

Title Page**Therapeutic Effects of Connective Tissue Manipulation on Wound Healing and Bacterial Colonization Count among Patients with Diabetic Foot Ulcer**

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Short Running Title: Connective tissue manipulation in diabetic foot ulcer

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Summary

This study investigated the therapeutic effects of connective tissue manipulation (CTM) in diabetic foot ulcer (DFU). A total of 20 participants (10 in CTM group and 10 in conventional treatment group (CG)) with DFU underwent the conventional DFU treatment. In addition, the CTM group received CTM twice per week for 6 weeks. The percentage wound area reduction (PWAR) and bacterial colonization count (BCC) in log₁₀ colony-forming units (CFU) per ml wound fluid was evaluated at baseline and six weeks. Results showed a significant change in PWAR in CTM ($p < 0.05$, $t = 3.82$, $Df = 9$, $CI L = 0.98$ $U = 3.81$) and CG ($p < 0.05$, $t = 2.97$, $Df = 9$, $CI L = 0.26$ $U = 1.98$). Mean reduction of BCC showed a significant reduction ($p < 0.05$), with percentage of BCC reduction higher in CTM group (6.45%) than CG (3.55%). The findings suggest CTM as an effective adjunct therapy for DFU to enhance conventional treatments.

Key words: Connective tissue manipulation; diabetes; ulcer; physiotherapy; rehabilitation

Introduction

Connective tissue manipulation (CTM) is a specialized manipulative therapy pioneered by Elisabeth Dicke from Germany in the 1930s (L. A. Holey, 1995a). This manual therapy technique utilizes a shear force at connective tissue interfaces which creates a stretch in both elastic and viscous components of the tissues (L. A. Holey, 1995b). CTM has mechanical and reflex effects in the peripheral vascular system which causes peripheral vasodilatation and increased blood flow to the peripheral areas (L. A. Holey, 1995b). The CTM strokes are usually applied by finger tips to the defined zones of the body called connective tissue zones producing therapeutic reflex effects on the tissues that shares the same segmental innervations with the connective tissue zones (Holey, 2000; L. A. Holey, 1995a; Reed and Held, 1988). Previous studies suggest that the peripheral vasodilatation achieved is due to the stimulation of autonomic system related cutaneovisceral reflexes and reticular plexus activity (Holey et al., 2011; Reed and Held, 1988) combined with regulation of sympathetic-parasympathetic balance (Castro-Sanchez et al., 2011; L. A. Holey, 1995b; Holey et al., 2011).

Diabetes is a serious health concern and around 15% of people develop diabetic foot ulcers (DFU) (Delmas, 2006; Levin, 1997). The ulcerated diabetic foot may result in ischemia, infection, (Levin, 1997) and contribute to 85% of lower limb amputations (Delmas, 2006). Therapeutic techniques to prompt revascularization to the ulcerated foot may minimize amputation risk and hospitalization (Levin, 1997). In DFU with an underlying pathology of deprived peripheral circulation to the wound, the therapeutic effects of CTM due to peripheral vasodilatation might be an effective adjunct treatment.

In DFU, local tissue ischemia causes tissue necrosis and the presence of critical bacterial contamination affects the healing status of the wound (Browne et al., 2001; Stojadinovic et al.,

2008). This higher bacterial burden hinders the healing mechanism by maintaining the inflammatory state, thereby delaying the formation of collagen tissue which is essential for the healing of the wound (Stojadinovic et al., 2008; Xu et al., 2007). In addition, impaired peripheral blood flow delays the delivery of immune cells and fibroblasts to the ischemic foot, further slowing wound healing (Schramm et al., 2006). If CTM does improve the vascular status of the diabetic ischemic foot, then the increased blood flow may improve oxygenation, the delivery of antibodies and medication in the affected foot subsequently reducing the bacterial count which, in turn should enhance wound healing.

Whilst the therapeutic effects of CTM have been reported in several studies (Castro-Sanchez et al., 2011; Ekici et al., 2009; Reed and Held, 1988), there is a paucity of conclusive evidence to support the theoretical basis and the clinical benefits of CTM in diabetic foot ulcer. Therefore the aim of this study was to investigate the therapeutic effects of CTM on wound healing in DFU, measured by the total bacterial contamination count (BCC) and the percentage wound area reduction (PWAR). The hypothesis of this study was that PWAR in DFU would be significantly higher if CTM was applied in addition to usual clinical care.

