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Comparison of platelet-rich fibrin and povidone-iodine in the treatment of chronic cutaneous ulcers in Port Harcourt, Rivers State

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Abstract

Background: Patients with chronic skin ulcers undergo several wound dressing protocols to achieve wound healing. Povidone iodine and Platelet Rich Fibrin (PRF) are agents that can be used to accelerate tissue healing and cell regeneration. This study aims to compare the effects of both agents on chronic cutaneous ulcers.

Materials and method: The study was a prospective study carried out at the outpatient clinic of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State. Ethical approval was sought and obtained from the hospital's ethical committee. Half of the patients with chronic cutaneous ulcers were dressed with PRF, while the other half were dressed with povidone-iodine. Each of the groups was followed up for 8 weeks. The data were recorded on a proforma, entered in Microsoft Excel 2010 and analyzed with Epi-info version 7.02.

Results: The presence of infection and slough were commoner in the cutaneous ulcers treated with povidone-iodine with statistically significant values. The PRF group showed earlier signs of re-epithelialization. The PRF group attained higher frequencies for evidence of healthy granulation tissue early in the study period, with more ulcers healing before the eighth week. The wound dimensions reduced more speedily in the PRF group. The majority of the patients in the I group experienced more pain during dressing changes.

Conclusion: Platelet Rich Fibrin (PRF) conferred superior healing capabilities to the chronic cutaneous ulcers of the patients studied despite their varying aetiologies in comparison to povidone-iodine.

Keywords: Platelet Rich Fibrin; Povidone iodine; Chronic cutaneous ulcers; Wound healing, pain; Epitheliazation, wound contraction

1. Introduction

A chronic ulcer is a breach in the epithelium which has lasted for and beyond 12 weeks1. Such ulcers often pose a serious challenge to the plastic surgeon due to their tendency to persist and reoccur. In the normal routine of the Plastic Surgeon, a lot of chronic wounds or ulcers are encountered which contribute significantly to the bulk of the patients cared for2. These chronic ulcers, are often of varying aetiologies2. Extrinsic and intrinsic factors interrupting the normal healing process often result in the chronicity of ulcers3. However, in our environment, poor management of wounds in their acute phase plays a major role4. These chronic wounds are characterized by uncoordinated and haphazard processes of wound healing which has a counter-effect on the healing process thereby perpetuating the wound5. These ulcers include venous leg ulcers, arterial ulcers, pressure ulcers, diabetic ulcers, neurotropic ulcers and traumatic ulcers.

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The long duration of these ulcers, and the need for frequent hospital visits may lead to loss of income and man-hours, increased hospital costs, negative self-image and loss of patient's confidence in his or her doctor. The psychological impact of such a chronic wound on the patient is significant as it lowers his self-esteem, and leads to social alienation1. Chronic wounds as stated in a work by Harding "represent a major health burden and drain on resources"6.

The various approaches and strategies that have been employed all over the world in managing these chronic ulcers are basically two-pronged. First is to address and treat the primary cause of the ulcer, and secondly, to help the wound heal1,3. The strategies include the use of several wound dressing agents, the choice of which is based on the wound characteristics such as the degree of wound exudation, presence of cavities, exposure of vital structures like bone, tendon, nerves etc. and presence of slough7. In addition, the patient's demographics play a role in the choice of wound dressing agent employed7.

Some of the wound dressing agents commonly used locally in my institution, University of Port Harcourt Teaching Hospital, are povidone-iodine, gentamicin ointment, honey, hydrogels, hydrocolloids, alginate-based dressings and recombinant epidermal growth factor preparations. These help in reducing the wound bioburden or directly provide growth factors that aid in wound healing. However, due to its wide availability, affordability and ease of procurement by the end-users, povidone-iodine is one of the commonest utilized8.

Povidone iodine is an antiseptic agent used for skin preparation before surgical procedures9. It is also widely used for the dressing of open wounds9. It is a chemical complex made up of povidone, hydrogen iodide and elemental iodine9. Povidone is a water-soluble polymer made from monomer N- vinylpyrrolidone10 while hydrogen iodide is a diatomic molecule and a hydrogen halide, which is used in organic and inorganic synthesis as a primary source of iodine and as a reducing agent11. Elemental iodine is a stable halogen and found in the thyroid hormone12. Povidone iodine works by interfering with the enzymes of the respiratory chain of microorganisms, causing irreversible damage to them13. It is also on the World Health Organization's List of Essential Medicines9.

The application of platelet rich fibrin in chronic ulcers would be of great interest in plastic surgery14. Platelet Rich Fibrin (PRF) is a second-generation platelet concentrate which has been used in several surgical sub-specialties to accelerate tissue healing and promote cell regeneration15. PRF is an autologous, thus natural source of growth factors that are useful in wound healing and tissue regeneration16. Choukroun17 and his associates in 2001 in France, developed the PRF protocol which they employed in oral and maxillofacial surgery for bone healing.

The PRF is a fibrin matrix which consists of platelet cytokines, growth factors and cells and serves as a slow release medium for essential growth factors facilitating the wound healing process. It has been widely employed in dental surgeries in combination with bone grafts15. Some of the advantages of its use are the simplicity, ease and affordable means of harvesting and processing it. The platelet rich fibrin is completely autologous and may be more acceptable by people14. We are unaware of any study comparing the effects of povidone iodine and PRF in Port Harcourt and Southern Nigeria. We therefore carried out this study to compare the effects of both agents on chronic wounds.

2. Materials and methods

2.1. Study location

This study was carried out at the Outpatient Department of the University of Port Harcourt Teaching Hospital (UPTH). Approval for this study was obtained from the Ethical committee of UPTH management. The University of Port Harcourt Teaching Hospital is a major tertiary care hospital with a 500-bedded facility located in Port Harcourt, Rivers State65. The state has a population of five million, one hundred and ninety-eight thousand, seven hundred and sixteen (5,198,716) people66. The outpatient department records about 500 new cases every week. Orthoplast Surgical Consultants is a specialist private hospital that cares for patients with both Plastic and/or Orthopaedic surgical needs. It is also located in Port Harcourt and about 15 new cases are seen every week.

The study was done on an outpatient basis and each patient was followed up for an 8 week period till the endpoint of research was reached as stated in details further below. Biodata and contact details of all the patients recruited for the study were collected for easy tracing and to encourage compliance.

