Araştırma Makalesi / Research Article

A method for preventing major amputation in patients with diabetic foot ulcer: negative-pressure wound therapy combined with intralesional epidermal growth factor

Diyabetik ayak ülseri hastalarında major amputasyonun önlenmesi için bir yöntem: negatif basınçlı yara tedavisi ile kombine intralezyonel epidermal growth faktör kullanılması

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Abstract

Background: Diabetic foot ulcer (DFU) is a serious health problem affecting 15% of patients with diabetes mellitus. Diabetic foot ulcers cause a decrease in the quality of life of patients like labor loss, long-term of stay in hospital, financial burden and also lower extremity amputation. Many methods have been applied to treat patients with DFU, but amputations have not been prevented. In this study, we aimed to present the effects of our treatment in DFU patients who had been major amputation (trans-metatarsal, tarsometatarsal, intertarsal, rare foot, and at the upper level of the ankle amputations) decision
Methods: In this study, we presented patients who had been referred to us from various hospitals with a decision for major amputation, but treated by us using intralesional epidermal growth factor therapy (EGF) combined with negative-pressure wound therapy (NPWT) to prevent below-knee amputation. NPWT (three times a week on alternate days up to the complete closing with skin tissue was achieved) combined with EGF (twice times a week for three days up to complete granulation tissue was achieved) therapy were applied to patients. The efficacy of treatment was assessed according to wound closure and amputation prevention.
Results: According to PEDIS classification, 27 (84.3%) out of 32 patients were the grade 3, and 5 (15.6%) were the grade 4 patients. Infection was present in 24 (75%) of the patients. Necrosis was present in 12 (37.5%) of these patients. The average wound size was 18 cm (10-25 cm). Complete skin closure was achieved in 27 patients (84.3%), and closure with 100% granulation was achieved in 5 patients (15.6%). None of the patients underwent major amputation. EGF [average of 10 injections (7-12 injections)] combined with NPWT [average of 11 sessions (8-13 sessions)] treatment were administered for 45 days on average. Infections were developed in three patients during one-year of follow-up approximately, and medical treatment was administered.
Conclusion: Our patients were in the high risk group for major amputation, which were referred from various hospitals with the recommendation of major amputation. We think that the regular application of NPWT combined with EGF can prevent major amputations.

Keywords: Diabetic foot, Amputation, Epidermal growth factor

Öz.

Amaç: Diyabetik ayak ülserleri (DAÜ), diyabetik hastaların %15'ini etkileyen bir sağlık problemidir. DAÜ, iş gücü kaybı, uzun süre hastanede yatış, maddi kayıp ve ayrıca alt ekstremite amputasyonu gibi nedenlerle hastaların yaşam kalitesinde düşüşe neden olmaktadır.
DAÜ'lerinin tedavisinde birçok yöntem uygulanmış fakat amputasyonların önüne geçilememiştir. Çalışmada major amputasyon (trans-metatarsal, tarsometatarsal, intertarsal ve diz altı amputasyonlar) kararı alınmış DAÜ hastalarına uyguladığımız tedavinin etkilerini sunmayı amaçladık.
Materyal ve Metod: Çalışmada, çeşitli sağlık kurumlarından hastanemize yönlendirilen ve majör amputasyon kararı alınmış fakat hastanemizde negatif basınçlı yara tedavisi (NBYT) ile kombine intralezyonel epidermal growth faktör (EGF) uygulanarak majör amputasyon yapılmadan tedavi edilen hastalar değerlendirildi. Hastalara EGF (haftada 3 kez veya cilt dokusu ile tam kapanma oluşuncaya kadar) ile kombine NBYT (haftada iki kez üç gün boyunca tam granulasyon dokusu oluşuncaya kadar) tedavileri uygulandı. Yara kapanması ve amputasyonu önlemeye yönelik tedavinin etkileri incelendi.
Bulgular: PEDİS sınıflamasına göre 32 hastanın 27'si (%84.3) grade 3, 5'i (%15.6) grade 4 DAÜ'ine sahipti. Hastaların 24'ünde (%75) infeksiyon mevcuttu. 22 hastada (%68.7) nekroz mevcuttu. Hastaların ortalama yara büyüklüğü 18 cm (10-25cm) idi. Uygulanan tedavi ile hastaların 27' sinde (%84.3) tam cilt kapanması, 5 hastada (%15.6) yara zemininin tamamının granülasyon dokusu ile kapanmasısağlandı. Hastaların hiç birine major amputasyon yapılmadı. EGF ortalama 10 injeksiyon (7-12 injeksiyon) ile kombine NBYT ortalama 11 seans (8-13 seans) tedavisi ortalama 45 gün uygulandı. Yaklaşık bir yıllık takip süresi içinde üç hastada enfeksiyon gelişti ve medikal tedavi uygulandı.
Sonuç: Hastalarımız, major amputasyon önerilerek çeşitli hastanelerden yönlendirilen, major amputasyon riski yüksek olan hastalardı. Çalışmanın sonucunda, NBYT ve EGF'nin birlikte düzenli olarak uygulanması ile major amputasyonların önlenebileceği kanısındayız.