Materials and Methods

This was a pilot randomized control trial conducted in the outpatient physiotherapy department of a university teaching hospital. A total of 26 subjects were recruited for this study. The inclusion criteria includes participants with both fasting blood glucose (≥ 110 mg dl⁻¹) (WHO, 1999) and 2 hours post glucose load (≥ 180 mg dl⁻¹)(WHO, 1999), presence of a non-infected DFU on the plantar aspect of the foot with Wagner classification (Wagner Jr., 1981; Wagner, 1987) of grade 1 and 2 (skin ulcer) or Texas grade (Lavery et al., 1996) 1A and below. All the participants included are undergoing conventional treatments such as orthotics, antibiotic

therapy, drugs therapy and exercise therapy. The exclusion criteria for this study includes participants with current haemodialysis, past surgical history of lower limb revascularization, participants who are unable to comply a minimum of four treatment sessions continuously, bleeding disorders such as hemophilia, sickle cell disease, thrombocytopenia, leukemia, or blood dyscrasia, glycosylated hemoglobin (HbA1c) level more than 9% and use of immunosuppressive agents. The participants who were accepted for the study were randomized into a CTM (intervention) group or usual (control) treatment group. Pieces of paper were numbered and placed into a closed box. The participants were instructed to draw one each and those who picked odd numbers were allocated to the CTM group and those with even numbers were allocated to the control group. Ethical approval was obtained from the Medical Research Secretariat Ethical Committee from a university teaching hospital with (reference NN-010-2008). Written informed consent was obtained from the participants prior to the participation in the study.

Outcome Measures

Demographic data was collected from the participants. Measurement of the PWAR and bacterial colonization count (BCC) were carried out before and after intervention. Acetate surface area tracing for PWAR measurement has been found to be a standard reliable method and hence was used in this study. The baseline PWAR of the wound surface measurement and the corresponding weekly PWAR of the wound surface area of the diabetic foot ulcer was measured using a standardized acetate tracing method (Harding, 1995).

One qualified staff nurse trained in wound surface area measurement with acetate tracing measured the circumference of the wound surface in all participants and was blinded to the applied interventions for the study participants. The margin of the wound was traced on to two

layered 0.25 cm² preprinted sterile acetate tracing, using a black, extra-fine, felt-tipped marking pen. The contact layer was discarded and the area of the wound calculated by counting each square that is more than half within the border of the wound (Harding, 1995). Finally, the total surface area inside each tracing was calculated by multiplying the number of squares with 0.25 cm as each complete square was equivalent to 0.25 cm². The participant was positioned in the same position (long sitting) at each measurement in order to define the edge of the wound accurately. PWAR is the percentage difference in wound surface area (WSA) from baseline to end of intervention and is calculated by

$$(\text{Baseline WSA} - \text{final WSA} \times 100 = \text{Final WSA})$$

BCC at the wound site is a widely used technique to evaluate the healing status of the diabetic foot ulcer (Hirsch et al., 2008; Xu et al., 2007). Therefore, BCC was used to analyze the mean percentage reduction of bacterial volume pre and post intervention from the diabetic ulcer site by taking a swab from the diabetic wound. The BCC in log₁₀ colony-forming units (CFU) per ml wound fluid from each group was obtained prior to intervention and six weeks after intervention to study the potential effects of CTM. The BCC in log₁₀ was obtained from the wound bed by taking a swab based on an established protocol (Levine et al. 1976). Before harvesting the bacterial culture specimen, the wound bed was adequately cleaned with saline to remove excess necrotic debris, and gently compressed around wound edges to elicit new drainage. The bacterial cultures were obtained from the diabetic foot wound using 1cm² wide sterile Dacron-tipped (DuPont, Wilmington, DE) applicators (swabs). The bacterial swabs were obtained by a trained staff nurse by rotating the swab 360 degrees over 1cm² of the healthiest granulation tissue wound tissue while applying gentle pressure to harvest both anaerobes and aerobes. A sufficient pressure was applied to the wound to avoid bleeding in the underlying tissue while obtaining the

swab. Care was taken not to spread exudates, pus, eschar, and heavily fibrous tissue into the wound bed area. The swabs were immediately coded and placed in 1.0 ml thioglycolate broth (transport medium) to prevent contamination and hand-delivered to microbiology laboratory at a university teaching hospital where cultures were immediately performed.

