Effectiveness of Low-Frequency Stimulation in Proprioceptive Neuromuscular Facilitation Techniques for Post-Ankle-Sprain Balance and Proprioception in Adults- a Randomized controlled trial

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Abstract

Background

Stretching is an important part of post-ankle-sprain rehabilitation, as well as an effective exercise for improving general ankle-joint performance. But the combination of stretching alongside muscle stimulation has not yet been extensively studied. Therefore the purpose of the present research is to compare the baseline, post- and follow-up effects of the proprioceptive neuromuscular facilitation (PNF) stretching technique combined with transcutaneous electrical nerve stimulation (TENS), as compared against the effects of the PNF stretching technique alone.

Methods

Sixty subjects with lateral ankle sprains were selected and randomly allocated to three groups: Experimental Group One (EG 1), Experimental Group Two (EG 2), and the Control Group (CG). Subjects in EG 1 received the PNF stretching technique combined with TENS. Subjects in EG2 received the PNF stretching technique alone. Both experimental groups received these treatments for 4 weeks (4 days/week); follow-up assessments were administered in the third and fifth weeks. CG received no treatment; outcome measures alone were assessed. Outcome measures comprised pain, flexibility, proprioception, range of motion, muscle strength, physical activity, and balance. A mixed-model ANOVA was used to analyze the effects of time factors and groups on these outcome measures.

Results

There was significant interaction (time and group), and the time effect for all the outcome measures \((p < 0.05)\). Physical activity, dorsiflexion, and balance in the medial, lateral, anterolateral, and anteromedial directions did not show a significant difference between the groups. EG 1 showed significant improvement for all the outcome variables between pre- and post-treatment and follow-up when compared to the other groups.

Conclusions

The present study showed that a 12-session treatment program of 3 weeks’ duration that combines PNF stretching with low-frequency TENS for post-ankle sprain subjects, compared against PNF stretching alone, produced significant improvements in balance, proprioception, strength, and range of motion. The study also showed that the treatment effect was sustained even after treatment was ceased after the follow-up assessment in the fifth week.

Trial Registration:

Human Research Ethics Committee approval for the trial (approval no.: ECM#2019-26)

Clinical trial was also registered in the Clinical Trials Registry – ISRCTN 18013941

Background

The ankle joint plays a vital role in collecting sensory feedback as well as in controlling balance and posture.\(^1\) Ankle sprain refers to a ligament tear in the ankle, and ligament sprains most commonly occur on the lateral side of the ankle in isolation.\(^2\) This type of sprain is a very common injury that may result from a wide range of activities.
Ankle sprains are easily diagnosed because the pain, tenderness and swelling is usually localized on the outside of the ankle for a patient who has twisted their ankle with inversion. Ankle sprain not only causes damage to the structure of the ligament, but also damages the surrounding structures such as muscles, tendons, and nerves in the ankle complex. Any such injury may lead to ligament laxity of the ankle joint, muscular weakness, and deficits. This injury leads to impairment of joint proprioception, balance, firing of ankle muscles, nerve conduction velocity, cutaneous sensation and muscle power, as well as restriction of the range of motion of the ankle, especially dorsiflexion. Rehabilitation of ankle injuries requires specific activities and exercises to improve and recover normal function of muscles and ligaments. The journey towards recovering normal function after the rehabilitation phase of ankle-post sprain is challenging.

The effectiveness of a rehabilitation program after injury or surgery often determines the success of future function and performance. For example, range of motion and muscle power should return to normal pre-injury levels such that functional activities may be performed normally as per pre-injury. Most patients with ankle sprain recover completely, but a minority of patients report consistent pain, fear of recurrence, and functional limitation. Intervention to curb and permanently recover from an ankle sprain is still much-debated, and is coupled with a lack of evidence for the effectiveness of treatment designed to build confidence for patients with chronic ankle sprain.

Because pain and restriction in range of motion are commonly reported, usage of transcutaneous nerve stimulation (TENS) is shown to relieve pain in clinical practice, especially when applied before stretching and therapeutic exercises. Stretching is used in various therapeutic procedures that are designed to increase the length of soft tissue structures that have been shortened due to pathological causes, thereby increasing the range of motion.

Tight muscles of the leg are passively stretched, isometrically activated, and then further stretched to increase the ankle dorsiflexion range of motion. Proprioceptive neuromuscular facilitation (PNF) is a stretch-training technique used to increase flexibility. PNF uses static stretching in combination with triggering isometric muscle contraction. PNF stretch uses muscle contraction to trigger neuromuscular activity, initiate a greater stretch, and increase range of motion. PNF techniques such as the “contract-relax” technique or the “hold-relax” technique can be used to achieve a range of motion increase beyond that of traditional stretching.

In rehabilitation, the complete care of ankle injuries must include regaining full ankle range of motion as well as improving muscle strength, proprioception, and balance. These goals can be achieved by modalities that include flexibility and strengthening exercises, proprioception, and balance training. A structured program of intervention, allowing for the significant effect of time and treatment, is essential for understanding ankle rehabilitation.

The purpose of this research is to compare baseline, post- and follow-up effects between the PNF-stretching technique combined with TENS versus PNF stretching alone. A second aim is to determine the treatment effect on pain, physical activity, balance, flexibility, proprioception, and strength between the groups.

This present research would provide in-depth knowledge about improving the functional status of adults with post-lateral-ankle sprain, and to assess and effectively identify pain, balance, and proprioception problems in order to improve the functional status of post-sprained-ankle patients.

**Methods**
The clinical trial was approved on 10/30/2019 by the Institutional Ethics Committee (ECM#2019-26) of King Khalid University, Saudi Arabia. A clinical trial was also registered in the Clinical Trials Registry – ISRCTN 18013941 (UK). The clinical trial registry was applied for on 11/28/2019 and was approved by 12/14/2019. The recruitment process was completed by January 2020, and the data collection was completed by April 2020.

