

Nursing Care on Type 2 Diabetes Mellitus with The Risk of Ineffective Peripheral Perfusion: A Case Study


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ABSTRACT

Background: Diabetes mellitus is a chronic disease characterized by hyperglycemia. Hyperglycemia can damage small blood vessels which affects the peripheral blood's flow with the resulting risk of ineffective peripheral perfusion

Objective: This study aimed to evaluate the effectiveness of foot massage, Buerger-Allen exercises, and foot exercises in improving peripheral perfusion in a patient with type 2 diabetes mellitus. Implementation was carried out for 7 days with foot massage therapy, doing Buerger Allen exercises and foot exercises.

Case: This research is a descriptive case study with the subject Mrs. S.

Results: The results show that a change of ABI value from pre-test of 0.71 (right leg) and 0.78 (left leg) to 0.9 (normal) for both legs at post-test. The NSS score also changes from 6 scores (moderate risk of neuropathy) to 0 (not at risk). The combination of BAE with diabetic foot exercises is very efficient because the muscles in the lower extremities can move maximally to increase blood vessel transport so that peripheral perfusion in the extremities increases.

Conclusion: Foot massage therapy can relax the leg muscles so that during physical exercises, vasodilation of blood vessels occurs which will improve the perfusion in peripheral tissues. In addition, managing the 5 pillars of DM is important in efforts to increase peripheral perfusion and reduce blood sugar levels.

Keywords: ABI; Buerger Allen Exercise; Foot Exercise; Foot Massage; Peripheral Perfusion

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INTRODUCTION

Diabetes Mellitus according to *the International Diabetes Federation* (IDF) Atlas 2021 is a chronic disease that occurs due to increased blood glucose levels (hyperglycemia) (IDF, 2021). Hyperglycemia is caused by the pancreas not being able to produce enough insulin. Hyperglycemia due to insulin resistance can cause a person to be at risk of small blood vessel damage (Sapra & Bhandari, 2023). Damage to the blood vessels will affect blood flow to the periphery which can cause blockage so that people with diabetes mellitus can be at risk of ineffective peripheral perfusion (Arifahyuni, 2022).

The International Diabetes Federation (IDF) Atlas in 2021 reported that 536.6 million people in the world aged 20-79 years suffer from DM. *The International Diabetes Federation* (IDF) also estimates that in 2030 there will be 642.7 million DM sufferers and an increase in 2045 of 783.2 million people aged 20-79 years. *The International Diabetes Federation* (IDF) Atlas in 2021 states that more than 90% of type 2 DM is the most common type of diabetes worldwide. Indonesia is the 5th country with the most diabetes mellitus sufferers, namely 19.5 million people after China, India, Pakistan and America (IDF, 2021).

The estimated number of Diabetes Mellitus sufferers in East Java is 863,686 of the population aged 15 years and over (Dinkes Ponorogo, 2023). The incidence of DM cases in Ponorogo Regency in 2022 was 15,773 cases (Dinkes Ponorogo, 2023). This number has increased from 2021, which was 15,513 cases (Dinkes Ponorogo, 2022) and 2020, which was 15,396 cases (Dinkes Ponorogo, 2021). The number of DM patients in the Sukosari Health Center work area is ranked 17th out of 31 health centers in Ponorogo Regency. The prevalence of DM in the Sukosari Health Center work area has increased every year. DM cases in the Sukosari Health Center work area in 2022 were 467 cases (Dinkes Ponorogo, 2023), while in 2021 it was 459 cases (Dinkes Ponorogo, 2022) and in 2020 it was 455 cases (Dinkes Ponorogo, 2021). The results of a preliminary study conducted by researchers on March 25, 2024 on 2 patients who were treated at the Sukosari Health Center General Polyclinic stated that they complained of numbness and tingling in both feet.

