Clinical Efficacy and cost effectiveness of Cerdak dressing over Saline gauze dressing among diabetic foot ulcers

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INTRODUCTION:

Ulceration of diabetic foot ulcers is common, and widely acknowledged to be a source of major distress and morbidity in a predominantly elderly population, as well as an enormous drain on health-care resources [1-3]. Not only diabetes makes the foot more liable to ulceration, but it also impairs the process of healing, and diabetic foot ulcers readily develop into chronic wounds. While the pathophysiology of chronic wounds remains poorly understood, there is no logical framework to underpin many strategies of care.

A number of factors have been implicated in the predisposition to non healing, slowly resolving wounds observed in this order, such as peripheral neuropathy; altered red blood cell physiology, at least in part because of glycation of cell membrane components; glycosylation of hemoglobin and subsequent altered tissue delivery of oxygen; impaired innate post response and immune mechanisms and peripheral micro vascular disease. Irrespective of the specific of the etiological factor it is established that effective reparative responses are impaired in diabetes leading to the development of chronic non healing wounds [4-6].

Detailed understanding of the wound healing in diabetes is very essential to develop new therapies for the healing process. Normal wound healing is characterized by an orderly series of cell and tissue responses that can be grouped into four major phases. The initial injury phase stimulated platelet aggregation and clot formation to attain haemostasis. The resident platelets then release cytokines and growth factors to attract the various cell types which are utilized in the second phase. In this inflammatory phase of healing, neutrophil recruited from the circulation released chemo tactic substances followed by macrophage arrival to suppress bacterial infection and remove dead tissue. The third phase, characterized by proliferation the wound bed is gradually replaced by granulation tissue which is produced mainly by fibroblast laying down extracellular matrix (ECM) assisted by the endothelial cells promoting angiogenesis. During the four and final stage of wound healing the granulation is further remodeled to increase wound tensile strength [7]. In chronic ulcers that occur in diabetes a persistent inflammatory phase is witnessed associated with delay in granulation tissue formation. Continued

bacterial infection and increased formation of advanced glycation end products (AGE's) may play an important role [8].

The paucity of the evidence base for the treatment of diabetic foot ulcers has been highlighted in several recent reviews [9-11]. The effectiveness of some of the more recently introduced therapeutic agents (including growth factor preparations and bioengineered human skin products) has been suggested in some (but not all) industry funded trials and yet remains to be confirmed.

Cerdak is a wound care product that consists of a non-woven fabric sachet filled with micro-porous ceramic granules [12].Cerdak not only covers and protects the wound from infection, in additions enhances the trapping and removal of the excess exudates into the granules thus creating a micro-moist environment, making a suitable condition for wound healing [13]. One of the salient features of Cerdak is its ability to heal with no or minimal scar. Cerdak's antibacterial property is mechanical in nature and it 'sucks' the microbes from the wound. It demonstrates a high water and endotoxin binding capacity. It also protects DNA damage by reactive oxygen species. It is also demonstrated by some studies that detoxification property of Cerdak could contribute to its high healing ability.

Though Cerdak has proved its efficacy in healing different wounds, its efficacy and cost effectiveness is yet to be proved in healing of diabetic foot ulcer especially in comparison with standard saline dressing which is used extensively in developing countries like India. Hence the aim of this study was therefore, to compare the efficacy and cost effectiveness of modern dressing material with promising therapeutics like Cerdak to the standard therapy like Saline dressing in healing of diabetic foot ulcer.

RESEARCH DESIGN AND METHODS:

A total of 60 (M: F; 32:28) subjects having type 2 diabetes with diabetic foot ulcer of 2-5cm2 size and attending podiatry clinic for the treatment of foot ulcers were recruited. Study protocol and procedures were approved by the Institutional Ethics Committee and all the study participants gave written informed consent prior to participating in the study. All the subjects were then randomly divided into 2 groups. The study groups were Normal Saline (control) and Cerdak (Microporous ceramic dressing). Swab culture was taken from the wound on day 1 and after 3 weeks. Photographs were taken at every visit. The wound size was evaluated on an OHP sheet. The wounds were graded according to Texas Classification. Tissue samples and blood samples were collected before the application of dressing, after 1 week and at the 3rd week of the enrollment and were stored immediately at -20^0C. Blood samples were centrifuged to obtain serum and plasma before freezing at -20^0C. Tissue samples were later used for

H&E Staining of the sections (5µ), Van Gieson Staining for Collagen, MMP2 and MMP 9 by Zymography. Serum samples were used for the estimation of hsCRP and IL-6 by ELISA procedure. Cost incurred by all the study subjects for healing of their diabetic ulcer and their basic medical details were captured from the medical records. Patients in both the groups were followed till the complete healing of the wound occurred and the healing time in days were recorded accordingly.

HAEMATOXYLIN AND EOSIN STAINING

The wound tissue samples were washed thoroughly with double distilled water to remove the adhering blood clots and fixed in 10% formalin. Formalin fixed samples were embedded in paraffin and sections of 5µ thickness were prepared for histological examination. The sections were deparaffinised in xylene, hydrated in descending alcohol series, accumulated in water and stained in aqueous Delta field's haematoxylin for 5 minutes. The stained sections were washed in running tap water, then dehydrated and counter stained in 0.1 percent eosin in 95% alcohol for sixty seconds. After further dehydration and cleaning, the sections were mounted in a synthetic resin medium and viewed under the light microscope.