2.2. Study design

The study was a prospective hospital-based prospective quasi-experimental and observational study.

2.3. Study population

The study patients were adult patients being managed for chronic cutaneous ulcers of various aetiologies, located on different parts of their bodies, for which they were undergoing wound care at the outpatient department of both health facilities.

2.4. Inclusion criteria

- Patients with chronic cutaneous ulcers at any site of the body with dimensions ≤ 6cm x 6cm, who were willing to undergo the study and had given consent.
- Female and male patients in the age group of 18-65 years.
- Ulcers more than 12 weeks duration.
- Diabetic patients (having 1-3 above) with controlled blood sugar.
- Sickle cell disease patients (having 1-3 above) with haemoglobin levels of≥ 8g/dl.

2.5. Exclusion criteria

- Patients who were below 18 years and above 65 years.
- Patients with cardiovascular instability.
- Patients with haemoglobin levels below 10g/dl and sicklers with < 8g/dl.
- Patients with extensive chronic wounds exceeding the preferred dimensions of ≤6cm x 6cm area.
- Patients who had not given consent for the study.
- Patients with a history of bleeding disorder.
- Patients with clinical features of malignant ulcers.
- Patients with chronic osteomyelitis.
- Patients with cellulitis.
- Diabetic patients with uncontrolled blood sugar.
- Very ill patients.

2.6. Sampling method

Stratified random sampling method67 was applied for subject selection for patients who met the inclusion criteria. The patients were separated into different homogenous clusters based on age, sex, similar aetiology and wound characteristics of their ulcers. Then a random sampling was done within each subset to derive the two test groups – Platelet Rich Fibrin (P group) and povidone iodine (I group).

2.7. Sample size calculation

The sample size was determined using the Leslie Kish formula below15:-

The sample size required: $N = Z2\ P(1-P)/d^2$

N : sample size

Z :normal standard deviation set at 1.96, corresponding to 95% confidence interval.

P = Annual prevalence of chronic wounds at the outpatient clinic in my centre.

= no. of new chronic wound patients at the general outpatient clinic in the past 3years

otal no. of patients visiting the general outpatient clinic over a given period (3 years) (i.e. 2014 – 2016)

d:(confidence interval) = 0.05

P:221 = 0.02499 Ω 0.025

8,842

$$N = \frac{(1.962\ 0.025\ (1 - 0.025)}{(0.05)2} = \frac{3.8416 \times 0.025 \times 0.975}{0.0025}$$

$$N = \frac{0.093639}{0.0025} = 37.45\ \Omega\ 37.5$$

N = 37.5 + 10% attrition (3.75) = 41.25

Approximated to 42 patients.

2.8. Ethical clearance/informed consent

- Ethical clearance was obtained from the hospital ethical committee before the study was begun.
- All consultants whose patients were enrolled in the study were informed and their co-operation solicited.
- Informed consent was also obtained from all the recruited patients and confidentiality assured and maintained.
- A photographic release permission consent was also obtained from the study subjects.

2.9. Scope of the study

This was a hospital-based study aimed at comparing the outcome of chronic cutaneous ulcers management using topical Platelet Rich Fibrin and povidone iodine.

3. Methodology

A careful history was taken and physical examination was done. Also, investigations and interventions (listed below) were carried out in order to identify those that met the inclusion criteria. Patients whose ulcers had the clinical features of a malignant ulcer were not subjected to further histological studies for purposes of this study and were not recruited. Further histological testing and appropriate management were taken up by the respective managing teams in the hospital. Patients who were recruited for the study were sorted and matched according to age, sex, wound size, aetiology of the wound and medical comorbidities to create different subsets.

Then, balloting within each subset of patients was done to select the patients into P (PRF) and I (povidone iodine) groups. 'I' patients received the conventional wound dressing on a weekly basis for their chronic wounds (normal saline to clean and, sofratulle and povidone iodine-soaked gauze dressing), while for the P patients, normal saline to clean, PRF gel, and sofratulle were used for their weekly wound dressing. Full details of the dressing protocols for each group are explained below. The dressings were adequately padded (equal layers of gauze and cotton wool for matched patients) to prevent strike-through. However, when the need arose for more frequent dressing changes in some patients whose dressings got soaked before one week, a dressing change was requested for, and this was documented as a research finding. The researcher was assisted in this study by three surgical residents and two nurses who had been trained in the method of wound dressing change, measurement of the wound size, proper assessment of healthy granulation tissue

(uniformly red/ pink with a velvet appearance and no slough/wound discharge), and technique of processing the blood samples to obtain PRF.

3.1. PRF gel administration

This was administered by me aseptically in the dressing room of the outpatient clinic. The protocol for PRF gel for chronic wounds was adopted from the Choukroun protocol43 for PRF gel administration. This consisted of withdrawal of 10 millilitres of the patient's blood collected into two plain vacutainer sample bottles, each containing 5 millitres (mls) of blood. The blood was spun immediately (within 2 minutes of collection) in a centrifuge (Figure 1: Eschmed Medical England 800D model) in the outpatient department at 3000 revolutions per minute (rpm) for ten minutes.

The blood then separated into 3 layers (Figure 2). The topmost layer was straw-coloured and contained the acellular (platelet-poor) plasma, the middle layer was the gel-like fibrin clot, while the red bottom layer was the red blood cells.

The top layer was decanted and using sterile forceps, the fibrin clot was extracted, separating it from the red blood cells layer with the aid of sterile scissors (Figures 3 and 4). The PRF gel was then smeared over the wound (Figures 5 and 6) which had been previously cleaned with normal saline and then further dressed with sofratulle (serving as a non-adherent dressing layer), dry gauze and crêpe bandage (occlusive dressing). An aseptic technique was maintained at all stages. This dressing was left on for a period of 7 days before it was changed. The other patients were on a weekly wound dressing with normal saline to clean, then dressed with sofratulle and povidone-iodine soaked gauze, gamgee and crepe bandage.

The PRF administration was done once weekly and the progress of the ulcers monitored clinically by measurements of the wound dimensions, clinical judgment of the state of the wound as regards, formation of healthy granulation tissue, re-epithelialization, presence of exudates or slough, pain during and after dressing changes.