**Participants**

After obtaining written informed consent, 69 subjects were recruited, consisting of both university students and staff. Of the initial group, 60 subjects had a unilateral lateral ankle sprain and were included in the study based on the following inclusion criteria: males who sprained their ankle at least 3 months before, aged between 18 to 40 years, and who are unable to bend their foot upwards on the post-sprain ankle as much as on the normal ankle, and who have been diagnosed by an orthopedic surgeon (Fig. 1). Excluded were subjects with general health issues, ankle fracture, dislocation, grade-3 ankle sprains, bony limitation, swelling, neuropathies, or any other neuromuscular pathologies.

**Design**

The study was a single-blind randomized controlled trial. Subjects were randomly allocated to three groups using block randomization, each group 20 subjects of the 3 blocks. Concealed allocation was achieved using a computer-generated table of block-randomized numbers. The random numbers were placed in sealed envelopes. The researcher opened the envelopes and proceeded with treatment according to the group assigned. Twenty subjects were randomly allocated to EG 1, EG 2, and the control group (CG).

EG1 received PNF (hold-relax technique) along with TENS; EG 2 received PNF stretching (hold-relax technique) only; and CG received no treatment. In all, there were 12 treatment sessions for the experimental groups, conducted four times per week, for three weeks, in the university clinic. All three groups were assessed at baseline, post-treatment in the third week, and follow-up was recorded in the fifth week. Outcome measures were tested by an independent evaluator not involved in providing treatment to subjects. The primary outcome measures were pain and range of motion, and the secondary outcome measures were flexibility, proprioception, muscle strength, physical activity, and balance.

**VAS**

The visual analog scale (VAS) was recorded using a handwritten mark on a 10 cm line representing a continuum from “no pain” to “worst pain.”(16)

**Flexibility (Knee to Wall Test)**

Subjects were asked to stand facing a wall with about 10 cm between their toes and the wall. Subjects were then asked to step back a distance of one foot behind the other foot. The knee was bent to the front until it touched the wall, and the subject was asked to keep that heel in full contact with the floor. If the knee could not touch the wall without the heel coming off the floor, the front foot was moved closer to the wall. This exercise was then repeated, and the distance from the tip of the big toe to the wall was measured.(17)

**Ankle Proprioception**

A digital dual inclinometer (Dueler IQ PRO Digital Inclinometer, J-TECH, Midvale, UT 84047, USA) was used to measure joint proprioception of the ankle. Subjects were asked to sit in a high sitting position with their eyes closed.
A dual inclinometer was strapped to the mid-shaft lateral face of the tibia, and the display unit was strapped to the middle of the third lateral border of the foot. The foot was brought to the targeted angle dorsiflexion, and the subject was asked to maintain the position for 10 seconds (in order to remember this position) and then to return to the neutral position. The subject was then asked to bring the foot actively to the target angle once again, as shown in Fig. 2. The measurement was taken during three consecutive trials from both angles separately (dorsiflexion and plantarflexion). Recorded mean values (in degrees) were used for analysis of both target positions. The error angle deviation from the target position set angle was used as the result value.

**Range of Motion (ROM)**

ROM was assessed using a flexi-plastic baseline (USA) standard universal goniometer for measuring dorsiflexion ROM and plantarflexion ROM. The subject was seated in the high sitting position, with the fulcrum centered over the lateral malleolus of the ankle and the stationary arm parallel to the fibula and tibia. The movable arm of the goniometer followed a line parallel to the 5th metatarsal of the foot. Subjects were asked to dorsiflex and then plantarflex their ankles from the foot-relaxed starting position (considered to be the zero-neutral position), and the average of the three trials was recorded.

**Muscle Strength**

Isometric muscle strength was measured using a strength dynamometer (Baseline, USA). Each participant was positioned in the supine position with their feet over the edge of a plinth. The strength dynamometer was positioned against the metatarsal heads on the plantar surface of the foot to measure the strength of the plantar flexors. The strength dynamometer was positioned on the dorsal aspect of the foot proximal to the metatarsal heads to measure the strength of the dorsiflexors. Each participant performed submaximal test movements in order to familiarize themselves with the movements prior to testing. Three repetitions were performed for both dorsi- and plantar flexors, with a minimum rest period of 10 seconds between each contraction. A single examiner performed all tests for each individual.

**The Foot and Ankle Disability Index (FADI) Score**

All subjects completed the FADI during three different sessions (pre, week 3, and week 5). The completed survey indicated the function of the injured ankle at each session. Each item was scored from 0 (unable to do) to 4 (no difficulty at all), based on 22 questions related to functional activities and four questions related to pain. The FADI has a total of 104 points and is scored as percentages. A total of 100% indicates no dysfunction at all.

**Star Excursion Balance Test (SEBT)**

The SEBT was measured with the subjects standing barefoot at the center of a grid with eight lines extending at 45° angles. Subjects were instructed to touch the farthest point on the line with their distal part of the foot while maintaining the posture. Each subject maintained a single-leg stance and used the contralateral leg to touch as far as possible along the chosen line. The examiner marked the point touched by the foot (distance in cm) from the center of the grid to the point touched by the big toe. Subjects then returned to a bilateral stance and maintained equilibrium. Leg length was measured while subjects were in the supine position, from the anterior superior iliac spine to the distal tip of the medial malleolus in order to normalize the reach distance.

A valid trial was measured in the same standing posture, and when any change was detected, the subject’s stance foot was repositioned to the center of the grid prior to beginning the next trial. The eight lines—anterolateral (AL), anteromedial (AM), posterolateral (PL) and posteromedial (PM)—were
constructed based on the direction shown in Fig. 3. Reach direction order was designed using a Latin square to avoid any order sequence effect that might contaminate the data. (24) Subjects performed two practice trials in each direction with a ten-second rest break between each reach trial. (25)

After evaluating the primary and secondary outcome measures, the subjects were provided with treatment as per their group protocol.

**PNF Stretching Method**

PNF technique (hold-relax) was applied for the calf muscle using agonist contraction, after stretching followed by isometric contraction of the tight muscle and followed by concentric contraction to the opposite tight muscle. (26) The hold-relax PNF protocol was performed, with each subject lying prone on a plinth and the therapist resisting the subject's plantar flexion. The therapist followed the fundamental principles of the PNF method in terms of manual contact, body position and body mechanics, verbal commands, and vision. (27) The subject was asked to perform isometric calf muscle contraction for 20 seconds, after which the therapist waited for four seconds before resuming the calf stretch, slowly and continuously, until the subject reported strong but tolerable discomfort and began to feel a stretching sensation. Once this benchmark had been reached, the stretch was maintained for approximately 30 seconds longer. This method was adopted from the previously published work by Esnault and Viel (28) and was performed four times per session on the affected lower limb.