Hyperglycemia in DM patients can inhibit blood supply to the extremities, increasing the risk of ineffective peripheral perfusion. Treatment of patients with ineffective peripheral perfusion can affect physical aspects, quality of life and cost of care. Furthermore, blood circulation at the capillary level can interfere with body metabolism. This will affect the length of treatment for patients with impaired peripheral perfusion, and requires more intensive observation of peripheral circulation and vital signs to determine the patient's body perfusion condition. This is characterized by cold feet, dry, scaly, and cracked skin. In addition, toenails can grow abnormally. These signs and symptoms can trigger serious complications such as *Occlusive Peripheral Disease* (arteriovascular occlusive disease) (K. Teo, 2023). Peripheral arterial disease is a chronic arterial occlusive disease of the lower extremities characterized by burning, pain, cramps, fatigue, or numbness in the calves, thighs, or buttocks when moving or doing activities (Barnes et.al., 2020).

Interventions to address the problem of ineffective peripheral perfusion risk in patients are by performing circulation care (I.02079) by identifying and treating local areas with limited peripheral circulation (Tim Pokja SIKI PPNI, 2018). Circulation care can be done with foot massage therapy and training patients with foot exercises combined with *Buerger Allen Exercise* to improve the adequacy of distal blood vessel blood flow. Based on the results of research conducted by Hijriana & Miniharianti (2022) foot massage therapy can improve circulation disorders and increase ABI values. *BAE exercises* performed every day will reduce complaints of ineffective peripheral perfusion risk such as pain, stiffness, tingling and numbness in the feet which are

characterized by an increase in ABI values and foot exercises performed 4 times a week for 30-40 minutes can provide optimal results (Widiawati & Kalpataria, 2020; Wijayanti & Warsono, 2022).

Another nursing intervention that can be done is hyperglycemia management (I.03115) by identifying and managing blood glucose levels (Tim Pokja SIKI PPNI, 2018). Hyperglycemia management can be done by checking blood sugar using a glucometer. Reducing the risk of peripheral perfusion and blood glucose stability can be supported by diet education interventions (I.12369) namely by teaching the amount, type and schedule of programmed food intake (Tim Pokja SIKI PPNI, 2018). Hasina et al's (2021) study showed that Buerger Allen Exercise (BAE) was effective in increasing peripheral blood vessel vascularization. The results showed that the average ABI value in the intervention group was 0.78 (mild obstruction) and the control group was 0.75 (mild obstruction) with a p value = 0.693, meaning there was no difference. And after being given BAE, the average ABI value for the intervention group was 0.99 (normal) and the control group was 0.70 (mild obstruction) with a p value <0.05, meaning there was a significant difference in the ABI value before and after being given BAE intervention in the intervention group and the control group. In this study, in addition to being given BAE, patients were also given leg exercises and foot massage. Based on the background, the researcher conducted a study (case study) on nursing care for patients with type 2 diabetes mellitus with a risk of ineffective peripheral perfusion.

CASE PRESENTATION

This research design uses a descriptive case study method with the subject of the study Mrs. S. Data collection begins with conducting an assessment, determining a nursing diagnosis, planning nursing actions, implementing and evaluating the results of the actions that have been taken. Data collection methods used interviews, observations, physical examinations and documentation studies. the research was conducted for 7 days, namely by conducting observations every day once using SLKI parameters: *Risk of Ineffective Peripheral Perfusion*; *Ankle-Brachial Index* (ABI) to assess peripheral perfusion and *Neuropathy Symptom Score* (NSS) as a parameter to assess the degree of diabetic neuropathy.

RESULTS

Patient with the initials Mrs. S is 55 years old, female, Muslim, last education is elementary school, occupation as a housewife, the patient lives with her husband in Dkh. T, Ds. S, Kec. B, Kab. P. The patient has suffered from DM for 15 years. Complaints felt by the patient during the assessment were numbness in both feet accompanied by tingling. Complaints are

felt when tired such as walking too long and standing after sitting. The acral feels cold and the skin color is pale on both feet. Random blood sugar examination 445 mg / dl. *Ankle-Brachial Index* (ABI) examination

obtained a value of 0.78 (*borderline perfusion*) and the score on the *Neuropathy Symptom Score* (NSS) questionnaire 6 (moderate).

