Original Research Article

Microbiologic Profile of Diabetic Foot Ulcers: A Trial of Honey versus Povidone Iodine Dressings

ABSTRACT

Diabetic foot ulcers (DFU), a debilitating complication of diabetes mellitus, are often infected. Infections impede wound healing and can lead to ulcer progression and possible mortality if poorly managed. Wound dressing is vital to DFU management, and supports the prevention and treatment of wound infections.

Aims: To compare the effect of honey and povidone iodine dressings on wound microbial colonization and infection for Wagner Grade 2 DFU.

Study design: This was a randomized controlled trial on the effects of honey and povidone iodine dressings on the microbiologic profile of Wagner grade 2 DFU at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt,spanning a year interval.

Methodology: Thirtypatients(13 females) aged 47 to 65 years with Wagner grade 2 diabetic foot ulcers were enrolled.Data on socio-demographics, and contaminating/infecting organisms were obtained from serial swab sample collection and microbiological investigation and analysed using Statistical Package for the Social Sciences (SPSS) 20.0. A p-value < 0.05 was considered significant.

Results: The mean HbA1c levels were 7.52±1.023% and 7.40±0.944% for the honey and povidone iodine groups respectively (p-value=0.727). Staphylococcus aureus, Escherichia.coli, Pseudomonas, Klebsiella, Proteus spp. were all cultured at baseline studies. S. aureus was the most prevalent throughout the study duration.

Conclusion: Polymicrobial ulcer contamination occurs commonly in DFU. Optimal wound care controls microbial activity, thereby promoting wound healing.

Keywords:Diabetic foot ulcer, wound dressing, povidone iodine, honey, microbiologic profile

1. INTRODUCTION

Foot ulcers are debilitating complications of diabetes mellitus, a disorder of abnormal glucose metabolism.[1] The prevalence of DM foot lesions is 0.9% to 8.3% in Nigeria.[2][3] About a sixth of diabetics develop diabetic foot ulcers (DFU) in their lifetime, with associated physical, psychological, and financial disability.[4][2]Over 80% of non-traumatic lower limb amputations are also attributable to DFU. [2][5] According to a 2004 analysis, chronic wounds cost about $9.7 billion in the US annually, making them the most expensive human skin condition in terms of direct medical costs.[6]

Neuropathy, angiopathy, traumaand impaired immunity act synergistically to produce ulcers and further impede the healing of these wounds [1][4] Excessive mechanical stress on the wound, tissue ischemia, or interstitial oedema usually

contribute to impair the healing of DFU [7]Autonomic dysfunction causes anhydrosis, dry and easily-cracked skin that permits bacterial invasion.[4]Advanced glycation end-products bind to tissues,initiate florid atherosclerosis, delay collateral vessel formation, cause thrombosis, and indirectly hinder the local antimicrobial mechanism, while impaired bacterial phagocytosis and cell-mediated immunity directly produce immunosuppression, infection.[4][2]Long-standing bacterial colonisation and infection by diverse pathogens are common complications of healing wounds.[7]

The Wagner Classification is a commonly used DFU grading system.[1][8] It considers the ulcer depth, occurrence of osteomyelitis, and the extent of tissue gangrene.[8] Wagner grade 2 ulcers are deep, with tendon, bone, ligament, or joint involvement. [1][8]

DFU infections are often polymicrobial.[2][4][9][10]*Streptococcus sp., Bacteroides, Pseudomonas, Proteus, Enterococcus, Escherichia coli, and Acinobacter*have been isolated severally, *S.aureus* being the most common. [11][12]Culture samples are best taken from deep tissues beneath the ulcers. [13] Culture-based approaches to microbial isolation is being replaced by highly sensitive molecular sequencing which targets the species-specific small subunit ribosomal RNA (16S rRNA) gene, and is able to characterize the wound microbiota based on microbial load, microbial diversity, and presence of pathogens. [10][14] However, this is not commonly available in developing countries. Studies have found quinolones, cephalosporins, gentamicin combined with metronidazole to be the most potent antibiotics against both gram-positive and gram-negative organisms in DFU and thus recommended them for empirical use.[9][15]

Wound healing is not necessarily impaired by bacterial colonization. [10][16]The administration of antibiotic based only on the isolation of microbe from the base of the wound is inappropriate and risks the development of multi-drug resistant microbial strains. [7][10] The clinical picture should be considered also.

