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PLATELET DERIVED GROWTH FACTOR IN DIABETIC LOWER EXTREMITY ULCER: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED STUDY IN INDIAN CONDITION

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ABSTRACT: Background-In September 2012, the world health organization reported a global prevalence of diabetes exceeding 300 million people, predicting a further 60-70% increase by the year 2030, which means India alone will have 100 million people by year 2030. Wound healing is problematic in diabetic patients. Encouraging results have shown that PDGF application is better than good wound care alone. But the evidence to demonstrate the safety and efficacy of PDGF in Diabetic ulcer is scanty. **Aim-**To Study the Efficacy of Platelet Derived Growth Factor in Diabetic Lower Extremity Ulcer. **Methods-**This study was a prospective, double blind, and placebo controlled study. A total of 29 patients with 35 ulcers were included and divided in case and control group. The study medication was administered in conjunction with standardized good wound care for 24 weeks or until target ulcer healed. **Results-**It was found that 74.2% of ulcers were neuropathic while 48.3% patients had foot deformity in this study. At the end of 24 weeks 100% ulcers in group receiving PDGF completely healed while group receiving Placebo 76.4% ulcers healed. Mean of time to achieve wound healing was 9 $\pm$ 7.1 weeks in ulcer receiving PDGF gel and they healed 50% faster as compared to ulcers receiving placebo. **Conclusion-** There was no relation of type, duration, ABI, neurological deficit, type of ulcer, foot deformity, wagner grade, edema, infection, duration of ulcer with time to achieve wound closure. This meant that PDGF was solely responsible for the ulcers to heal 50% faster.

INTRODUCTION: According to WHO report, India today heads the world with over 32 million diabetic patients [1]. In September 2012, the world health organization reported a global prevalence of diabetes exceeding 300 million people, predicting a further 60-70% increase by the year 2030, which means India alone will have 100 million people by year 2030 [2]. As a result, a parallel increase in the incidence of diabetic lower extremity ulcer is expected. At least 15% people with diabetes eventually develop a lower extremity ulcer of some sort [3].

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Numerous risk factors for the development of foot ulcers have been suggested, the most important being peripheral sensory neuropathy followed by peripheral vascular disease. The proportion of neuropathic, neuroischemic and purely ischemic lesions in diabetes is 54%, 34%, and 10% respectively [4].

Over 100 physiologic factors contribute to wound healing deficiencies in individuals with diabetes. These include decreased or impaired growth factor production, angiogenesis response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte and fibroblast migration and proliferation, number of epidermal nerves, bone healing and balance between the accumulation of ECM components and there remodeling by the MMP's [5]. The main reason is related to loss of balance between metalloproteinase (MMPs) and

MMP inhibitor [6]. At the present diabetic foot ulcers were treated with some physical therapies such as vacuum assisted closure [7, 8, 9], high voltage pulsed current electrical stimulation [10], hyperbaric oxygen therapy (HBOT) [11], negative pressure wound therapy (NPWT) [12]. Some biological therapies were also evaluated in diabetic foot ulcer treatment. Some growth factors such as Epidermal growth factor [13, 14], Granulocyte colony stimulating factor [15], Nerve growth factor [16], Vascular endothelial growth factor [17], activated platelet rich plasma [6] were evaluated in diabetic foot ulcers.

Platelet derived growth factor (PDGF) is a dimeric protein, composed of 2 disulfide – linked polypeptide chain. It exists in 3 different isomers, the heterodimer PDGF –ab consisting of an a and b chain, and 2 homodimers, consisting of 2a or 2b chains (pdgf –aa and pdgf –bb) it has been shown in preclinical and clinical studies to promote the formation of granulation tissue at the wound site and to stimulate wound healing [18]. Microscopic examination of the wounds treated with topical PDGF showed a marked increased intensity of the inflammation phase of the wound healing cascade characterized by an increased presence of neutrophils, monocytes and fibroblasts grossly. It is hypothesized that PDGF positively promotes angiogenesis indirectly through its activities on other inflammatory cells [19].

Encouraging results have shown, that PDGF is better than good wound care alone [20, 21, 22]. In patients receiving PDGF, significant cases achieved complete healing compared to good wound care group. The average time for healing was shorter with greater reduction in the ulcer size [23]. Clinical trials conducted in western countries have demonstrated the safety and efficacy of PDGF in the management of diabetic ulcer but only few trials are conducted in India hence the need for this study in our setup.

MATERIAL AND METHODS: This study was a prospective, parallel group, double blind, and placebo controlled study. Total number of 32 patients with 38 chronic diabetic lower extremity ulcers were included in the study group. An informed consent was taken from all the patients before starting a treatment.