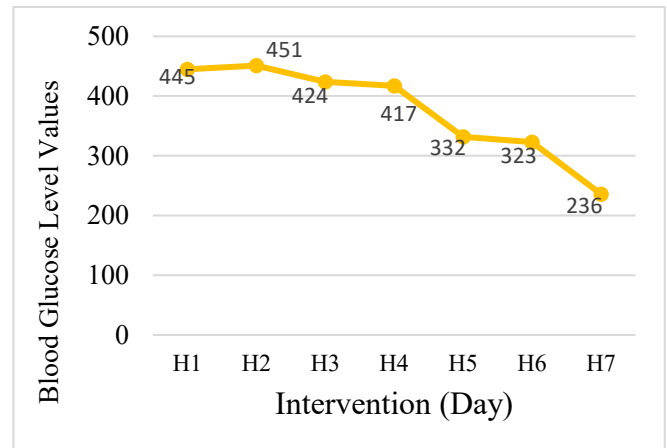
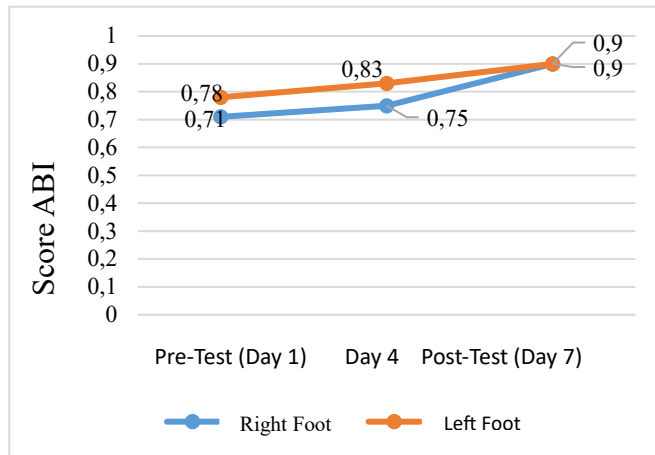


Figure 1. ABI Values Before and After Foot Massage, BAE and Diabetic Foot Exercises

Figure 2. Blood Glucose Levels

DISCUSSION

Patient Profile and Clinical Background

Patient with the initials Mrs. S is 55 years old, female, Muslim, last education is elementary school, occupation as a housewife, the patient lives with her husband in Dkh. T, Ds. S, Kec. B, Kab. P. The patient has suffered from DM for 15 years and has a Body Mass Index (BMI) of 23.37 kg/ m². A person aged ≥45 years has an increased risk of developing DM and glucose intolerance due to degenerative factors, namely decreased body function to metabolize glucose (Susilawati & Rahmawati, 2021). According to researchers, patients aged 55 years are at risk of developing DM because at that age, the body experiences decreased function to metabolize glucose.

Most sufferers of type 2 DM are female. Women are at greater risk of diabetes mellitus because physically women have a greater chance of increasing their body mass index (Komariah & Rahayu, 2020). In line with this theory, female patients with a Body Mass Index (BMI) of 23.37 kg/ m² and have the opportunity to experience an increase in body weight.

Complaints felt by the patient during the assessment were numbness in both feet accompanied by tingling. Complaints were felt when tired such as walking for too long and standing after sitting. In addition, the results of the acral examination showed cold sensations and pale skin color in both feet. A person with diabetes mellitus is at risk of experiencing blood circulation disorders due to poor blood circulation because the blood is too thick and contains a lot of sugar. Narrowing and blockage of

blood in the peripheral part, namely the feet, can be manifested by numbness and tingling (Permatasari et.al, 2020). Researchers argue that patients experience signs and symptoms of the risk of ineffective peripheral perfusion such as numbness and tingling in the lower extremities. This occurs because the blood vessels are not smooth due to the accumulation of glucose so that blood viscosity occurs which causes the flow to be slow and the feet feel numb and tingling.