**Group 1: Experimental Group 1 (EG 1)**

This group received PNF stretching, as described above, in the PNF stretching method combined with TENS (Trans Med from Enraff Nonius). Two electrodes (4 × 8 cm) were used for this procedure. One was placed distal to the popliteal fossa, and the other was placed 5 cm distal to the proximal electrode, directly on the calf muscle of the affected leg as shown in Fig. 4. The TENS device unit was adjusted to deliver a biphasic current with a symmetrical waveform at 5 Hz for 15 seconds, tuned for a 3-second ramp-up time, and a 30-second rest time with a 250-microsecond pulse duration. The intensity was set to the maximum tolerance limit by each subject. This method was adopted from the previously published work by Albert Pérez-Bellmunt et al. (29) Each subject underwent this procedure four times per session on the affected lower limb, and the total intervention was 30 minutes. This combination method was applied to achieve stronger isometric contraction.

**Group 2: Experimental Group 2 (EG 2)**

For this group, the total intervention was 30 minutes and involved PNF stretching alone. The PNF stretching protocol was carried out as previously described in the PNF stretching method.

**Group 3: Control Group (CG)**

Assessments alone were administered to the control group.

**Statistical Analysis:**

The sample size was determined by G*Power software v. 3.0.10. A mixed-model ANOVA was used to see the time effect, group effect, and time × group interaction effect between the three groups. Statistical Package for the Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA) was used for analysis, with the level of significance set at \( p < 0.05 \).

**Results**
The normality of distribution of all variables was verified using the Shapiro-Wilk test \((p < 0.01)\). Pooled means and standard deviations of all outcome variables as well as \(p\) values showed no significant difference, except for the SEBT in the anterior direction, as shown in Table 1.

Mixed-model ANOVA showed a significant difference between the group’s time, time \(\times\) group interaction for pain, flexibility, proprioception, range of motion, muscle strength, physical activity, and balance. Meanwhile, dorsiflexion ROM, as well as balance in the medial, lateral, anterolateral and anteromedial directions, did not reveal a significant difference between the groups, as shown in Tables 2 and 3. Statistical significance was indicated at \(p < 0.05\), and the confidence interval was set at 95%. The time \(\times\) group interaction effects are shown in Figs. 5 and 6 for all variables. There was a significant change for all outcome measures in post and follow-up for EG 1 compared to EG 2 and CG, as shown in Tables 4 and 5. CG did not show any significant change in time, group, or time \(\times\) group interaction effect for the variables.

**Pain**

Assessments of pain between the groups revealed a significant time effect, group effect, and time \(\times\) group interaction effect \((p \leq 0.00)\), as shown in Table 2. EG 1 showed a significant decrease in pain after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (81.4%, 20%, and 85.1%, respectively) compared to EG 2 (28.2%, 0%, and 28.2%, respectively).

**Flexibility**

Assessments of flexibility between the groups also revealed a significant time effect, group effect, and time \(\times\) group interaction effect \((p \leq 0.00)\), as shown in Table 2. EG 1 showed a significant increase in flexibility after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (34.3%, 1.4%, and 36.3%, respectively) compared to EG 2 (11.9%, 5.3%, and 5.9%, respectively).

**Proprioception**

Mixed-model ANOVA revealed a significant time effect, group effect, and time \(\times\) group interaction effect \((p \leq 0.01)\) for proprioception assessed in dorsiflexion and plantar flexion, as shown in Table 2. EG 1 showed a significant increase in dorsiflexion proprioception after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (76.7%, 20%, and 81.3%) compared to EG 2 (13.8%, 0%, and 13.8%, respectively). EG 1 showed a significant increase in plantar flexion proprioception after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (89.7%, 0%, and 89.7%, respectively) compared to EG 2 (13.6%, 2.63%, and 11.3%, respectively).

**Range of Motion (ROM)**

Dorsiflexion and plantar flexion ROM between the groups revealed significant time effect and time \(\times\) group interaction effect \((p \leq 0.00)\). The group effect was significant for plantar flexion ROM \((p \leq 0.00)\) but was not significant for dorsiflexion ROM \((p \leq 0.15)\), as shown in Table 2. EG 1 showed a significant increase in dorsiflexion ROM after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (16.9%, 5%, and 17.6%, respectively) compared to EG 2 (4.8%, 3.4%, and 1.2%, respectively). EG 1 showed a significant increase in plantar flexion ROM after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (19.5%, 0.2%, and 19.7%, respectively) compared to EG 2 (3.6%, 1.5%, and 2%, respectively).

**Muscle Strength**
Dorsiexor and plantar flexor muscle strength between the groups revealed a significant time effect, group effect, and time × group interaction effect (p ≤ 0.00), as shown in Table 2. The EG 1 showed a significant increase in dorsiexors' strength after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (25.2%, 0%, and 25.2%, respectively) compared to EG 2 (4.9%, 0.8%, and 4%, respectively). The EG 1 showed a significant increase in plantar flexors strength after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (29.4%, 0%, and 29.4%, respectively) compared to EG 2 (4.1%, 1.3%, and 2.7%, respectively).

**Physical Activity**

Physical activity between the groups revealed significant time effect and time × group interaction effect (p ≤ 0.00); however, the group effect was not significant (p ≤ 0.09), as shown in Table 2. EG 1 showed a significant increase in physical activity score after treatment in the pre-to-post, post-to-follow-up, and pre-to-follow-up periods (4.3%, 0.2%, and 4.5%, respectively) compared to EG 2 (0.8%, 0%, and 0.8%, respectively).

