"A Comparative Study of Methylene Blue Guided Debridement Vs Conventional Debridement In Diabetic Foot Ulcer"

1. Dr. Thangapandi MS[1].,

Assistant professor of surgery, Govt. Theni Medical College, Theni

2. Dr.Karthikeyan , *Junior Resident*

I.   Introduction

"I cried because I had no shoes until I met a man who had no feet"-Helen Keller

In terms of grief, losing a part of the body may be on par with that of losing a loved one be it a digit or an entire leg. This loss is usually unexpected and traumatic especially for a piece of oneself which has been well known and been neglected through-out life, has gone forever or changed. Amputation is not something which anyone could find in the brain's dictionary of words. It becomes of utmost importance in this fast-paced life of ours to prevent unnecessary amputations especially in the wake of chronic diseases like Diabetes Mellitus. In India, as opposed to western countries, mean age at diagnosis of diabetic foot and mean age at amputation was significantly lower. Advanced atherosclerotic diseases, impaired cerebral / peripheral / renal circulation seen in older patients reported in Western may be the reasons adversely affecting mortality and contralateral limb amputation rate. Above knee to below knee amputation ratio was 1:2 vs. 1:17 in Western vs. Indian series and the most frequently performed amputation was the above knee amputation among diabetics in Western population.

An essential part of the body designed for efficient weight distribution and locomotion; the foot is often ignored by people with diabetes mellitus resulting in complications. "Diabetic Foot" was the umbrella term used to describe complications of the foot due to diabetes. Recently the term "diabetic foot" has been replaced with "diabetic foot syndrome (DFS)." Serious impacts on morbidity and mortality of diabetes mellitus population is seen with DFS which causes multiple foot complications, among which foot ulceration is the most dangerous which leads to amputation. Statistics from several studies show that a lower limb is lost every 30 s among diabetes mellitus population. Due to lack of awareness, management resources, and economic constraints the suffering among Indians might have been high.

Treatment of foot problems among diabetic individuals is a resource draining affair in terms of manpower allocation and financial burdened with a significant impact on family members[3]. The impact of this situation is multiplied several folds especially in places with limited access to health care and diabetic foot awareness is low. Socioeconomic concerns are particularly prevalent among patients dependent on their caregivers throughout the treatment and the resulting high costs of the treatment. In the U.S. it was estimated thatthe average cost of a treatment of a hospitalized patient with a diabetic foot ulcer was

36.00   dollars annually[4].

The current diabetic scenario in India is less draught with major complications due to majority of population being affected in of the younger generation with adapting western lifestyles. However, the scenario is bound to change with the increasing duration of diabetes, out of pocket expenditures, poverty, onset of antimicrobial resistance, increased smoking, barefoot walking and improper treatment by unqualified personnel. With India having become the "Capital of Diabetes" it is no wonder that it might also emerge as the capital for diabetes related amputations if steps are not taken in this regard.

This study attempts to highlight one of the methods of surgical debridement of diabetic foot ulcer by using methylene blue as a stain to remove devitalized tissue and at the same time preserving healthy normal tissue.

AIM

TO COMPARE METHYLENE BLUE GUIDED DEBRIDEMENT Vs CONVENTIONAL DEBRIDEMENT IN DIABETIC FOOT ULCER
MATERIALS AND METHOD

STUDY DESIGN   :   Quasi experimental study

STUDY AREA :   Department of General Surgery,

Government Theni Medical College and Hospital, Theni
STUDY DURATION   :   JANUARY 2019 to JANUARY 2020

STUDY POPULATION :   Patients attending General Surgery department,

Theni Medical College and Hospital with a history of Diabetes and Foot Ulcer

Inclusion Criteria:
- Patients between 18 to 80 years of age
- Both sexes
- Diabetes mellitus and Diabetic Foot Ulcer

Exclusion Criteria:
- Patients not willing for informed consent or participation
- Patients with peripheral vascular disease
- Patients with grade IV and V ulcers by Wagner's classification

II.   Methodology:

Study Tools – Collected using questionnaire Patient demographics
Details of patient like name, age, sex, address, occupation, and per capita income are collected
History
History of the following parameters are obtained
1. Onset of ulcer – trauma / spontaneous
2. Site of ulcer
3. Duration of Ulcer - rapid progress / slow progress
4. Diabetic history – duration / type / type of treatment
5. History of alcohol and tobacco

Foot Examination
The following parameters were noted – dryness of skin, site edema, site erythema, fissures of heel, scratch marks, signs of infection, deformities

Examination of Foot Ulcer

- Side : Right / Left / Bilateral
- Site : Dorsum of foot / dorsum of leg / sole / multiple
- Size : length x height (mm)
- Shape : Circular / oval / irregular
- Margins : erythematous/ edematous/ indurated
- Edge : Punched out /sloping/undermined
- Floor : Slough covered/Blackish discoloration/granulation
- Others : Slough, periulcer edema, exposed tendon

Pulse examination
Digital examination of pulses of lower limb on both sides is done. Palpation of pulses of the following blood

vessels are done
1. Femoral artery
2. Popliteal artery
3. Posterior tibial artery
4. Dorsalis pedis artery

Investigations
Hemoglobin, packed cell volume (PCV), total count, differential count, red blood cell count, platelet count, erythrocyte sedimentation rate (ESR), bleeding time, clotting time, blood group, Blood urea, serum creatinine, fasting and post- prandial blood sugar (FBS & PPBS)

Doppler Studies
Doppler studies of lower limb vessels to assess patency of the blood vessels and to rule out possibility of peripheral vascular disease
X Ray of Foot
Done for all patients to rule out osteomyelitis and monitor the progression of the disease

MRI of ankle joint
Done in selected patients developing Charcot's arthropathy and in those with severe foot deformities
Pus for culture and sensitivity
Done for all patients to start on appropriate antibiotic regimen. Antimicrobial susceptibility testing of aerobic isolates was performed by the Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI)[116]
For wound debridement, the selected patients were subjected to admission and procedure was done in operation theatre under strict aseptic precautions. Written informed consent was obtained prior to the procedure from the patient. Visual inspection was done and grading of the ulcer prior to debridement done. Methylene blue applied using cotton tip and stained devitalized tissue was meticulously removed. Post procedure proper wound dressing was given to ensure wound healing. In the control group wound inspection was done and surgical debridement done without methylene blue using sharp instrumentation and hydrogen peroxide.
Follow Up – Period 6 weeks

The following characteristics of the ulcer were used as parameters for outcome analysis for those having undergone wound debridement with and without methylene blue.
1. Presence of necrotic tissue by visual evaluation – visual score

a. Score 1: 76 – 100% necrotic tissue covering the ulcer

b. Score 2: 51-75% necrotic tissue covering the ulcer

c. Score 3: 26 – 50% necrotic tissue covering the ulcer

d. Score 4: 11 -25% necrotic tissue covering the ulcer

e. Score 5: 0-10% necrotic tissue covering the ulcer

f. Score 6: no necrotic tissue covering the ulcer

2. Presence of granulation tissue by clinical evaluation – visual score

a. Score 1: no granulation tissue covering the ulcer

b. Score 2: pink/dull, < 25% granulation tissue covering the ulcer

c. Score 3: Beefy red, 25 – 74% granulation tissue covering the ulcer

d. Score 4: Brightly red, 75 -100% granulation tissue covering the ulcer

3. Wound surface area by scale measurement

STATISTICAL ANALYSIS:

Results were analyzed using SPSS software 16.0. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD and results on categorical measurements are presented in Number (%). Differences in the quantitative variables between groups were assessed by means of unpaired t test. Comparison between groups was made by non-parametric Mann-Whitney test. Chi square test was used to analyze categorical variables and multivariate analysis was done to test dependent variables. p value of < 0.05 using a two tailed test was taken as statistically significant. Analysis of covariance was done and Kaplan meyer curve analysis and Hazard ratio calculated.

