a very low fertility (less than 14% of the wild-type) (1,2), indicating that these oncogenes are essential for their fertility. In other words, longevity trades fertility (1,2).

Unfortunately, however, any chemical compounds such as LY3023414 which block the oncogenic PI-3 kinase-AKT signalling cannot be used clinically for promoting the longevity, simply because this pathway is essential for heart development/function as well (2,5). Tiny "experimental" invertebrates such as *C. elegans* and
**Drosophila** have no cardiovascular system. Fortunately, in 2021, PAK1-deficient mutant of mice was proven to live significantly longer than the wild-type without any complication on either heart or brain (6). In addition, mice treated with rapamycin, a TOR-inhibitor, have been shown to live longer than the control mice (7). However, this drug has been used mainly to suppress the immune response against grafted organs (2). Therefore it may be rather risky for ordinary people, in particular during pandemics of COVID and other deadly viruses.

2. Natural chemicals, that prolong the lifespan of *C. elegans*, inhibit melanogenesis by blocking PAK1

Using *C. elegans* as a target, a number of natural longevity promoters have been identified. Among are curcumin (CC), caffeic acid (CA), caffeic acid phenethyl ester (CAPE), and melatonin (8-11). Interestingly, all these longevity promoters are known to inhibit melanogenesis, without affecting directly the enzymatic activity of tyrosinase which is responsible for biosynthesis of melanin from tyrosine (12-15).

Since the first three chemicals (CC, CA and CAPE) at least have been known to block PAK1, during 2015-2017 we examined whether melanogenesis of melanoma (B16F10) requires PAK1 or not. We found that treatment of melanoma with si-RNA specific for PAK1 (silencing *PAK1* gene) clearly reduces the melanin synthesis to a half of the control cells only when cells are activated with either serum (PDGF) or alpha-MSH (16), indicating that the "induced" melanogenesis depends on PAK1, although the "basic" melanogenesis without PDGF or alpha-MSH does not (Figure 1A).

3. *Lactobacillus rhamnosus* extends the lifespan of *C. elegans* and inhibits melanogenesis

In 2016, to our great surprise, researchers found that *C. elegans* fed with *Lactobacillus rhamnosus* which is used for fermentation of Xinjiang cheese lives 40% longer than the worm fed with the standard *E. coli*. In 2020, other researchers found that an extract from a Chinese traditional herb mixture called "ChiBai" fermented with *Lactobacillus rhamnosus* inhibits alpha-MSH-induced melanogenesis of B16F10 melanoma by suppressing the tyrosinase gene expression (18) which depends on PAK1 (Figure 1B, 16). These two independent findings altogether indicate that both longevity-promoting and anti-melanogenic activities of this bacterium closely link to each other, and perhaps suggesting its PAK1-blocking activity. Incidentally, in 2021, another group found that *Lactobacillus rhamnosus* inhibits COVID fibrosis in part by producing butyric acid (19), which is known to inhibit HDAC (histone de-acetylase), thereby blocking PAK1 (20,21) that is responsible for inflammation, melanogenesis, oncogenesis and so many other diseases (for a review. 22).

4. *Bacillus subtilis* also extends the lifespan of *C. elegans* and inhibits melanogenesis.

It is well known that vitamin D3, a PAK1-blocker, is also anti-melanogenic and extends the healthy lifespan of *C. elegans* by 40% at 1 mg/mL (23,24). Interestingly, another vitamin called K2 or menaquinone 7 (Figure 2 left), derived from a traditional Japanese soybean product called "Natto" (fermented by *Bacillus subtilis* natto), also blocks PAK1 and is anti-melanogenic (25), although its longevity-promoting activity has not been tested as yet. In 2019, however, it was found that *C. elegans* fed with *Bacillus subtilis*, instead of the standard *E. coli*, at 20°C has 30% lesser size (number of eggs laid) than the *E. coli*-fed, and far more resistant to heat-shock at 34°C than

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**Figure 1.** (A), “Melanogenic” signaling pathway (PDGF-PAK1). (B), Blocking melanin synthesis. PAK1-blockers do not inhibit directly tyrosinase, but suppress its gene expression.

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which was developed a century ago by a Japanese monk (Kaiun Mishima) using *L. helveticus*, inhibits the inducible melanogenesis of B16F10 melanoma by suppressing PAK1-dependent tyrosinase gene expression (33,34). Thus, it is quite possible that this popular fermented milk could contribute to both COVID prevention/therapy and the longevity eventually (for review, 35).

