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The Effectiveness of Hypochlorous Acid Solution on Healing of Infected Diabetic Foot Ulcers

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Abstract:
Introduction: Wound cleansing remains a corner stone in the management of diabetic foot ulcer. Hydrogen Peroxide (H_2O_2) and Povidone Iodine are topical antimicrobial agents but known to be toxic to cells involved in the wound healing cascade. The biggest challenge for the physicians and nurses is searching for a safe, non-cytotoxic and effective wound cleansing as Hypochlorous acid (HOCL). This study aimed to compare the efficacy of HOCL versus H_2O_2 followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers. **Patients and methods:** Design: used a randomized clinical trial design to compare the efficacy of HOCL versus H2O2 followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers. Setting: This study was carried out in outpatient plastic and vascular surgery clinic at Assiut university Hospital. Subjects: A random selection was performed on 60 patients with infected diabetic foot ulcers divided equally to control and study group. Methods: HOCL was used for the study group, as irrigate the wound with HOCL in a concentration; sterile Nacl 0.9% to HOCL 50.5% at ratio 8:2 and leave for 5 minutes, before covered the wound with sterile dressing. While H_2O_2 followed by Povidine Iodine used for the control group and the results were compared. Bacterial cultures were obtained before start washing, after five days thin each five days to the end of the study for the two groups. **Results:** HOCL was able to be effective against Candida, Proteus, Klebsella, Psudomonas, and Methicillin resistant staphylococcus aureus (MARSA) compare to H2O2 and Povidine iodine. There is a statistically significant difference between using HOCL and H2O2 and Povidine iodine as a washing therapy in reducing bacterial count, wound pain, odor, discharge and improve wound healing in diabetic foot ulcer. **Conclusion:** Hypochlorous acid is a potent antimicrobial cleanser against a wide range of microorganisms. Hypochlorous Acid is safe, low cost, painless, easy to perform, and improve wound healing or rapidly prepared diabetic foot ulcer for skin flap or graft. **Recommendation:** The study recommended use of HOCL as a virtuous diabetic wound care cleanser. Further research is needed on a larger scale to validate the effectiveness of Hypochlorous acid as a wound care agent in septic diabetic foot ulcers.
Keywords: Hypochlorous Acid, Hydrogen Peroxide, Infected diabetic foot ulcer, Povidine Iodine.

1. Introduction

Diabetic foot ulcer is one of the most serious complications of diabetic and is the leading cause of non-traumatic lower limb amputations.(Sampson & Sampson 2008) Factors that affect development and healing of diabetic foot ulcers include the presence of ischemia or infection, the degree of metabolic control, and continuing trauma to feet from excessive plantar pressure or poorly fitting shoes (Yazdanpanah et al. 2015).

If a standardized treatment approach is applied with a multidisciplinary foot care team, major amputations can be avoided in about 95% of patients with infection.(Wu et al. 2015)

A critical part of diabetic wound bed preparation including treating infection and quantitative reduction of bacteria to a level that is treatable by the immune system, through aggressive wound cleansing with a prepared non-cytotoxic wound cleanser.

Hydrogen Peroxide, and Povidone Iodine are topical antimicrobial agents but known to be toxic to cells involved in the wound healing cascade so that impede healing (Dumville et al. 2013). While HOCL is characterized by an influx of immune cells that destroy and remove bacteria, cellular debris, and necrotic tissue (O'Meara et al. 2013) .

HOCL is a weak acid formed by the dissolution of chlorine in water. Its conjugate base HOCL– is the active ingredient in bleach and the chemical species responsible for the microbiocidal properties of chlorinated water. However, in mammalian systems it is also responsible for destroying many pathogens (Winterbourn 2009; Dennis et al. 2011)

Innate immune cells can sense pathogens both chemotactically and by direct physical contact, resulting in Phagocytosis by Neutrophil (Harrison & Schultz 2011). Once Phagocytosis is accomplished, HOCL which is generated in activated neutrophils by Myeloperoxidase-mediated peroxidation of chloride ions, act as a bactericidal activity, through selective inhibition on RNA and DNA synthesis of the bacteria, and so it prevents replication of bacteria. Which will create an optimum local and systemic conditions for diabetic foot ulcer healing with reducing pain, malodour and improve healing (Sampson & Muir 2002;Morrison 2010; Malli 2010)).

Wound management is an area of nursing practice that has a presence in all of the specialties within

the health-care setting, basic nursing care practice encompassing dressings which mainly a nursing practice and infection control but also managing infection requires careful attention to properly diagnosing the condition, obtaining appropriate specimens for culture, thoughtfully selecting empirical and then definitive antimicrobial therapy, quickly determining when surgical interventions are needed and providing all other necessary types of wound care (Morison & Ovington 2010; Wilkie 2011).

The nurse and physician should be careful closely monitor, observing and recording of characteristics of the wound, not to be focus on the wound only but to the extent that the patient is not treated as total person, also should alert to changing facial expressions during dressing to do not affect the patient psychologically(Bale 2012; Minnis 2001).