**Balance**

Mixed-model ANOVA revealed a significant time × group interaction and time effect for balance in all eight directions (p ≤ 0.00). The group effect was significant in the anterior, posterior, postero-lateral, and postero-medial directions at p ≤ 0.05, as shown in Table 3. In anterior, posterior, medial, lateral, antero-lateral, antero-medial, postero-lateral, and postero-medial directions, EG 1 showed a significant increase in balance after treatment (5.4%, 4.2%, 4.5%, 3.4%, 6%, 5.3%, 3.9%, and 5.6%, respectively) compared to EG 2 (1.3%, 1.2%, 1.2%, 0.4%, 0.7%, 1.3%, 0.7%, and 0.8%, respectively).

**Discussion**

Studies have reported that approximately 3–34% of ankle sprain patients report pain, sprain recurrence, a “giving-way” sensation, limitation in physical activities, swelling, and lack of confidence to participate in sports activities. When such complaints persist for more than six months, the condition may be defined as “chronic.” Approximately 20% of the post-ankle-sprain population complains of instability.\(^{(30)}\) The present study was conducted among subjects who had experienced ankle sprains in the past that were followed by a limitation in effectively using the ankle. The aim of this randomized controlled trial was to compare the effectiveness of combining TENS with a PNF-stretching regimen for subjects with post-lateral ankle sprains. After three weeks of treatment, the between-group comparisons showed significant improvement in outcome metrics for the TENS and PNF-stretch group than for the PNF-stretch group and control group, when evaluated at the fifth week after beginning treatment.

A systematic review conducted by John M et al.\(^{(8)}\) suggests that FADI can be used to assess function, SEBT to evaluate balance, and muscle reaction time to assess proprioception for people with chronic ankle sprains. In the present study, as suggested by the previous literature, VAS was used for pain and SEBT for balance; proprioception was assessed using a dual inclinometer; ankle strength was measured using a strength dynamometer, the knee-to-wall test was used for flexibility; and FADI scores for physical activity were used as subjective and objective measures to observe the effectiveness of post-ankle-sprain treatment.

Several reports have been published about stretching conditions and methods of application that also examined time parameters\(^{(31)}\). Some of these studies reported that a minimum of 2 minutes’ stretching of the gastrocnemius significantly produces an ROM increase and a decrease of stiffness in the muscle-tendon unit.\(^{(32)}\) Many studies have also used various types of stretching techniques and methods to address musculoskeletal problems and the impacts those problems have on sports performance and physical condition testing.\(^{(33)}\) Furthermore, many
protocols based on the published results of previous studies have been found to contradict the results of those studies; therefore, the authors of this study selected a PNF-stretching protocol from previously published research. (34)

The results of a randomized controlled trial for children with hamstring syndrome who were given stretching combined with TENS indicated that said combination produced better results than a protocol without TENS combinations. (35) Another study applied TENS combined with PNF stretching for the hamstring muscles in volleyball players, following a very similar design. (36) Considering the evidence indicating that TENS could play an important role in stretching programs, the present study contributes to the existing evidence in favor of PNF combined with TENS for the calf muscles, and results of the present study support these earlier works.

The present study used TENS in combination with PNF stretching, and found significant improvement in both balance and strength, based on the hypothesis that the application of TENS to the foot and ankle significantly increases balance and postural control. Kang et al (37) suggested that TENS, when applied directly to the skin overlying the gastrocnemius (calf muscle), is effective in improving balance for healthy adults. TENS not only improves strength, but also increases joint position sense and balance. (38) TENS causes elicited muscle contractions and also allows for activation of a greater proportion of type-II muscle fibers compared to volitional exercise at comparable intensity. (39–41)

Application of TENS causing stimulation of cutaneous sensory nerve endings in the foot showed no immediate effect on either ankle proprioception or leg-muscle strength. A recent study concluded that TENS has a distracting impact on balance, (38) which conflicts with the results of the present study, which found a significant improvement in balance and proprioception in post-treatment and follow-up assessments among EG 1 subjects. It can be held that EG 1 showed a significant difference and improvement attributable to the autogenic inhibition reflex, which is the reflex produced when a Golgi organ registers an increase in muscle tension. In our case, contraction in the PNF later provokes a reflex relaxation of the muscles. (42) This reflex may be triggered more when using the TENS because of its unique electrical stimulation that can trigger a tetanic contraction, simultaneously improving muscle strength and increasing tension in the Golgi organ.

Various studies have provided mixed conclusions on the effect of PNF stretching and the permanence of ROM gains. For example, one study reported that ROM improvements were not immediately significant 6 minutes after 5 repetitions of PNF stretching. (43) Another study, however, concluded that even after a single repetition of PNF stretching, ROM was significantly higher than baseline values. This improvement was evident 90 minutes after cessation of intervention for the muscle groups stretched. (44) Several other studies have also noted that ROM increments decrease quite sharply once intervention ceases, (43, 45) and therefore recommend that PNF stretching should be conducted at least once or twice weekly. In the present study, EG1 and EG 2 were treated with four PNF stretching sessions per week for three weeks, and study results clearly showed that the follow-up assessment for EG 1 PNF stretching combined with TENS produced a significant increase in ROM. This result also aligned with available studies.

The results of the present randomized controlled trial suggest that the use of TENS low-frequency currents improved the results of PNF stretching when applied directly to the calf muscles—and therefore, that the results obtained with this stretching modality are significant. The study was conducted with male subjects only. A comparison study with females to understand the response to PNF stretching, with and without low-frequency electrical stimulation, should also be examined. The present study was only conducted for subjects participating in recreational sports; results may differ for professional athletes.
Conclusions

The present study showed that a 12-session treatment program, spread over 3 weeks, with PNF stretching combined with TENS on post-ankle-sprain subjects, produced significant improvements in balance, proprioception, strength, physical activity and range of motion, while also yielding reducing pain. It was also demonstrated that the treatment effect was sustained even after cessation of treatment to the follow-up assessment in the 5th week. For this reason, this treatment procedure will likely be helpful in rehabilitating post-ankle-sprain patients by improving overall function and helping build confidence in capacity for physical activity.