The cornerstone of DFU treatment is multidisciplinary care with a focus on patient education, regular foot assessment, glycaemic control, and aggressive intervention (debridement, antibiotic therapy, and regular dressing). [2][6][17] Wound dressings should relieve symptoms, provide mechanical and antimicrobial protection, absorb exudates, control odour, be cost-effective and ultimately promote healing.[4][7][18][19] The choice of dressing is based on the wound features i.e. appearance and exudate.[18] There is however no model dressing for DFU.[6][18]

Honey is a supersaturated sugar solution prepared by bees, from nectar or other plant secretions.[20][21][22] The earliest medical literature gives evidence of honey's therapeutic benefits.[7] It contains carbohydrates - usually glucose and fructose(80–85%), water (15–17%), protein(0.1–0.4%), ash(0.2%), and small amounts of enzymes, amino acids, vitamins, and phenolic antioxidants. [21][22][23]It also contains hydrogen peroxide, and inhibin, and has high acidity(pH 3.2 - 4.2) which all impede bacterial growth.[19][11][24] Honey prevents biofilm development, reduces the ulcer's bioburden by debriding necrotic tissues, reduces inflammation and pain, and promotes granulation and epithelisation while minimizing scarring. [7][11][19][25] It deodorizes infected wounds by providing local bacteria with glucose and fructose as substitutes for amino acids from dead cells and serum, leading to the production of lactic acid instead of malodourous substances such as amines, ammonia, and sulphur. [26]Studies show that honey produced faster healing and wound size reduction than other conventional dressings.[7]Honey is potent against *Staphylococcus aureus (S. aureus), Pseudomonas aeroginosa, Klebsiella pneumoniae, Escherichia coli,* methicillin-resistant *S. aureus,* etc.[11][27]It is cheap, thus reducing patients' financial burden.[11] [41,42]

Povidone-iodine contains a loose blend of iodine and a non-ionic surfactant.[28][29][30] It easily penetrates cell membranes, and interrupts microbial protein synthesis, respiratory chain enzymes, lipid membrane, and nucleic acid activity.[29][30] Its potency is greatest at 0.1%–1% dilution due to weakened bonds between the carrier polymer and iodine molecules. This increases the quantity of free elemental iodine in solution which is lethal to bacteria, viruses, protozoa, fungi, amoebic cysts, and protozoa.[3][11] It eradicates all common nosocomial infection-causing microbes in under 20–30 seconds. [30] Povidone-iodine is also effective against bacterial biofilm, has a good safety profile and is widely tolerated.[30][31] Despite its extensive use, resistance or antibiotic cross-resistance to it is yet unreported since it acts on multiple bacterial targets.[30][31]

In an established infection, bacteria impede healing by depriving host cells of nutrients and oxygen, produce cytotoxic substances, and inhibit immune responses, leading to local hypoxia, thrombosis, tissue death, and ulcer deterioration.[10][16]Therefore, an understanding of practical, affordable, and accessible dressing materials that control infection would significantly improve DFU management.[7][11]

2. MATERIAL AND METHODS

2.1 Study Design

This randomized controlled study compared the microbiologic profiles of organisms contaminating healing Wagner Grade 2 DFU in patients receiving povidone iodine dressings versus honey dressings in UPTH between April 1st, 2017, and April 30th, 2018.

2.2 Sample Size Determination

The sample size for the study was calculated using the formula for comparison of groups [32];

$$n = \frac{2\,(Z\alpha + Z\beta)^2 s^2}{d^2}$$

where n= minimum sample size; $Z\alpha$ = significance level of 95%; corresponds to a value of 1.96;$Z\beta$ = power of 80%; corresponds to a value of 0.84; *S* = standard deviation; standard deviation of the rate of healing among patients with diabetic foot ulcers using honey dressing from a similar study was 0.94[14]*; d* = level of precision of 0.5.

$$n = \frac{2\,(1.96 + 0.84)^2\,(0.94)^2}{(0.5)^2} = \frac{2\,(7.84)\,(0.884)}{0.025} = 55.41$$

Allowing for an attrition of about 10%, the sample size was rounded up to 60.