Anahtar kelimeler: Diyabetik ayak, Amputasyon, Epidermal büyüme faktörü

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Introduction

Diabetic foot ulcer (DFU) is a serious health problem affecting 15% of patients with diabetes mellitus (1). Diabetic foot ulcers cause a decrease in the quality of life of patients like labor losslong-term length of stay in hospital and huge financial burden and also lower extremity amputation (2,3). Diabetic foot ulcers account for 50-70% of the causes of non-traumatic amputation (4). Bone and joint infections caused by DFU requires antibiotic therapy, local debridements, resections and amputations (5).

Epidermal growth factor (EGF) is a molecule originally produced from the submandibular glands of mice, with strong mitogenic properties in polypeptide structure (6). Intralesional EGF has been started to be used for medical treatment in Turkey since 2012 (7). EGF stimulates the progression of cells into the wound area, the formation of granulation tissue, the activation and proliferation of myofibroblasts, and the induction of wound contraction, and the migration of epithelial cells into the ulcer area (8). Negative-pressure wound treatment (NPWT) is a non-invasive treatment method. In this method, a controlled negative-pressure system is used to decrease the wound drainage, sources of infection, edema, bleeding of the wound and to stimulate the formation of granulation tissue (9). In addition, the NPWT method also provides dilatation of arterioles, local blood circulation, and angiogenesis in the wound bed (10).

In our study, we have presented the patients who have the risk of below-knee amputation. These patients have treated by intralesional EGF combined with NPWT (EGF+NPWT).

Materials and Methods

Records of patients with DFU, who had been referred by various clinics for below-knee amputation (infection covering the entire foot and the presence of cellulite extending to the bottom of the knee, presence of necrosis and infection at the minor amputation site, presence of osteomyelitis in calcaneus and talus bones), however treated without major amputation (below-knee amputation) in the General Surgery Clinic between January 2017 and January 2018, were reviewed retrospectively. Patients were evaluated according to their demographic characteristics, duration of diabetes and duration of the development of diabetic foot, wound characteristics, infection findings, treatment moralities, duration of treatment, and complications.

Inclusion criteria of the study

PEDIS grade 3 and grade 4 patients older than 18 years and less than 65 years of age, who received treatment without success in wound closure previously, and had been referred with an major amputation decision from various clinics, were included in the study.

Exclusion criteria of the study

Patients who were pregnant, diagnosed with cancer, multiple comorbidities (other than diabetes mellitus), immunosuppressive treatment, renal failure or risk of renal failure. Akut kidney injury was defined using the the RIFLE system (Risk, Injury, Failure, Loss of function, and End-stage renal disease). The risk was defined by 50% increase in serum creatinine level, 25% decline in GFR, urine excretion falls below 0.5 mL/kg per hour over 6 hours (11).The kidney injury was defined by twofold increase in serum creatinine level, urine excretion falls below 0.5 mL/kg per hour over 12 hours (11). Vascular or dermatological foot ulsers and patients with charkot joint were excluded from the study. Failure to detect dorsalis pedis and tibialis posterior pulsations on physical examination, and no flow or monophasic flow seen with hand Doppler, were evaluated as PAD. Detection of occlusion with doppler ultrasonography examination and monophasic flow in peripheral vessels were evaluated as PAD. Ankle-brachial index (ABI) <0.95 was assessed as PAD (12). The range of 0.95-1.30 was considered normal (12). This patients were first treated by interventional radiology.