List Of Abbreviations

VAS, Visual Analogue Scale, BMI, Body Mass Index, ROM, Range of Motion, MS, Muscle Strength, EG 1, Experimental Group 1, EG 2, Experimental Group 2, CG, Control Group, SEBT, Star excursion balance test, PNF, proprioceptive neuromuscular facilitation, TENS, transcutaneous electrical nerve stimulation.

Declarations

Ethics approval and consent to participate: The clinical trial was approved on 10/30/2019 by the Institutional Ethics Committee (ECM#2019-26) of King Khalid University, Saudi Arabia, written consent form to participate in the research was individually collected from all the participants.

Consent for publication: Not Applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: All the authors declare that they have no competing interests.

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Authors’ contributions: KAA and SPS are responsible for the conception and design of study, acquisition and analysis of data. Along with IA, KR, VNK, RSR, JST they drafted the article and approved the final manuscript after critical revision. IA, VNK and SPS contributed towards acquisition of data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Tables

Table: 1 Data (Mean and standard deviation) for experimental groups and control group variables
| VARIABLES                  | EXPERIMENTAL GROUP 1 (EG 1) PNF+TENS (n=20) | EXPERIMENTAL GROUP 2 (EG 2) PNF (n=20) | CONTROL GROUP (CG) (n=20) | p-value |
|---------------------------|---------------------------------------------|----------------------------------------|--------------------------|---------|
| AGE                       | 25.8±5.7                                    | 25.7±5.6                               | 25.9±6.2                 | 0.991   |
| HEIGHT IN METERS          | 1.6±0.0                                     | 1.7±0.0                                | 1.7±0.0                  | 0.276   |
| WEIGHT                    | 65.9±14.1                                   | 72.6±15.4                              | 68.9±13.6                | 0.346   |
| BMI                       | 23.0±5.5                                    | 24.5±4.5                               | 23.1±4.2                 | 0.573   |
| LEG LENGTH (CM)           | 83.4±10                                     | 85.0±5.4                               | 86.3±6.3                 | 0.483   |
| SEBT ANTERIOR             | 78.2±2.5                                    | 68.5±3.9                               | 70.5±5.9                 | 0.00*   |
| SEBT POSTERIOR            | 92.4±3.1                                    | 91.2±4.6                               | 90.7±5.4                 | 0.494   |
| SEBT MEDIAL               | 95.7±3.5                                    | 96.9±3.7                               | 96.6±3.5                 | 0.576   |
| SEBT LATERAL              | 89.1±5.9                                    | 92.9±4.7                               | 91.6±5.5                 | 0.090   |
| SEBT ANTERO-LATERAL       | 74.9±4.7                                    | 75.1±3.9                               | 75.8±4.1                 | 0.091   |
| SEBT ANTERO-MEDIAL        | 84.2±5.8                                    | 83.1±5.7                               | 83.8±5.7                 | 0.843   |
| SEBT POSTERO-LATERAL      | 95.1±3.0                                    | 95.6±2.9                               | 94.9±2.8                 | 0.711   |
| SEBT POSTERO-MEDIAL       | 96.9±2.6                                    | 96.8±2.8                               | 96.9±3.3                 | 0.998   |
| VAS                       | 2.7±1.0                                     | 2.3±1.0                                | 2.2±0.9                  | 0.270   |
| KNEE TO WALL (CM)         | 7.5±1.8                                     | 6.7±0.8                                | 7.0±0.8                  | 0.180   |
| DORSI-PROPRIOCEPTION       | 2.1±1.2                                     | 1.8±1.0                                | 1.9±1.0                  | 0.599   |
| PLANTAR-PROPRIOCEPTION     | 2.5±1.7                                     | 2.2±1.9                                | 2.4±1.2                  | 0.722   |
| DORSI-ROM                 | 15.9±1.5                                    | 16.4±1.8                               | 16.7±1.7                 | 0.336   |
| PLANTAR-ROM               | 36.0±3.9                                    | 38.5±3.3                               | 37.3±4.0                 | 0.224   |
| DORSI-MS (KG)             | 11.1±1.8                                    | 11.2±0.8                               | 11.1±0.8                 | 0.971   |
| PLANTAR-MS (KG)           | 13.7±1.7                                    | 14.5±0.9                               | 14.1±1.4                 | 0.020   |
| PHYSICAL ACTIVITY SCORE   | 94.3±2.5                                    | 94.7±3.5                               | 95.3±3.8                 | 0.665   |

Abbreviations: VAS, Visual Analogue Scale, BMI, Body Mass Index, ROM, Range of Motion, MS, Muscle Strength

*Significant (p < 0.01)

