Glycosylated Hemoglobin (HbA1c) Levels and Wound Healing in Diabetic Foot Ulcers–An Observational Study

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Abstract

Background: Altered glycemic levels (HbA1c) can influence the healing process of diabetic foot ulcers (DFU), prevalent among type-2 Diabetes Mellitus (DM) patients. The objective of this study was to assess the rate of wound healing in DFU corresponding to HbA1c levels in type 2 diabetes individuals.

Methodology: The present observational study was conducted in 90 DFU patients with single Wagner grades 1 or 2 foot ulcer. Depending on the HbA1c levels; Group 1: $< 7\%$, Group 2: 7-8% and Group 3: $> 8\%$. Photographs were taken using the Tissue Analytics software interface installed on an android smartphone, on day 0 and day 14. The software then calculated and yielded the length, width, and area of the ulcer. Culture and antibiotic sensitivity of foot ulcer was done. Chi-square test was used for group comparisons and $p<0.05$ was considered significant

Results: The mean HbA1c level was 8.76±2.75% and the duration of DM was 118.87±83.52 months in all the subjects. The range of DM duration was 0.08 to 30 years (Confidence Interval: 8.45 to 11.36). Among the subjects, 44.44% had ulcers for 1-4 weeks. A significant negative correlation (rho= -0.55) between HbA1c and rate of wound healing ($p<0.0001$) was observed using Spearman rank correlation. Wagner grade was significantly associated with group ($p=0.001$). Neuropathy, Peripheral Vascular Disease (PVD) and bacterial infection at the site was seen in 44.40%, 31.10% and 53.1% patients.

Conclusion: The duration required for wound healing increased with rise in HbA1c.

Keywords: Diabetic foot, foot ulcer, Glycated haemoglobin A, wound healing.

Introduction

Diabetic Foot Ulcer (DFU) is one of the most common causes for hospitalization of a diabetic patient which could result in amputation if untreated. In India, the onset of diabetes is observed at a young age and is associated with complications regardless of the duration of diabetic illness. [1] Complications of diabetes are divided into microvascular (diabetic neuropathy, nephropathy and retinopathy) and macrovascular (peripheral vascular diseases, coronary artery diseases and

stroke). [2] DFU patients have been observed to carry a risk of developing vasculopathy and neuropathy with a short duration of diabetes in India. [1] Worldwide DFU incidence is 9.1 to 26.1 million per annum. [3] Foot ulcers can be averted by a frequent screening of high risk patients. An infection of the ulcer can be avoided by awareness, control of blood sugar level, removal of dead tissue from wound, progressive dressing, surgery and progressive treatment. [4,5] Risk factors associated with DFU are increasing age, female gender, peripheral neuropathy, peripheral arterial diseases, history of diabetes and elevated Glycated Hemoglobin (HbA1c). [6,7] Modality of DFU consists of debridement of infection, antibiotic treatment, subsequently accurate vascular reformation. A negative wound pressure therapy could be the appropriate treatment method for DFU.[8] Wagner grading system delineates the severity of ulcer, existence of gangrene or osteomyelitis. [9]

Assessment of HbA1c in diabetic patients is advantageous over the routine blood glucose or Oral Glucose Tolerance Test (OGTT).[10,11] The benefits of HbA1c testing is that it does not require any prior preparation and it can be done at any time of a day. HbA1c provides an average glucose level for three months which is beneficial over repeated testing of blood glucose. Foot ulcer healing is delayed with elevated HbA1c, total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels. Further HbA1c is a known reliable diagnostic tool for the prediction of DFU. [8,12] Body Mass Index (BMI) and HbA1c are two risk factors associated with diabetes and their raised levels can predict poor healing process of DFU. These two factors signify the restrain over the complications of diabetes. [1] Tissue analytic software assess the wound healing through an electronic health record (HER) plug-in, which is powered by Artificial Inelegancy (AI) software solution. The company recently adopted an interlinked platform with selected partners, that indicates the cutting-edge of wound care instruments, characterized with picturization and examine oxygenation of tissue under the skin, briefing of bioburden and prejudice the DFU.