Adjustment for population <10,000 using finite population correction[32]
Adjusted sample size = $\frac{n_0\,N}{n_0 + (N-1)}$
n_0 = minimum sample size = 60

N = Total population of DFU from the review of records (UPTH, 2016) = 47

Therefore, the adjusted sample size = $\frac{60 \times 47}{60 + (47-1)}$ = 26.6 approximated to 30

Hence, a total sample size of 30 comprising 15 patients per group was involved in the study.

2.3 Eligibility Criteria

The study particitants were diabetics with Wagner Grade 2 foot ulcers, aged 30-65 years (lower risk of co-morbidities).

2.3.1 Inclusion Criteria

They also fulfilled the following criteria:

a. Ankle brachial pressure index (ABPI) >0.9,
b. Serum albumin concentration >35g/dl.
c. Oxygen saturation of ≥92% by pulse oximetry

2.3.2 Exclusion Criteria

a. Patients who refused to give, or withdrew their consent.
b. Patients with multiple co-morbidities, severe immunosuppression, malignancy or chemotherapy, haemoglobinopathies, steroid therapy, and neutrophil count under 2000/mm^3.

2.4 Study Procedure

2.4.1 Randomization

The subjects were allocated into groups by simple randomizationusing an opaque envelope containing papers labelled “A” or “B”. A paper was randomly selected from the opaque envelope for each of the eligible subjects, who were then

assigned to the group labelled on the paper. Group A received honey dressing while Group B received povidone iodine dressing.

2.4.2 Blinding

The researcher was blinded to the dressing options to avoid bias and ensure the validity of the outcome measures. He was absent at the removal of old dressings, returned to assess the wound/ foot parameters, and left before the application of new dressings by trained nurses.

2.4.3Details of the Study

Honey obtained from a single source and 10% povidone iodine solution were used. All subjects received suitable antibiotics and their ulcers were surgically debrided by the researcher or trained orthopaedic residents. Tissue specimens excised from the ulcer bed were instantly sent for microscopy, culture and sensitivity analysis. Optimum glycaemic control was maintained under the supervision of a physician.

Dressing was performed daily. The wound was first cleansed with 0.9% saline, covered with honey or povidone iodine-soaked gauze supported by dry sterile gauze, and then bandaged.
A weekly wound assessment was performed by the researcher who was blinded to the material of the dressing. A wound swab was also taken from the ulcer bed and sent for analysis by a physician medical microbiologist. The assessment ended 6 weeks after the initial debridement or when the wound had healed, whichever occurred first.

The consumables used were honey, 10% povidone iodine (Betadine®), 0.9% saline, sterile cotton swabs, sterile gauze, crepe bandages, sterile swab sticks, sterile gloves and culture media (sheep blood agar, MacConkey agar, and Robertson cooked meat medium).