VAN GIESON'S STAINING

The deparaffinised and hydrated sections were brought down to water. Weigert's iron haematoxylin was used to stain the nuclei and washed thoroughly in distilled water, then stained in Van Gieson's solution for two to five minutes. Rinsed in distilled water and placed in 95% alcohol. Finally, it was dehydrated in absolute alcohol, cleared in xylene, mounted in a synthetic resin medium and viewed under the light microscope.

MICROBIAL ANALYSIS

Culture specimens were obtained from the ulcer base after debriding the ulcer with sterile instruments and collected in transport medium for aerobic isolation. The samples were cultured and bacteria were identified by standard techniques. Gram-negative bacilli were identified by preliminary biochemical identifications by the use of mannital motility, triple sugar iron agar, blood agar, MacConkey agar, indole, Simmons's citrate and urease test. Among the Gram-positive cocci, *Staphylococcus* is differentiated from *Streptococcus* by performing the catalase test. *Staphylococcus* is catalase positive and *Streptococcus* is catalase negative. The pathogenic species of *staphylococcus* is differentiated from the non-pathogenic *staphylococci* by the coagulase test. *Staphylococcus aureus* is coagulase positive *Staphylococci* and *Staphyloccous* epidermis is coagulase negative *Staphylococci*.

PREPARATION OF TISSUE EXTRACT

A 5 mm punch biopsy of tissue samples from the diabetic foot ulcer of study subjects were collected in cold 0.9% saline, washed immediately to remove all adhering blood, placed immediately on dry ice and transferred to a freezer and stored at -80^{0}C. Samples were further processed by mincing the pieces with a scalpel and extracting at 4°C with 500 µl of homogenizing buffer that contained 50 mM Tris (pH .4), 150 mM NaCl, 1% Triton X-100 using a glass homogeniser. The homogenates were transferred to 1.5 ml eppendorf tubes, centrifuged at 13,000 Xg for 10 minutes and the supernatant aliquoted and stored at -80°C for further analysis. Aliquots from each biopsy extract were analyzed individually for MMP and serine protease activities by Gelatin Zymography using SDS-PAGE.

ESTIMATION OF PROTEIN

Total protein content of all the assay samples (plasma and haemolysate were quantified by the method of Bradford.

Principle

Coomassie brilliant blue, a dye, exists in 2 different colour forms, red and blue. The red form is converted into blue form upon binding of the dye to protein. The protein-dye complex has a high extinction coefficient thus leading to a great sensitivity in measurement of the protein. The binding of dye to protein is a very rapid process and the protein-dye complex remains dispensed in the solution for relatively long time. The blue colour is measured at 595 nm spectrophotometrically.

Reagents

1. Bradford's reagent

50 mg of Coomassie brilliant blue (G-250) in 25 ml of 95% ethanol and 50 ml of ortho phosphoric acid was dissolved and made up to 500 ml with double distilled water. It was then filtered with Whatman filter paper and stored at 4°C.

2. Standard bovine serum albumin (BSA)

100 mg of crystalline BSA was dissolved in 100 ml of double distilled water to give a standard solution containing 1 mg/ml. Standard solutions were suitably diluted to have 5-50 µg concentration.

Procedure

Samples and standards of varying concentration was treated with Bradford's reagent and incubated in a water bath at 37°C for 15 min. The blue colour developed

was read at 595 nm spectrophotometrically. The values were expressed as mg/ml.

GELATIN ZYMOGRAPHY

Principle

With zymography, proteolytic species are separated on the basis of molecular size by electrophoresis through a sodium dodecyl sulphate – polyacrylaramide gel within which a substrate for the enzyme of interest (gelatin) is copolymerised. The position and activity of each enzyme in the gel is visualized by its ability to degrade the substrate, as indicated by areas of clearing after staining of gels with a protein stain, Commassie blue. Since in this method enzymes that have the same substrate specificity are separated on the basis of molecular weight, it was of importance to study the MMP (gelatinases; MMP-2 and MMP-9). First, it allowed the MMP-2 (72 KD) gelatinase to be distinguished from the MMP-9 (92 KD) species. Second, the zymography process itself (a denaturation of the enzyme for the electrophoresis and a renaturation before incubation for activity determination) activated the various proenzyme forms of the enzymes and could be detected.

Procedure

Proteins from tissue extracts and serum (20 or 40 μg) were separated by SDS-PAGE under the non reducing condition on 8 or 10% polyacrylamide gels as specified, containing 1 mg/ml gelatin without prior heating or reduction at 4°C. The gels were then incubated in renaturing buffer [2.5% (v/v) Triton x-100] to extract SDS for 1 hour in an hybridization chamber, which were then incubated overnight to allow the enzymes to degrade gelatin at 37^0C in enzyme buffer (50 mM Tris-HCl pH 7.8, containing 150 mM NaCl and 10 mM $CaCl^2$) with gentle agitation. The gels were stained with Coomassie Brilliant Blue R-250 and destained in 30% methanol and 10% acetic acid. Zones of enzymatic activity appeared as clear bands against a blue background and the gels were photographed.

For confirmation that the activity was caused by metalloproteinases, identical gels were incubated in the presence of 10 mM Methylene diamine tetraacetic acid (EDTA) for EDTA inhibition of MMP's.