RESULTS AND OBSERVATION

This study involved 100 participants with diabetic ulcer of foot. All participants provided informed consent before getting enrolled in the study. Follow up period was till the ulcer criteria for cure were fulfilled

Demographics

The mean age of the study participants was 56.71 years (± 12.676). The minimum age is 21 years while the maximum age is 82 years. Among the 100 study participants 29 were females and 71 were males. The mean age group of males is 55.59 years (± 14.11) and that of females is 57.17 years (± 12.12).

Table 1: Gender distribution among study participants

Age group	Male		Female	
	n	Percentage	n	percentage
< 26 years	1	1.4	1	3.4
26 - 40 years	6	8.5	5	17.2
41 - 55 years	29	40.8	9	31.0
56 -70 years	25	35.2	10	34.5
> 70 years	10	14.1	4	13.8
Total	71	100	29	100

The mean difference between male and female participants is 1.583 and this difference in means is not statistically significant. (p =0.574), thereby stating that the composition of age groups among male and female participants was similar. Among males the majority of the study population were in the age group of 41 − 55 years (40.8%) while female participants were mostly of the 56 − 70 years age group (34.5%).

Diabetes and comorbidities

Among study participants 6% had Type 1 diabetes and 94% had Type 2 diabetes. Table 2: Diabetes distribution among study participants

Diabetes	Male				Female			
	n	Duration mean (±SD)	OHA	Insulin	n	Duration OHA mean (± SD)		Insulin
Type I	2	102 (±42.46)	-	2	3	87(± 38.41)	-	3
Type II	69	70.91 (± 56.1)	62	7	26	84.7 (± 53.0)	25	1
Total	71	71.79 (±55.79)	62	9	29	85.03 (±50.65)	25	4

87% were on Oral Hypoglycemic agents while 13% of patients were on Insulin therapy. The mean duration of diabetes among the study participants was 76 months (± 54 months). Among males 60 (84.5%) were smokers while 51 were alcoholics (71.8%). The mean duration of smoking was 17.25 (± 10.7) months while the mean duration a male was alcoholic was 11.48 (± 9.6) months. Comorbidities

Fig 13: Distribution of comorbidities among study participants

Distribution of comorbidities among study participants
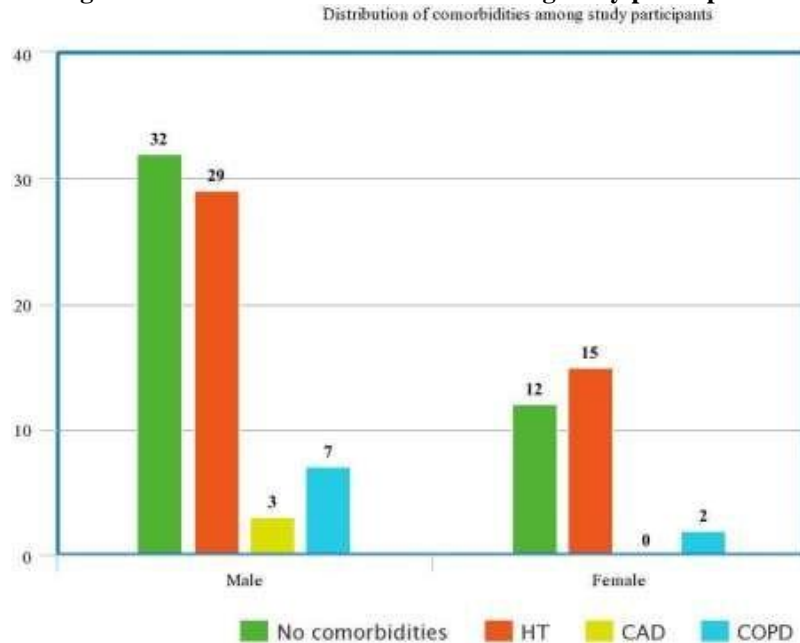
* HT – hypertension, CAD – Coronary artery disease, COPD – Chronic obstructive pulmonary disease
Ulcer characteristics

49% of study participants had reported a spontaneous onset of diabetic foot while 51% had a traumatic onset. Pain was present among 29 participants.
Serosanguinous discharge was the most common type seen in 68% of individuals while serous and purulent discharge was seen in 16% of individuals respectively. While 85% of study participants had taken some form of treatment, 15% had not provided any form of treatment for their diabetic ulcer.

Fig 14: Distribution of site of ulcer among study participants
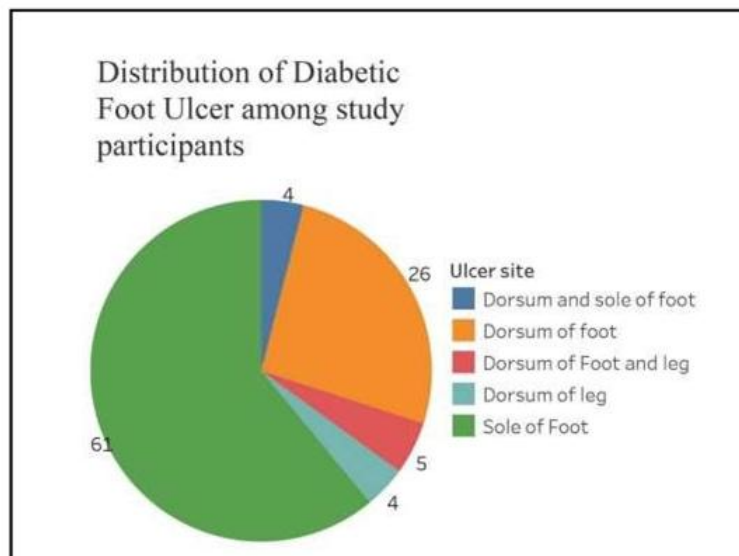
Majority of the ulcers are in the sole of the foot for both male and female subjects was in the sole of the foot, while the least commonest site was the dorsum of foot and leg. This shows that pressure component has an important role to play in the progression of Diabetic Foot Ulcer.