Intervention

During the 6 weeks of the study, all participants underwent the indicated standard conventional DFU treatment which includes foot orthotics, antibiotic therapy, exercise therapy, wound dressing and sonotron. In addition to the conventional treatment, the CTM groups were given CTM treatment twice per week. The CTM treatment was rendered twice a week for a period of 6 weeks over the skin area known as the arterial zone of legs, which is around the sacrum and borders of iliac crest for all the participants. The technique was administered for a duration of 15 minutes using finger tips by establishing a contact between the middle finger of the therapist's hand and the skin of the patient. The technique was performed by flexion of the distal interphalangeal joint of middle finger to take up 'slack' in the superficial tissues on the patients' skin so that the desired strata of tissue is stimulated over the connective tissue zones. In the first two CTM sessions, preparatory techniques such as skin technique and superficial technique were given. Preparatory techniques were performed for gentle lifting movements of the layers of skin away from the fascial layer to reduce skin tension. The fascial technique which places traction at the skin/fascial interface to stimulate the autonomic endings supplying the horizontal circulatory plexus to induce autonomic reflex effects was applied for the subsequent sessions. No complications or adverse incidents were reported. Two qualified and experienced physiotherapists who trained in CTM randomly performed the treatment for all the patients. The therapists who administered the CTM therapy remained blinded to the results of outcome

measures. All the participants received the same protocol of CTM intervention. The participants' blinding was performed by not disclosing the real benefits of the CTM intervention at the time of application of the technique. Instead the participants were briefed that the back massage was given to them in order to relax their mind and well-being. Thus, the participants remained blinded to the actual aim of the study until completing the study and the real benefit of the CTM was explained to participants only at the end of the study.

Statistical analysis

The collected data were analyzed with SPSS version 20. Descriptive analysis was performed to evaluate the demographic and clinical characteristics of the subjects. The differences in PWAR at the baseline was examined using independent sample t-test between the experimental (CTM plus conventional treatment) and control (conventional treatment) groups. Mann-Whitney U test were used to analyze the baseline differences of BCC. The result of PWAR after six weeks of CTM intervention was analyzed using paired t-test in CTM group and control group. Wilcoxon signed rank test was used to analyze the BCC results between baseline and six weeks in both intervention group (A) and control group (B). The significance level was set at $p < 0.05$ (5%).

Results

A total of 34 participants were assessed to be eligible for this study, but 26 participants met the inclusion criteria and participated in the study. However, only 20 participants completed the full 6 weeks of the study period and were taken into account for data analysis. The flow of the study participants and drop outs were explained in the study flow chat (Fig.1). Table 1 shows the demographic and clinical characteristics of the subjects. All twenty subjects who participated in

this study were diagnosed with DM type II and hence there was no DM type I subjects participated in this study.

Regarding PWAR, the analysis showed that the baseline mean surface area was not significantly different between the CTM and control groups ($p > 0.05$, see Table 1) The results from Paired t-test showed a significant difference in relation to the change in PWAR between baseline and six weeks in both the CTM ($p < 0.05$, $t = 3.82$, $Df = 9$, $CI L = 0.98$ $U = 3.81$) and control group ($p < 0.05$, $t = 2.97$, $Df = 9$, $CI L = 0.26$ $U = 1.98$) (Table 2). However, the PWAR was 57% in the CTM group and 28% in the control group after 6 weeks of intervention.

About BCC, there was no significant difference of measures among the participants at the baselines between the CTM and control group ($p > 0.05$, see Table 1). Post six weeks analysis of results using Wilcoxon signed rank test showed there was significant reduction of BCC in both the CTM group ($p < 0.05$, $Z = -2.81$, $df = 10$) and control group ($p < 0.05$, $Z = -2.80$, $df = 10$) (Table 3). However, in comparison with the baseline BCC, the post 6 weeks analysis of CTM treatment in addition to conventional treatment caused a 6.4% mean reduction of bacterial burden on wound surface in CTM group when compared with 3.5% reduction of bacterial burden in control group.