HIGH SENSITIVITY C REATIVE PROTEIN [HS CRP] & INTERLEUKIN -6 [IL-6] ESTIMATIONS

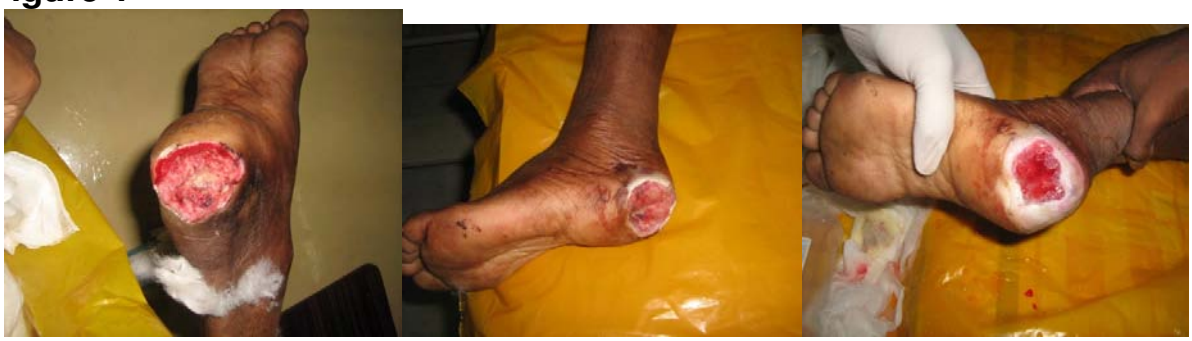
Blood was collected in clot activator vacutainer tubes for the measurement of IL-6 and hsCRP. Serum samples were obtained following centrifugation at 2000 rpm for 10 min, and stored at -20^0C until analysis. Estimation for hsCRP and IL-6 were performed using solid phase enzyme linked immunosorbent assay (Quantikine hsCRP ELISA & IL-6 ELISA; R&D systems, Inc Minneapolis, USA) in duplicate using a commercially

available kit. The coefficient of mean variations in the samples were <5%. The minimum detectable levels with the kits were less than 5pg/ml. No significant cross reactivity or interference were observed with these assay kit. Intra- and inter-assay coefficients of variation were <5% for both high sensitivity CRP and Interleukin -6 and all samples were analysed on a single run. Absorbances were read at 540nm and correction absorbances were read at 450nm.

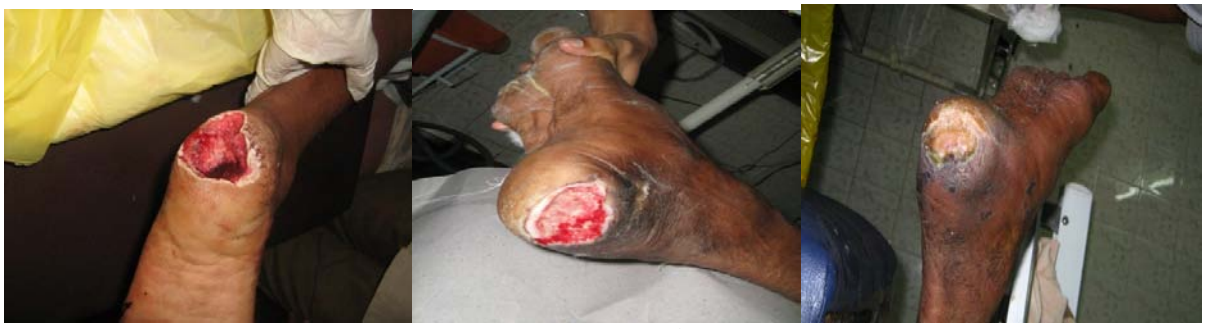
RESULTS:

Out of total 60 type 2 diabetic subjects enrolled in the study, 26 subjects were of 2B grade and 16 were of 3B grade of Texas classification. While 40% of Normal Saline group, 50% ofCerdak group were of 2B grade and others were of 3B grade of Texas classification [Figure1].

Figure 1



Wounds treated with Normal Saline



Wounds treated byCerdak dressings

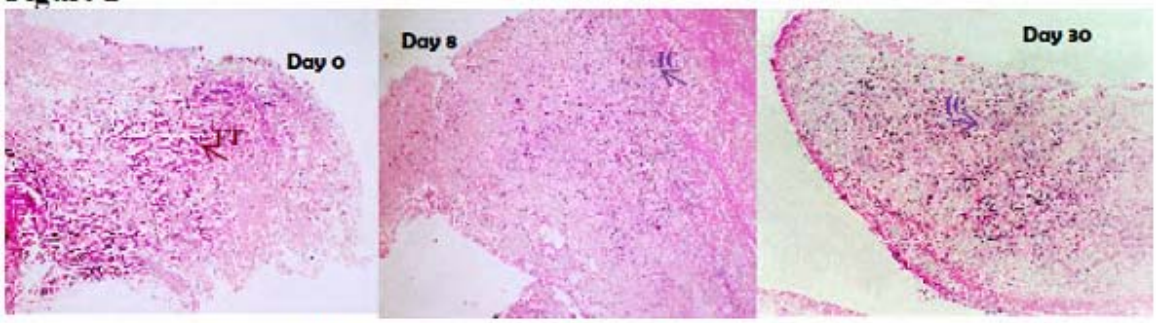
As per Haematoxylin and Eosin (H&E) stains of patients tissue samples of different study groups, normal saline group showed moderate fibrosis on pre application day (day 0) leading to abundant fibrous exudates with less granulation and more inflammation in majority of cases by 1 week of dressing application (day 8). There was formation of active granulation tissue only by one month of dressing application (day 30). Van Gieson (VG) sections on pre application (day 0) of Normal saline group showed grade 0-1 collagen. It took 1 month of dressing application (day 30) to reach

grade 1-2.