Fig 15: Distribution of DFU among male and female participants
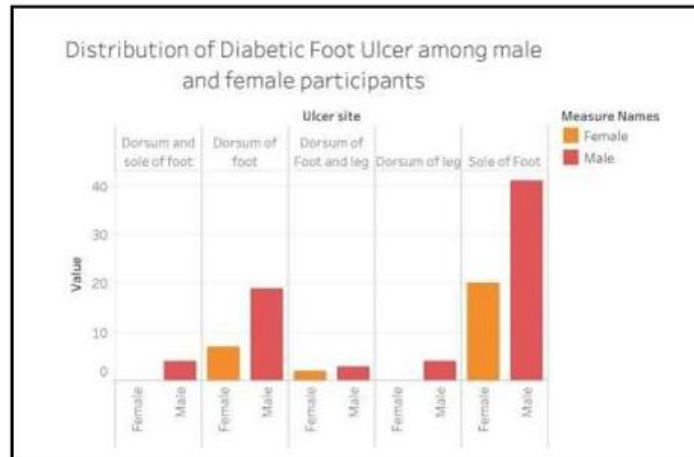
Wagner Classification of Ulcers

Table 3: Classification of DFUs among study participants - Wagner

Wagner	Grade I	Grade II	Grade III	Grade IV	Grade V
Participants	68	28	3	-	-

Majority of the patients had Wagner's Grade I ulcer, while grade II was seen in 28% of participants and grade III in 3%. Grade IV and V were not included in the study.

Shape of the Ulcer
The shape of the ulcer was oval in most patients with length more than the width but in others the ulcer was irregular either due to slough or granulation formation or due to previous surgical debridement
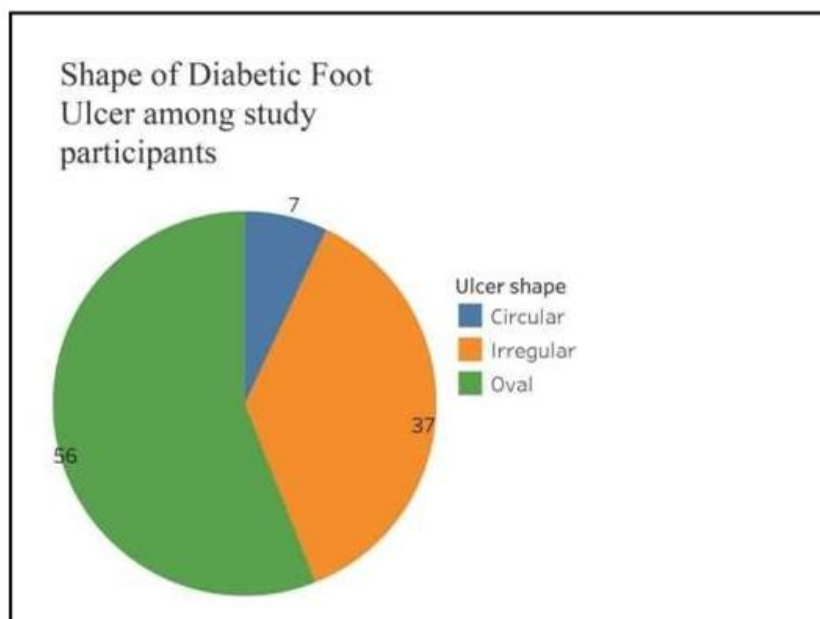

Fig 16: Graph showing distribution of ulcer shapes among study participants

When stratified for gender, the ulcer shape had similar distribution pattern like the overall study group.
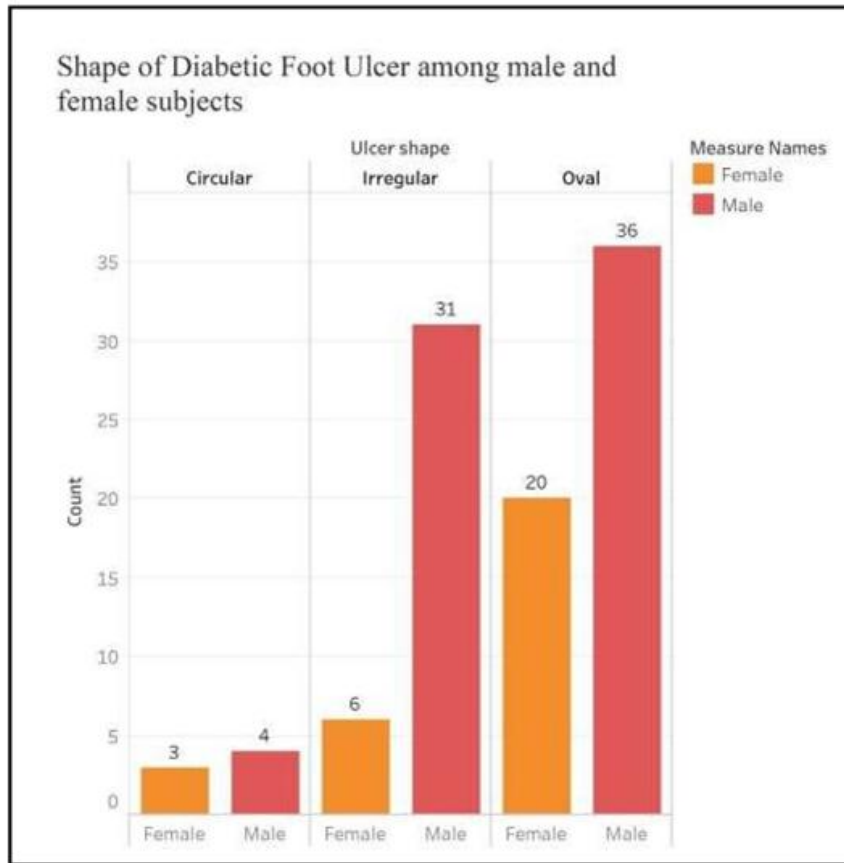

Fig 17: Distribution of ulcer shapes between male and female participants Ulcer margin and edge

Majority of the subjects had an indurated ulcer margin thereby showing an ongoing inflammation in the vicinity of the ulcer. Edema of the ulcer margin was seen in few patients. Majority of the participants had sloping ulcer edge thereby indicating an ulcer which shows some sign of granulation and healing.
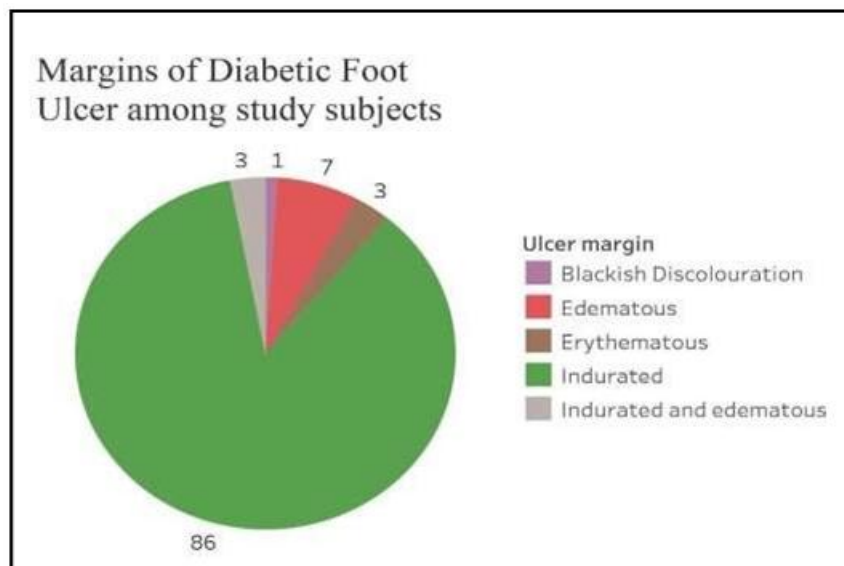