Figure 1 Eschmed Medical Centrifuge

3.2. Povidone iodine administration

10% commercial preparation of povidone iodine (Betadine) diluted with normal saline to get a 2% solution (1:5) was used. This was sourced from the hospital General Outpatient Department Pharmacy. The patients in the I group were dressed with normal saline, sofratulle and povidone iodine occlusively.

3.3. Routine for all study subjects

All wounds were dressed under sterile condition and all slough were mechanically removed at the commencement of the study. Some patients however, required repeat debridement in the course of the study. The patients were assessed on a weekly basis and their progress was charted in a proforma (see appendix).

Measurement of the dimensions of the ulcers was done using a sterile rule to measure the wound dimensions (the widest diameter or length of the longest side). This was documented every week. Clinical photographs were taken and compared. Furthermore, the cost of the dressings was calculated and documented. In addition, pain assessment during and after dressing changes was carried out using the visual analog pain scale for illiterates and the numerical rating pain scale for literate patients.

In order to ensure adequate haemoglobin levels and provide vitamins and elements essential for wound healing, all patients were placed on high dose multivitamins – caps Vitamin A 20,000 I.U. daily, caps Vitamin E 2,000 I.U. daily, tabs Vitamin C 500mg b.d, tabs Vitamin B complex ii b.d, tabs Fersolate 200mg t.d.s (except for sicklers) and tabs Folic acid ii daily (except those above 50 years).

Patients were followed up for a period of 8 weeks and the endpoint of the study was either a re-epithelialized ulcer or a non-healing chronic ulcer which had converted to a healing ulcer at the end of the 8 week study period. This was evidenced by a greyish/bluish hue of advancing epithelial cells at the edges of the wound and markedly reduced wound dimensions of up to two-thirds of the initial wound size.

3.4. List of consumables for wound dressing

- Sofratulle
- Gauze pieces
- Crêpe bandage
- Normal saline infusion
- Surgical gloves
- Disposable sterile rules.

3.5. Quality assurance statement for povidone iodine and platelet rich fibrin

Povidone iodine as a wound dressing agent has become a staple across various units in UPTH due to its effectiveness in eradicating and controlling wound infection. There have been no observed adverse effects in patients using it. It is also widely available in the hospital and local pharmacies. The brand that was used was Betadine which is a 10% solution of Povidone Iodine which was diluted with normal saline sourced from the hospital pharmacy to a 2% dilution.

PRF use is relatively new but based on published works, there has been no untoward effect from its use. It is completely autologous; hence eliminating concerns of hypersensitivity reactions and is potentially cost-effective.

3.6. Investigations and interventions conducted on patients

The following investigations were carried out in order to ensure safety of the patient

- and rule out complications like osteomyelitis.
- Full blood count (fortnightly)
- Bleeding time and clotting time (once at commencement of the study).
- Fasting blood sugar (at inception for all patients and weekly for diabetics).
- Relevant radiological investigations (at inception of the study).
- Cost of investigations was borne by the researcher.

3.7. Data collection

The cutaneous ulcers were assessed and data were recorded on a proforma (included as an appendix).

3.8. Data analysis

The data collected was entered in Microsoft Excel 2010. Data analysis was done using Epi-info version 7.02 (Centre for Disease Control (CDC), WHO). Descriptive statistics was done utilizing frequency and percentages for categorical variables and means and standard deviation for continuous variables. Inferential statistics analysis was carried out using Chi-square (x2) and Student T-test. Chi-square (x2) was used to compare categorical variables and Student T-test for continuous variables. A p-value of ≤ 0.05 was considered statistically significant.

4. Results

A total of 54 patients were recruited for the study and followed up for 8 weeks during the 9-month study period, assessing wound characteristics, degree of pain and need for analgesia, level of wound exudation, frequency of dressing change and cost of dressings. There were no adverse effects on the study patients as strict asepsis was maintained and haematological indices were closely monitored and tested. In addition, the reduced dressing frequency decreased the amount of blood lost during dressing changes.

Most (25.93%) of the patients studied were within the 50-59 years age bracket, were mostly male and were either businessmen or civil servants (Table 1). The least frequency was observed in those between 18-19 years. The ulcers were mostly diabetic ulcers with foot ulcers predominating (Table 2).

The group that was dressed with povidone iodine (I) showed greater levels of wound exudation (Table 9), which consequently increased their need for repeat dressings (Table 10), These findings were observed more at the beginning of the study, however, the numbers of these patients reduced as the study progressed but the values were still statistically significant (Tables 9, 10).

The ulcers of the patients in the P group had statistically lower infection rates (Table 3) than the patients in the I group. In addition, there was a lesser frequency in the presence of slough in this group (Table 4) and presence of foul odour in the wound prior to dressing changes (Table 8).

It was noted that the I group patients incurred more costs towards their wound management (Table 11) as they had a greater need for analgesia during dressing changes and had more frequent dressing changes (Table 10). There were statistically significant lower frequencies in degree of pain experienced during dressing changes in the P group than in the I group (Table 12).

The effect on wound healing time and skin cover requirements were assessed using evidence of re- epithelialization (Table 5), evidence of healthy granulation tissue (Table 6) and size of the wound over the weeks (Table 7). The results were statistically lower for the P group.

Table 1 Socio-demographic characteristics of study patients

Variables	**Frequency n=54**	**Percentage (%)**
Age group of respondents (in years)		
≤ 19 years	3	5.56
20-29 years	10	18.52
30-39 years	11	20.37
40-49 years	10	18.52
50-59 years	14	25.93
60-69 years	6	11.11
Mean	*42.44±13.76 years*	
Gender		
Male	41	75.93
Female	13	24.07
Occupation		
Business/Trader	14	25.93
Civil servant	12	22.22
Artisan	11	20.37
Student	6	11.11
Retired	4	7.41
Farmer	2	3.70
Unemployed	5	9.26

As can be observed in Table 1, the patients' ages ranged from 18 to 65 years with a mean age of 42.44 ± 13.76 years. The lowest frequency, 3 (5.56%) was seen in the ≤ 19 years age group while the highest frequency, 14 (25.93%), was in the 50-59 years age bracket. The patients were predominantly male, 41 (75.93%), being mostly business men or traders -14 (25.93%) and civil servants, 12 (22.22%). The lowest frequencies were observed in the farmers 2, (3.70%) and in the retired civil servants - 4 (7.41%).