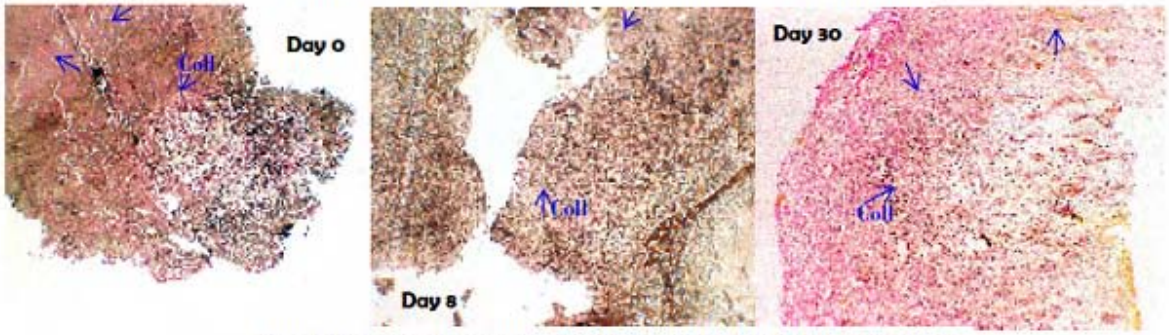
In Cerdak group, H&E sections of pre application (day 0) showed granulation tissue and less inflammation. Some subjects in this group showed gangrenous changes and prominent scaring on pre application day. While on 1 week of dressing application (day 8) showed prominent inflammation. On one month of dressing application (day 30), there was ulcer with active granulation. VG sections of Cerdak group showed gradual increase in collagen formation which was minimal on pre application day (day 0) to grade 1 on 1 week of dressing application (day 8) leading to grade 3 collagen formation after 1 month of dressing application (day 30).

Findings of our study showed Cerdak removes excess exudates from the wound and creates a micro-moist environment, making a suitable condition for wound healing. Cerdak also had better collagen formation with greater reduction in inflammatory cells during healing days resulting in decreased days of healing. Whereas, Normal Saline had minimal collagen formation, high grade of inflammation during the healing days with maximum exudates formation resulting in increased days of healing [Figure 2].

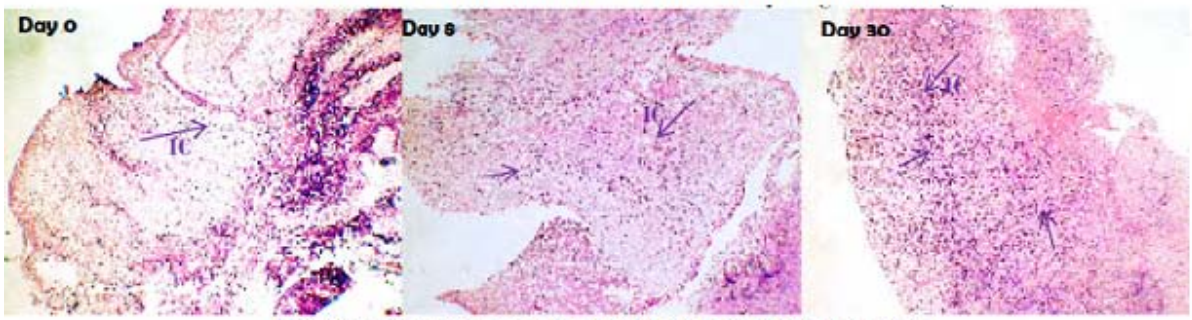
Figure 2



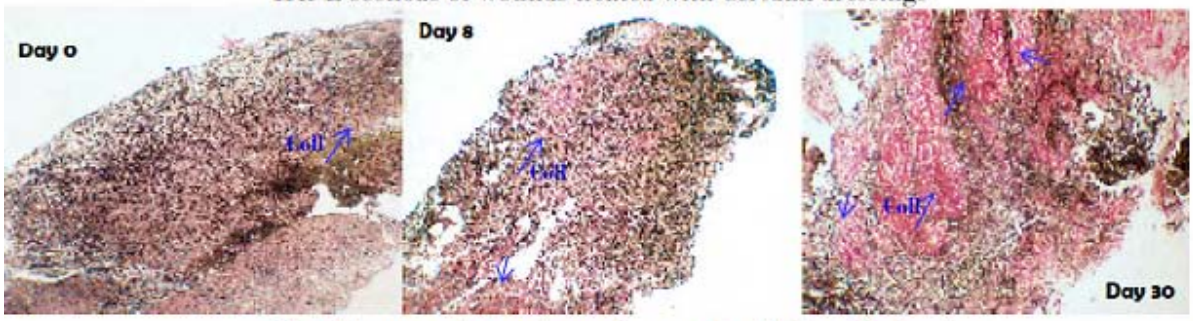
H& E sections of wounds treated with Saline dressings



Van Gieson sections of wounds treated with Saline dressings



H& E sections of wounds treated with Ceredak dressings



Van Gieson sections of wounds treated with Ceredak dressings

FT: Fibrous tissue; IC: Inflammatory cells, GT: Granulation tissue; Coll: Collagen