A paucity of studies regarding a compelling association between DFU and HbA1c level was observed. It has been reported that inconsistent HbA1c levels of diabetic patients have a greater wound healing activity with a baseline control. [13,14] Moreover, after accommodating confounding factors, there has been no correlation between HbA1c level and healing of DFU. [14] Contradicting these outcomes there has been a strong association between elevated HbA1c and delay in the healing of DFU. [4,6,15] Hence, this study proposed to assess the DFU healing rate and its association with corresponding HbA1c levels.

Material and Methods

This prospective observational study was conducted for one year from January 2017 to December 2017 in the department of Surgery at a tertiary care hospital. Using Cohen's convenience method with Effect size of 0.335, 80% power and 5% significance level, sample size was calculated and 90 DFU patients were enrolled in the study. Convenience method of sampling technique was applied for the sample selection. DFU patients who consented for the hospital stay for a minimum of 15 days, patients above 18 years, 1 or 2 Wagner grades with single foot ulcer and only new cases of DFU, irrespective of their Diabetes mellitus duration were included in the study. Exclusion criteria of the study was Wagner grade strike 3 or more, foot ulcer with gangrene, osteomyelitis of foot, diabetic ketoacidosis, immunodeficiency states, hemoglobinopathies, autoimmune diseases like malignancy and patients receiving unconventional treatment for DFUs. The study was approved by Institutional ethical

committee and the informed consent was obtained from the study participants.

Baseline characteristics of the cohort such as age, gender, duration of diabetes, history of hypertension and duration of foot ulcer was collected. During the clinical examination of patients Wagner grade, neuropathy, and Peripheral Vascular Disease (PVD) were recorded. [2.16,17] Ulcer characteristics such as shape, location, edge, margins, floor of ulcer were noted on – Day 0 (beginning of study) and Day 14 (end of study) were examined by a qualified General Surgeon. Diagnostic part of the study culture and antibiotic sensitivity of foot ulcer was conducted, for this wound discharge or deep tissue culture during debridement was carried out before inclusion into study.

For the estimation of HbA1c level among DFU patients, 2 cc of blood sample was collected in an ethylenediamine tetra acetic acid (EDTA) anticoagulant tube and sent to the hospital laboratory. In the laboratory HbA1c level was measured by High Performance Liquid Chromatography (HPLC). [18] Depending on the HbA1c level of patients, they were categorised into three groups: the first group comprised of patients with less than 7%, the second group included patients with 7-8% and in the third group, patients with HbA1c level greater than 8% were included.

Photographs were taken using the Tissue Analytics software interface which was installed on an android smartphone. The photograph was taken after placing a green circle sticker of 1 cm^2 area next to the wound (used as a scale) on Day 0 and Day 14. The software, then calculated and produced the length, width, and area of the ulcer.

The initial wound area on Day 0 (x) and the final wound area on Day 14 (y) were noted in all the three HbA1c groups (Figure-1). From this data the wound area reduction and rate of wound healing was calculated as follows: rate of wound healing or area reduction per day = x-y/15 [18], where, x = wound area on D_0, y = wound area on D_{14}. All the data was collected from the patients and documented in Microsoft Excel sheets.

Wound dressing was done with normal saline daily for a period of 15 days and antibiotics were administered based on the wound culture and sensitivity report. All the patients were accompanied with insulin therapy during the hospital stay.

Statistical analysis was done using R 3.6.1 software. Descriptive analysis of the data was done using Excel sheet. Continuous variables were represented by mean ± standard deviation. Categorical variables were represented by frequency tables. Categorical variables were compared using Chi-square test and continuous variables between groups were compared using ANOVA. A p-value<0.05 was considered statistically significant.