Discussion

This study is the first reported randomized controlled pilot study on the therapeutic effects of CTM on wound healing among patients with DFU. The wound surface area showed significant improvement between the initial and final values of PWAR in both the groups. However, the PWAR in the CTM group was 57% compared to 28% in the control group. This trend suggests that CTM can be clinically significant as an adjunct physiotherapeutic intervention to conventional DFU management. In the current study, the swab technique was chosen to quantify

BCC instead of the tissue biopsy technique. Although skin/tissue biopsy might be an appropriate option, several clinical challenges guided our action to choose swab technique. The process to quantify BCC by performing tissue biopsy requires an invasive procedure, is potentially traumatic to the patient which raises ethical issues, needs expert manipulation, warrants specialized equipment and causes increased workload for the microbiology laboratory. Furthermore, evidence demonstrates a correlation between surface cultures obtained through swab technique and tissue biopsy cultures (Levine et al. 1976, Bowler 2001). Therefore, the swab technique was used to quantify BCC as opposed to skin/tissue biopsy technique.

Regarding BCC, currently, the medical and research communities realize that the static progression of chronic wounds may be influenced significantly by the diversity of bacterial populations (Dowd et al., 2008; Wolcott et al., 2009). Therefore, the total number of micro bacteria existing in the wound was taken into account in this study, instead of particular species of the bacteria. Evidence suggests that most of the acute and chronic wound infections involve mixed populations of both aerobic and anaerobic microorganisms. In addition, in view of the poly microbial nature of the DFU, it is suggested that the treatment of infection could be based on a better understanding on the general microbiology of the wounds in addition to precisely define the causative micro organism in wound infection. Therefore, the bacterial identification of the samples to ascertain ecological relationships with wound healing duration was not attempted in the current pilot study. While it would be interesting to see the ecological relationships to wound healing, it is proposed to be conducted in a future large scale CTM study on wound healing.

After the six week study, there was a significant reduction of bacterial burden in both the CTM and the control group between the baseline and six weeks post intervention period. However the

reduction was significantly greater in the CTM group (6.4%) when compared to the control group (3.5%). Therefore it could be suggested that CTM in addition to conventional diabetic wound care was more effective in diminishing the bacterial growth in DFU subjects than the conventional treatment alone. This data finding was consistent with the conceptual explanation that a wound environment not conducive to healing can be retarded by high bacterial load itself (Xu et al., 2007). The secretion of metalloproteinase and their tissue inhibitors from the bacteria disturbs the pattern of inflammatory cytokines and growth factors and therefore are detrimental to local tissue healing (Wang et al., 2005). Therefore, an improvement in bacterial colonization count is a crucial factor to facilitate the wound healing and hence these effects are clinically significant.

The results obtained in this study can be compared with a previous study that supports the therapeutic effects of CTM. Their study reported improved blood circulation to the lower limbs after 15 weeks of CTM among patients with Type II Diabetes (Castro-Sanchez et al., 2011). It further reported on improved oxygen saturation followed by CTM among diabetic patients (Castro-Sanchez et al., 2011). Thus, the therapeutic effects obtained in our study could be supported by the findings of Castro et al, 2012 as we opine that improved blood circulation and oxygen saturation might have facilitated wound healing among our study participants. It is also to be noted that CTM in the current study was applied only for six weeks when compared to the fifteen weeks CTM by Castro et al, 2012. Nevertheless, CTM showed evidence of therapeutic effectiveness to facilitate healing of diabetic foot ulcer in the current study irrespective of the differences in duration of intervention. On the other hand, what is the optimum duration for CTM intervention may be of interest to the clinicians and it needs to be explored in future studies.

The results of this study showed that CTM had therapeutic effects in healing of diabetic ulcer. The possible mechanisms of action have been questioned that how these effects have been achieved by CTM and by what physiological mechanism. CTM potentially creates traction at the skin/fascial interfaces, which are rich in autonomic nerve endings (L. A. Holey, 1995a). It is thought to influence the autonomic nervous system through enhanced parasympathetic function, causing reflex vasodilatation and increased circulation to the peripheral extremities (Holey, 2000; Holey et al., 2011). This could be a rationale for our findings as the PWAR is significantly reduced in CTM group as compared to the control group.