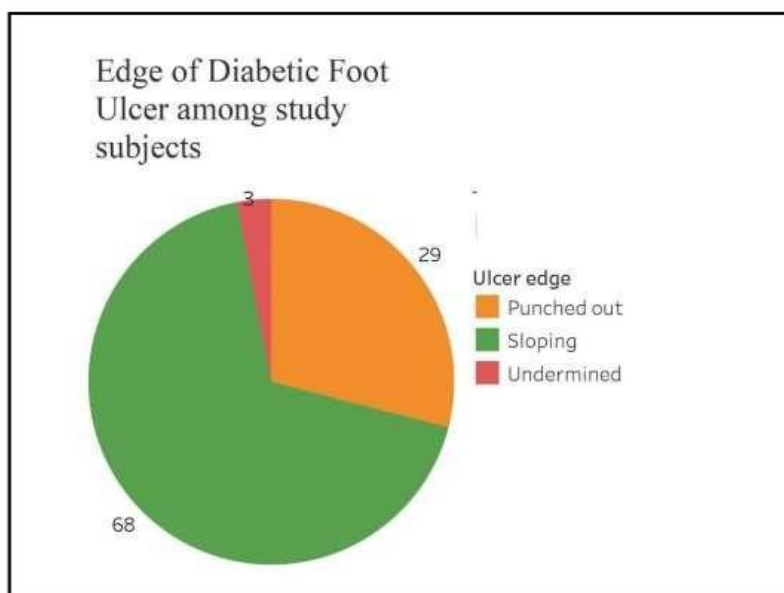**Fig 18: Types of margins of DFU among study participants**

Fig 19: Types of ulcer edges among study participants

Table 4: Ulcer characteristics and gender distribution

Ulcer Characters	Male (n = 71)		Female (n=29)	
	n	%	n	%
Onset of ulcer				
Spontaneous	38	53.5	11	37.9
Traumatic	33	46.5	18	62.1
Pain	22	31.0	7	24.1
Discharge				
Serous	10	14.1	6	20.7
Serosanguinous	47	66.2	21	72.4
Purulent	14	19.7	2	6.9
Treatment	59	83.1	26	89.7

Male (n = 71)   Female (n=29)

The onset of the ulcer was traumatic among majority of women while among men it was spontaneous. The type of discharge was serosanguinous in most of the participants.
Bacterial isolates and antibiotic sensitivity

Doxycycline is the antibiotic of choice for DFUs infected with non-fermenting gram - ve rods and Klebsiella whereas for Pseudomonas it is erythromycin
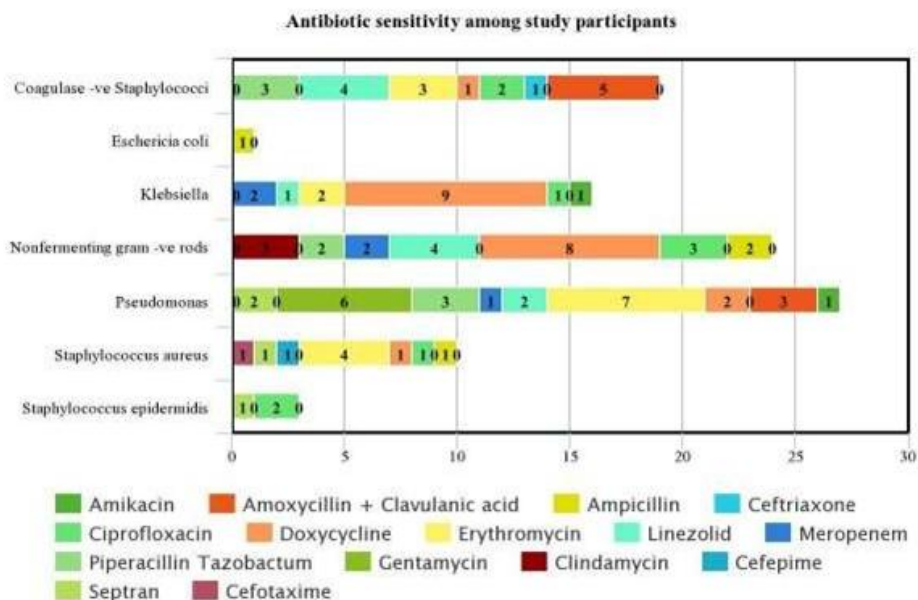
Fig 20: Antibiotic sensitivity pattern among study participants

Table 5: Descriptive statistics – start of study

Parameters	Group A (methylene blue n=47)		Group B (no dye n= 53)		
	Mean	SD	mean	SD	p value
Age (years)	56.11	± 13.1	57.25	± 12.4	0.656
Diabetes duration (months)	64.06	± 44.6	85.89	± 60.4	0.045
Ulcer length (cms)	7.74	± 2.36	6.89	± 2.08	0.056
Ulcer width (cms)	4.09	± 0.75	3.85	± 0.66	0.097
Ulcer depth (cms)	0.70	± 0.51	0.85	± 0.41	0.113
Ulcer size (cms)	28.19	± 15.5	24.70	± 11.3	0.197
Ulcer duration (months)	58.09	± 41.2	29.06	± 21.1	$p >$ 0.001
Ulcer area (cm^2)	28.97	± 13.4	23.77	± 11.4	0.38

The above table shows the descriptive statistics between the two groups based on usage of methylene blue dye for surgical debridement shows that most of the parameters are similar at the start of the study and there was no difference statistically between the two groups.
Demographics among groups

Table 6: Demographic patterns among the study and control groups

Parameters	Group A (methylene blue n=47)		Group B (no dye n= 53)	
	frequency (n)	percentage (%)	frequency (n)	percentage (%)
Age group				
< 26 years	1	2.1	1	1.9
26 - 40 years	6	12.8	5	9.4
41 - 55 years	21	44.7	17	32.1
56 -70 years	11	23.4	24	45.3
> 70 years	8	17.0	6	11.3
Gender				
Male	36	76.6	35	66.0
Female	11	23.4	18	34.0
Diabetes Type				
Type I	1	2.1	4	7.5
Type II	46	97.9	49	92.5
Onset of Ulcer				
Spontaneous	24	51.1	25	47.2
Traumatic	23	48.9	28	52.8
Pain	15	31.9	14	26.4
Discharge				
Serous	10	21.3	6	11.3
Serosanguinous	29	61.7	39	73.6
Purulent	8	17.0	8	15.1