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**Conflict of Interest:** The authors have no conflicts of interest to disclose.

**References**


Effect of corticosteroids in patients with COVID-19 early stage pneumonia and risk of disease progression: An uncharted territory

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SUMMARY Corticosteroids are one of the few drugs that have shown a reduction in mortality in coronavirus disease 2019 (COVID-19). In the RECOVERY trial, the use of dexamethasone reduced 28-day mortality compared to standard care in hospitalized patients with suspected or confirmed COVID-19 requiring supplemental oxygen or invasive mechanical ventilation. No benefit in patients not requiring respiratory support at randomization was observed. However, we believe that the use of corticosteroids in patients with COVID-19 pneumonia might not be subject to a decision based solely on oxygen needs. Evidence has shown that 30% of COVID-19 patients in its initial phases will progress to acute respiratory distress syndrome, particularly patients in whom laboratory inflammatory biomarkers associated with COVID-19 disease progression are detected. We postulated that corticosteroids in patients with COVID-19 in its initial phases and risk of progressing to severe disease might lead to a decrease in the development of acute respiratory distress syndrome, and thereby reduce death.

To the Editor,

Corticosteroids were the first group of drugs that showed clinical benefit in coronavirus disease 2019 (COVID-19) patients. In the RECOVERY Trial, conducted during the first SARS-CoV-2 wave, the use of dexamethasone resulted in lower 28-day mortality in patients hospitalized with COVID-19 who were receiving supplemental oxygen or invasive mechanical ventilation compared to standard care, but not among those receiving no respiratory support at randomization (1). This finding was supported by a prospective meta-analysis of seven randomized clinical trials by the WHO Rapid Evidence Appraisal for COVID-19 Therapies Working Group, which also confirmed that administration of corticosteroids in critically ill patients with COVID-19 was associated with a lower 28-day all-cause mortality, compared to usual care or placebo (2). Although current COVID-19 Treatment Guidelines do not recommend the use of corticosteroids in patients without oxygen needs (3-5), the optimal timing for initiating corticosteroids in hospitalized COVID-19 patients remains unclear. Therefore, we read with interest the recent study by Aggarwal et al. (6) in Drug Discoveries & Therapeutics.

In this study, the authors evaluated retrospectively the role of corticosteroids in preventing hypoxia in symptomatic COVID-19 patients with peripheral capillary oxygen saturation ≥ 94% on room-air. A total of 140 consecutive COVID-19 patients were included. Progression to hypoxia was significantly higher in patients who received corticosteroids before day 7 of symptoms (36.7% vs. 14.3%). Kaplan-Meier curves showed that patients with early corticosteroid intake had an increased risk for oxygen requirement at 30 days (HR: 4.38, 95% CI = 1.84-10.4, p = 0.001) compared to delayed corticosteroid administration. Findings from this observational study provide real-world evidence demonstrating a significantly increased risk of progression to acute respiratory failure in symptomatic COVID-19 patients who received early treatment with corticosteroids. However, we believe that definitive conclusions regarding the use of corticosteroids in COVID-19 patients without additional oxygen needs should be further discussed.

Even though RECOVERY trial showed that dexamethasone was not effective in reducing mortality
in patients with SARS-CoV-2 pneumonia without the need for supplemental oxygen and patients with symptoms for less than 7 days, we postulate that the use of corticosteroids in patients with COVID-19 pneumonia should not be based solely on oxygen needs or time from symptoms onset. Evidence has shown that 30% of COVID-19 patients in its initial phases will progress to severe life-threatening disease, largely due to acute respiratory distress syndrome (ARDS) (7). Inflammatory parameters are one of the most significant risk markers to identify patients with a high risk of COVID-19 disease progression and mortality (Table 1). Corticosteroids might have a different effect in patients with COVID-19 in its initial phases depending on the degree of underlying inflammation. Laboratory biomarkers such as C-reactive protein, D-dimer, or lactate dehydrogenase are strictly related to COVID-19 severity and they could be helpful for risk stratification, identifying patients at high-risk of ARDS who might potentially benefit from early treatment with corticosteroids, attenuating the cytokine storm, thereby preventing the progression to ARDS and death (8,9). Unfortunately, the RECOVERY trial did not differentiate between patients with elevated inflammatory parameters and without them among patients without oxygen needs. Hence, it is possible that the benefit of glucocorticoids in this subgroup of patients was underestimated. In accordance with this hypothesis, in the previously mentioned study by Aggarwal et al. (6), the risk of developing hypoxia was higher in patients with a high C-reactive protein (OR: 1.03, 95% CI: 1.02-1.06, p < 0.001). However, the analysis might be limited by a small sample size.