There was no difference in age and duration of diabetes of study subjects of both the groups. Other biochemical parameters like HbA1c, ESR, total count, Urea and serum creatinine were also similar in both the study groups. Total cholesterol was significantly lower in normal saline compared to Cerdak groups [mean ± SD; Normal saline Vs Cerdak; 113.2 ± 51.6 Vs 162.2 ± 53.1; p<0.0001]. However triglycerides levels were non significantly higher and HDL-cholesterol levels were significantly lower in normal saline group compared to Cerdak groups [26.4 ± 15.4 Vs 35.1 ± 13.9; p = 0.027]. There was no statistical difference in serum albumin and liver functions tests of both the groups. Positive family history for diabetes was higher among Cerdak group when compared with normal saline groups. Among other diabetic complications, almost all the subjects suffered with peripheral neuropathy in both the groups, while hypertension was found to be the second common complication in all the study subjects. There was no statistical difference in complications among both the groups. [Table 1]

Maximum number of cases had ulcer at their forefoot in both the study groups [Normal saline Vs Cerdak; 66.7% Vs 70%], Mid foot infection was slightly more among normal saline group [13.3% Vs 10%], while hind foot infection was similar in both the study groups [13.3% Vs 13.3%]. Results of culture and sensitivity test showed there was no difference in infection rate and infection type between both the groups. Normal saline group had 30% cases with no growth, 26.7% cases with gram positive infection and 40% cases with gram negative infection and just 3.3% cases with both gram positive and gram negative infection. While Cerdak group had 40% cases having no growth, 20% cases with gram positive growth, 33.3% cases with gram negative infection and 6.7 % cases with both gram positive and negative infection. [Table 2]

There was significant contraction seen in the size of the ulcer in both the study groups depicting the healing process. However Cerdak group depicted better contraction rate. There was no statistical difference in initial length and breadth of the wound size among both the groups. However, final length and breadth decreased significantly in both the groups, retaining Cerdak group with statistically significant reduction compared to normal saline group. [Mean ± SD; Final length: Normal Saline Vs Cerdak; 5.5 ± 4.4 Vs 2.8 ± 2.5; p = 0.013, Final Breadth: 3.4 ± 2.03 Vs 2.1 ± 1.9; p = 0.024]. [Table 2]

Among normal saline group only 13.3% cases were healed while 80% cases underwent SSG. There were 6.7% cases that required flap over closure of the wound. Among Cerdak group 46.7% cases healed and 10% went for suturing, while 36.7% and 6.7% cases went for SSG and flap over process for wound healing respectively. There were no cases that underwent amputation in both the groups. Statistical significance was observed in healed cases among both the groups with Cerdak group having more number of cases that healed, similarly normal saline showed with statistical significance increase in number of cases that underwent SSG. [Table 3]

Saline dressings were either done once or twice daily, wherein Cerdak dressing was done once in 2 or 3 days. Table 7.3 also depicts the dressing interval of both the study groups. Subjects treated with normal saline required more number of dressings with more frequency compared to Cerdak group. Number of days of hospitalization was also less in Cerdak group. On the contrary, median healing days were less in Cerdak group compared to normal saline group. [Table 3]

Baseline hsCRP and IL-6 levels were similar in both the study groups. There was tremendous reduction in hsCRP levels seen in Cerdak group compared to normal saline groups from baseline to 30th day of treatment [mean ± SD; normal saline Vs Cerdak; 19.3±6.4 Vs 9.3±4.9; $p<0.05$]. Similarly IL-6 levels also reduced maximum and significantly in Cerdak group compared to normal saline groups [mean ± SD; 29.1±5.6 Vs 13.9±8.7; $p<0.05$]. [Figure 3a & b]

Figure 3a: Mean hs CRP levels among study groups at different time points.

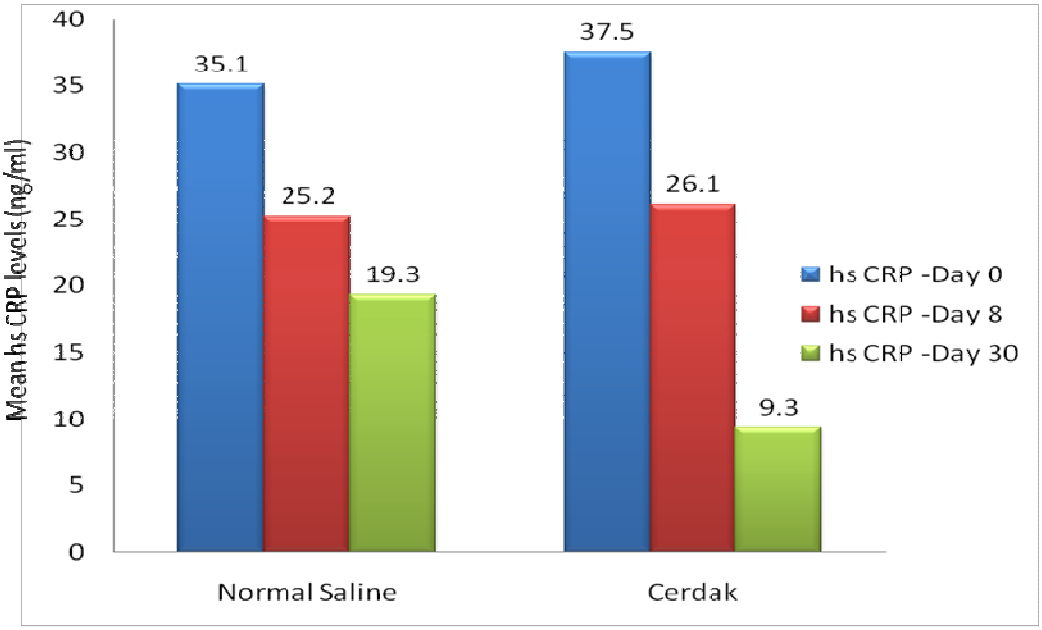


Figure 3b: Mean IL-6 levels among study groups at different time points.