Table 2 Relevant Medical History of the Study Patients

Variables	**Frequency n=54**	**Percentage (%)**
Diagnosis		
Diabetic foot ulcer	16	29.63
Diabetic hand ulcer	2	3.70
Post-traumatic ulcer (RTA, assault)	8	14.81
Post-infective ulcer	7	12.96
Venous ulcer	6	11.11
Sickle cell ulcer	5	9.26
Post-burns ulcer	7	12.96
Post-surgical wound breakdown	3	5.56
Smoking		
Yes	10	18.52
No	44	81.48
Alcohol intake		
Yes	21	38.89
No	33	61.11
Diabetes Mellitus		
Yes	18	33.33
No	36	66.67
Sickle Cell Disease		
Yes	5	9.26
No	49	90.74
Site of ulcers		
Lower limbs	34	62.96
Other parts of the body	20	37.04
Previous intervention		
Yes	28	51.85
No	26	48.15
Type of previous intervention (n=28)		
Debridement	16	57.14
Suturing	9	32.14
STSG	3	10.71
Duration of Ulcers		
≤4	12	22.22
5-9	24	44.44
10-14	8	14.81

≥ 15	10	18.51
Mean	*11.89±16.85 months*	
Previous Healing		
Yes	17	31.48
No	37	68.52
Analgesic use during dressing		
Yes	11	20.37
No	43	79.63
Analgesic use after dressing		
Yes	30	55.56
No	24	44.44

In Table 2, it can be seen that the aetiology of the ulcers was mostly Diabetes mellitus associated, with diabetic foot ulcers having the highest frequency of 16 (29.63%), followed by post-traumatic ulcers, 8 (14.81%) and post-infective and post burns ulcers, each with a frequency of 7 (12.96%). The least frequency was observed in the post-surgical wound breakdown patients, 3 (7.14%). A greater majority 34 (62 of the ulcers were observed in the lower limbs.

A total of 18 (33.33%) patients had Diabetes mellitus while 5 (9.26%) patients had sickle cell disease. 10 (18.52%) patients smoked while 21 (38.89%) took alcohol. 28 (51.85%) patients had previous interventions out of which 16 (28.57%) were previous wound debridements. 3 (10.71%) patients had had split thickness skin grafting (STSG) while 9 (32.14%) had undergone wound suturing.

Majority, (44.44%) of the ulcers had lasted for 5-9 months with a few (14.81%)

that had lasted for a duration of 10-14 months. The mean duration for all the ulcers was 11.89 ± 16.85 months. As can also be observed in Table 2, 37 (68.52%) patients had no previous healing of their ulcers, while 17 (31.48%) patients' wounds had healed previously. Prior to the commencement of the study, 11 (20.37%) patients required analgesics during dressing changes while 30 (55.56%) required some form of analgesics after dressing change.

Table 3 Comparison of Presence of Infection in Chronic Wounds Using PRF and Povidone Iodine

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**Chi-square (X2), df**	***p-value***
Week 1				
Yes	13 (48.15)	19 (70.37)	1.92, 1	0.166
No	14 (51.85)	8 (29.63)		
Week 2				
Yes	2 (7.41)	15 (55.56)	14.94, 2	*0.001**
No	24 (88.89)	12 (44.44)		
Healed	1 (3.70)	0 (0.0)		
Week 3				
Yes	0 (0.0)	12 (44.44)	15.99, 2	*0.0003**
No	23 (85.19)	14 (51.85)		
Healed	4 (14.81)	1 (3.70)		

Week 4				
Yes	0 (0.0)	9 (33.33)	11.68, 2	*0.003**
No	21 (77.78)	16 (59.26)		
Healed	6 (22.22)	2 (7.41)		
Week 5				
Yes	0 (0.0)	3 (11.11)	6.23, 2	*0.04**
No	18 (66.67)	21 (77.78)		
Healed	9 (33.33)	3 (11.11)		
Week 6				
Yes	0 (0.0)	6 (22.22)	9.30, 2	*0.01**
No	16 (59.26)	17 (62.96)		
Healed	11 (40.74)	4 (14.81)		
Week 7				
Yes	0 (0.0)	2 (7.41)	15.31, 2	*0.001**
No	8 (29.63)	20 (74.07)		
Healed	19 (70.37)	5 (18.52)		
Week 8				
Yes	0 (0.0)	4 (14.81)	10.61, 2	*0.005**
No	8 (29.63)	15 (55.56)		
Healed	19	8		

*Statistically significant (p<0.05)

From Table 3 above, infection rates were high in both study groups in the first week; 13(48.15%) for the P group and 19 (70.37%) for the I group. However, the pattern rapidly changed for the P group with infected wound cases falling to 2 (7.41%) in the second week, and this continued to fall over the succeeding weeks. The rates for the I group, however, declined gradually over the first few weeks till the fifth week, when the infection rates dropped more markedly.

Table 4 Comparison of the Presence of Slough in Chronic ulcers between PRF and Povidone iodine Groups

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**Chi-square (X2), df**	**p-value**
Week 1				
Yes	19 (70.37)	19 (70.37)	0.00, 1	1.00
No	8 (29.63)	8 (29.63)		
Week 2				
Yes	3 (11.11)	18 (66.67)	17.84, 2	0.001*
No	23 (85.19)	9 (33.33)		
Healed	1 (3.70)	0 (0.0)		
Week 3				
Yes	0 (0.0)	12 (44.44)	15.99, 2	0.0003*

No	23 (85.19)	14 (51.85)		
Healed	4 (14.81)	1 (3.70)		
Week 4				
Yes	0 (0.0)	7 (25.93)	9.23, 2	0.01*
No	21 (77.78)	18 (66.67)		
Healed	6 (22.22)	2 (7.41)		
Week 5				
Yes	0 (0.0)	4 (14.81)	7.11, 2	0.03*
No	18 (66.67)	20 (74.07)		
Healed	9 (33.33)	3 (11.11)		
Week 6				
Yes	0 (0.0)	3 (11.11)	7.71, 2	0.02*
No	15 (55.56)	20 (74.07)		
Healed	12 (44.44)	4 (14.81)		
Week 7				
Yes	0 (0.0)	2 (7.41)	15.31, 2	0.001*
No	8 (29.63)	20 (74.07)		
Healed	19 (70.37)	5 (18.52)		
Week 8				
Yes	0 (0.0)	4 (14.81)	10.61, 2	0.005*
No	8 (29.63)	15 (55.56)		
Healed	19 (70.37)	8 (29.63)		

*Statistically significant ($p<0.05$)

The presence of slough in the ulcers of the patients in the P group declined rapidly from 19 (70.37%) in the first week to 3 (11.11%) in the second week, and to 0 (0.0%) in the third week. This level was maintained till the endpoint of the study as can be seen in Table 4. For the I group, the rates also dropped sharply from the second week onwards from 18 (66.67%) to 7 (25.93%) in the fourth week. The findings here are statistically significant.