Patients who received medical treatment (hyperbaric oxygen theraphy, NPWT, EGF, ozone therapy, wound care materials) in the last month were not included in the study.

Criteria for evaluation of the patients before the treatment

With the recommendation of endocrinology, blood glucose regulation was provided in all patients before the treatment. Antibiotic for the treatment were started according to wound culture results. Broad-spectrum antibiotics were administered to the patients until obtaining culture results. Doppler ultrasonography examination was performed to differentiate DFU with peripheral arterial and venous foot ulcers(In order to make the treatment more effective for patients with severe venous insufficiency, firstly medical treatment was applied by cardiovascular surgery.) and medical treatment was started by cardiovascular surgery. ABI (ankle-brachial index) indices were calculated (Ankle-brachial index (ABI) <0.95 was assessed as PAD). In addition, cilastasole (Pletal® 100mg AI pharma, Istanbul, Turkey) was started to be administered to each patient with the recommendation of a cardiovascular surgeon. Peripheral artery angiography was performed in patients when necessary (In these patients, failure to detect dorsalis pedis and tibialis posterior pulsations on physical examination, and no flow or biphasic flow seen with doppler ultrasonography). Peripheral neuropathy was assessed by neurology through a neurological examination, including a monofilament test. Recommendations of dermatology department were used for foot care. Presence of osteomyelitis was investigated

by magnetic resonance imaging of all patients. Increased signal intensity of the bone marrow on fat-suppressed T2-weighted images and focally decreased bone marrow signal intensity on T1-weighted images and relative to areas of normal bone marrow were accepted as osteomyelitis (13). First, surgical debridement was performed in all patients, and infected and necrotic tissues were removed. Minor amputation (toe amputation) was performed as necessary. After this phase, the treatment has started.

Evaluation of the wound

DFU was evaluated on admission of the patents according to PEDIS classification (table 1).. The presence of infection and necrosis was evaluated by clinical observation and physical examination. The presence of purulent discharge around the wound or foot, redness, endurance, abscess, edema was evaluated as the presence of infection.

EGF application

EGF (Heberprot-P, Has Biotec) was administered three times a week, intralesionally to the sides and base of the wound. 75 µg recombinant human epidermal growth factor was used (7). Heberprot-P was used after diluting with 5 ml saline (7). Intravenous anti-histamine was administered to the patient prior to application in order to prevent an allergic reaction (7). First, the patients' infection was controlled and then epidermal growth factor was injected to the patients.

NPWT application

NPWT (KCImanufactured by KCI Medical Ltd., England) was applied to all the patients until discharge and inflammation stopped. NPWTA maximum of 125 mmHg pressure was applied continuously (12). NPWT was changed every 2-3 days (12). Between each change, it was closed with wet dressing with normal saline for at least 6 hours to allow ventilation and normalization of blood circulation. During this time, EGF applications were performed to the non-infected wounds NPWT was applied again at least 3 hours after EGF application (figure 1).

Evaluation of the success of the treatment

In our study, the best result means that the closure of the wound completely with the skin. Based on the rate of closure of the wound surface with granulation tissue, wounds were evaluated as less than 25% closure (no treatment response), 26-50% closure (minimal response to treatment), 51-75% closure (partial response to treatment), more than 75% closure (complete response to treatment) (7).

Major amputations were the amputations which were made trans-metatarsal, tarsometatarsal, intertarsal, rare foot, and at the upper level of the ankle amputations. Amputations performed under these levels were considered as minor amputations (eg finger amputations).

Termination of the study

Achieving a complete closure, ability to close the wound with graft or flap, major amputation necessity, septic condition, and duration of treatment more than 90 days required termination of the study for respective patients.