The most common age group in the methylene blue dye group was 41-55 years while the 56-70 years age group was seen commonly in the no dye group. Male and female distribution was similar in both the groups

Foot examination Characteristics
Dryness of skin and scratch marks were seen with increased frequency in both the groups. However, signs of infection were seen more predominantly in the no dye group. The most common ulcer site in both the groups was sole of foot. Ulcer shape in no dye group was predominantly oval while no such findings were seen in the group using methylene blue dye. Indurated ulcer margin with punched out edges were seen commonly among both the groups. There was no difference between the side of ulcer in group A but ulcers were commonly seen in the right side among participants of Group B

Table 7: Ulcer parameters distribution between two groups

Parameters	Group A (methylene blue n=47)		Group B (no dye n = 53)	
	Freq (n)	percentage (%)	Freq (n)	percentage (%)
Dryness of skin	32	68.1	45	84.9
Edema	15	31.9	8	15.1
Erythema	16	34.0	14	26.4
Heel pressure	28	59.6	40	75.5
Scratch marks	36	76.6	47	88.7
Signs of infection	27	57.4	39	73.6
Deformity	1	2.1	3	5.7

Ulcer side					
Right		24	51.1	:	60.4
Left		23	48.9	:	39.6
Ulcer site					
Dorsum & sole of foot		1	2.1	3	5.7
Dorsum of foot		13	27.7		24.5
Dorsum of foot and leg		4	8.5	1	1.9
Dorsum of leg		3	6.4	1	1.9
Sole of foot		26	55.3	:	66.0
Ulcer shape					
Oval		20	42.6	36	67.9
Circular		5	10.6	2	3.8
Irregular		22	46.8	15	28.3
Ulcer margin					
Erythematous		2	4.3	1	1.9
Edematous		2	4.3	5	9.4
Indurated		41	87.2	‹	84.9
Indurated & edematous		1	2.1	2	3.8
Blackish discoloration	1		2.1	0	0
Ulcer edge					
Punched out	27		57.4	‹ 77.4	
Sloping	17		36.2	: 22.6	
Undermined	3		6.4	0	0
Ulcer floor					
Slough covered		2	4.3	53	100
Blackish discoloration	45		95.7	0	0
Granulation		7	14.9	1	1.9
Peri ulcer edema	14		29.8	7	13.2
Ulcer slough	45		95.7	53	100
Exposed tendon	14		29.8	16	30.2
Necrotizing fasciitis		1	2.1	0	0

Additional findings like necrotizing fasciitis was seen in one patient in the Dye group. Exposed tendons were seen in 29.8% of the Dye group and 30.2% of the no dye group participants
Wagner's Classification of ulcer between study groups
Based on ulcer classification by Wagner, 65% of the group A participants had grade I ulcer and 69.81% of the Group B participants had Grade I ulcer. Grade III ulcers were not seen in group B

Table 8: Wagner's classification of ulcers between two study groups

Wagners Grade	Group A (47)		Group B (53)	
Grade I	31	65.95%	37	69.81
Grade II	13	27.66%	16	30.19
Grade III	3	6.38%	0	0

*Grade IV and V were not present as they were part of the exclusion criteria

Examination of peripheral pulses

Table 8: Peripheral pulses examination

Parameters	Group A (47)		Group B (53)	
	Freq (n)	percentage (%)	Freq (n)	percentage (%)
Right femoral				
Normal	47	100	53	100
Weak	0	0	0	0
Absent	0	0	0	0
Left Femoral				
Normal	47	100	53	100
Weak	0	0	0	0
Absent	0	0	0	0
Right Popliteal				
Normal	47	100	53	100
Weak	0	0	0	0
Absent	0	0	0	0
Left Popliteal				
Normal	47	100	53	100
Weak	0	0	0	0
Absent	0	0	0	0
Right Posterior Tibial				
Normal	41	87.2	38	71.7
Weak	6	12.8	15	28.3
Absent	0	0	0	0

Left Posterior Tibial				
Normal	41	87.2	42	79.2
Weak	6	12.8	10	18.9
Absent	0	0	1	1.9
Right Dorsalis Pedis				
Normal	36	76.6	27	50.9
Weak	10	21.3	16	30.2
Absent	1	2.1	10	50.9
Left dorsalis pedis				
Normal	32	68.1	33	62.3
Weak	14	29.8	15	28.3
Absent	1	2.1	5	9.4
Though pulses were absent	clinically	doppler studies were	done	to rule out the

possibility of a vascular deficit. This could be due to interobserver variations or on the thickness of the skin surrounding the ulcer area

Blood Parameters between two groups

Table 9: Blood parameters among study participants

Parameters	Group A (47)		Group B (53)		
	Mean	SD	mean	SD	p
Hemoglobin (mg/dl)	10.71	± 1.39	11.3	± 1.4	0.037
Packed Cell Volume (%)	38.93	± 1.61	37.7	± 0.9	<0.001
RBC count (cells/cc)	317830	± 23543	314132	± 20817	0.465
platelet count (cells/cc)	295383	± 41215	283406	± 35501	0.121
Bleeding time (min)	2'35"	± 0'24"	2'34"	± 0'19"	0.816
Clotting time (min)	7'08"	± 0'50"	8'05"	± 0'49"	<0.001
Urea (mg/dl)	40.5	± 9.01	48.04	± 11.38	0.004
Creatinine (mg/dl)	1.15	± 0.18	1.2	± 0.29	<0.001
FBS (mg/dl)	216.23	± 51.44	228.77	± 45.38	0.198
PPBS (mg/dl)	432.04	± 122.97	472.74	±112.2	0.086
Except for a few parameters	like creatinine, PCV, Hb,		Clotting time and Urea		all other

parameters had no statistical difference between the two groups.

Fig 21: Bacterial isolates from pus culture
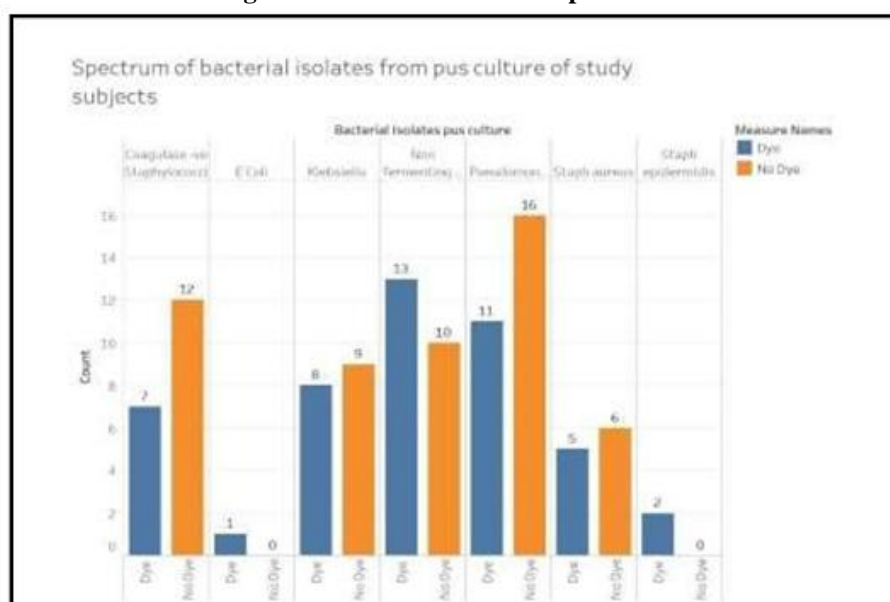
Pseudomonas was the most common bacterial isolate among group b while Non fermenting gram negative bacteria was the most common isolate in group A.