Cold acral and pale skin conditions are manifestations of a lack of oxygen carried by the blood to the periphery (Zulqifni & Suandika, 2022). This is in accordance with Mrs. S's condition where the acral feels cold and the skin is pale on both feet. This is due to the condition of blood vessels that are not smooth due to glucose accumulation so that oxygen transport to the peripheral area is not optimal.

Nursing Diagnosis and Rationale

Researchers found subjective data, namely the patient said his feet felt numb and tingling, and said the complaint was felt when walking too long and changing from sitting to standing. Examination of the acral felt cold and the skin color was pale on both feet. The patient also said he rarely did physical exercise. Objective data found were composmentis awareness, examination of vital signs; BP: 140/90 mmHg N: 87 x / minute S: 36.7°C RR: 18 x / minute, GDA examination: 445 mg / dl, ABI examination: 0.71 on the right leg and 0.78 on the left leg (*borderline perfusion*) or mild risk and NSS questionnaire score: 6 (moderate risk of neuropathy).

According to Tim Pokja SDKI PPNI (2017), the definition of the risk of ineffective peripheral perfusion (Diagnosis 1) is the risk of experiencing decreased blood circulation at the capillary level which can disrupt the body's metabolism. The risk of ineffective peripheral perfusion is characterized by paresthesia, extremity pain (intermittent claudication), capillary filling >3 seconds, decreased or impalpable peripheral pulses, cold palpable acral, pale skin color, edema, slow wound healing, *ankle-brachial index* <0.90 , femoral bruit and decreased skin turgor. Research by Hijriana & Miniharianti (2022), foot massage therapy can improve circulatory disorders and increase ABI values. Foot massage therapy can increase blood flow. Good blood flow will support the supply of oxygen and nutrients to nerve cells so that it can reduce symptoms that lead to complications of diabetic neuropathy in patients with type 2 diabetes mellitus.

Researchers also found another nursing diagnosis, namely unstable blood glucose levels related to insulin resistance (Diagnosis 2). According to Tim Pokja SDKI PPNI (2017), unstable blood glucose levels are variations in blood glucose levels up/down from the normal range. Instability of blood glucose levels is characterized by fatigue or lethargy, high blood/urine glucose levels, dry mouth, increased thirst and increased urine volume. Non-pharmacological interventions can provide education about the five pillars of DM (diet, medication, blood sugar checks, exercise, education).

Researchers stated that DM patients may experience problems with the risk of ineffective peripheral perfusion due to hyperglycemia which causes blood viscosity. Thus, blood flow slows down which can be manifested by symptoms of numbness and paresthesia or tingling, cold acral and pale skin color in the lower extremities.

Intervention Strategies and Scientific Basis

foot massage interventions, *buerger allen exercises* and diabetic foot gymnastics to overcome the problem of ineffective peripheral perfusion risk in patients. According to the SLKI PPNI Working Group Team (2019), peripheral perfusion increased with the criteria of normal ABI value results ($\geq 0.9-1.3$) and the *Neuropathy Symptom Score* (NSS) questionnaire with indicators: Negative symptoms: numbness and tingling in the feet decreased.

According to the SIKI PPNI Working Group Team (2018), circulatory care is carried out by checking peripheral circulation such as peripheral pulses, edema, capillary refill, color, temperature, *ankle-brachial index*, identifying risk factors for circulatory disorders, monitoring heat, redness, pain or swelling in the extremities, providing foot care and recommendations regarding proper diet.

Researchers conducted ABI examinations by comparing systolic pressure in the arms and legs and using the NSS questionnaire as a screening for whether the patient had neuropathy complications. Furthermore, patients were given foot massage interventions, *buerger allen exercises* and diabetic foot exercises to overcome the problem of ineffective peripheral perfusion risk in patients. The intervention was carried out for 7 days which was expected to help reduce patient complaints such as numbness and tingling in the feet.