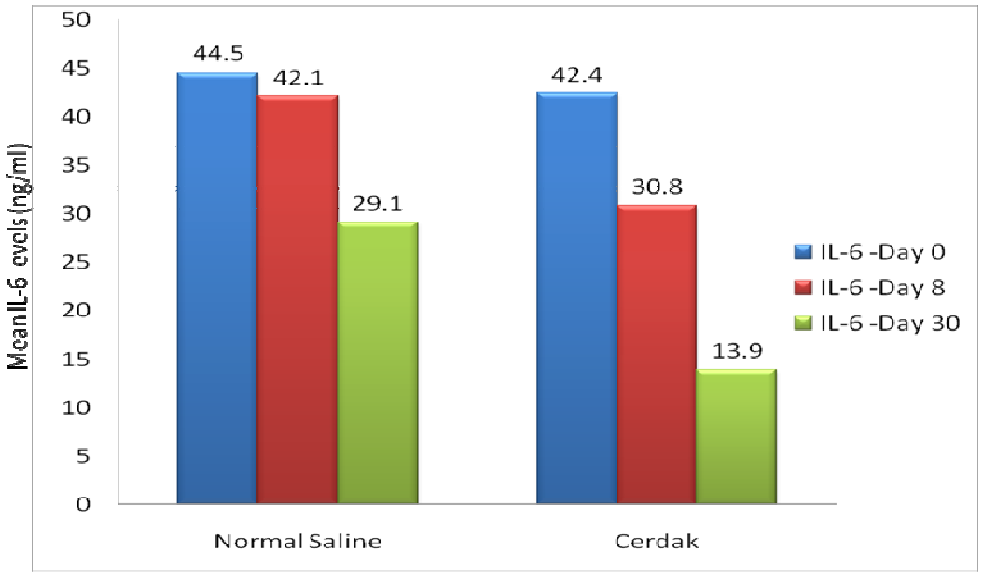
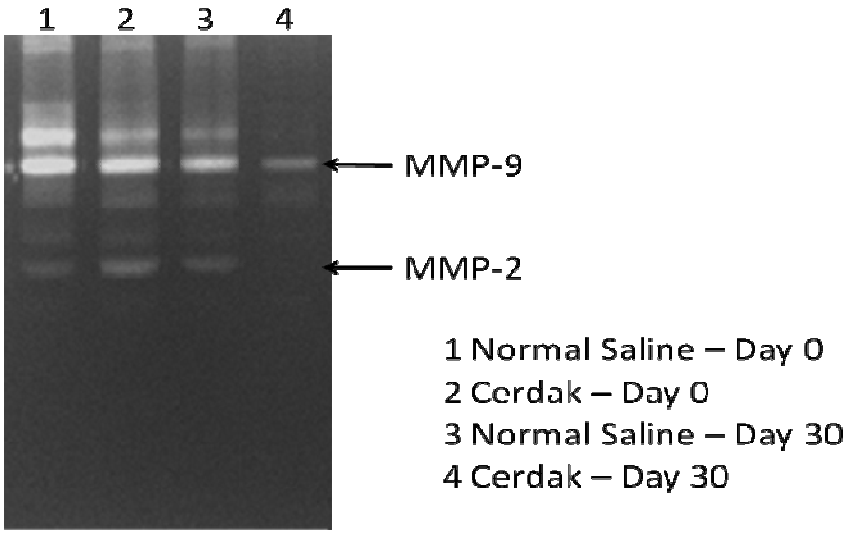


Table 4 shows the direct and indirect cost (in Rupees) incurred in treating diabetic foot ulcer using normal saline and Cerdak dressing materials. Total median direct cost incurred in treating diabetic foot ulcer by normal saline was 78134 (36057-115029) and by Cerdak was 66463 (35555-98270) and showed statistical difference of p = 0.003.Cerdak group had lower median cost incurred in treating diabetic foot ulcer. Total indirect median cost incurred was 2235 (700-16300) when treated with saline dressing and lower when treated with Cerdak dressing was 1980 (500-13000), however there was no statistical difference seen between the two groups for indirect cost. Though the cost of Cerdak dressing material was more compared to normal saline, but cost incurred for other dressing materials, hospitalization, medicine and accommodation was found to be significantly higher among cases using normal saline.

MMP-2 and MMP-9 were higher in both the study groups on the pre application day (day 0). However, Normal saline group had more intensity of MMP-9 on pre application day compared to Cerdak study groups. There was reduction in the intensity of MMP-9 expression observed in Cerdak compared to normal saline. MMP-2 expressions were not that intensive in both the study groups on pre application day (day 0). MMP-2 expression was not prominent on pre application day (day 0) but existed with 1 month of dressing application. In Cerdak group, there was resolving of MMP-2 expression after one month of dressing application compared to pre application day [Figure 4].

Figure 4: SDS- PAGE gel picture showing MMP-9 and MMP-2 of the samples from both the study groups on pre application and after one month of application time point.