Table 5 Comparison of Evidence of Re-epithelialization between the PRF and Povidone iodine groups

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**Chi-square (X2), df**	**p-value**
Week 1				
Yes	2 (7.41)	1 (3.70)	0.00, 1	1.00
No	25 (92.59)	26 (96.30)		
Week 2				
Yes	15 (55.56)	5 (18.52)	9.67, 2	0.01*
No	11 (40.74)	22 (81.48)		
Healed	1 (3.70)	0 (0.0)		

Week 3				
Yes	22 (81.48)	9 (33.33)	21.47, 2	0.001*
No	1 (3.70)	17 (62.96)		
Healed	4 (14.81)	1 (3.70)		
Week 4				
Yes	21 (77.78)	12 (44.44)	15.45, 2	0.0004*
No	0 (0.0)	12 (44.44)		
Healed	6 (22.22)	3 (11.11)		
Week 5				
Yes	18 (66.67)	20 (74.07)	5.03, 2	0.08
No	0 (0.0)	3 (11.11)		
Healed	9 (33.33)	4 (14.81)		
Week 6				
Yes	15 (55.56)	22 (81.48)	3.09, 1	0.004*
Healed	12 (44.44)	5 (18.52)		
Week 7				
Yes	8 (29.63)	21 (77.78)	10.72, 2	0.001*
Healed	19 (70.37)	6 (22.22)		
Week 8				
Yes	9 (33.33)	19 (70.37)	6.0, 1	0.01*
Healed	18 (66.67)	8 (29.63)		

*Statistically significant ($p<0.05$)

In Table 5, the P group patients showed earlier signs of re-epithelialization from the second week with values of 15 (55.56%) compared to the 5 (18.52%) observed in the I group. .For both study populations, there were increasing rates of re-epithelialization with early cases of healing seen in the P group in the second week and in the I group in the third week. Nevertheless, the P group had statistically significant rates for all the weeks except the fifth week.

Table 6 Comparison of the evidence of healthy granulation tissue between the PRF and Povidone Iodine Groups

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**Chi-square (X2), df**	***p-value***
Week 1				
Yes	8 (29.63)	2 (7.41)	3.07, 1,	*0.08**
No	19 (70.37)	25 (92.59)		
Week 2				
Yes	19 (70.37)	6 (22.22)	14.76, 2	*0.001**
No	7 (25.93)	21 (77.78)		
Healed	1 (3.70)	0 (0.0)		
Week 3				

Yes	22 (81.48)	9 (33.33)	21.47, 2	*0.001**
No	1 (3.70)	17 (62.96)		
Healed	4 (14.81)	1 (3.70)		
Week 4				
Yes	21 (77.78)	18 (66.67)	7.23, 2	*0.03**
No	0 (0.0)	6 (22.22)		
Healed	6 (22.22)	3 (11.11)		
Week 5				
Yes	18 (66.67)	22 (81.48)	3.32, 2	0.190
No	0 (0.0)	1 (3.70)		
Healed	9 (33.33)	4 (14.81)		
Week 6				
Yes	15 (55.56)	22 (81.48)	3.09, 1	0.079
Healed	12 (44.44)	5 (18.52)		
Week 7				
Yes	9 (33.33)	22 (81.48)	10.91, 1,	*0.001**
Healed	18 (66.67)	5 (18.52)		
Week 8				
Yes	8 (29.63)	18 (66.67)	6.0, 1	*0.01**
Healed	19 (70.37)	9 (33.33)		

*Statistically significant (p<0.05)

Healthy granulation tissue was observed at statistically significant values for the P group from the first to the fourth weeks and then, in the seventh and eighth weeks, as depicted in Table 4.6. The highest values were recorded in the third week for the P group – 22 (81.48%) and a similar value for the I group from the fifth week onwards.

Table 7 Rate of reduction in the size of wound (cm) in the PRF and povidone iodine patient groups

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**t-test**	***p-value***
Week 1	4.51±1.05	4.63±1.07	0.38	*0.702*
Week 2	3.80±1.33	4.54±1.29	2.06	*0.04**
Week 3	3.39±1.42	4.35±1.47	2.32	*0.03**
Week 4	2.96±1.41	4.30±1.44	3.17	*0.003**
Week 5	2.77±1.46	4.23±1.42	3.22	*0.003**
Week 6	2.69±1.54	4.22±1.40	3.09	*0.004**
Week 7	3.44±0.83	4.20±1.16	1.69	*0.102*
Week 8	3.06±0.94	4.20±0.86	3.02	*0.005**

*Statistically significant (p<0.05)

There was a marked difference in the sizes of the wounds as observed for the P group in Table 7 from the second week till the endpoint of the study. The decline was gradual for the ulcers of the I group.

In Table 8, there was a noticeable reduction of the foul odour from the wounds of patients in the P group from the second week onwards compared to the patients' wounds in the I group. The highest rate of foul odour was observed in the third week - 19 (70.37%) in the I group but there was a marked reduction from the fifth week onwards.