Implementation and Monitoring

Implementation carried out on patients is foot massage therapy, *buerger allen exercise* and diabetic foot gymnastics. Foot massage therapy is done every day. After foot massage therapy, patients feel more relaxed and ABI values increase. Based on the results of research conducted by Hijriana & Miniharianti (2022) *foot* massage therapy can improve circulation disorders and increase ABI values. Foot massage therapy can increase blood flow. Good blood flow will support the supply of oxygen and nutrients to nerve cells so that it can reduce symptoms that lead to complications of diabetic neuropathy in patients with type 2 diabetes.

In line with the research conducted by Hijriana & Miniharianti (2022), according to Mrs. S's condition where the patient was given foot massage therapy every day and it affected the ABI value. The ABI value when not given foot massage therapy indicated *borderline perfusion* or mild risk. 0.71 on the right leg and 0.78 on the left leg. After implementation, the ABI values in both patient legs increased on the 4th day to 0.75 on the right leg and 0.83 on the left leg. On the 7th day *post-test*, the ABI values of both legs were in the normal range or not at risk (0.9).

Buerger Allen exercise is a leg movement exercise that is useful for increasing peripheral perfusion and ABI values. *BAE exercises* performed every day will reduce complaints of ineffective peripheral perfusion risk such as pain, stiffness, tingling and numbness in the feet which is indicated by an increase in ABI values (Wijayanti & Warsono, 2022). Patients were given *Buerger Allen exercise* for 7 days and the results showed that the ABI value increased in the normal range (0.9). In addition, complaints of peripheral perfusion risk such as numbness and tingling in the feet decreased as evidenced by a decrease in the NSS questionnaire score, namely 6 (moderate risk of neuropathy) in the *pre-test*, on the 4th day it dropped to 4 (mild risk) and becomes 0 (no risk) on the *post-test*.

Leg exercise is a physical exercise performed to improve blood circulation in the legs, increase ABI values and lower blood glucose levels. DM causes blood viscosity due to hyperglycemia so that blood circulation is disrupted which causes patients to

experience the risk of ineffective peripheral perfusion (Arifahyuni & Retnaningsih, 2024). Leg exercises performed 4 times a week for 30-40 minutes can provide optimal results (Widiawati & Kalpataria, 2020). In line with this theory, patients do leg exercises with a frequency of 4 times a week with a duration of 30 minutes, the results of complaints of numbness and tingling are reduced, ABI values increase and blood glucose levels decrease. On the 2nd day, the patient's blood glucose increased because the patient drank coffee with sugar in the morning.

Outcome Evaluation: ABI, NSS, and Blood Glucose Ankle-Brachial Index (ABI)

Evaluation on *pre-test* day 1, day 4 and *the post-test* on day 7 conducted at the patient's home showed changes in ABI values with the risk of ineffective peripheral perfusion. ABI examinations on *the pre-test* and day 4 still showed *borderline perfusion results*. On *the post-test* on day 7, After the patient underwent foot massage, *BAE* and foot exercises, the ABI values of both patient's legs increased to the normal range.

Based on the results of research conducted by Hijriana & Miniharianti (2022) foot massage therapy can improve circulation disorders and increase ABI values. Foot massage therapy can increase blood flow. Good blood flow will support the supply of oxygen and nutrients to nerve cells so that it can reduce symptoms that lead to complications of diabetic neuropathy in patients with type 2 diabetes mellitus.

Buerger Allen exercise and diabetic foot exercise are physical exercises that can increase ABI values. According to research conducted by Putri (2020) *BAE* physical exercise and foot exercise can improve lower extremity perfusion as evidenced by an increase in ABI values. *Buerger Allen exercise* utilizes the force of gravity which helps empty the veins and fill the arteries alternately so that it can improve blood circulation in the lower extremities. Meanwhile, diabetic foot exercise is done by moving the joints and ankles which makes the muscles actively press the blood vessels so that vasodilation occurs. The combination of *BAE* with diabetic foot exercise is very efficient because the muscles in the lower extremities can move maximally to increase blood vessel transportation so that peripheral perfusion in the extremities increases.