Results

The mean age of the study subjects was 56.4±11.41 years across the groups. By Chi-square test, gender was equally distributed across the groups (p=0.3928) and a male (74.44%) preponderance was noticed. The range of DM duration was 0.08 to 30 years (95% Confidence Interval: 8.45 to 11.36). HbA1c was significant among all the three HbA1c groups (p< 0.0001). Using ANOVA, the mean duration of DM (in months) (p=0.5283), the duration of foot ulcer (p= 0.4265) and the site of ulcer (p=0.0506) were found to be insignificantly correlated between the three groups, whereas Wagner grade was significantly co-related with group (p=0.001). (Table-1)

Using two-way ANOVA with interaction, the status of neuropathy did not significantly affect the rate of wound healing (p=0.3436). Effect of HbA1c levels on wound healing was not significantly different over the status of neuropathy (Group: Neuropathy

interaction; p=0.6610). The mean rate of wound healing significantly differed in at least 2 groups (p<0.0001). By ANOVA test, the presence of PVD did not significantly affect the rate of wound healing in the foot ulcer subjects (p=0.8934) and there was no significant interaction effect between PVD and Group (p=0.7147) (Table 2). Wound infection was reported in 53.1% of the study population. However, the presence of infection did not significantly affect the rate of wound healing (p=0.3930) and there was no significant interaction effect between Culture and the Groups (p=0.3261) (Table-2).

A significant negative correlation (rho= -0.55) between HbA1c and rate of wound healing among foot ulcer subjects (p<0.0001) was observed using Spearman rank correlation (Figure-2).

Table 1: Association of the baseline characteristics between the groups.

Factor		Group I HbA1c < 7%	Group II HbA1c 7-8%	Group III HbA1c >8%	Total	p-Value
Age (in years)		55.93±12.77	56.5±10.55	56.77±11.19	56.4±11.41	0.96
Age group	≤30	1 (3.33%)	1 (3.33%)	0 (0%)	2 (2.22%)	0.4713
	31-40	1 (3.33%)	0 (0%)	4 (13.33%)	5 (5.56%)	
	41-50	9 (30%)	8 (26.67%)	5 (16.67%)	22 (24.44%)	
	51-60	9 (30%)	9 (30%)	8 (26.67%)	26 (28.89%)	
	>60	10 (33.33%)	12 (40%)	13 (43.33%)	35 (38.89%)	
Gender	Male	25 (83.33%)	21 (70%)	21 (70%)	67 (74.44%)	0.3928
	Female	5 (16.67%)	9 (30%)	9 (30%)	23 (25.56%)	
HbA1c (%)		6.49±0.39	7.6±0.29	12.2±2.01	8.76±2.75	< 0,0001*
Duration of DM (in months)		105.4±83.16	121.77±88.32	129.43±79.88	118.87±83.52	0.5283
Duration of Diabetic mellitus category (in years)	0-3	8 (26.67%)	7 (23.33%)	4 (13.33%)	19 (21.11%)	0.8371
	4-8	10 (33.33%)	8 (26.67%)	8 (26.67%)	26 (28.89%)	
	9-13	3 (10%)	5 (16.67%)	9 (30%)	17 (18.89%)	
	14-18	6 (20%)	5 (16.67%)	5 (16.67%)	16 (17.78%)	
	19-23	2 (6.67%)	3 (10%)	3 (10%)	8 (8.89%)	
	≥24	1 (3.33%)	2 (6.67%)	1 (3.33%)	4 (4.44%)	
Duration of Ulcer	<1 week	11 (36.67%)	10 (33.33%)	7 (23.33%)	28 (31.11%)	0.4265
	1- 4 weeks	15 (50%)	11 (36.67%)	14 (46.67%)	40 (44.44%)	
	>4 weeks	4 (13.33%)	9 (30%)	9 (30%)	22 (24.44%)	
HTN	Yes	15 (50%)	14 (46.67%)	14 (46.67%)	43 (47.78%)	0.9564
	No	15 (50%)	16 (53.33%)	16 (53.33%)	47 (52.22%)	
Site of foot ulcer	PLANTAR	14 (46.67%)	12 (40%)	21 (70%)	47 (52.22%)	0.0506
	DORSUM	16 (53.33%)	18 (60%)	9 (30%)	43 (47.78%)	
Wagner grade	1	16 (53.33%)	13 (43.33%)	8 (26.67%)	37 (41.11%)	0.001*
	2	14 (46.67%)	17 (56.67%)	22 (73.33%)	53 (58.89%)	

HTN: Hypertension, *p< 0.05 is Significant

Table 2: Comparison of risk factors across the groups.