STUDY PROTOCOL

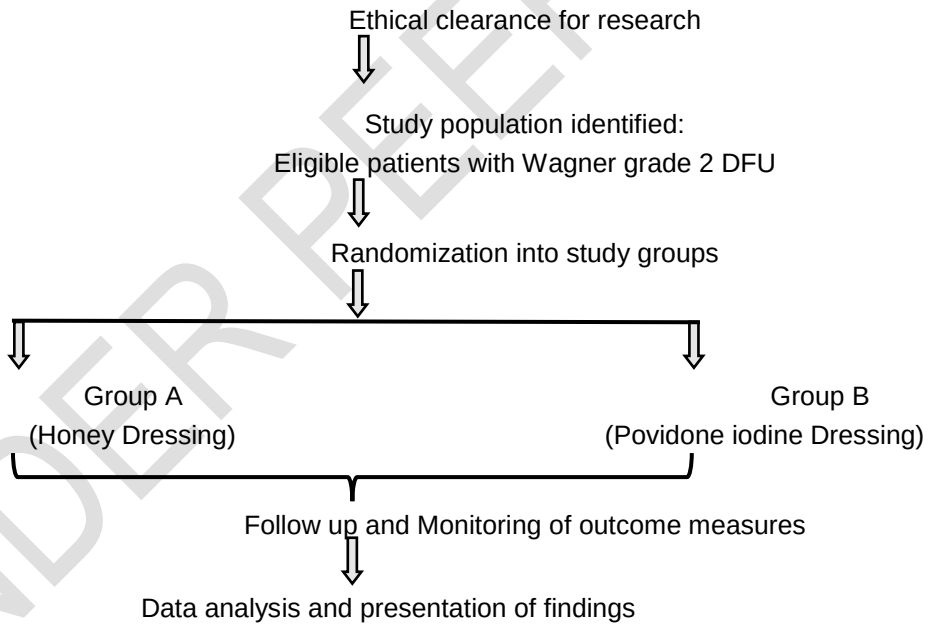


Figure 1: Diagrammatic Presentation of Study Protocol

2.5 Data Analysis

Data analysis utilized the IBM ® Statistical Package for the Social Sciences (SPSS) version 20. Data were presented as tables and charts. Qualitative variables such as age categories were expressed as frequencies and proportions while quantitative variables such as HbA1c were summarized as means ± standard deviation. Student's t test was used to compare the differences in means between the groups for data with normal distribution (such as HbA1c). Chi square test

or Fisher's exact test was used to compare the differences in proportions between the groups. A p value < 0.05 was considered statistically significant.

3. RESULTS

The study had 17 males and 13 females, aged 47-65 years with a mean 55.53±5.041 years and 54.93±5.298 years in the honey and povidone iodine groups respectively. There was no significant difference in age (p-value=0.266) and sex (p-value=0.713) between both study groups as depicted in Table 1.

Table 1: Demography of study groups

	Groups in the study					
	Povidone Iodine		Honey		Total	
Variables	**N=15**	**n (%)**	**N=15**	**n (%)**	**N=30**	**n (%)**
Age category						
45-49 years	3	(20.0)	4	(26.7)	7	(23.3)
50-54 years	4	(26.7)	0	(0.0)	4	(13.3)
55-59 years	5	(33.3)	7	(46.7)	12	(40.0)
>60 years	3	(20.0)	4	(26.7)	7	(23.3)
	Fisher's exact test=4.432;p-value=0.266					
Sex						
Female	7	(46.7)	6	(40.0)	13 (43.3)	
Male	8	(53.3)	9	(60.0)	17 (56.7)	
	Chi-square=0.136;p-value=0.713					

The HbA1c range was 6.3% - 10.2% in the povidone iodine group, and 6.7%-9.9% for the honey group. The mean HbA1c was 7.52±1.023% and 7.40±0.944% for the honey and povidone iodine groups respectively. This difference was not significant (p-value=0.727).

Staphylococcus aureus was the most prevalent organism isolated (63.3%) in the initial wound swab. Diverse organisms were cultured from three ulcers (10%) at the initial swab. In all the weeks of follow-up, *S. aureus* remained the most prevalent organism as shown in Table 2.

Table 2: Distribution of microbiological organisms identified across the study period

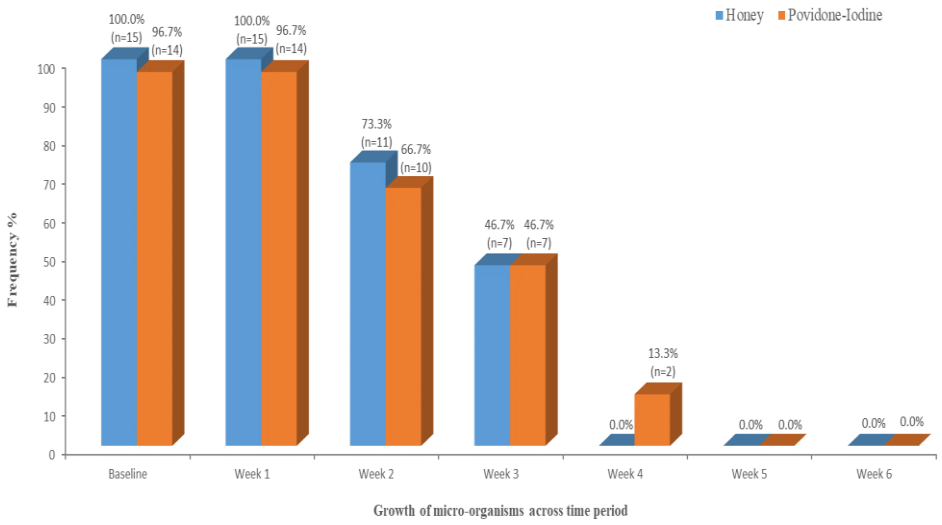
	Groups in the study		
	Honey	Povidone-Iodine	Total
Variables	**N=15 n (%)**	**N=15 n (%)**	**N=30 n (%)**
Baseline*			
S.aureus	8 (53.3)	11 (73.3)	19 (63.3)
E.coli	3 (20.0)	2 (13.3)	5 (16.6)
Pseudomonas	2 (13.3)	1 (6.7)	3 (10)
Klebsiella	2 (13.3)	0 (0.0)	2 (6.7)
Proteus	2 (13.3)	0 (0.0)	2 (6.7)
Week 1*			
S.aureus	11 (73.3)	11 (73.3)	22 (73.3)
E.coli	2 (13.3)	3 (20.0)	5 (16.7)
Klebsiella	1 (6.7)	0 (0.0)	1 (3.3)
Proteus	1 (6.7)	0 (0.0)	1 (3.3)
Week 2			
S.aureus	10 (66.7)	9 (60.0)	19 (63.3)
Pseudomonas	1 (6.7)	1 (6.7)	2 (6.7)