In line with research conducted by Putri (2020), patients after being given foot massage therapy, *buerger allen exercise* and foot gymnastics showed changes in ABI values. Patients did physical exercises enthusiastically so that on the 4th and 5th days they started doing *BAE exercises* and foot gymnastics independently. Physical exercise can train the muscles in the lower extremities so that it

can smooth blood circulation which can make ABI values increase to the normal range.

Neuropathy Symptom Score (NSS) Questionnaire

Evaluation on *pre-test* day 1, day 4 and *post-test* day 7 also shows changes in the NSS questionnaire score against the risk of neuropathy experienced by patients. On *the pre-test* and day 4 showed that patients were at risk of neuropathy complications. On *the post-test* on day 7, after foot massage, *BAE* and foot exercises, the patient no longer felt symptoms of neuropathy.

Physical exercise in patients with type 2 diabetes will cause metabolic changes. Physical exercise will increase blood flow so that more insulin receptors and become more active. Someone who rarely does physical exercise, over time, high blood glucose levels will damage the nerves. Thus, DM patients are at high risk for complications of diabetic neuropathy which is characterized by symptoms of numbness and tingling (Wanjaya et.al, 2020).

Researchers stated that patients before being given foot massage therapy, *buerger allen exercise* and foot gymnastics experienced symptoms of neuropathy such as numbness and tingling in the feet. After doing physical exercise, on the 7th day the patient no longer felt symptoms of neuropathy. Physical exercise in patients with diabetes mellitus can reduce the risk of ineffective peripheral perfusion which can lead to complications of neuropathy. Physical exercise can open capillary blood vessels so that there are more insulin receptors so that blood glucose levels can decrease where high glucose conditions will risk damaging nerves.

Evaluation of the acral and color of the lower extremities also changed. On day 1, cold acral and pale skin color were found on both feet. After leg exercises, the evaluation on day 4 changed where both of the patient's feet were no longer pale and returned to normal (no cyanosis, redness or *jaundice*) but the acral was still cold. On day 7, the acral results were found to be warm and the skin color was normal on both feet.

In line with research conducted by Zulqifni & Suandika (2022) stating that cold acral and pale skin color are due to lack of oxygen in the blood. This is in accordance with the patient's condition where before the intervention was found cold acral and pale skin color on both feet. Researchers stated that physical exercise can smooth blood flow so that oxygen transportation to the periphery can increase. After the patient underwent leg exercise intervention, the oxygen supply in the blood of the patient's lower extremities increased so that the acral became warm and the skin color returned to normal (not pale, no cyanosis, redness or *jaundice*).

Blood Glucose Levels

Evaluation for 7 days also showed changes in the patient's random blood glucose levels. Interventions given to patients with foot massage therapy, *buerger allen exercise* and foot gymnastics resulted in a significant decrease in blood glucose levels. In addition, patients also carried out 5 pillars of DM management.

Physical exercise in patients with type 2 diabetes mellitus can control blood glucose. When doing physical exercise, the muscles will contract, triggering the release of glucose transporters to take glucose in the muscles without depending on insulin to be carried to the cells. In addition, there will be an increase in blood flow so that the capillaries will open more. When the capillaries open more, the insulin receptors are more numerous and more active. Thus, blood glucose levels will decrease by themselves (Istiqomah & Yuliyani, 2022).

In line with this theory, patients before doing physical exercise had high blood glucose levels as evidenced by GDA examination on the first day, which was 445 mg/dl. Physical exercise performed by patients every day lowered blood glucose levels. Patients were able to do physical exercise enthusiastically and independently so that the muscles contracted which caused the capillary blood vessels to open. The capillary blood vessels are more open so that blood flow becomes smooth and produces more insulin receptors so that glucose levels decrease.