DISCUSSION:

Treatment methods for diabetic foot ulceration often entail burdensome regimes including pressure relief - often in the form of a below knee cast or custom footwear - daily dressing changes and frequent trips to various health professionals. Although these treatment regimes are essential for healing and prevention of further complications, they can last for many months and have a profound effect on activities of daily living and quality of life.

Ulceration usually occurs when one or more of the following are present: peripheral neuropathy (particularly loss of feeling/ protective sensation), foot deformity, and minor trauma [2, 14]. Less frequently, diabetic foot ulceration may present as a symptom of deeper infection such as cellulitis, an abscess, or osteomyelitis. Another less frequent precursor to ulceration is peripheral vascular disease, as impaired circulation contributes significantly to non-healing of ulcers and subsequent risk for amputation [15].

Timely resolution of diabetic foot ulceration is essential if further tissue loss and infection are to be avoided. Current guidelines recommend the use of pressure relieving devices, appropriate dressings to promote healing and prevent infection, and where appropriate, debridement, drainage and revascularization [16, 17]. In addition, optimization of glycaemic control and patient education are important factors in achieving successful ulcer healing [15].

Despite the need for speedy resolution in order to avoid greater complications, it is not uncommon for diabetic foot ulcers to reach a point where progress slows or even stops altogether. Commonly referred to as ’delayed’ or ’non-healing’ this phenomenon contributes significantly to the chronicity associated with diabetic foot ulcers, and can occur despite appropriate treatment and management of all contributing factors. There are several factors that have the potential to contribute to a delay in wound healing, including poor patient compliance with treatment regimes, poorly controlled glycaemic (blood sugar) levels and poor tissue oxygenation [16]. Also implicated in the delayed or non-healing of diabetic foot ulcers, is the impaired immune response to injury commonly seen in people with diabetes that frequently results in poor, or no progress, after initiation of the inflammatory phase of healing (the initial healing phase involving the arrival of blood cell types and removal of bacteria from the site). This ultimately affects formation of granulation tissue (new tissue containing all the cellular components for skin formation), which is a pre-requisite to epithelialisation or complete skin healing [17].

There are some literature that mentions the clinical relevance of wound bed preparation, with specific reference to moisture and bacterial balance. The whole wound healing cascade described is dependent on the availability of cellular elements and controlled inflammation.

The action of the ceramic devices can be described as a pulsed action in which moisture is locked away from the wound environment, intending the presence of stagnated factors and cellular elements produced by the body in exudates, which are permanently retained by the wound. The pulsed action ensured that fresh exudates, with fresh elements are present in the wound bed. The control of exudates also lead to control over microorganisms and all other foreign or dead objects that is not contributing to the healing process of the wound.

Apart from their role in Extra Cellular Matrix remodeling, MMPs have other important functions, including regulation of cell growth and differentiation. Specifically with regard to wound healing, they can alter cell motility; affect cell-cell interactions, and release growth factors and cytokines to affect cellular proliferation and growth [18-22]. These many functions of MMPs further reinforce the concept that they are key players in wound repair. Increased circulating and tissue glucose level can alter wound healing by a complex interplay of metabolic signals leading to the activation of the pathways that

increase inflammation [23]. In some cells, hyperglycemia activates the mitogen-activated protein kinase or protein kinase C pathways to stimulate cytokine production and promote inflammation. High glucose levels may also indirectly affect MMPs by formation AGEs which accumulate during prolonged hyperglycemia and which are also pro-inflammatory [24]. These pathways can collectively affect wound healing by enhancing inflammation and thereby affecting remodelling of ECM [25-28].

Several studies have shown in chronic wounds, including some diabetic wounds, elevated expression and activation of MMPs -2 &-9 [26-30]. Whether these effects are cell type specific and how they change as the diabetic wound heals has not been studied in detail. There is also a relative paucity of data regarding the pattern of expression of MMPs in human diabetic foot ulcers [26, 30-34]. In our study we not only determined the expressions of MMP 2 & 9 but also verified the comparison of different dressings reducing MMP levels. Findings of our study once again proved Cerdak dressing is more influential in reducing MMP 2 & 9 levels in diabetic foot ulcers which ultimately helps in faster wound healing and thereby reducing its associated complications. Other studies also prove Cerdak has disinfective property leading to faster and hygienic wound healing process.