Week 3			
S.aureus	7 (46.7)	7 (46.7)	14 (46.7)
Week 4			
S.aureus	0 (0.0)	2 (13.3)	2 (6.7)

**Some of the DFUs in the groups had more than one micro-organism isolated. Weeks 5 and 6 reported no growth of micro-organisms*

At baseline, microorganisms were isolated from all patients in group A and all but one (96.7%) patient in group B. By week 4, none of the patients in the honey group (0.0%) had organisms isolated while two of the patients in the povidone iodine group (13.3%) had organisms isolated. By weeks 5 and 6, no microbial organism was isolated from the patients in both groups as seen in Figure 2.

Figure 2: Presence of growth of micro-organisms across time



Before hospital presentation, the wound care product most commonly used was antibiotic powder (23.3%). 13.3% of patients used multiple products while 10% used none. The difference between both study groups was not significant (p-value=0.658). See Table 3.

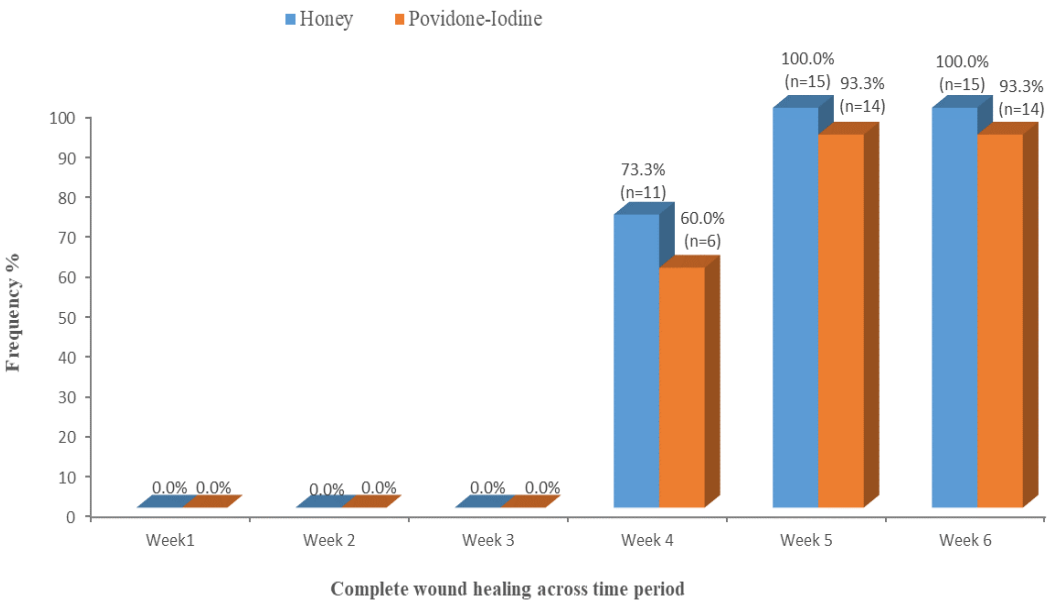
Table 3: Distribution of wound care products used by patients before hospital presentation