Evaluation on the 7th day obtained the results of complaints of ineffective peripheral perfusion risk resolved with the efforts that the patient had made, namely the management of the 5 pillars of DM. Compliance with the 5 pillars of DM affects changes in the risk of peripheral perfusion experienced by patients. Patients who carry out the management of the 5 pillars of DM will help the process of increasing peripheral perfusion. In line with research conducted by Hasina et al., (2022) according to the patient's condition where before receiving comprehensive management of the 5 pillars of DM, namely education, diet planning, physical exercise, treatment and blood glucose monitoring, complaints were obtained regarding the problem of ineffective peripheral perfusion risk such as numbness, tingling, cold extremities and pale skin color on both feet. During 7 days of treatment, the patient carried out the management of the 5 pillars of DM by getting education about the disease and the importance of physical exercise, complying with the diet, doing physical exercises such as foot massage therapy, *buerger allen exercise* and foot gymnastics, routinely taking Glibenclamide 1x5 mg every day and regular blood glucose monitoring.

Implications

This study has important implications in various aspects. Practically, the results indicate the need to

improve health education for DM patients, particularly physical activity exercises such as foot massage therapy to relax leg muscles and increase blood vessel vasodilation, which will improve perfusion in peripheral tissues. Furthermore, managing the five pillars of DM is crucial in efforts to improve peripheral perfusion and reduce blood sugar levels. In a clinical context, this study emphasizes the importance of a multidisciplinary approach involving healthcare professionals, active family involvement, health cadres to support DM patients, and community support. Routine monitoring, such as technology-based medication reminders, is also recommended. From a policy perspective, this study can encourage local governments to allocate resources for programs to improve adherence to the five pillars of DM. This study also provides a basis for further research exploring factors influencing physical exercise adherence and can be used as a guide in developing training modules for healthcare workers.

Limitations and Recommendations

This case study research has several limitations, such as the use of descriptive methods with a single patient. Data collected through the NSS questionnaire and ABI measurements on respondents are susceptible to subjective bias, and this study did not explore other factors that could influence peripheral perfusion results. To address these limitations, researchers conducted measurements periodically over seven days. Similar research should also be conducted on several respondents to improve the accuracy of the results. Education on the five pillars of diabetes mellitus (DM) is provided to patients to further enhance understanding and awareness regarding health, especially diabetes. In addition, multidisciplinary collaboration involving families and health workers at the community health center (Puskesmas) and village health posts (Ponkesdes) needs to be strengthened, and validation of measurement tools such as the NSS questionnaire is necessary to improve its suitability.

CONCLUSIONS

The patient rarely did physical exercises such as *Buerger Allen Exercise (BAE)* and diabetic foot exercises. Lack of physical exercise caused the patient to experience *borderline perfusion* with an ABI value of 0.71 on the left foot and 0.78 on the right foot and a random blood glucose examination of 445 mg/dl. In addition, the NSS questionnaire score obtained by the patient showed a score of 6 (moderate risk of neuropathy). Nursing implementation was carried out on Mrs. S for 7 days with foot massage therapy, doing *Buerger Allen exercise* and training diabetic foot exercises. In addition, education was given on DM management with 5 pillars (health education, meal

planning or diet, physical activity, medication, and blood glucose monitoring). Nursing evaluation showed that the ABI value increased before the intervention was given, namely 0.71 on the right foot and 0.78 on the left foot (*borderline perfusion*) and after doing physical exercise on the 4th day the ABI value increased to 0.75 on the right foot and 0.83 on the left foot. In the *post-test* on the 7th day, the ABI value became 0.9 (normal) on both feet. Complaints such as numbness and tingling, cold extremities and pale skin color of both feet were resolved. In addition, the results of blood glucose tests tended to decrease every day.

Declaration of Interest

In the process of conducting research until the publication of research results, there is no conflict of interest between the researcher and related parties.

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Authors' Contributions

All authors contributed substantially to the conceptualization, design, data curation, formal analysis, interpretation, writing, review, and editing of the paper. All authors approve the final version to be published.

Data Availability

The resulting datasets were then subjected to analysis during the study and are currently available from the corresponding author on reasonable request.

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