Estimating the impact of corticosteroid treatments in COVID-19 patients during its initial phase is challenging. We believe that there is a need for randomized, blinded, placebo-controlled clinical trials for evaluating the impact of corticosteroids in this specific subpopulation of patients with COVID-19 pneumonia.

Table 1. Laboratory biomarkers associated with COVID-19 disease progression (8,9)

<table>
<thead>
<tr>
<th>Elevations in</th>
<th>Decrease in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer</td>
<td>&gt; 1,000 ng/mL</td>
</tr>
<tr>
<td>LDH</td>
<td>&gt; 245 units/L</td>
</tr>
<tr>
<td>CRP</td>
<td>&gt; 100 mg/L</td>
</tr>
<tr>
<td>Ferritin</td>
<td>&gt; 500 mcg/L</td>
</tr>
<tr>
<td>Troponin</td>
<td>&gt; 2 × the upper limit of normal</td>
</tr>
<tr>
<td>CPK</td>
<td>&gt; 2 × the upper limit of normal</td>
</tr>
<tr>
<td>Decrease in:</td>
<td>Absolute lymphocyte count &lt; 800/microL</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; LDH, lactate dehydrogenase; CPK, creatine phosphokinase.

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Arsenic intoxication with renal failure managed with hemodialysis alone: A case report

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SUMMARY Arsenic has widespread use in agriculture, in alternative medicine and in treatment of certain malignancies, therefore it is vital to timely recognize and treat arsenic toxicity in a suspected patient. Hemodialysis conventionally is thought to play only a supportive role in managing arsenic toxicity but it can be life-saving when chelation is not possible or available. A middle-aged female with a history of non-dialysis-dependent chronic kidney disease (CKD) was brought to the emergency with altered sensorium. On presentation, she was hemodynamically stable with pallor and exfoliating lesions on palms, hyperkeratotic lesions on soles and hyperpigmented macules on the trunk. Investigations revealed pancytopenia and deranged kidney function tests. In view of skin lesions, the toxicological analysis was sent which revealed high levels of Arsenic (594 and 2,553 mcg/L in blood and urine respectively). Thus, a diagnosis of metabolic encephalopathy with the underlying cause being uremic or/and arsenic intoxication was made. Considering renal failure, she was managed with thrice-weekly hemodialysis. Chelation was not possible due to unavailability of agents during lockdown in Coronavirus disease (COVID-19) pandemic. Following dialysis, there was a significant improvement in sensorium, skin lesions, and pancytopenia depicting the utility of hemodialysis in such cases. Thus, hemodialysis is an effective and perhaps underutilized modality in the treatment of arsenic intoxication with impaired renal function.

Keywords Arsenic, arsenic poisoning, treatment, hemodialysis, chronic kidney disease (CKD)

To the Editor,

Arsenic is an important component of medicinal, agricultural, and industrial usage. At least 140 million people in 50 countries are exposed to high arsenic levels in drinking water including India, Bangladesh, Argentina, Chile, Mexico, and the United States of America (1). With increasing use of arsenic in alternative medicine and in treatment of myelodysplastic syndrome, acute promyelocytic leukemia, multiple myeloma, and other malignancies, it is important to recognize and treat arsenic toxicity in suspected patients (2). The use of chelating agents in intoxicated patients with renal failure can be deleterious due to lack of their urinary elimination. The role and efficacy of hemodialysis as standalone therapy in management of arsenic intoxication in renal failure patients is not much known.

A 31-year-old lady from central India with a history of non-dialysis-dependent chronic kidney disease (CKD) for the past four months was brought to emergency with complaints of drowsiness and irrelevant talking for the past six hours. On presentation, she had a pulse rate of 136/min, blood pressure 150/100 mm Hg, respiratory rate 24/minute, and GCS E4V2M1. There was pallor along with exfoliating skin lesions over both palms, diffuse warty hyperkeratotic lesions on soles, blotchy diffuse hyperpigmentation on the forehead and perioral area, and multiple discrete hyperpigmented macules over the chest and abdomen (Figures 1A and 1B).