Table 8 Comparison of the Presence of foul odour between the PRF and povidone iodine groups

Time	**Topical PRF Freq (%) n=27**	**Povidone iodine Freq (%) n=27**	**Chi-square (X2), df**	***p-value***
Week 1				
Yes	16 (59.26)	16 (59.26)	0.00, 1	1.00
No	11 (40.74)	11 (40.74)		
Week 2				
Yes	0 (0.0)	18 (66.67)	27.26, 2	*0.001**
No	26 (96.30)	9 (33.33)		
Healed	1 (3.70)	0 (0.0)		
Week 3				
Yes	0 (0.0)	19 (70.37)	29.43, 2	*0.001**
No	22 (81.48)	7 (25.93)		
Healed	5 (18.52)	1 (3.70)		
Week 4				
Yes	0 (0.0)	10 (37.04)	12.4, 2	*0.002**
No	21 (77.78)	14 (51.85)		
Healed	6 (22.22)	3 (11.11)		
Week 5				
Yes	0 (0.0)	5 (18.52)	6.92, 2	*0.03**
No	18 (66.67)	18 (66.67)		
Healed	9 (33.33)	4 (14.81)		
Week 6				
Yes	0 (0.0)	2 (7.41)	5.60, 2	0.06
No	15 (55.56)	20 (74.07)		
Healed	12 (44.44)	5 (18.52)		
Week 7				
No	9 (33.33)	21 (77.78)	9.08, 1,	*0.003**
Healed	18 (66.67)	6 (22.22)		
Week 8				
No	9 (33.33)	18 (66.67)	4.74, 1	*0.003**
Healed	18 (66.67)	9 (33.33)		

*Statistically significant ($p<0.05$)

Table 9 Comparison of the Presence of exudate in the PRF and Povidone iodine groups

Time	Topical PRF Freq (%) n=27	Povidone iodine Freq (%) n=27	Chi-square (X2), df	*p-value*
Week 1				
Yes	26 (96.30)	26 (96.30)	0.00, 1	1.00
No	1 (3.70)	1 (3.70)		
Healed				
Week 2				
Yes	2 (7.41)	26 (96.30)	42.73, 2	*0.001**
No	24 (88.89)	1 (3.70)		
Healed	1 (3.70)	0 (0.0)		
Week 3				
Yes	1 (3.70)	18 (66.67)	23.47, 2	*0.001**
No	23 (85.19)	8 (29.63)		
Healed	3 (11.11)	1 (3.70)		
Week 4				
Yes	0 (0.0)	8 (29.63)	10.42, 2	*0.01**
No	21 (77.78)	17 (62.96)		
Healed	6 (22.22)	2 (7.41)		
Week 5				
Yes	0 (0.0)	8 (29.63)	10.53, 2	*0.005**
No	19 (70.37)	16 (59.26)		
Healed	8 (29.63)	3 (11.11)		
Week 6				
Yes	0 (0.0)	5 (18.52)	9.27, 2	*0.01**
No	15 (55.56)	18 (66.67)		
Healed	12 (44.44)	4 (14.81)		
Week 7				
Yes	0 (0.0)	3 (11.11)	15.65, 2	*0.0004**
No	8 (29.63)	19 (70.37)		
Healed	19 (70.37)	5 (18.52)		
Week 8				
Yes	0 (0.0)	2 (7.41)	9.72, 2	*0.01**
No	8 (29.63)	17 (62.96)		
Healed	19 (70.37)	8 (29.63)		

*Statistically significant ($p<0.05$)

The values for wound exudation were higher in the I group than in the P group from the second week till the study ended. These values illustrated in Table 4.9 were statistically significant.

Table 10 Comparison of the Need for Repeat Dressings in the PRF group and Povidone iodine groups.

Characteristics	Topical PRF Freq (%) n=27	Povidone iodine Freq (%) n=27	Chi-square (X2), df	*p-value*
Twice weekly	0 (0.0)	10 (37.04)	22.74, 2	*0.001**
Thrice weekly	0 (0.0)	6 (22.22)		
No	27 (100.0)	11 (40.74)		

Table 10 above shows that the I group patients had more frequent dressing changes, with 10 (37.04%) patients on twice weekly dressing changes and 6 (22.22%) on thrice weekly wound dressing changes. Notably, some of these patients were converted back to the once weekly dressing changes as the study progressed. There were no repeat dressing changes for the PRF patients.

Table 11 Comparison of the Cost of dressing (N) between the PRF and Povidone iodine groups

Time	Topical PRF Freq (%) n=27	Povidone iodine Freq (%) n=27	t-test	*p-value*
Week 1	2608.52±227.61	3295.56±611.64	5.47	*0.001**
Week 2	2531.35±60.39	3290.00±615.66	6.25	*0.001**
Week 3	2515.00±0.00	3101.54±462.67	5.94	*0.001**
Week 4	2515.00±0.0	3060.00±437.32	5.70	*0.001**
Week 5	2515.00±0.0	3090.00±490.56	4.96	*0.001**
Week 6	2515.00±0.0	3024.78±385.96	4.91	*0.001**
Week 7	2515.00±0.0	3055.91±406.02	3.73	*0.001**
Week 8	2515.00±0.0	3074.21±408.95	3.82	*0.01**

*Statistically significant (p<0.05)

Table 11 shows that the cost of dressings were significantly higher in the I group from week 1 to week 8. For the P group, the highest cost was in the first week, with dressings averagely costing ₦2608.00 and ₦2515.00 in the later weeks. In contrast to the P group, the I group spent an average of ₦3295.56 in week 1and ₦3074.21 in week 8.

Table 12 Comparison of Pain assessment during dressing for the PRF and Povidone iodine groups

Time	Topical PRF Freq (%) n=27	Povidone iodine Freq (%) n=27	Chi-square (X2), df	*p-value*
Week 1				
Mild	2 (7.41)	1 (3.70)	0.98, 2	0.613
Moderate	18 (66.67)	16 (59.26)		
Severe	7 (25.93)	10 (37.04)		
Week 2				
Mild	16 (59.26)	2 (7.41)	20.22, 3	*0.0002**
Moderate	10 (37.04)	20 (74.07)		
Severe	0 (0.0)	5 (18.52)		
Healed	1 (3.70)	0 (0.0)		