Results

Of the 32 patients, 28 (87.5%) were male, 4 (12.5%) were female (table 2). The median age of 59±10.4 years. All the patients had been treated at various hospitals unsuccessfully, and below-knee amputation had been recommended (15 of the patients (46.8%) had an infection covering the entire foot and the presence of cellulite extending to the bottom of the knee. 9 of the patients (28%) had a necrosis and infection at the minor amputation site, 8 of the patients (25%) had an osteomyelitis in calcaneus and talus bones). Of the patients, 18 (56.2%) had hypertension, 2 (6.2%) had chronic obstructive pulmonary disease. In other hospitals, sterile gauze and saline had been applied in 12 of the patients (37.5%), 7 of them (21.8%) had been treated with hyperbaric oxygen therapy, 5 (15.6%) had been treated with EGF alone, and 8 (25%) had been treated with NPWT method. There was no adequate closure or clinical deterioration in the treatment of these patients. Twenty-six patients (81.2%) had received an insulin treatment, and 6 patients (18.7%) had received an oral antidiabetic treatment. Oral antidiabetic treatments were stopped. All of the patients received an insulin treatment. Duration of diabetes were 20 years, and duration of diabetic foot infection for 2.9 years on average. Nine patients had minor amputations (4 patient fingers, 4 patients had ray amputation, 1 patient had chopard amputation) at an external institution, but below-knee amputation has been recommended for them due to clinical deterioration. Other patients underwent medical treatment modalities in respective clinics.

According to PEDIS classification, 27 (84.3%) of the patients were the grade 3, and 5 (15.6%) were the grade 4 patients. Average duration of treatment was 45 days. DFU was in the fingers in 7 patients (31.2%), in the soles of 8 patients (25%), in the foot lateral in 4 patients (12.4%), in the heel in 4 patients (12.4%) and in the amputation site in 9 patients (28%). Infection was present in 24 (75%) of the patients. Necrosis was present in 12 (37.5%) of these patients. The average wound size was 18 cm (10-25 cm). Complete skin closure was achieved in 27 patients (84.3%), and closure with 100% granulation was achieved in 5 patients (15.6%). None of the patients underwent major amputation. NPWT was administered for 36±4.2 days on average. After patients' seroma, inflammation, discharge and edema were improved, EGF+NPWT administration was replaced by EGF administration. This duration was 9 days on average. Allergic reactions and maceration were observed in 7 patients (21.8%) due to NPWT application. NPWT treatment was interrupted and corrected with topical treatment. Chills and Tremble were

observed in 16 patients (50%), nausea in 5 patients (15.6%) and pain at the application site in 5 patients (15.6%). In addition, transient blood glucose level was elevated in 26 patients (81%) within 2 hours after application, and no intervention was required.

Table 1. The PEDIS classification system

Grade	Perfusion	Extent	Depth	Infection	Sensation	Score
1	No PAD	Skin intact	Skin intact	None	No loss	0
2	PAD, No CLI	<1cm^2	Superficial	Surface	Loss	1
3	CLI	1-3 cm^2	Fascia, muscule, tendon	Abcess, fasciitis, septic artritis		2
4		>3 cm^2	Bone or joint	SIRS		3

PAD, peripheral arterial disease; CLI, critical limb ischemia

Table 2. Demographical characteristics and clinical outcomes of the patients.

Demographical and clinical features of the patients (n:32)	
Age (mean±SD years)	59 ±10.4
Gender (%)	
Females	4 (12.5)
Males	28 (87.5)
Duration of diabetes (year)	20 (8-32)
Median (25th 75th percentile)	
Duration of diabetic foot ulcer (year)	2.9 (1-7)
Infection (%)	24 (75)
Necrosis (%)	12 (37.5)
Wound size (cm^2) median (25th 75th percentile)	18 (10-25)
Wound localization (%)	
Sole	8 (25)
Heel	4 (12.4)
Phalanges	7 (31.2)
Footlateral	4 (12.4)
Amputation stump	9 (28)
Osteomiyelitis (%)	20 (62.5)
Meanduration of treatment (25th 75th percentile) (day)	45 (40-52)
PEDIS classification, n (%)	
Grade 1	0 (0)
Grade 2	0 (0)
Grade 3	27 (84)
Grade 4	5 (16)
Outcome, n (%)	
Complate skin closure (%)	27 (84.3)
Complete response (granulation tissue>75%)	5 (15.6)
Partial response (granulation tissue 51-75%)	0 (0)
No response	0 (0)
Average length of follow-up (month)	11.4

n: Number of cases, SD: Standart deviation

Figure 1. The treatment with EGF+NPWT system in infected and ischemic low extremity. Firstly minor amputation was applied to the foot.