Out of these 32 patients with 38 ulcers, 3 patients with 3 ulcers were lost to follow up. Out of which one patient opted for surgical treatment and did not follow up. We were left with 29 patients with 35 ulcers.

Inclusion criteria were patients with diabetes, wagner's stage I, II, III, target ulcer > 4 weeks duration. Exclusion criteria were radiological evidence of underlying osteomyelitis, ulcers resulting from any other cause (e.g. electrical, chemical, radiation etc.), any concomitant disease (e.g. connective tissue disease), any medication affecting healing (e.g. steroid), pregnant women, ankle brachial index <0.4, poor nutritional status (<6.5 gm% total proteins and albumin < 3.5 gm %). Ulcers were defined as break in continuity of epithelium of skin. The lower extremity neuropathic ulcers were randomized. If the patient had one ulcer it was randomized either for treatment group or for control group. If the patient had two ulcers one was randomized for treatment to treatment group and the other for control group. Before randomization the target ulcer was debrided. Eligibility for randomization was: full medical history, complete examination, radiographs and Doppler of lower extremity with other relevant investigations. Once eligibility was confirmed, particulars of target ulcers like surface area were measured. The ulcer was classified according to Wagner's grading. Thereafter these ulcers were randomized to

1. Ulcers treated with placebo gel
2. Ulcers treated with PDGF gel

Both the placebo gel and PDGF gel were provided by the same manufacturers and had similar packing. The placebo gel had identical vehicle component of the gel formulation containing active drug .Randomization was done by the same person every time not included in the study. Two separate chits with numbers namely 1 and 2 were put in two different and similar opaque envelopes and different envelopes of the same kind were used every time. Same person not included in the study opened the envelop every time and number which was inside envelop was given to the ulcer, either 1 or 2, where 1 represented ulcers treated with placebo gel, and 2 represented ulcer treated with PDGF Gel. The number thus obtained was then

written on the sticker and the sticker was then applied on placebo gel tube or PDGF gel tube accordingly, by the dresser. The study medication was administered in conjunction with standardized good wound care for 24 weeks or until target ulcer healed.

The wounds were covered with approx1.5mm layer of PDGF gel and with moist saline dressing. Adequate control of infection was done by giving oral or injectable antibiotics and debridement done where required. The intended period of treatment was 24 weeks/complete healing whichever was earlier.

At each follow up visit at an interval of 1 week for 8 weeks and then every 2 weeks till 12 weeks and after that every 4 weeks for 24 weeks, area of target ulcer was assessed clinically for granulation, percentage decrease in size and culture sensitivity. Primary efficacy criteria for wounds that closed or healed 100% was scored 1 and less than 100% was scored 2. Additional secondary criteria included time to achieve complete wound closure, percentage reduction in ulcer area and total wound evaluation. An efficacy evaluation at treatment period of 10 weeks and 24 weeks was also made.

Statistical Analysis: Association between drug used and wound healing was calculated using the Chi Square test. Comparison of all the other discrete variables was done using Chi Square test. Statistical significance was determined by a p value < 0.05.

RESULTS: Placebo gel was applied on 17 (48.6%) ulcers and Pdgf was applied on 18 (51.4%) ulcers (**Fig. 1**).

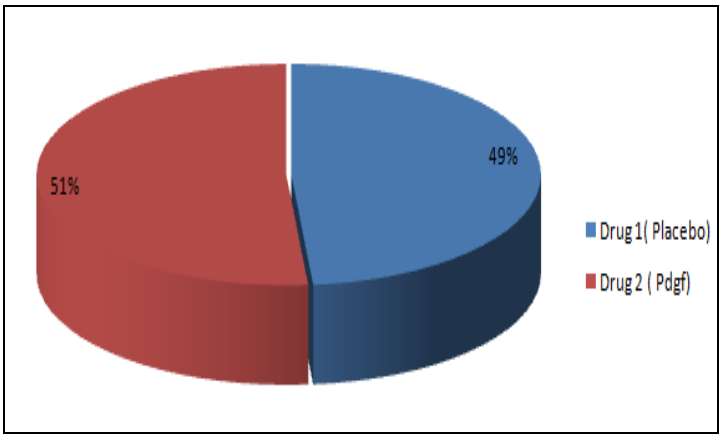

FIG. 1: DISTRIBUTION OF ULCER ACCORDING TO DRUG USED

In this study Mean age was 56.09. There was no preponderance of ulcer for the limb to be involved. Male to female ratio was 1.6:1. (41.4%). There was no correlation between gender and results ($p>0.05$). There were 96.5 % patients with 34 ulcers who had type 2 Diabetes Mellitus. Most of the ulcers in patients had duration of diabetes less than 10 years. In our study duration of diabetes mellitus had no significant role in time to achieve wound closure ($p>0.05$).Regular treatment for Diabetes Mellitus was taken by 89.7% patients. We did not come across any study which shows any correlation between regularity of treatment of diabetes mellitus and development of ulcers. In our study there was no correlation between regularity of treatment and time to achieve wound closure ($p>0.05$).