Association between Ulcer surface area and type of debridement

Table 10 shows the association between ulcer size and the surgical debridement methodology adopted over the 6 weeks follow up period. During the follow up period serial measurements of ulcer dimensions were done to assess the outcome properties using the methylene blue guided surgical wound debridement

The mean difference in ulcer area between base line value and at week 6 for the people in group A was 13.85 mm and this difference was statistically significant (p<0.00001) using student t test for paired samples. Similarly the mean difference in ulcer area for group B between baseline value and at week 6 was 20.28 and this difference is statistically significant at 95% CI levels (p<0.0001)

Table 10 :Follow up of both groups for 6 weeks period

	Base Line	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group A	28.97	34.01	35.74	37.81	38.73	38.64	37.05
Group B	26.77	28.11	30.05	32.46	35.91	42.02	42.81
Mean difference	2.20	5.9	5.69	5.35	(-2.82)	(-3.38)	(-5.76)
p value	0.138	0.028	0.044	0.076	<0.001	<0.001	<0.001

As the follow up period increases the size of the ulcer increases and this is shown by the statistical analysis wherein till 3 weeks the difference in ulcer size between the two groups are statistically significant but as the weeks of follow up increases the difference starts to reduce and the statistical association becomes stronger when the follow up period extends beyond 3 weeks as evident by the p values.

This could be due to the extensive wound debridement or due to the ulcer progression. In Group A the baseline ulcer surface area was much bigger compared to the baseline values of Group B.

Fig 22: Ulcer surface area pattern among the study groups during follow-up
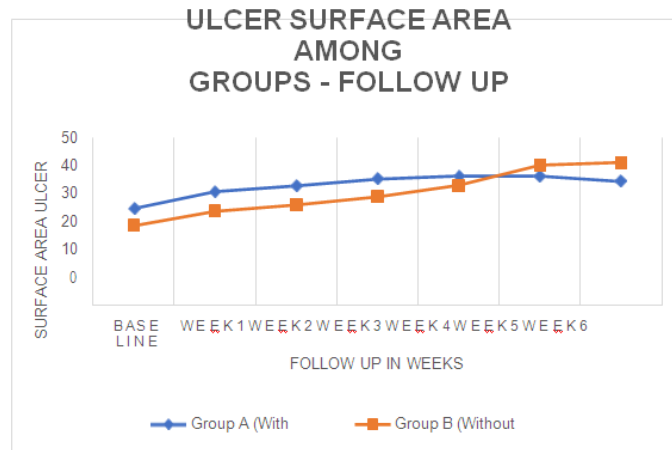
Analysis of Covariance

Analysis of Covariance with base line surface area as the covariate shows that the association between surface area and the type of surgical debridement employed is statistically significant (F=20.032, p< 0.0001) with 95% confidence limits interval.

Fig 23: Analysis of Covariance between wound surface area and type of debridement
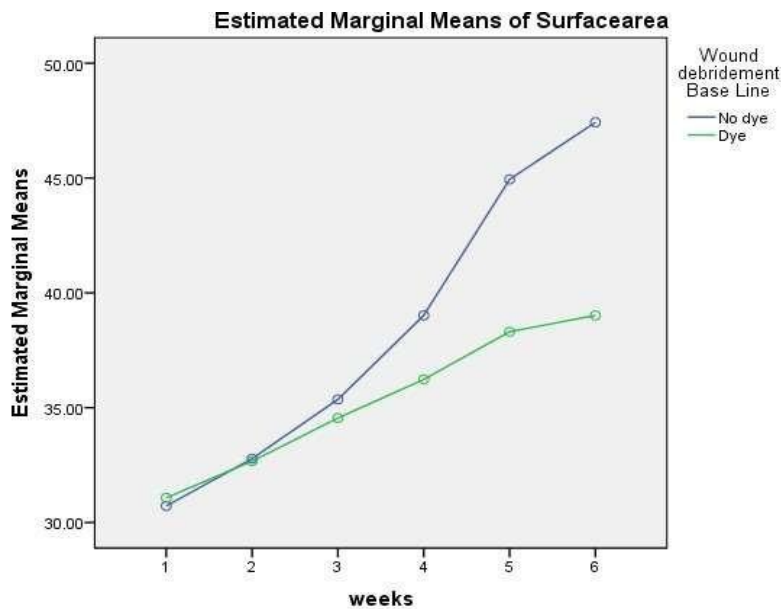
Also, from the plot it can be clearly seen that using methylene blue to aid surgical debridement is associated with reduction in ulcer surface areas.

Association between granulation tissue formation and type of surgical debridement
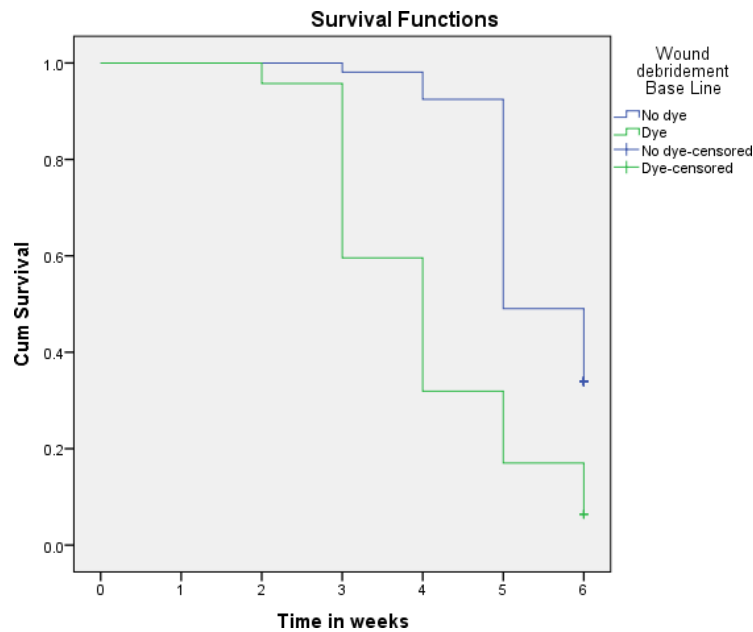