During 11.4 months follow-up infections were detected again in 3 patients and controlled by antibiotic treatment. Also, we have amputated of one patient (below-knee amputation) due to osteomyelitis in the calcaneus.

Discussion

In the study, we presented the results of NPWT and EGF methods applied to our patients with diabetic foot ulcers. This practice was not common in the literature. The patients we presented had been referred from various clinics and hospitals with a recommendation of below-knee amputation particularly, who have been survived the major amputation with EGF+NPWT application. Its success in PEDIS grade 3-4, ischemic, infected patients with high-risk of amputation was surprising about this treatment method. Despite this, retrospective nature of the study, limited number of patients, lack of comparisons, and evaluation of wound healing clinically rather than histologically are the limitations of the study. These limitations need to be taken into account when reading this manuscript.

PDGF (Platelet-Derived Growth Factor) is a growth factor that triggers VEGF (Vascular Endothelial Growth Factor) for angiogenesis (15). It is known that it is necessary for new tissue formation in bone and soft tissue (16). EGF is found in the structure of platelets, macrophages and fibroblasts (17). In one study, it has been shown that the amount of EGF and thus epithelialization have been significantly stimulated following an acute injury (18). It has been shown in a study conducted in the diabetic mouse model by Erba et al. that NPWT provided a significant PDGF, EGF, TGF-β (transforming growth factor-beta) activation in the wound (19). In the study, PDGF and EGF expression was found to form a granulation tissue rich in collagen on the wound surface (19). Leptin is a recently identified cellular factor that stimulates new angiogenesis, inflammation and immunological function (20). Leptin has been reported to have a critical role as an autocrine/paracrine regulator in normal wound healing in wounded tissues (21). Studies have shown that NPWT is effective on leptin and HB-EGF (Heparin-binding epidermal growth factor-like growth factor), albeit not very noticeable (22). Jacob et al. showed that NPWT administration stimulates growth factor production, which in turn increases angiogenesis and collagen deposition (23). VEGF is a growth factor effective on endothelial cells and stimulating angiogenesis (24). Labler et al. showed that the amount of VEGF in the wound fluids of patients treated with NPWT was much higher than that of treated

with wet dressing (25). It has been shown that VEGF, EGF, PDGF, ANG-2 (angiotensinogen) levels increase significantly in the stress environment created by NPWT (22).

In our study, we were able to increase the growth factors required for complete wound healing and angiogenesis with NPWT application and to remove infected fluids from the environment to provide the appropriate environment for wound healing. In addition, we performed intralesional EGF to accelerate epithelization. Both methods were routinely used methods in our clinic. We applied both treatment methods at the same time in diabetic foot wounds, especially in the high-risk group for amputation.

One of the most feared consequences of diabetic foot ulcers is extremity amputations. DFU amputation rates in studies in Turkey were reported to be 50%, 37% and 28% (26,27,28). These high rates were obtained as a result studies carried out by orthopedics and endocrinology clinics. In a study conducted in Sweden, 34% of patients with diabetes with severe foot infections underwent minor amputation, whereas 8% underwent major amputation (29). Our major amputation rate in our clinic does not exceed 9%. According to various studies, risk factors for lower extremity amputation due to DFU were as follows: being over 60 years, gangrene, ABI <0.8, white blood cell count >15000, higher CRP level, higher level of sedimentation, and presence of staphylococcus infection (28, 30). In our study, the median age of patients was 59 years. Necrosis was present in 12 patients (37.5%) and 3 patients (9.3%) had necrotizing fasciitis. The mean ABI index of the patients was 0.78. The average value of the blood leukocyte was 16200 (normal values: 3.5-10.5 K/µL). The mean CRP value was 1.27 (normal values: 0-0.8). Patients were at risk for amputation at first evaluation. Yet, as a result of evaluating the patients with diabetic foot ulcer in our clinic by multidisciplinary treatment approach, NPWT + EGF therapy was started by avoiding all these risk factors or by taking all these risk factors under control in a time as short as a week. With this practice, we believe that we are increasing the treatment chance of patients.