Week 3				
Mild	23 (85.19)	3 (11.11)	40.18, 3	*0.001**
Moderate	0 (0.0)	20 (74.07)		
Severe	0 (0.0)	3 (11.11)		
Healed	4 (14.81)	1 (3.70)		
Week 4				
Mild	21 (77.78)	0 (0.0)	46.0, 3	*0.001**
Moderate	0 (0.0)	23 (85.19)		
Severe	0 (0.0)	1 (3.70)		
Healed	6 (22.22)	3 (11.11)		
Week 5				
Mild	18 (66.67)	0 (0.0)	42.92, 3	*0.001**
Moderate	0 (0.0)	22 (81.48)		
Severe	0 (0.0)	1 (3.70)		
Healed	9 (69.23)	4 (14.81)		
Week 6				
Mild	14 (51.85)	2 (7.41)	32.56, 3	*0.001**
Moderate	0 (0.0)	19 (70.37)		
Severe	0 (0.0)	1 (3.70)		
Healed	13 (48.15)	5 (18.52)		
Week 7				
Mild	8 (29.63)	2 (7.41)	29.36, 3	*0.001**
Moderate	0 (0.0)	18 (66.67)		
Severe	0 (0.0)	1 (3.70)		
Healed	19 (70.37)	6 (22.22)		
Week 8				
Mild	8 (29.63)	2 (7.41)	23.17, 3	*0.001**
Moderate	0 (0.0)	15 (55.56)		
Severe	0 (0.0)	1 (3.70)		
Healed	19 (70.37)	9 (33.33)		

*Statistically significant ($p<0.05$)

At the commencement of the study, more patients, 18(66.67%), in the P group experienced moderate levels of pain than in the I group as can be seen in Table 12 above. In week 2, their pain level had decreased to mild in 16 (52.38%) patients in the P group. This mild level was observed uniformly for all of the study patients in the P group till the endpoint of the study was attained. For the I group more of the patients experienced moderate levels of pain from the beginning till the study ended.

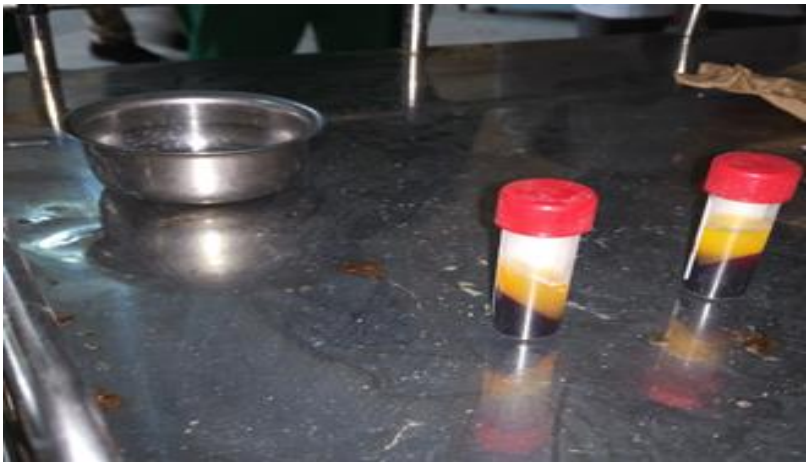

Figure 2 Centrifuged sample

Figure 3 and 4 PRF held between a pair of forceps

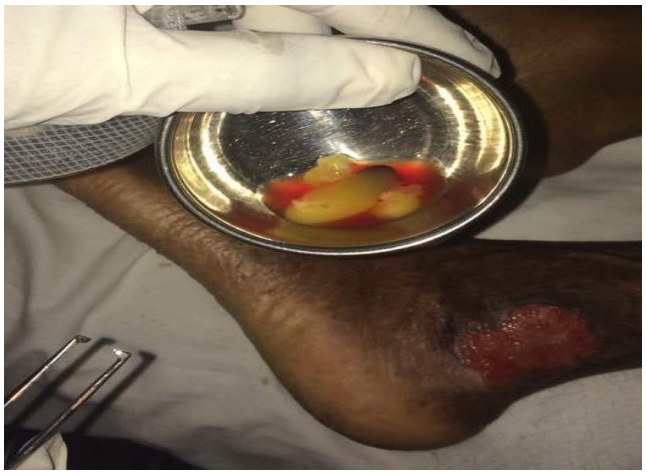

Figure 5 PRF just before application

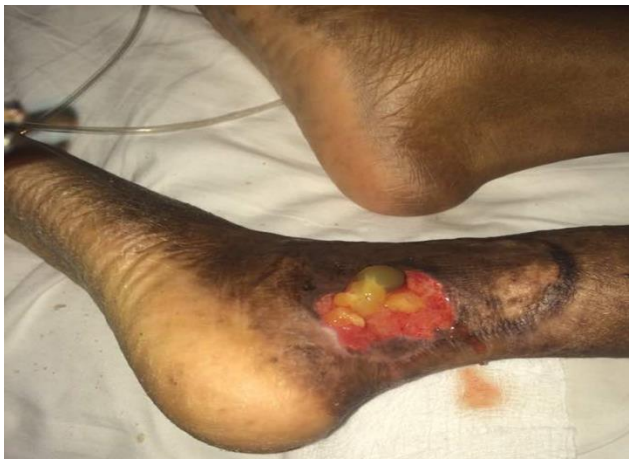

Figure 6 PRF after application

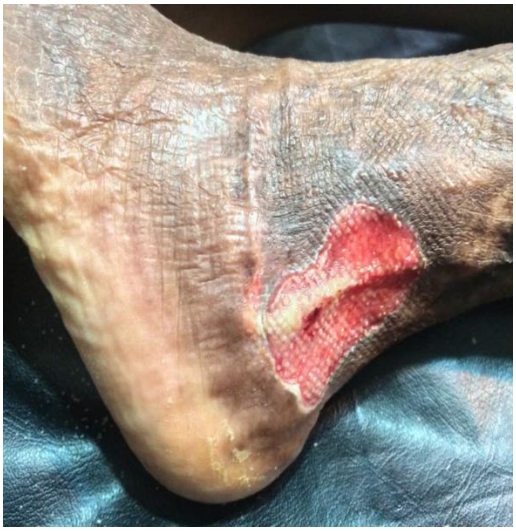

Figure 7 Ulcers after week of PRF dressing

5. Discussion

The management of chronic ulcers demand significant time and resources from both the Plastic surgeon and the patient18. Several strategies have been used to facilitate the wound healing process which include the use of antimicrobial agents such as povidone iodine and more recently, the application of platelet rich fibrin (PRF) which concentrates growth factors and platelet cytokines at the wound, accelerating healing13,14. The desire to search for more effective means to accelerate wound healing in the resource-challenged environment of our hospital, motivated this study.