There are some limitations to this study. Two therapists carried out the CTM treatment and standardization of technique was not monitored, although both had undergone the same training in the technique. A small sample size and lack of between group comparisons were other limitations in the study. As it was designed to be a pilot study with a small number of samples, subjects, a power analysis and between group comparisons were not performed. Nevertheless, the mean and standard deviation obtained from this pilot study will be used for power analysis and sample size calculation in a full larger study in future. Future trials are required with larger sample size across various types of diabetes to validate the clinical effects of CTM on DFU in clinical practice.

Conclusion

The result from this preliminary study provides support for the use of CTM as an effective adjunct therapy for DFU to enhance conventional treatments. Also, the longer term clinical effectiveness of these findings needs further investigation.

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References

- Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *ClinMicrobiol Rev.* 2001 Apr;14(2):244-69.
- Browne, A.C., Vearncombe, M., Sibbald, R.G., 2001. High bacterial load in asymptomatic diabetic patients with neurotrophic ulcers retards wound healing after application of Dermagraft. *Ostomy. Wound. Manage.* 47, 44–49.
- Castro-Sanchez, A.M., Moreno-Lorenzo, C., Mataran-Penarrocha, G.A., Feriche-Fernandez-Castany, B., Granados-Gamez, G., Quesada-Rubio, J.M., 2011. Connective tissue reflex massage for type 2 diabetic patients with peripheral arterial disease: randomized controlled trial. *Evid.Based. Complement. Alternat.Med.* 2011, 804321. doi:10.1093/ecam/nep171
- Delmas, L., 2006. Best practice in the assessment and management of diabetic foot ulcers. *Rehabil.Nurs.*31, 228–34.
- Dowd, S.E., Sun, Y., Secor, P.R., Rhoads, D.D., Wolcott, B.M., James, G.A., Wolcott, R.D., 2008. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiol.*8, 43. doi:10.1186/1471-2180-8-43
- Ekici, G., Bakar, Y., Akbayrak, T., Yuksel, I., 2009. Comparison of Manual Lymph Drainage Therapy and Connective Tissue Massage in Women With Fibromyalgia: A Randomized Controlled Trial. *J. Manipulative Physiol. Ther.* 32, 127–133. doi:10.1016/j.jmpt.2008.12.001
- Harding, K.G., 1995. Methods for assessing change in ulcer status. *Adv. Wound Care* 8, suppl 37–42.
- Hirsch, T., Spielmann, M., Zuhaili, B., Koehler, T., Fossum, M., Steinau, H.-U., Yao, F., Steintraesser, L., Onderdonk, A.B., Eriksson, E., 2008. Enhanced susceptibility to infections in a diabetic wound healing model. *BMC Surg.* 8, 5. doi:10.1186/1471-2482-8-5
- Holey, E.A., 2000. Connective tissue massage: a bridge between complementary and orthodox approaches. *J. Bodyw. Mov. Ther.* 4, 72–80. doi:10.1054/jbmt.1999.0125
- Holey, L.A., 1995a. Connective tissue manipulation towards a scientific rationale. *Physiotherapy* 81, 730–739. doi:10.1016/S0031-9406(05)66573-X
- Holey, L.A., 1995b. Connective tissue zones: An introduction. *Physiotherapy* 81, 366–368. doi:10.1016/S0031-9406(05)66758-2
- Holey, L.A., Dixon, J., Selfe, J., 2011. An exploratory thermographic investigation of the effects of connective tissue massage on autonomic function. *J. Manipulative Physiol. Ther.* 34, 457–462. doi:10.1016/j.jmpt.2011.05.012
- Wagner F.W.Jr., 1981. The dysvascular foot: a system for diagnosis and treatment. *Foot Ankle:* 64–122. doi:10.1016/j.fcl.2010.03.004