Risk factors		Group I	Group II	Group III	Total	p-value
NEUROPATHY	Yes	10 (33.33%)	14 (46.67%)	16 (53.33%)	40 (44.44%)	0.6610
	No	20 (66.67%)	16 (53.33%)	14 (46.67%)	50 (55.56%)	
PVD	Yes	7 (23.33%)	13 (43.33%)	8 (26.67%)	28 (31.11%)	0.7147
	No	23 (76.67%)	17 (56.67%)	22 (73.33%)	62 (68.89%)	
CULTURE	Yes	10 (33.33%)	16 (53.33%)	22 (73.33%)	48 (53.33%)	0.3261
	No	20 (66.67%)	14 (46.67%)	8 (26.67%)	42 (46.67%)	

PVD: Peripheral Vascular Disease

A: Group 1

B: Group 2

C: Group 3

Figure 1: Clinical photographs of ulcer on Day 0 (left hand side) and Day 14 (right hand side)
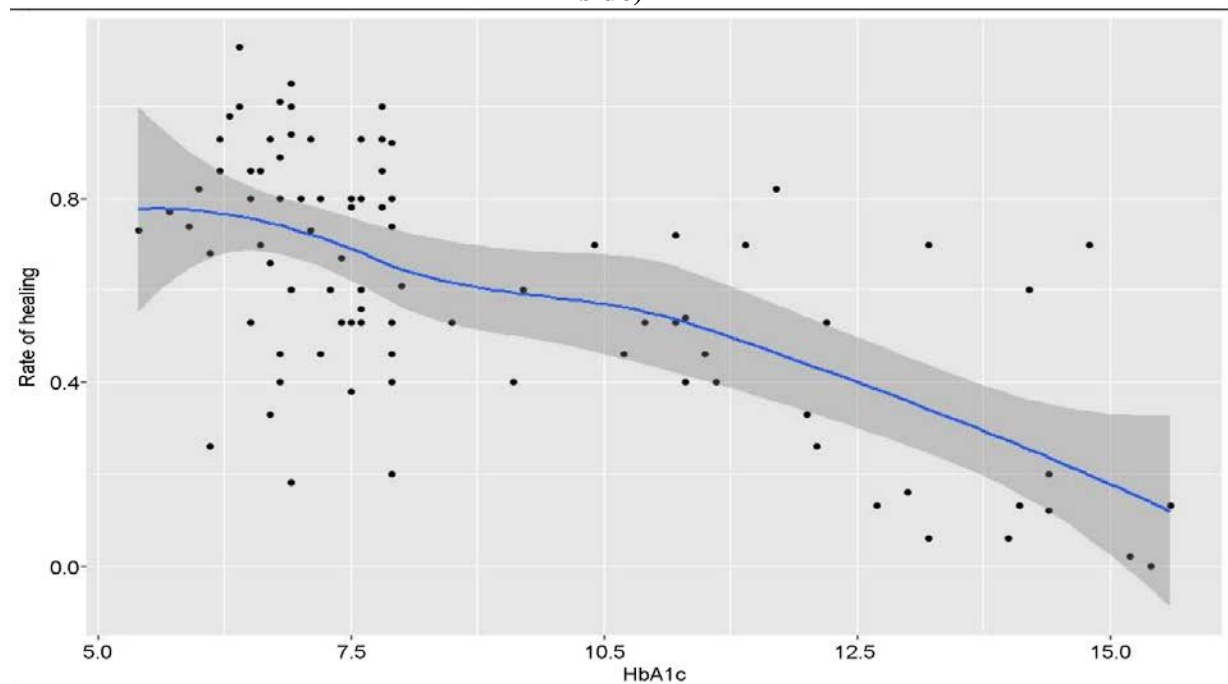