From the study it was observed that the patients in the P group had lower cases of infected wounds, presence of slough, foul odour and decreased wound exudation than the I group. This finding may be attributed to the effects of various chemokines and growth factors in PRF, one of which is Human beta-defensin-3 (hBD-3). According to Bayer et al,17"Human beta-defensin-3(hBD-3) is an antimicrobial peptide inducibly expressed in human keratinocytes especially upon wounding". Other immunity-conferring growth factors are also elaborated in the centrifugation process and corroborated by several authors14. In a similar study carried out on chronic wounds utilizing Leucocyte – Platelet Rich Fibrin (L-PRF) as a dressing material by Pinto et al18, it was observed that the foul odour emitted from some of the chronic ulcers of their study group reduced dramatically with PRF use.

The earlier and faster rates of re-epithelialization, as evidenced by a greyish-blue hue of advancing epithelial cells at the wound edges, seen in the PRF group, as compared to the povidone iodine group may be attributed to the superior ability of PRF in tissue regeneration and enhancing keratinocyte proliferation 14,19. However, another study carried out by Bayer et al20 on the effect of platelet-released growth factors on primary human keratinocytes did not attribute these positive clinical findings to increased keratinocyte proliferation by the platelet concentrates.

It was also observed that the patients in the P group had higher frequencies and faster rates for formation of healthy granulation tissue. This finding corroborates those documented by earlier researchers like Desai 14 who noted that PRF accelerated wound healing and granulation tissue formation and also possessed anti-inflammatory properties. The term 'modified secondary intention wound healing' was coined from their utilization of PRF to heal wounds.

In tandem with the other positive signs of accelerated wound healing noticed in the P group, was also the remarkable sustained decrease in size of the ulcers. The dimensions of the ulcers reduced with a similar progression consistently throughout the duration of the research and the frequencies for healing were higher in the P group. The results obtained were statistically significant from the second week onwards and corroborates similar findings by Pinto et al18.

The cost of using PRF to dress the ulcers was significantly lower due to the simplicity and autologous nature of the procedure. In addition, the need for repeat/ more frequent dressings observed with the I group also directly increased the cost of wound treatment in this group. The lower cost of using PRF to dress chronic ulcers was a significant finding noted by several other researchers 18,19. For the purposes of this study, equal amounts of dressing consumables were

prescribed for all the patients at the beginning, but some patients in the I group required more dressing materials and analgesia during dressing change which increased their costs. In the period studied, table 11 showed that the P group spent more money than the I group. For example, an average of ₦2608 was spent by the I group in week 1 while the P group spent an average of ₦3295 in the same week. The reason for the higher cost was probably due to the need for more pieces of gauze to pad the wounds; the need for analgesia and the cost of the brand of Povidone iodine (Betadine@1L) used, which cost ₦7000 and averaged at ₦875 per week whereas the cost of spinning the blood for PRF synthesis cost only ₦600 per week. Conclusively, PRF use in dressing chronic ulcers has been determined to be significantly cheaper than using povidone iodine.

The effect of PRF on pain modulation was evident with statistically significant numbers of the patients in the P group recording mild levels of pain as the study progressed from the second to eighth weeks. This finding confirms the pain-modulating action of PRF on wounds as observed by Lokman et al21, in a study they carried out which showed that PRF reduced both post-operative pain, trismus and number of analgesics taken, in studied patients who underwent surgery for an impacted third molar. The decreased need for analgesia was also a research finding by Pinto et al[18] which is a remarkable feature for a wound healing/dressing agent as it may ensure patient acceptance and compliance.

In this study, majority, 14 (25.93%) of the study subjects fell within the 50-59 years age bracket. This finding differed from previous findings by Guo and Di Petro 22 and also by Gould 71 et al where it was found out that the incidence of chronic wounds was higher in elderly patients (above 65 years) with ulcers like diabetic ulcers and venous ulcers being commoner in older patients because of a higher incidence of co-morbidities. A study carried out by Shubhangi 23 also noted the higher incidence of chronic wounds as the age increased. The lower incidence in the patients in the above 60 years group in this study may be from the fact that the average life expectancy of the Nigerian male/female is 55/56years24.

More males than females were recruited for the study which may be due to the fact that acute wounds have been shown to heal more slowly in males, possibly leading to chronicity in males than in females.[25] This may be due to the effects of oestrogen which has been demonstrated by several researches to hasten wound healing. These researches were carried out on post-menopausal women or ovariectomized rats26. On the other hand, androgens impaired wound healing as documented by Ashcroft and Mills27. There were more business men/traders and civil servants who were recruited for the study. It is well known that educated people, who are relatively better informed than illiterates tend to seek orthodox care more and that may be the reason for higher levels of chronic cutaneous ulcers seen in these patients.

The study in addition, demonstrated a total higher incidence of diabetes mellitus-associated ulcers with the least frequent aetiology being post-surgical wound breakdown. Diabetes mellitus incidence rate in the country has been increasing together with diabetes mellitus associated complications like the diabetic foot ulcers77-80, which constituted the largest single group in this study.

6. Conclusion

PRF demonstrated superior capabilities in the healing of chronic ulcers of various aetiologies compared to povidone iodine. Its potential to re-initiate healing in indolent ulcers and induce the formation of healthy granulation tissue promptly was remarkable. In addition, the process of dressing the wounds once weekly with PRF was relatively pain-free and cheaper compared to conventional dressings while the patients experienced less wound exudation, decreased presence of slough and reduction of foul odour from their wounds.

Limitations of the study

- The povidone iodine group and PRF group comprised of different individuals with different innate healing capabilities which may indirectly have affected their wound healing rates.
- The ulcers were from different aetiologies.
- The chronic wounds occurred in different sites of the body which may have affected their wound healing rates.
- The study was time bound.

Recommendations

- PRF may be adopted as a routine wound healing agent in chronic wound management in resource poor hospitals.
- A repeat of the study may be done comparing PRF with more advanced wound care products to determine if similar results will be obtained.

- Studies to compare the strength of the newly formed skin in both study groups may also be informative.
- Trials to determine the effect of PRF on wounds of patients prone to keloid formation are advocated.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Ethical approval was sought and obtained from the hospital's ethical committee.

Statement of informed consent

Informed consent was obtained from each participant who presented for the study.

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