Normal ankle brachial index was found in most of the patients. Peripheral pulsation was palpable in all the patients. Most of the ulcers (74.2%) were neuropathic in this study. There was no relation of type of ulcer with the results ($p>0.05$).

Infection was found to be more in group receiving drug 2 (pdgf). There was no significant correlation between Infection and time to achieve complete wound healing ($p>0.05$).

Most of the ulcers which were in the study were of 4-24 weeks of duration with mean of 15.4 $\pm$15.5 weeks. Mean of time to achieve wound healing was 9 $\pm$ 7.1 weeks. Ulcer receiving PDGF gel healed 50% faster as compared to ulcers receiving placebo (**Table 1**).

TABLE 1: TIME TO ACHIEVE WOUND HEALING

Time to achieve wound Healing (weeks)	Drug I		Drug II		Total
	No of ulcer	%age	No of ulcer	%age	
1-5	5	29.4	10	55.6	15
6-10	3	17.7	6	33.3	9
11-24	5	29.4	2	11.1	7
Did not heal	4	23.5	0	0	4
Total	17	100	18	100	35

P=0.032

Score of 1 was achieved by 100% ulcers in the group receiving drug 2 (pdgf) at the end of ten weeks whereas, in group receiving drug 1 (placebo) 64.7% of ulcers achieved score of 1. At the end of 24 weeks 100% ulcers in group receiving drug 2 (pdgf) achieved score of 1 and in group receiving drug 1 (placebo) 76.4% ulcers achieved score of 1. Percentage healing in ulcers was 50% better in group receiving drug 2 (pdgf).

There was no relation of age, gender, type of diabetes mellitus, duration of diabetes mellitus, regular/irregular treatment of diabetes mellitus, body mass index, ankle brachial index, neurological deficit, type of ulcer, foot deformity, Wagner grade, edema, granulation, infection, duration of ulcer, area of ulcer, absolute lymphocyte count, glycemic control, total protein and albumin with time to achieve wound closure. This means that PDGF was solely responsible for the ulcers to heal 50% faster.

Majority of ulcers had area <25cm^2 with the mean of 31.43 $\pm$61.4 cm^2. Sum of area of all the ulcers which received drug 1 (placebo) was 523 cm^2, and sum of area of all the ulcers which received drug 2 (pdgf) was 577 cm^2 (**Fig. 2**).

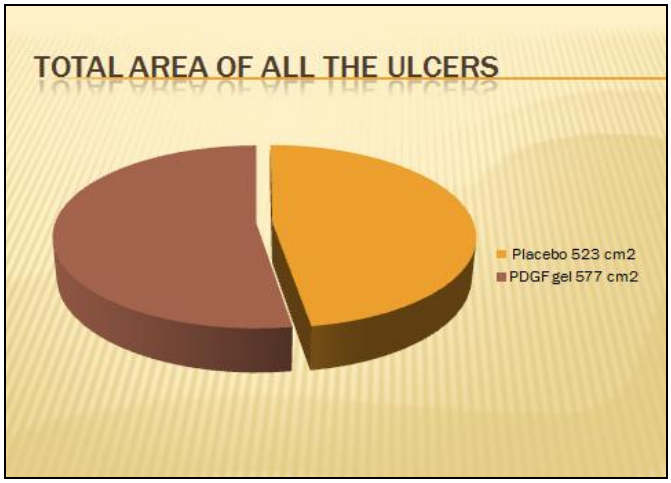

FIG.2: DISTRIBUTION OF TOTAL AREA OF ALL THE ULCERS ACCORDING TO DRUG USED

DISCUSSION: Mean age which was in 5th decade in our study was almost similar to most of the studies. Majority of patients were in the 6th decade according to Margolis et al (2001) [24]. The lower age of patients presenting with diabetic lower extremity ulcer can be attributed to lifestyle changes due to urbanization which increases the risk of developing diabetic lower extremity ulcers. Male to female ratio was 1.6:1. (41.4%). this finding was similar to the study done by Hardikar et al (2005) [25]. Higher incidence of males over females could be due to the fact that females have a largely indoor existence in our society. Most of the ulcers in patients had duration of diabetes less than 10 years. This was similar to the study done by Ogbera et al (2005) [31] which had average duration of diabetes 8.4 years $\pm$ 5.4 in patients developing diabetic ulcers. A study done by Boyko et al (1999) [26], also known as the Seattle diabetic foot study showed average duration of diabetes in patients developing diabetic ulcers to be 12.9 years $\pm$ 9.6.This could be attributed to the fact that neuropathy and foot deformity develop after many years of diabetes mellitus which is the leading cause of developing ulcers in diabetics.