Figure 2: Correlation between HbA1c and rate of wound healing

Discussion

Glycemic control is impacted by the foot ulcer healing process and the severity of diabetes. Duration of the wound healing corresponds to HbA1c level.[6] Old age, peripheral arterial disease, neuropathy, female gender, long duration of diabetes and increased HbA1c levels influence DFU. [7] HbA1c level indicates the severity of diabetes, and subsequently it represents the risk of incidence of DFU. Additionally, increased HbA1c level is accompanied by partial and slow healing of DFU. [12] However, an Indian study reported that the DFU was observed among younger age group with a short duration of type-2 diabetes mellitus. This study reported that, the mean age of all participants was quite low when compared to studies conducted by Christman et al., and Xiang et al. [13,19] This study reported that there was a male predominance as compared to the female patients which was in line with studies conducted by Xiang et al., and Kumar et al. [1,13] A delay and incomplete healing of foot ulcer was associated with elevated HbA1c level in diabetic patients. HbA1c levels regulates the incidence of DFU and it can be used as a diagnostic tool to monitor severity of diabetes and its associated complications. [6,12] One more study reported that, DFU healing rate was efficient in the HbA1c level 7-8% group with well controlled compared to less than 7% and above 8%.[13] The mean HbA1c levels for the first-two groups was slightly higher than Christman et al., study and the total mean of three groups in this study was quite lower than the Younis et al., study.[7,19] Further, the duration of DM was lower than the Xiang et al., and Fesseha et al., studies for the first-two groups and for the mean of all subjects, respectively. [13,14] The duration of foot ulcer in Xiang et al., study for all the groups of HbA1c levels was more than 4 weeks, where as in our study only 13-30% of study population had a history of foot ulcer for more than 4 weeks in all the groups of HbA1c level including the total number of subjects. [13] In our study, when the baseline HbA1c was studied in

relation to the duration of the ulcer (in weeks), an interesting correlation was found. The number of patients with DFU at 1-4 weeks was comparable in Group 1(n=15; 50%) and Group 3 (n=14; 46.67%). When the wound healing was assessed at 4 weeks, Group 1(n=4; 13.33%) and Group 2 and 3(n=9; 30%) were observed indicating a fewer patients in Group 1 than Group 2 and 3. The effectiveness of HbA1c as a prognostic tool in the wound healing of DM is challenged with these proportionate results. The small size of the sample could be attributed to this commensurate result. Older studies have given conflicting views on this regard, which could possibly explain this observation. Fesseha et al., observed that the baseline HbA1c levels had no association with wound healing. [14] Guo and Dipietro explained the relationship between poor wound healing and hyperglycaemic subjects. [19] The state of hyperglycaemia could possibly lead to the accumulation of oxidative stress and release reactive oxygen species. Murine studies have concluded that the end products of glycation could deteriorate wound healing. [20] Hypertensive DFU patients were lower in this study as compared to Xiang et al., and Fesseha et al., studies for all the groups of HbA1c levels and the group of healing status, respectively. [13,14]

Tissue analytics provides softer solution which consists of AI to develop good quality data from clinical images automatically and objectively. The software has advantages of data availability, security, compliance, management, enhances favourable outcomes and is cost effective. [21] In Younis et al., study, patients with dorsal foot ulcer were less than the present study and patients with planter foot ulcers were more. [7] In our study, Wagner grade 2 patients' percentage was greater than Xiang et al., and Kumar et al., studies for all the classes of HbA1c and the total number of patients. [1,13] For Wagner grade 1, the number of patients were lower in our study as compared to Kumar et al., study. [1] The patients of neuropathy with ulcer were higher in Younis et al., and Dhatariya et al., studies compared to our study. [6,7] In Fesseha et al., study, the total number of patients with PVD was slightly higher than our study. [14] There is a need to educate the diabetic patient about DFU, importance of hygiene, self-assessment of foot temperature, usage of proper footwear, and good diabetes control. [4]

This study re-validated the findings that there was a negative correlation between HbA1c level and the severity of DFU among diabetic patients. HbA1c level was significant with the study groups (p< 0.0001). Wound culture was positive in two groups of HbA1c and with the total number of patients. In all the three groups, more prominent strains of bacteria found were Streptococcus pyrogens, Staphylococcus, Escherichia Coli (E-coli), Methicillin Resistant Staphylococcus Aureus (MRSA) and Pseudomonas. Limitations of this study was the absence of a long-term follow-up of foot ulcer patients as it was restricted to hospital stay of 15 days. Additionally, the identification of bacterial growth from ulcer and their sensitivity to antibiotics was not pursued.

Conclusion

HbA1c is the routine diagnostic tool to monitor the known type 2 DM patients, and the same was used to assess the rate of wound healing among DFU patients in this study. The study concluded that the HbA1c was a good indicator for wound healing process of DFU.

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