Table 1: Biochemical details of study subjects from both the groups

Variables	Normal Saline	Cerdak	p value
N	30	30	--
M:F	12:18	20:10	--
Values are mean ± SD [Test of significance: paired T test]			
Age (years)	52.3 ± 9.9	54.6 ± 10.1	
Dur-DM(years)	10.9 ± 7.03	9.03 ± 6.6	0.303
HbA1c (%)	10.8 ± 2.0	10.3 ± 1.9	0.334
ESR mm/hr	98.3 ± 30.9	102.7± 32.5	0.619
Total Count	12526 ± 4920	12845 ± 4236	0.802
Urea (mg/dl)	33.3 ± 16.0	29.83 ± 14.5	0.255
Sr. Creat (mg/dl)	0.96 ± 0.37	0.95 ± 0.51	0.883
Total Chol (mg/dl)	113.2 ± 51.6	162.2 ± 53.1	<0.0001
Triglycerides (mg/dl)	124.3 ± 78.1	147.1 ± 74.3	0.259
HDL-Chol (mg/dl)	26.4 ± 15.4	35.1 ± 13.9	0.027
LDL-Chol (mg/dl)	90.9 ± 20.8	95.1 ± 38.5	0.593
SGOT	18.1 ± 7.8	19.8 ± 8.7	0.445
SGPT	18.9 ± 6.5	23.2 ± 16.8	0.219
Total Protein	6.62 ± 2.3	6.8 ± 2.39	0.706
Sr. Albumin	3.1 ± 1.1	3.3 ± 1.2	0.403
Sr. Globulin	3.7 ± 1.4	3.6 ± 1.3	0.835
Values are n (%) [Test of significance: □2 test]			
FH-DM (years)	21(70)	23 (77)	0.085; 0.770
Smoking	4 (13.3)	4 (13.3)	0.144; 0.704
Presence of other diabetic complications			
Peripheral neuropathy *Diab Nephropathy* *Diab Retinopathy* *CAD* *IHD* *Hypertension* *Dyslipidemia*	29 (97) 6 (20) 10 (33.3) 1 (3.33) 0 16 (53.3) 9 (30)	30 (100) 3 (10) 5 (16.6) 2 (6.7) 4 (13.3) 12 (40) 7 (23.3)	0.000; 1.000 0.523; 0.407 1.422; 0.233 0.000; 1.000 2.411; 0.121 0.603; 0.438 0.085; 0.770

Table 2: Wound details of the study groups

Variables	Normal Saline	Cerdak	$\chi 2$; p Value
N	**30**	**30**	
Values are n (%)			
Site of Ulcer			
Forefoot	20 (66.7)	21 (70)	0.0; 1.000
Midfoot	4 (13.3)	3 (10)	0.0; 1.000
Hindfoot	4 (13.3)	4 (13.3)	0.002; 0.962
Forefoot & Midfoot	2 (6.7)	1 (3.3)	0.0; 1.000
Midfoot & Hindfoot	0	1 (3.3)	0.0; 1.000
Culture & Sensitivity			
No growth	9 (30)	12 (40)	0.29; 0.588
Gram positive	8 (26.7)	6 (20)	0.093; 0.760
Gram Negative	12 (40)	10 (33.3)	0.072; 0.789
Both	1 (3.3)	2 (6.7)	0.0; 1.000
Values are mean ± SD			
Initial Length (cm)	7.7 ± 4.2	6.3 ± 1.9	0.131
Final Length (cm)	5.5 ± 4.4	2.8 ± 2.5	0.013
p value by paired 't' test	**0.004**	**<0.0001**	**--**
Initial Breadth (cm)	5.3 ± 1.6	4.8 ± 1.5	0.245
Final Breadth (cm)	3.4 ± 2.03	2.1 ± 1.9	0.024
p value by paired 't' test	**<0.0001**	**<0.0001**	**--**
Vales are n (%)			
Wound closure by			
Healing	4 (13.3)	14 (46.7)	6.4; 0.011
Suturing	0	3 (10)	1.4; 0.236
SSG	24 (80)	11 (36.7)	9.9; 0.002
Flap over	2 (6.7)	2 (6.7)	0.3; 0.605
Amputation	0	0	--

Table 3: Wound dressing details of the study group

Variables	Normal Saline	Cerdak	$\chi 2$; p value
N	**30**	**30**	**--**
Values are n (%)			
Dressing Interval			
Daily	7 (23.3)	9 (30)	0.085; 0.770
Twice daily	23 (76.7)	-	31.4; <0.0001
Once in 2 days	-	19 (63.3)	24.9; <0.0001
Once in 3 days	-	2 (6.7)	0.517; 0.472
Values are median (Range)			
Dressing Interval (days)	2 (1-2)	3 (3-4)	<0.0001
No.of days dressing material used	23.5 (5-68)	21 (4-61)	0.002
No. of days of hospitalization	2 (1-3)	1 (1-2)	0.262
Healing Days	28.o (11-98)	23.5 (8-92)	0.080

Table 4: Cost incurred in treating diabetic foot ulcer

Variables	Normal Saline	Cerdak	Median Test p value
N	**30**	**30**	**--**
Cost of dressing material (Rs)	18 (18)	165 (18-355)	<0.0001
Cost of other dressing material (Rs)	9570 (4646-22782)	4214 (782-13514)	<0.0001
Hospitalization cost (Rs)	57800 (16810-69100)	34500 (20200-70000)	0.003
Lab Cost (Rs)	1225 (900-4900)	1200 (770-11000)	1.000
Medicines Cost (Rs)	12600 (781-22500)	11000 (1630-21440)	0.003
Footwear cost (Rs)	750 (750-2495)	800 (750-2200)	0.056
Total Direct Cost (Rs)	**78134 (36057-115029)**	**66463 (35555-98270)**	**0.003**
Loss of wages (Rs)	1000 (370-5200)	1000 (500-3000)	0.052
Cost of food (Rs)	900 (300-5000)	890 (450-3000)	0.709
Accommodation cost (Rs)	4900 (1020-4900)	1100 (250-1200)	0.047
Travel Cost (Rs)	1100 (1500-4100)	2500 (500-4500)	0.181
Total Indirect Cost (Rs)	**2235 (700-16300)**	**1980 (500-13000)**	**0.781**

CONCLUSION:

Cerdak, as found by our study can be considered beneficial due to several reasons such as, it describes effective fundamental principles required for effective wound bed preparation, the ease of applications, less frequent application, requiring less number of other dressing materials, attaining better result faster and complete healing of wound and having lower direct and indirect cost involved for treating a diabetic foot ulcer.

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