In addition, smoking has been reported to be a risk factor for amputation and play an important place in the development of DFU (26). Of the patients, 21 (65.6%) were chronic smokers. At the initial evaluation of these patients, 85.7% quit smoking by the treatment provided by family medicine clinic for smoking cessation. Patients were ensured to quit smoking during their treatment and control. This is because even though infections are tried to be controlled for DFU, more than 85% of amputations is carried out due to untreatable infections (31). In our study, 24 (75%) of the patients had infection due to DFU. Nine patients (28%) had osteomyelitis. These patients were also the patients presented with necrosis and fasciitis. In our clinic, 4 of these patients had finger amputations, 2 had ray amputation, and 1 had chopard amputation. Other patients underwent wound and bone debridement. Staphylococcus aureus was isolated in 85% of the patients and antibiotic treatments were rapidly regulated. The infection was controlled by appropriate and fast minor amputation and debridement as well as starting an early antibiotic treatment. Antibiotics were administered for at least 10 days during their hospitalization. Antibiotic treatments of patients with osteomyelitis lasted for at least 21 days. Some studies indicate that a long conservative treatment increases the rate of healing of osteomyelitis (5). Therefore, antibiotic therapy was continued for a long time in patients with osteomyelitis. Osteomyelitis is a complication of infection in patients with DFU (32, 33). Osteomyelitis is known to develop in deep ulcers that do not respond to local wound care (32, 33). Early diagnosis and effective treatment of in osteomyelitis due to diabetes have an important role in the prevention of lower extremity amputations (34, 35). When we compare diabetic feet with osteomyelitis and diabetic feet with soft tissue infection, those with osteomyelitis were at high risk for amputation (34, 35). In the diagnosis of osteomyelitis, MRI (Magnetic Resonance Imaging) sensitivity is 90% and specificity is 80% (36). We evaluated patients in terms of osteomyelitis using MRI in our study.

Approximately 50% of patients with DFU also has peripheral arterial disease (37). Peripheral arterial disease, which causes foot lesions in diabetic patients, usually starts early with a rapid and aggressive course (38). There is also a high thrombogenic potential in patients with diabetes (39). The American Diabetes Association (ADA) has recommended the use of low-dose aspirin to reduce cardiovascular risk in patients with diabetes (40). In our clinic, cilostazol was administered instead of aspirin for the antiagregant treatment. In our study, cilostazol 100 mg was administered to all patients with diabetic foot ulcer daily.

Patients in the study undergone 45 days of treatment on average. This period did not include the period of evaluation of patients by other disciplines such as cardiovascular surgery, endocrinology, cardiology, neurology and infectious diseases. In a study conducted with 80% of Wagner's grade 3 patients, complete granulation was achieved in 23.6 days and complete wound closure in 44 days using only EGF (43). In another study using EGF, reepitilization has been reported to be achieved in 66 days (44). This study has reported that EGF reduce amputation rate (44). It has been reported that 90% of Wagner's grade 3-4 patients had achieved adequate granulation tissue in 46.6 days using EGF (45). In a study with Wagner 2-3 patients, diabetic wounds have been reported to be closed in 23.3 day on average with the NPWT system (46). In another study, granulation tissue has been achived in 32.9 days on average (47), whereas this duration has been reported as 57.4 days in another

study (48). Patients evaluated in our study were PEDIS grade 3-4 patients who had infected, necrotic, osteomyelitis, having a planned below-knee amputation actually. EGF+NPWT application, 84.3% of patients achieved complete skin closure, not just granulation tissue. The mean duration of application for EGF+NPWT was 36 days, followed by EGF treatment alone. Compared with the literature, this is a good duration for our patients in the high-risk group for amputation.

The adverse effects seen in our patients after EGF and NPWT application were consistent with the literature (7, 47). However, unlike the literature, transient blood glucose elevations were detected in our 81.2% of our patients after EGF administration.

In our study there are no comparison groups. If we look at the rate of major amputation we can see that it is a more successful method than the methods applied in other health institutions.

Conclusion

We believe that it may be more infrequent to use the EGF and NPWT combination instead of using the EGF or NPWT system alone in patients with necrotic, infected diabetic foot ulcer and osteomyelitis who are at risk for major amputation or who require major amputation. Although other health institutions' surgeons decided to underwent tomajor amputation as a result of their own treatment, our method of recovering patients from amputation after EGF+NPWT treatment showed that it became successful. But, we need prospective and comparative studies to demonstrate the superiority of EGF+NPWT over other treatments.

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