- Lavery, L. a, Armstrong, D.G., Harkless, L.B., 1996. Classification of diabetic foot wounds. *J. Foot Ankle Surg.* 35, 528–531. doi:10.1016/S1067-2516(96)80125-6
- Levin, M., 1997. Diabetic foot wounds: pathogenesis and management. *Adv. Wound Care* 10, 24–30.
- Levine, N.S., LT Col., Lindberg, R.B., Mason, A.D., Pruitt, B.A., Colonel. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. 1976. *The Journal of Trauma.* 16(2): 89-94.
- Reed, B. V, Held, J.M., 1988. Effects of sequential connective tissue massage on autonomic nervous system of middle-aged and elderly adults. *Phys. Ther.* 68, 1231–4.
- Schramm, J.C., Dinh, T., Veves, A., 2006. Microvascular changes in the diabetic foot. *Int. J. Low. Extrem. Wounds* 5, 149–59. doi:10.1177/1534734606292281
- Stojadinovic, A., Carlson, J.W., Schultz, G.S., Davis, T.A., Elster, E.A., 2008. Topical advances in wound care. *Gynecol. Oncol.* 111, S70–80. doi:10.1016/j.ygyno.2008.07.042
- Wagner, F.W.J., 1987. The diabetic foot. *Orthopedics* 10, 163–172.
- Wang, Y.Y., Myhre, A.E., Pettersen, S.J., Dahle, M.K., Foster, S.J., Thiemermann, C., Bjornland, K., Aasen, A.O., Wang, J.E., 2005. Peptidoglycan of *Staphylococcus aureus* induces enhanced levels of matrix metalloproteinase-9 in human blood originating from neutrophils. *Shock* 24, 214–218.
- WHO, 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. [WWW Document]. URL http://www.staff.ncl.ac.uk/philip.home/who_dmc.htm. (accessed 6.21.11).
- Wolcott, R.D., Gontcharova, V., Sun, Y., Zischakau, A., Dowd, S.E., 2009. Bacterial diversity in surgical site infections: not just aerobic cocci any more. *J. Wound Care* 18, 317–323. doi:10.12968/jowc.2009.18.8.43630
- Xu, L., McLennan, S. V, Lo, L., Natfaji, A., Bolton, T., Liu, Y., Twigg, S.M., Yue, D.K., 2007. Bacterial load predicts healing rate in neuropathic diabetic foot ulcers. *Diabetes Care* 30, 378–380. doi:10.2337/dc06-1383

Tables

Table 1. Demographic and clinical characteristics of diabetic subjects

Characteristic of study subjects		n=20				Total (n)	%	P value
		CTM		Control				
		n	%	n	%			
Gender	Male	6	60.0	4	40.0	10	50.0	0.37
	Female	4	40.0	6	60.0	10	50.0	
Diabetes type	Type I	0	0	0	0	0	0	NA
	Type II	10	100	10	100	20	100	
		M	SD	M	SD			
Age		55.50	1.96	55.70	1.82			0.63
Diabetes duration (Yrs)		12.00	1.98	15.10	2.53			0.26
HbA1c		7.19	0.35	7.71	0.29			
Baseline PWAR		4.15	3.91	4.00	3.28			0.17
Baseline BCC		3.891	0.266	3.999	0.221			0.44

M=Mean, SD= Standard deviation, n=number, NA= not applicable, CTM =connective tissue manipulation, PWAR = percentage wound area reduction, BCC= Bacterial colonization count

Table 2. Analysis of PWAR (Cm²) in CTM and Control group

PWAR	Pre Mean ± SD	Post Mean ± SD	Mean Difference	P value	t	CI	Effect size
CTM Group	4.15 ±3.91	1.75±2.49	2.40±1.42	<0.05	3.82	0.98-3.81	0.73
Control Group	4.00 ± 3.28	2.68±2.67	1.32±0.61	<0.05	2.97	0.26-1.98	0.44

CTM =Connective tissue manipulation, PWAR = Percentage wound area reduction, CI= Confident interval, SD= standard deviation

Table 3. Analysis of BCC (log CFU) in CTM and Control group

BCC	Pre Mean ± SD	Post Mean ± SD	Mean Difference	P value	z- score	Effect size
CTM Group (log CFU)	3.89±0.26	3.64±0.34	0.25±0.08	p <0.05	-2.81	0.81
Control Group (log CFU)	3.99±0.22	3.85±0.27	0.14±0.05	p <0.05	-2.80	0.57

CTM =Connective tissue manipulation, BCC=Bacterial colonization count, SD= standard deviation