The patient was intubated in the emergency for airway protection. Initial investigations revealed pancytopenia (hemoglobin 4.7 g/dl, total leukocyte count 2,000/mm³, platelet count, 60,000/mm³), normal electrolytes, and deranged renal function (blood urea, 314 mg/dL and serum creatinine, 11.3 mg/dL). Point-of-care ultrasonography revealed bilateral atrophic kidneys with raised echotexture and loss of corticomedullary differentiation which was consistent with chronic renal dysfunction. Other investigations like non-contrast computed tomography (NCCT) head and cerebrospinal fluid examination (CSF) were within normal limits. Urgent hemodialysis was done in view of uremic encephalopathy. Considering skin lesions, toxicological
analysis of blood and urine samples for heavy metals was sent which revealed blood arsenic concentration of 594 mcg/L [normal < 62 mcg/L] and urine arsenic concentration of 2,553 mcg/L [normal < 35 mcg/L].

A detailed evaluation was done to find the source of arsenic intoxication: blood, urine, and hair samples of the family members were tested, groundwater sample from home was analyzed, and a detailed history was sought but no environmental/occupational source of arsenic exposure could be identified. For managing arsenic intoxication, chelating agents were considered. Dimercaptosuccinic acid (DMSA) was relatively contraindicated owing to its unfavorable extracorporeal removal by hemodialysis while British Anti-Lewisite (BAL) had a risk of redistribution to the central nervous system thus exacerbating neurological dysfunction. Lockdown due to Coronavirus disease (COVID-19) pandemic created logistical issues in procuring dimercaptopropanesulfonic acid (DMPS). Consequently, regular hemodialysis was initiated thrice weekly with 4-hour sessions of middle flux hemodialysis (using ELISIO® dialyzer membrane on Fresenius 4008S Dialysis Machine). The patient's arsenic levels decreased progressively (Table 1 and Figure 2) while her sensorium, pancytopenia, and cutaneous lesions improved. She was extubated and discharged on thrice-weekly hemodialysis.

At follow-up after 3 months, her skin lesions and overall condition had improved considerably (Figures 1C and 1D).

Arsenic is a metalloid that binds to sulfhydryl groups and interferes with numerous enzyme systems involving cellular respiration, DNA synthesis, and repair. In acute poisoning, gastrointestinal complaints (like vomiting, abdominal pain, and diarrhea), renal injury, acute encephalopathy, and garlic odor in breath are observed. In chronic intoxication, peripheral neurologic and dermatological manifestations are prominent. Chronic arsenic exposure is also associated with the development and progression of chronic kidney disease and various malignancies including skin, lung, kidney, bladder, and prostate (3-6).

The characteristic dermatological manifestations like melanosis and hyperkeratosis can provide significant diagnostic clues towards arsenic intoxication. Arsenical hyperkeratosis appears predominantly on palms and soles and is the most sensitive marker for the detection of arsenicosis at an early stage (7). In this case, high arsenic levels, altered sensorium, and acute renal failure favored acute intoxication while the presence of dermatological manifestations suggested chronic exposure. An
environmental or occupational source of arsenic exposure couldn't be identified after detailed history and analysis of various samples (including groundwater and those of family members) for arsenic intoxication, probably suggesting that it was homicidal in nature.

In acute intoxication, decontamination and supportive care form the mainstay of therapy along with chelation and hemodialysis when indicated. The available chelating agents include BAL (dimercaprol) and DMPS (both given parenterally), and DMSA (given orally). BAL remains initial agent of choice in patients with reduced consciousness or decreased gastrointestinal motility as it can be given intramuscularly. In the setting of impaired renal excretion in CKD patients, the use of chelators can be deleterious since the arsenic mobilization induced by chelation can lead to aberrant organ deposition, particularly in the central nervous system. In renal failure, DMPS appears to be the treatment of choice because of its favorable properties allowing arsenic clearance during extracorporeal blood purification (8). Hemodialysis is also an effective tool in the treatment of arsenic intoxication as it leads to a considerable reduction in blood arsenic levels (9,10). One of the studies has also demonstrated the effective role of plasma exchange in the treatment of arsenic poisoning (11).

In most published studies, arsenic intoxication has been managed with chelation along with hemodialysis in patients with renal failure (8). This is the first case report demonstrating meaningful clinical benefit with hemodialysis alone in patients with renal injury and arsenic intoxication. The improvement in sensorium, the reversal of skin lesions, and pancytopenia show that hemodialysis may be used more than just rescue therapy especially in patients with renal failure. The trend in values of arsenic concentration in dialysate fluid effluent, blood and urine suggest that hemodialysis can be effective as a standalone modality in treating arsenic intoxication when the use of chelators is not possible. It also supports that hemodialysis may be considered upfront in emergency management of patients with arsenic intoxication till the chelating agents are arranged.

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