Most of the ulcers (74.2%) were neuropathic in this study. Our findings are similar to those of Mam et al (1998) [27], who also found the presence of non neuro-ischaemic cases among diabetic individuals. The probable cause for the presence of such cases might be the subclinical vascular compromise or subclinical neuropathic involvement which could not be picked on the basis of clinical parameters employed in our study.

Infection was found to be more in group receiving drug 2 (pdgf). Our findings were almost similar to the study done by wieman et al (1998) [21] which found that group receiving placebo had infection in 30% ulcers whereas 23% of ulcers were infected in group receiving PDGF. Inspite of more infection in

group receiving PDGF all the ulcers healed whereas there was less number of infected ulcers in group receiving placebo but still there were 4 ulcers which did not heal in this group. Percentage healing in ulcers was 50% better in group receiving drug 2 (pdgf). Our findings were similar to Wieman et al (1998) [21], Margolis et al (1999) [28], Embill et al (2000) [29], Nagai et al (2002) [30], Lone et al (2014) [7] and Hardikar et al (2005) [25]. Time to achieve wound Closure, percentage reduction of size was statistically significant (p>0.05) (**Fig.3**).

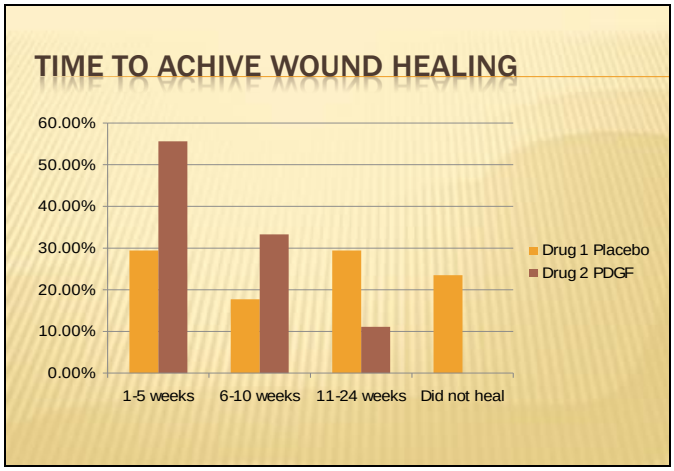

FIG. 3: CORRELATION OF TIME TO ACHIEVE WOUND HEALING WITH TYPE OF DRUG USED AND PERCENTAGE OF ULCER HEALED

Our study had larger mean of area of ulcer. We have included even the larger ulcers in this study which were excluded in the previous studies. In our study we took larger ulcers but it did not seem to have any significant relation on results (p>0.05). Even the larger ulcers healed much earlier in the study period when applied PDGF. We did not came across any study which has included area of ulcer (**Fig.4**).

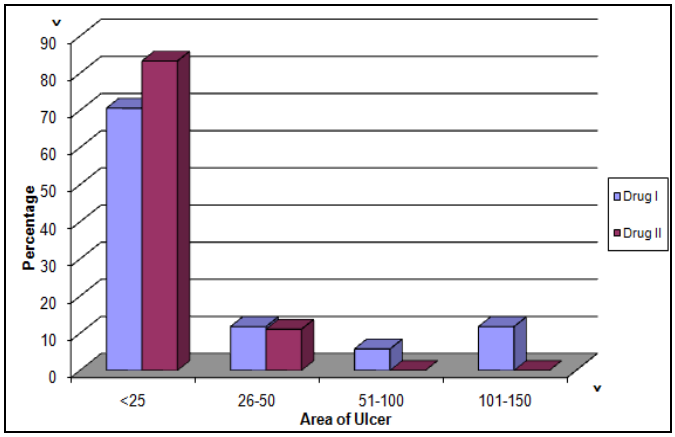

FIG. 4: DISTRIBUTION OF AREA OF ULCER HEALED AND DRUG USED

The aim of our study was to study the efficacy of platelet derived growth factor in diabetic lower extremity ulcer, and we found platelet derived growth factor to be effective in healing of these ulcers. Although in our study we found that infection in the wounds did not play a part in healing of ulcer when platelet derived growth factor was applied but still we recommend that wound infection to be taken care of first in all the wounds through proper repeated debridement and use of antibiotics before use of platelet derived growth factor.

Limitation of study: Inspite of having many diabetic patients with ulcers presenting in our opd the sample size appears to be small as compared to generalized population due to exclusion criteria, in order to get almost identical types of ulcers in which no other factor play a role in healing. However healing of ulcers receiving PDGF was significantly faster as compared to ulcers receiving placebo.

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