Table 2: Summary of statistical results of the Mixed ANOVA and pooled means and standard deviations of all variable measures
| VARIABLES                  | EG 1 (TENS+PNF) | Mean (Standard Deviation) | Mixed ANOVA (p value) | EG 2 (PNF) | CG Control Group |
|---------------------------|-----------------|---------------------------|-----------------------|------------|------------------|
|                           | Baseline        | 3rd week                  | 5th week              |            |                  |
|                           |                 |                           |                       | Interaction (group and time) effect η_p² (p value) | Group (G) effect η_p² (p value) | Time (T) effect η_p² (p value) |
| Pain VAS (cm)             | EG 1            | 2.7±1.0                   | 0.5±0.6               | 0.4±0.5    | 0.608 (0.00)*    | 0.192 (0.002)* | 0.674 (0.00)* |
|                           | EG 2            | 2.3±1.0                   | 1.6±0.8               | 1.6±0.8    | 0.661 (0.00)*    | 0.482 (0.00)* | 0.661 (0.00)* |
|                           | CG              | 2.2±0.9                   | 2.0±0.8               | 2.0±0.8    | 0.438 (0.00)*    | 0.141 (0.00)* | 0.390 (0.00)* |
| KNEE TO WALL (CM)         | EG 1            | 7.5±1.8                   | 10.1±1.2              | 10.2±1.2   | 0.520 (0.00)*    | 0.244 (0.00)* | 0.439 (0.00)* |
|                           | EG 2            | 6.7±0.8                   | 7.5±0.9               | 7.2±1.0    | 0.505 (0.00)*    | 0.166 (0.00)* | 0.390 (0.00)* |
|                           | CG              | 7.0±0.8                   | 7.1±0.8               | 7.1±0.8    | 0.438 (0.00)*    | 0.141 (0.00)* | 0.390 (0.00)* |
| DORSI-PROPRIOCEPTION      | EG 1            | 2.1±1.2                   | 0.5±0.5               | 0.4±0.5    | 0.655 (0.00)*    | 0.086 (0.00) # | 0.622 (0.00)* |
|                           | EG 2            | 1.8±1.0                   | 1.5±1.0               | 1.5±1.0    | 0.729 (0.00)*    | 0.148 (0.00)* | 0.693 (0.00)* |
|                           | CG              | 1.9±1.0                   | 1.8±1.0               | 1.8±1.0    | 0.741 (0.00)*    | 0.298 (0.00)* | 0.736 (0.00)* |
| PLANTAR-PROPRIOCEPTION    | EG 1            | 2.5±1.7                   | 0.2±0.5               | 0.2±0.5    | 0.594 (0.00)*    | 0.218 (0.00)* | 0.541 (0.00)* |
|                           | EG 2            | 2.2±1.9                   | 1.9±1.0               | 1.9±1.1    | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | CG              | 2.4±1.2                   | 2.4±1.3               | 2.4±1.2    | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
| DORSIFLEXION-ROM          | EG 1            | 15.9±1.5                  | 18.6±1.8              | 18.7±1.8   | 0.729 (0.00)*    | 0.148 (0.00)* | 0.693 (0.00)* |
|                           | EG 2            | 16.4±1.8                  | 17.2±1.9              | 18.7±1.8   | 0.741 (0.00)*    | 0.298 (0.00)* | 0.736 (0.00)* |
|                           | CG              | 16.7±1.7                  | 16.8±1.9              | 16.8±1.8   | 0.741 (0.00)*    | 0.298 (0.00)* | 0.736 (0.00)* |
| PLANTARFLEXION-ROM        | EG 1            | 36.4±3.9                  | 43.5±3.7              | 43.6±3.9   | 0.594 (0.00)*    | 0.218 (0.00)* | 0.541 (0.00)* |
|                           | EG 2            | 38.5±3.3                  | 39.9±3.4              | 39.3±3.0   | 0.594 (0.00)*    | 0.218 (0.00)* | 0.541 (0.00)* |
|                           | CG              | 37.3±4.0                  | 37.5±4.1              | 37.5±4.3   | 0.594 (0.00)*    | 0.218 (0.00)* | 0.541 (0.00)* |
| DORSIFLEXION-MS           | EG 1            | 11.1±1.8                  | 13.9±1.7              | 13.9±1.7   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | EG 2            | 11.2±0.8                  | 11.7±0.7              | 11.6±0.6   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | CG              | 11.1±0.8                  | 11.3±0.8              | 11.3±0.8   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
| PLANTARFLEXION-MS         | EG 1            | 13.2±1.7                  | 17.1±1.7              | 17.1±1.7   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | EG 2            | 14.5±0.9                  | 15.1±0.9              | 14.9±1.0   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | CG              | 14.1±1.4                  | 14.3±1.5              | 14.2±1.5   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
| PHYSICAL ACTIVITY SCORE   | EG 1            | 94.3±2.5                  | 98.4±1.9              | 98.6±1.7   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | EG 2            | 4.7±3.5                   | 5.3±3.4               | 95.3±3.4   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |
|                           | CG              | 5.3±3.8                   | 5.5±3.7               | 95.5±3.7   | 0.678 (0.00)*    | 0.078 (0.098) # | 0.651 (0.00)* |

Abbreviations: VAS, Visual Analogue Scale, ROM, Range of Motion, MS, Muscle Strength, EG 1, Experimental Group 1, EG 2, Experimental Group 2, CG, Control Group.

*Significant effect (p < 0.05) #Non-significant

Table 3: Summary of statistical results of the Mixed ANOVA and means and standard deviations of variable measure.
### Table 4: Comparison of outcome effect between the groups and p-value at baseline, post and follow-up for the variables