Fig 23: Kaplan Meyer Curve analysis showing the median time required for stage 4 granulation based on type of surgical debridement

Based on Kaplan Meyer curve analysis the median time required for stage 4 granulation formation in a Diabetic ulcer was 4 weeks in case of surgical debridement with methylene blue dye in comparison with a median time of 5 weeks compared to surgical debridement without dye. This difference was found to be statistically significant within 95% confidence interval limits (Log rank Test – Chi square value of 31.77, $p < 0.0001$)

Association between necrotic tissue and type of surgical debridement

The median time required for complete elimination of necrotic tissue in a Diabetic ulcer on the basis of Kaplan Meyer curve survival analysis was 4 weeks in case of surgical debridement with methylene blue dye in comparison with a median time of 6 weeks compared to surgical debridement without dye. This difference was found to be statistically significant within 95% confidence interval limits (Log rank Test

– Chi square value of 25.31, $p < 0.0001$)

Fig 24: Kaplan Meyer curve showing the median time required for complete elimination of necrotic tissue between different surgical debridement techniques
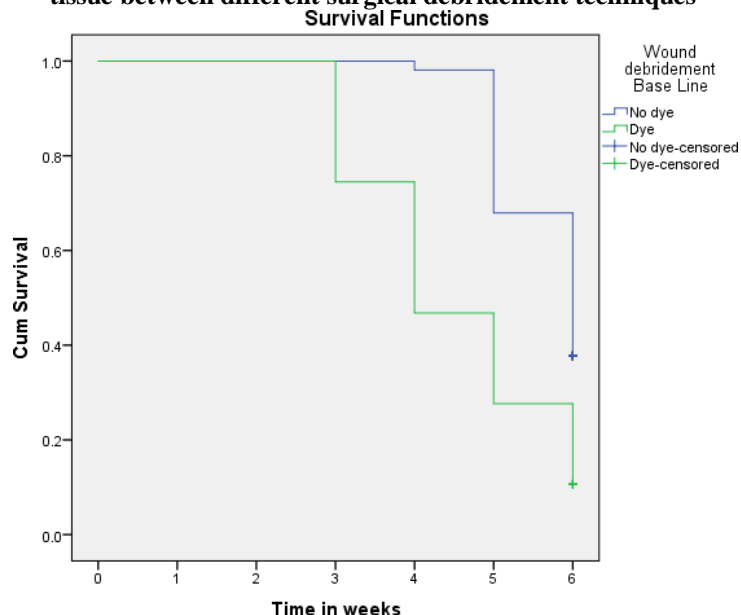
III. Discussion

The increasing pandemic of Diabetes Mellitus over the years has led to the occurrence of microvascular and macrovascular complications among patients at a dramatic proportion. In a country like India where majority of the people reside in rural areas, the occurrence of these complications often goes unnoticed. One such microvascular complication which can cause mortality and long-term morbidity among the diabetic individuals is diabetic foot ulcer. The increased prevalence of DFUs are due to worldwide prevalence of Diabetes and due to increased life expectancy of the patients. Untreated diabetic foot ulcer can lead to amputations and it is one of the most common indication of lower limb amputations in developing countries. Though several techniques exist for the management of diabetic foot ulcer, they are cumbersome and difficult to use in the rural set up. In this study by using a methylene blue stain as a guide for debridement of devitalized tissue it was possible to achieve good wound healing of DFU in comparison to the conventional sharp surgical debridement and wound dressing. By using this technique unnecessary removal of normal tissue can be reduced which in turn reduces the average ulcer size when followed up over time.

Demographic details of patients:

The mean age of the study participants was 56.71 years with the minimum age being 21 years and the maximum being 82 years. This was similar to the studies by Jiang et al in China wherein the study participants mean age was 64 years and majority of them were in the 55 to 64 years age group with a male to female ratio of 2.2 to 1. (117)

Table 10: Comparison between different studies

	Base Line	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group A	28.97	34.01	35.74	37.81	38.73	38.64	37.05
Group B	26.77	28.11	30.05	32.46	35.91	42.02	42.81
Mean difference	2.20	5.9	5.69	5.35	(-2.82)	(-3.38)	(-5.76)
p value	0.138	0.028	0.044	0.076	<0.001	<0.001	<0.001

The difference in the age groups reflects the earlier onset of diabetes and the occurrence of complications at a younger age. Male population is high among the studies in India and China which has most of the areas under agriculture and majority of the people may be bare foot walkers or due to ill-fitting shoes.

Diabetic profile among study participants

The mean duration of Diabetes among study participants was 14.47 years of which Type I had a mean duration of 17.83 years while among Type II diabetes it was 14.25 years. This shows that it takes an average of 15 years to develop a complication like Diabetic foot ulcer. These findings were similar to studies by Oyibo with a mean duration of 16.6 years from Manchester center and 14.72 years from San Antonio center.(119) Similarly a study by Young et al on prediction of Diabetic neuropathic foot ulceration using vibration perception thresholds had a mean duration of diabetes of 12.4 years. This vast difference in the duration of diabetes shows that in addition to the duration of diabetes there are several factors that are involved in the progression of a Diabetic foot ulcer.

Majority of patients were on oral hypoglycemic agents (87%) while only 13% received insulin. There was contrasting differences in the usage of Insulin by patients of this study when compared to other studies. A study by Ogbera et al from Saharan Africa shows an increased usage of Insulin when compared to OHAs.(120) Similarly a study by Chellan et al in India showed a higher proportion of people using insulin combinations (33%). The reason for this difference in patterns of usage of OHAs and Insulin could be due to the reason that the present study is taken in a government setup where majority of the patients are below poverty line and of lower socio-economic grade. This results in reduced acceptance for injectable medications like insulin which has to be administered on a daily basis which is often painful and requires a person to administer it. Due to these reasons there is a generalized stigma from usage of insulin which leads to poor control of blood sugar and thereby promote the progression of DFUs.

Comorbidities among study participants

84.5% of the male participants were smokers and 71.8% were alcoholics. the mean duration of smoking was 17.25 years while the mean duration of alcohol consumption was 11.48 years. This was high compared to other studies which have demonstrated that smoking is a significant covariate factor in diabetic foot ulcers progressing to amputation.(117,121,122) Comorbidities like Hypertension and CAD was seen in 34% and 3% of individuals respectively. However, the comorbidities like hypertension and CAD were much higher in studies done elsewhere. Jiang et al in his study on epidemiology of Type 2 Diabetic foot problems and predictive factors for foot amputation in China had a higher prevalence of hypertension (74.4%) and ischemic heart disease (37.5%).(117) of CAD is questionable and further detailed studies are needed to support this view. Similarly a study by Pemayun et al on risk factors for lower extremity amputations in patients with diabetic foot ulcers has shown a lower percentage of Hypertension (20.2%) and CAD (13.8%) respectively. These variations lead to the conclusion that Hypertension whether acting as a co-vaiate in progression of small vessel disease or that diabetic foot ulcer predicts future development

Ulcer Characteristics

In this study about 51% of the study participants had a traumatic onset of ulcer. This reflects the tendency of the rural subset of population using ill fitting shoes and bare foot walking and lack of awareness regarding foot care like moistening the sole, proper trimming of nails etc.