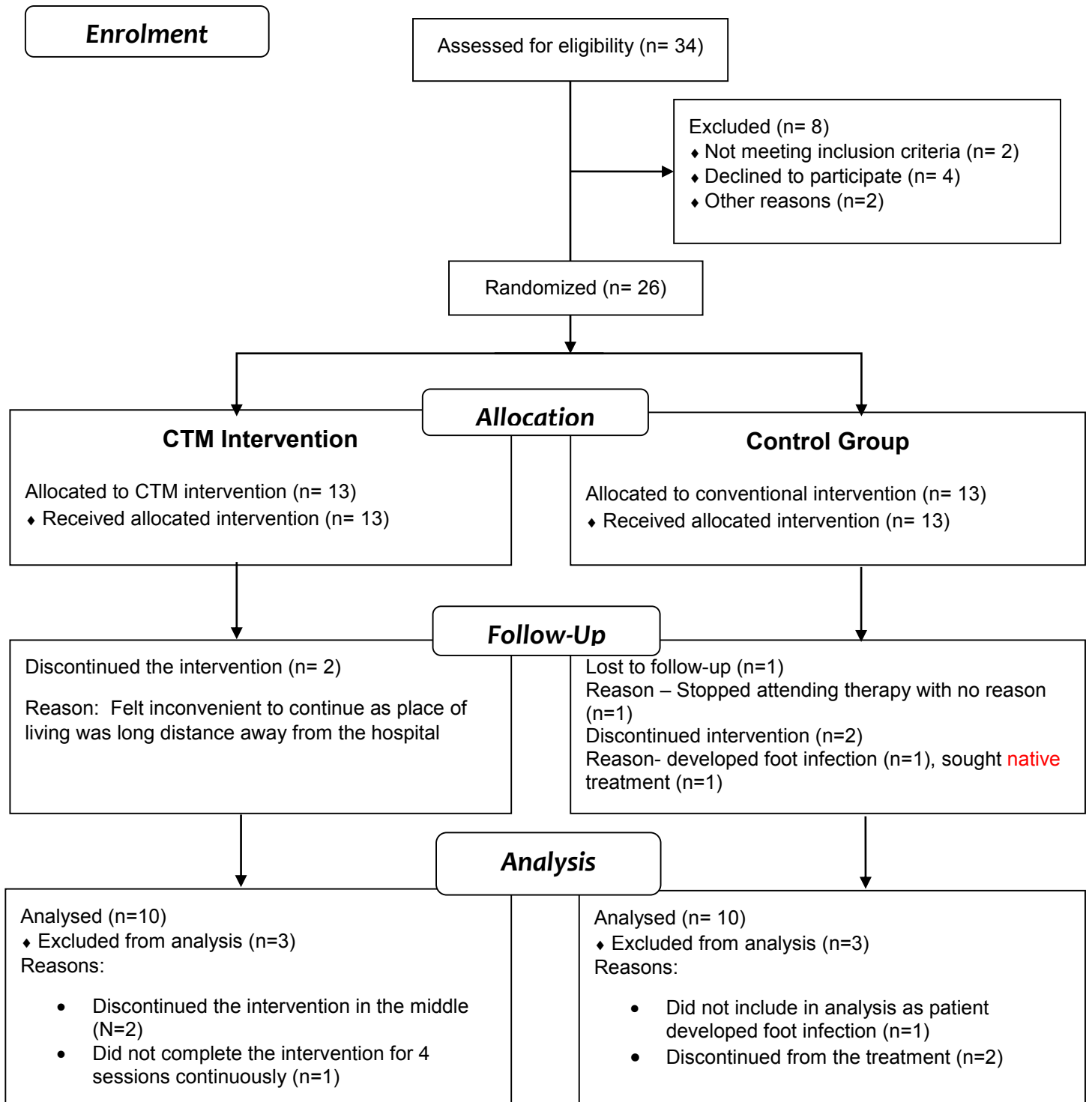


Fig 1. Flow chart of the study population throughout the course of the study

Legends:

Table 1. Demographic and clinical characteristics of diabetic subjects

Table 2. Analysis of PWAR (Cm2) in CTM and Control group

Table 3. Analysis of BCC (log CFU) in CTM and Control group

Fig 1. Flow chart of the study population throughout the course of the study