| BALANCE          | EG 1 (TENS+PNF) | Mean (Standard Deviation) | Interaction (group and time) effect $\eta^2_p$ (p value) | Mixed ANOVA (p value) |
|------------------|-----------------|---------------------------|---------------------------------------------------------|----------------------|
|                  | Group          | Baseline | 3rd week | 5th week | Group (G) effect $\eta^2_p$ (p value) | Time (T) effect $\eta^2_p$ (p value) |
|                  | EG 1           | 78.2±2.5 | 81.9±3.1 | 82.5±2.6 | 0.613 (0.00)* | 0.593 (0.00)* |
|                  | EG 2           | 68.5±3.9 | 70.2±4.1 | 69.4±4.2 | 0.655 (0.00)* | 0.136 (0.01)* |
|                  | CG             | 70.5±5.9 | 70.7±7.0 | 70.8±7.3 | 0.727 (0.00)* | 0.043 (0.28)# |
| SEBT ANTERIOR    | EG 2           | 68.5±3.9 | 70.2±4.1 | 69.4±4.2 | 0.655 (0.00)* | 0.136 (0.01)* |
|                  | CG             | 70.5±5.9 | 70.7±7.0 | 70.8±7.3 | 0.727 (0.00)* | 0.043 (0.28)# |
| SEBT POSTERIOR   | EG 1           | 92.4±3.1 | 96.1±3.1 | 96.3±3.1 | 0.607 (0.00)* | 0.033 (0.38)# |
|                  | EG 2           | 91.2±4.6 | 92.4±4.5 | 92.3±4.7 | 0.607 (0.00)* | 0.033 (0.38)# |
|                  | CG             | 90.7±5.4 | 90.9±5.3 | 91.0±5.2 | 0.607 (0.00)* | 0.033 (0.38)# |
| SEBT MEDIAL      | EG 1           | 95.7±3.5 | 99.8±4.1 | 100.1±4.1 | 0.655 (0.00)* | 0.136 (0.01)* |
|                  | EG 2           | 96.9±3.7 | 98.3±3.5 | 98.1±3.7 | 0.655 (0.00)* | 0.136 (0.01)* |
|                  | CG             | 96.6±3.5 | 96.8±3.3 | 96.7±3.4 | 0.655 (0.00)* | 0.136 (0.01)* |
| SEBT LATERAL     | EG 1           | 89.1±5.9 | 92.1±5.9 | 92.2±5.7 | 0.607 (0.00)* | 0.033 (0.38)# |
|                  | EG 2           | 92.9±4.7 | 93.9±4.2 | 93.4±4.1 | 0.607 (0.00)* | 0.033 (0.38)# |
|                  | CG             | 91.6±5.5 | 91.8±5.4 | 91.8±5.1 | 0.607 (0.00)* | 0.033 (0.38)# |
| SEBT ANTERO-     | EG 1           | 74.9±4.7 | 77.0±4.6 | 77.3±4.7 | 0.653 (0.00)* | 0.001 (0.96)# |
| LATERAL          | EG 2           | 75.1±3.9 | 76.4±3.8 | 75.6±4.0 | 0.653 (0.00)* | 0.001 (0.96)# |
|                  | CG             | 75.8±4.1 | 76.1±4.0 | 76.1±4.0 | 0.653 (0.00)* | 0.001 (0.96)# |
| SEBT ANTERO-     | EG 1           | 84.2±5.8 | 88.1±5.9 | 88.7±5.8 | 0.562 (0.00)* | 0.062 (0.15)# |
| MEDIAL           | EG 2           | 83.1±5.7 | 84.3±5.7 | 84.2±5.4 | 0.562 (0.00)* | 0.062 (0.15)# |
|                  | CG             | 83.8±5.7 | 84.1±5.8 | 84.1±5.8 | 0.562 (0.00)* | 0.062 (0.15)# |
| SEBT POSTERO-    | EG 1           | 95.1±3.0 | 98.6±2.4 | 98.9±2.4 | 0.678 (0.00)* | 0.118 (0.02)* |
| LATERAL          | EG 2           | 95.6±2.9 | 96.7±3.2 | 96.3±3.4 | 0.678 (0.00)* | 0.118 (0.02)* |
|                  | CG             | 94.9±2.8 | 95.1±2.9 | 95.1±2.9 | 0.678 (0.00)* | 0.118 (0.02)* |
| SEBT POSTERO-    | EG 1           | 96.9±2.6 | 102.1±4.0 | 102.3±4.0 | 0.633 (0.00)* | 0.181 (0.00)* |
| MEDIAL           | EG 2           | 96.8±2.8 | 98.3±3.2 | 97.7±3.3 | 0.633 (0.00)* | 0.181 (0.00)* |
|                  | CG             | 96.9±3.3 | 97.2±3.0 | 97.1±3.0 | 0.633 (0.00)* | 0.181 (0.00)* |

Abbreviations: SEBT, Star excursion balance test, EG 1, Experimental Group 1, EG 2, Experimental Group 2, CG, Control Group.  
*Significant effect ($p < 0.05$) #Non-significant
| VARIABLES          | Group1-EG-1 (TENS+PNF) | Group2- EG-2 (PNF) | Group3-CG | Baseline | Post (3rd week) | Follow-up (5th week) |
|--------------------|------------------------|--------------------|-----------|----------|----------------|---------------------|
|                    | effect $\eta_p^2$     | Sig.               | effect $\eta_p^2$ | Sig.     | effect $\eta_p^2$ | Sig.                |
| VAS                | EG 1 x EG 2            | 0.036              | 0.23      | 0.404    | 0.00*          | 0.447              | 0.00*               |
|                    | EG 1 x C               | 0.060              | 0.12      | 0.523    | 0.00*          | 0.552              | 0.00*               |
|                    | EG 2 x C               | 0.003              | 0.75      | 0.055    | 0.14           | 0.044              | 0.19                |
| KNEE TO WALL       | EG 1 x EG 2            | 0.065              | 0.11      | 0.567    | 0.00*          | 0.642              | 0.00*               |
|                    | EG 1 x C               | 0.030              | 0.28      | 0.654    | 0.00*          | 0.677              | 0.00*               |
|                    | EG 2 x C               | 0.021              | 0.36      | 0.052    | 0.157          | 0.002              | 0.08                |
| DORSIFLEXION       | EG 1 x EG 2            | 0.024              | 0.34      | 0.298    | 0.00*          | 0.339              | 0.00*               |
| PROPRICEPTION       | EG 1 x C               | 0.012              | 0.49      | 0.398    | 0.00*          | 0.435              | 0.00*               |
|                    | EG 2 x C               | 0.002              | 0.76      | 0.020    | 0.381          | 0.020              | 0.381               |
| PLANTARFLEXION     | EG 1 x EG 2            | 0.015              | 0.45      | 0.497    | 0.00*          | 0.485              | 0.00*               |
| PROPRICEPTION       | EG 1 x C               | 0.001              | 0.83      | 0.545    | 0.00*          | 0.569              | 0.00*               |
|                    | EG 2 x C               | 0.011              | 0.52      | 0.044    | 0.195          | 0.043              | 0.20                |
| DORSIFLEXION       | EG 1 x EG 2            | 0.023              | 0.35      | 0.126    | 0.02*          | 0.227              | 0.00*               |
| -ROM               | EG 1 x C               | 0.058              | 0.13      | 0.204    | 0.00*          | 0.205              | 0.00*               |
|                    | EG 2 x C               | 0.007              | 0.60      | 0.014    | 0.47           | 0.003              | 0.74                |
| PLANTARFLEXION     | EG 1 x EG 2            | 0.080              | 0.07      | 0.204    | 0.00*          | 0.276              | 0.00*               |
| -ROM               | EG 1 x C               | 0.015              | 0.45      | 0.368    | 0.00*          | 0.362              | 0.00*               |
|                    | EG 2 x C               | 0.025              | 0.33      | 0.089    | 0.06           | 0.057              | 0.13                |
| DORSIFLEXION       | EG 1 x EG 2            | 0.001              | 0.83      | 0.406    | 0.00*          | 0.432              | 0.00*               |
| -MS                | EG 1 x C               | 0.000              | 0.91      | 0.472    | 0.00*          | 0.483              | 0.00*               |
|                    | EG 2 x C               | 0.001              | 0.85      | 0.062    | 0.12           | 0.051              | 0.16                |
| PLANTARFLEXION     | EG 1 x EG 2            | 0.177              | 0.00*     | 0.355    | 0.00*          | 0.393              | 0.00*               |
| -MS                | EG 1 x C               | 0.078              | 0.08      | 0.427    | 0.00*          | 0.446              | 0.00*               |
|                    | EG 2 x C               | 0.021              | 0.37      | 0.080    | 0.07           | 0.061              | 0.12                |
| PHYSICAL ACTIVITY  | EG 1 x EG 2            | 0.003              | 0.72      | 0.252    | 0.00*          | 0.286              | 0.00*               |
|                    | EG 1 x C               | 0.022              | 0.36      | 0.198    | 0.00*          | 0.228              | 0.00*               |
|                    | EG 2 x C               | 0.007              | 0.61      | 0.001    | 0.82           | 0.001              | 0.82                |