The most common location of the ulcer was in the sole of the foot followed by the dorsum of foot both of which accounted for 86% of the cases. This was comparable to studies done by Madanchi et al and Ogbera et al wherein the most common location of DFUs were in the sole and dorsum of foot.(36,120) Skin abnormalities were seen in our study subjects of which Dryness of skin (77%) and scratch marks (86%) were the most common. These findings can be compared on similar terms to studies by El-Nahas et al among Egyptian diabetic individuals which show skin changes like dryness and scratch marks present commonly among the subjects.(123) In addition to neuropathy which can increase skin hardness as a reactive phenomenon, others like non-enzymatic glycation of collagens, ageing, autonomic neuropathy and inappropriate footwear.(124) Another study by Thomas et al has suggested that foot sole hardness and foot sole thickness play an important role in the development of plantar ulcers.(125)

Bacteriological isolates and Antibiotic sensitivity

The most common bacteria isolated was Klebsiella which was sensitive to Doxycycline followed by Pseudomonas which was sensitive to erythromycin. Higher generation antibiotics like Linezolid, Meropenem, Clindamycin and Piperacillin Tazobactam were required only in a small percentage ofparticipants especially in those with Klebsiella, Coagulase negative Staphylococci, Non fermenting gram-negative rods and Pseudomonas.

Table 11: Comparison between bacterial isolates from different studies

	Common bacteria isolated	Antibiotic sensitivity	Study location
	Klebsiella	Doxycycline	
This study	Pseudomonas	Erythromycin	South India
Abdul razzak et al**(126)**	Staph aureus Pseudomonas	Amoxycillin + Clavulanic acid	United Arab Emirates
Ako Nai et al**(127)**	E coli Staph aureus	Quinolones Penicillins	Nigeria
Shankar et al**(128)**	G-ve aerobes Pseudomonas	Ciprofloxacin Gentamycin	South India
The varied	antibiotic sensitivity	of the commonly isolated organisms in	

different parts of the world and within same country show that the widespread pattern of colonization of bacteria in DFUs and the need for routine pus culture and sensitivity to ensure adequate wound healing.

Ulcer surface area and type of surgical debridement

The analysis of ulcer surface area in Group A who have undergone surgical wound debridement using methylene blue shows that the ulcer size has increased following this method with a difference of 8.08 cm between baseline ulcer values and at week 6 with a rise of 2.12 cm every week. The ulcer dimension in Group B participants who have undergone wound debridement using standard protocol show an increase of 19.04 cm between base line and week 6 values with an increase of 3.12 cm every week. Though the difference in average increase between group A and B is very small as 1 cm every week this could be the deciding factor in better would healing by preserving normal tissue and removing devitalized tissue which is possible with color guided surgical debridement.

Granulation Tissue and Type of surgical debridement

The median time required for stage 4 granulation formation was 4 weeks for Group A while it was 5 weeks in case of Group B. This clearly shows that by adopting methylene blue staining techniques, there is complete removal of necrotic tissue and early appearance of granulation tissue. A cut off value of 4 weeks for formation of granulation tissue has been suggested by several authors as an indicator for good wound healing. Margolis et al. in her study described the predictive capacity of 61% area reduction at 4 weeks by formation of granulation tissue.[129] Sheehan [115], through his work also proposes a 50% wound closure at 4 weeks by granulation tissue as a good surrogate for final healing and thus a decision point in a foot ulcer management algorithm. A similar cut off value was also proposed by Snyder et al.[130]

These findings suggest that usage of a colour guided endpoint for surgical wound debridement offers several advantages to healing of DFUs in comparison to conventional sharp surgical dissection which includes

1.   Appropriate removal of devitalized tissue and avoiding unnecessary removal of normal tissue
2.   Earlier onset of granulation tissue and greater than 50% of wound coverage in half the span of time

IV.   Conclusion

With the increase in the prevalence of Diabetic Foot Ulcers in a developing country like India, due to several reasons like poor access to healthcare services, poverty, wearing ill- fitting shoes, earlier onset of diabetic microvascular complications etc. the need for a strategic approach for the management of this condition has become the need of the hour. Though there are several advances in the management of DFUs and despite several new techniques being added every year, the search for an ideal method is yet to be complete. Advanced techniques employing cumbersome procedures though have a good outcome in terms of wound healing and reduced deformities, their application in real life scenarios is challenging. This study by employing a simple technique of using a colour guided surgical wound debridement with methylene blue has been successful in reducing the wound healing time. In comparison with conventional methods of wound debridement which tends to remove excess of normal tissue or leave behind devitalized tissue, this technique of ours has demonstrated better surgical outcome,the median time required for stage 4 granulation formation was 4 weeks for methylene blue guided debridement while it was 5 weeks in case of conventional debridement. Further studies involving larger sample size and employing randomization techniques are needed to find the effect of confounding factors and effect modifiers.

References

[1].   Hazari A, Maiya GA. Clinical Biomechanics and its Implications on Diabetic Foot [Internet]. Singapore: Springer Singapore; 2020 [cited 2020 Jun 28]. Available from: http://link.springer.com/10.1007/978-981-15- 3681-6
[2].   Bakker K, van Houtum WH, Riley PC. 2005: The International Diabetes Federation focuses on the diabetic foot. Curr Diab Rep. 2005 Nov;5(6):436– 40.
[3].   Bilgiç E, Yılmaz E, Akan O. Diabetic foot preventation from amputation with epidermal growth factor: A Case Report. Int J Med Rev Case Rep. 2019;(0):1.
[4].   Temple ME, Nahata MC. Pharmacotherapy of Lower Limb Diabetic Ulcers. Journal of the American Geriatrics Society. 2000 Jul;48(7):822–8.
[5].   American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus.
[6].   Diabetes Care. 2014 Jan 1;37(Supplement_1):S81–90.
[7].   EBERS PAPYRUS, EBBELL B. The Papyrus Ebers, the greatest Egyptian medical document. Translated by B. Ebbell. Levin & Munksgaard: Copenhagen; Oxford University Press: London; 1937.
[8].   Roglic G, World Health Organization, editors. Global report on diabetes. Geneva, Switzerland: World Health Organization; 2016. 86 p.
[9].   Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2019. Diabetes Care. 2019 Jan 1;42(Supplement 1):S13–28.
[10].   Liapis CD, Balzer K, Benedetti-Valentini F, Fernandes e Fernandes J, editors. Diabetic Foot. In: Vascular Surgery [Internet]. Berlin, Heidelberg: Springer; 2007 [cited 2020 Jun 28]. p. 501–21. (European Manual of Medicine). Available from: https://doi.org/10.1007/978-3-540-30956-7_45