Products used	Groups in the study		Total
	Honey	Povidone-Iodine	
	n (%)	n (%)	n (%)
Iodine	1 (6.7)	3 (20.0)	4 (13.3)
Cetrimide	1 (6.7)	3 (20.0)	4 (13.3)
Gentian violet	2 (13.3)	1 (6.7)	3 (10.0)
Eusol	2 (13.3)	0 (0.0)	2 (6.7)
Hydrogen peroxide	2 (13.3)	1 (6.7)	3 (10.0)
Antibiotic powder	3 (20.0)	4 (26.7)	7 (23.3)
Combined products	3 (20.0)	1 (6.7)	4 (13.3)
Nil	1 (6.7)	2 (13.3)	3 (10.0)

Total	**15 (100.0)**	**15 (100.0)**	**30 (100.0)**

Fisher's exact test=5.846; p-value=0.658

Figure 3: Distribution of subjects with complete wound healing across the study period



4. DISCUSSION

The difficulty in finding patients who met the inclusion criteria - those whose wounds were supposed to heal as close to normal as possible, highlights the burden of DM complications, especially DFU. This indicates, as some writers have suggested, that most diabetics may already be prone to wound sepsis and other complications at their first presentation with ulcers. [1][3][17]

Both the honey and povidone iodine groups were comparable in terms of demographic characteristics, and HbA1c level, thus ruling them out as possible confounders. DFU chronicity and risk of infection correlate positively with poor glycaemic control as observed in studies. [33][34][35][36]Ignorance and socio-cultural influences such as belief in the supernatural causes of DFU contribute to late presentation as noted by authors such as Ogbera et al., in whose study 78% of respondents held such views.[37][38] This is still fairly common in the authors' practice

Staphylococcus aureus was the most prevalent organism isolated (63.3%) in the initial wound swab, possibly from self-contamination from patients' peri-ulcer normal flora, while one ulcer (3.3%) yielded no growth probably because the patient had self-administered antibiotics prior the sample collection. Several workers predominantly cultured *S.aureus* from DFUs in their studies.[11][12][15][39] In line with recent research also, 3 ulcers (10%) contained polymicrobial organisms at the first swab. [11][12][15]In contrast, Shukrimi*et al*[11] in a similar prospective comparative study in Malaysia, isolated more *Streptococcus sp*. (30%) than *Staphylococcus sp.* (16%), with (20%) of the ulcers harboring polymicrobial infections, while Mohammed *et al* reported a predominance of monomicrobial infections (77.3%) and *Candida albicans* in Egypt for unclear reasons[40]. This could be due to the differences in organisms that are prevalent in the different environments. Similarly, the majority of the ulcers yielded no growth towards the point of complete wound healing. Holubova et al surmised that antibiotic therapy was unnecessary because honey eradicated all, including the virulent microorganisms in ulcers with local signs of infection.[7]

Foot ulcers that were previously treated with cetrimide, iodine, hydrogen peroxide, or those that had no prior treatment before debridement in the honey group had an average healing time of 4.00±0.00 weeks. Accordingly, ulcers in the povidone iodine group that had previously received hydrogen peroxide, iodine, and gentian violet treatment healed in about 5.00±0.00 weeks. There was no significant difference in wound healing between both study groups based on the

individual wound care products used prior to this study. These products also did not appear to have influenced the nature of the colonizing microbiota in the course of the study. This is probably because before debridement, the ulcer beds contained varying amounts of slough, scab, and necrotic tissue that were excised giving rise to fresh ulcer beds that were not exposed to the applied substances. Furthermore, healing becomes progressive after debridement. [2][3][27]

This study's strength is in its design as a randomized controlled trial, which aims to offer significant information regarding the impact of honey versus povidone iodine on the microbiologic profiles of DFU. Nevertheless, because this was a single-center study, its generalizability might be limited; as a result, multi-center investigations are advised.

5. CONCLUSION

Microorganisms, most commonly *Staphylococcus aureus* readily colonize DFU at different stages of their healing, with multiple species often co-exiting within the ulcer.The mere presence of organisms in an ulcer does not necessarily impede its healing.

CONSENTAND ETHICAL APPROVAL

Ethical approval was obtained from the research and ethics committee of the UPTH before the commencement of the study. Written informed consent was obtained from all study participants after being given adequate information on the nature, scope, and reason for the research. Anonymity and confidentiality were upheld in the study. Participation in the study was voluntary, and patients' withdrawal from the study did not affect their medical care.

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