Abbreviations: VAS, Visual Analogue Scale, ROM, Range of Motion, MS, Muscle Strength, EG 1, Experimental Group 1, EG 2, Experimental Group 2, CG, Control Group.

*Significant effect (p < 0.05)

Table 5: Comparison of outcome effect between the groups and $p$-value at baseline, post and follow-up for the variables
| BALANCE         | Group1-EG-1 (TENS+PNF) | Group2-EG-2 (PNF) | Group3-CG | Baseline effect $\eta^2_p$ | Sig. | Post (3rd week) effect $\eta^2_p$ | Sig. | Follow-up (5th week) effect $\eta^2_p$ | Sig. |
|-----------------|--------------------------|-------------------|----------|-----------------------------|------|----------------------------------|------|-------------------------------------|------|
| SEBT ANTERIOR   | EG 1 x EG 2              | 0.691             | 0.00*    | 0.731                       | 0.00*| 0.784                            | 0.00*|                                     |      |
|                 | EG 1 x C                 | 0.432             | 0.00*    | 0.595                       | 0.00*| 0.650                            | 0.00*|                                     |      |
|                 | EG 2 x C                 | 0.040             | 0.21     | 0.003                       | 0.75 | 0.020                            | 0.38 |                                     |      |
| SEBT POSTERIOR  | EG 1 x EG 2              | 0.024             | 0.34     | 0.187                       | 0.00*| 0.206                            | 0.00*|                                     |      |
|                 | EG 1 x C                 | 0.035             | 0.24     | 0.270                       | 0.00*| 0.286                            | 0.00*|                                     |      |
|                 | EG 2 x C                 | 0.002             | 0.78     | 0.023                       | 0.34 | 0.017                            | 0.41 |                                     |      |
| SEBT MEDIAL     | EG 1 x EG 2              | 0.025             | 0.32     | 0.040                       | 0.21 | 0.065                            | 0.11 |                                     |      |
|                 | EG 1 x C                 | 0.017             | 0.42     | 0.146                       | 0.01*| 0.173                            | 0.00*|                                     |      |
|                 | EG 2 x C                 | 0.001             | 0.82     | 0.047                       | 0.18 | 0.036                            | 0.24 |                                     |      |
| SEBT LATERAL    | EG 1 x EG 2              | 0.115             | 0.03*    | 0.029                       | 0.29 | 0.014                            | 0.47 |                                     |      |
|                 | EG 1 x C                 | 0.047             | 0.17     | 0.001                       | 0.86 | 0.002                            | 0.80 |                                     |      |
|                 | EG 2 x C                 | 0.016             | 0.43     | 0.045                       | 0.19 | 0.028                            | 0.30 |                                     |      |
| SEBT ANTEROLATERAL | EG 1 x EG 2           | 0.063             | 0.11     | 0.005                       | 0.65 | 0.035                            | 0.24 |                                     |      |
|                  | EG 1 x C                 | 0.101             | 0.04*    | 0.010                       | 0.54 | 0.019                            | 0.39 |                                     |      |
|                  | EG 2 x C                 | 0.008             | 0.58     | 0.001                       | 0.84 | 0.003                            | 0.73 |                                     |      |
| SEBT ANTEROMEDIAL | EG 1 x EG 2          | 0.009             | 0.57     | 0.102                       | 0.04*| 0.140                            | 0.01*|                                     |      |
|                   | EG 1 x C                 | 0.001             | 0.85     | 0.111                       | 0.03*| 0.140                            | 0.01*|                                     |      |
|                   | EG 2 x C                 | 0.004             | 0.70     | 0.000                       | 0.19 | 0.000                            | 0.93 |                                     |      |
| SEBT POSTEROLATERAL | EG 1 x EG 2        | 0.007             | 0.59     | 0.096                       | 0.05 | 0.169                            | 0.00*|                                     |      |
|                  | EG 1 x C                 | 0.002             | 0.78     | 0.299                       | 0.00*| 0.352                            | 0.00*|                                     |      |
|                  | EG 2 x C                 | 0.018             | 0.41     | 0.065                       | 0.11 | 0.039                            | 0.21 |                                     |      |
| SEBT POSTEROMEDIAL | EG 1 x EG 2         | 0.000             | 0.95     | 0.031                       | 0.27 | 0.009                            | 0.56 |                                     |      |

Abbreviations: SEBT, Star excursion balance test, EG 1, Experimental Group 1, EG 2, Experimental Group 2, CG, Control Group.

*Significant effect (p < 0.05)
**Figure 1**

Flow Chart enrollment for the study
Figure 2

Proprioception of ankle in dorsiflexion and plantar flexion

Figure 3

Star excursion balance test
Figure 4

Application of TENS
Figure 5

Star Excursion Balance Test (SEBT) Group and time interaction of outcome variables. Abbreviations: anterior (A), posterior (P), medial (M), lateral (L), antero-lateral (AL), antero-medial (AM), postero-lateral (PL) and postero-medial (PM).
Figure 6

Group and time interaction of outcome variables, Abbreviations: VAS, Visual Analogue Scale, ROM, and Range of Motion.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2010Checklist.doc