Significant of the study

Recent reports from doctors and nursing staff in plastic and vascular surgery clinic at Assiut University Hospital pointed out increasing incidence of diabetic ulcers infection that associated with substantial morbidity, discomfort, reduced physical and mental quality of life, which need for wound care, antimicrobial therapy, and often surgical procedures. Furthermore, foot infection remains the most frequent diabetic complication requiring hospitalization and the most common precipitating event leading to lower extremity amputation. The number of patients with diabetic foot ulcers that following up to plastic and vascular surgery clinic of Assiut University Hospital at 2015 was 7551 cases according to the Hospital statistical record.

2. Patients and methods

2.1 Aim of the study:

This study aim to compare the efficacy of Hypochlorous Acid versus Hydrogen Peroxide followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers.

2.2 Research Hypothesis:

Infected diabetic foot ulcers that were washed by Hypochlorous Acid solution will have fewer exudates, odorless, and faster healing. Also patient will have less pain and good life style than those wounds that were washed by Hydrogen Peroxide followed by Povidine Iodine.

2.3 Research Design:

A randomized clinical trial design was utilized in this study. This design used to compare the efficacy of Hypochlorous Acid versus Hydrogen Peroxide followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers.

2.4 Setting:

This study was carried out in outpatient plastic and vascular surgery clinic at Assiut university Hospital.

2.5 Subjects:

Simple random samples of 60 patients who were followed up in outpatient plastic and vascular surgery clinics at Assiut university Hospital between March 2016 and November 2016. They were randomly assigned into two equal groups, study and control group (30 patients each). The study group used HOCL solution in dressing, while the control group used routine hospital care H2O2 followed by Povidine Iodine for wound care.

2.5.1 Inclusion criteria:

The patients had been selected according to the following criteria:

- Age more than 22 years and less than 60 years.
- Both sex, conscious and alert.
- Infected diabetic foot ulcers.

2.6 Tools of data collection:

2.6.1 Tool I: Structure interview questionnaire: it was developed and utilized by the researcher based on literature review to assess data for patients about: Socio-demographic data as (age, gender, occupation, and medical data as laboratory investigations…..etc.

2.6.2 Tool II: Diabetic foot ulcer assessment sheet:

This sheet was developed by the researcher to initial wound assessment using an observation sheet which includes wound bed exudates, color, amount and odor, frequency of wound pain, and condition of the wound.

2.6.3 Tool III: Evaluation sheet: it includes two parts:

2.6.3.1 Part One: This sheet was developed by the researcher to evaluate: Infection signs and symptoms which include exudates' amount, color and odor, and level and frequency of pain. It was used at the initial assessment of the infected diabetic foot ulcer, at start, at every dressing and at the end of the 30 days which the end of the study.

2.6.3.2 Part Two: Microbiological measurement was assessed by using a sterile swab that was pressed on the wound to express underlying fluid and exudates. It was taken for determining types of bacteria and also for microorganism count at the start, and every 5 days until the end of the study.

3. Methods

3.1 Administrative approval: A written approval was obtained from the director of outpatient plastic and vascular surgery clinic at Assiut University Hospital to carry out the study, the aim of the study was explained to them to obtain their cooperation.

3.2 Tools development: The study tools were developed by the researchers after extensive review of the relevant literature.

3.3 Validity: This tool was tested for content validity by seven experts in the field of nursing and surgical specialists. Modifications were done accordingly, and then the tool was designed in its final format.

3.4 Ethical consideration: Consent: Oral consent was obtained from patients to participate in this study. The researchers initially introduced themselves to all potential subjects and they were assured that the collected data were absolutely confidential. They were informed that participation is voluntary and they can withdraw at any time of the study.

3.5 Pilot study: A pilot study was conducted before starting data collection on six patients who were included in the sample to test the clarity, and applicability of the tool and to estimate the time required to fill the sheet. Modifications were done as needed.

4. Data collection:

The data collection was done in the following phases:

4.1 Assessment phase:

The researcher did interview with the patients individually and got their oral consent to participate and they answered the questions in the interview questionnaire. Initial assessment of the wound condition was done and recorded.

4.2 Implementation phase:

Hypochlorous acid was used as antiseptic wash for the study group, while Hydrogen Peroxide followed by Povidine Iodine for the control group. A standardized sheet recording patient's details include age, sex, clinical history, wound assessment and special investigations. Clinical response was recorded and reported. The wound condition also was recorded by a serial of photographs and special investigations before, during and after completion of the treatment.

Exudates: A sterile swab was taken from the exudates for quantitative and type of microorganism at the start, and every 5 days till the end of the treatment regimens. Odor was assessed and recorded as none, before, at, or after dressing removal. The nature and amount of exudates were also assessed and recorded as high in which the dressing was soaked with discharge, and the patient need twice daily dressing, moderate, when the patient needed once daily dressing , or low exudates, when dressing was changed every other day.

Color: exudates color was assessed as purulent (highly amount of pus and dead tissue), sanguimous (bloody purulent), serosanguimous (bloody stained serous exudates), serous (clear serous fluid), and none.

Pain: All wounds were assessed by the researcher every visit in study and control group according to standard of care. At each visit, the degree of pain was assessed using the developed questionnaire and pain tolerance scale, patients were asked to report their pain as it happens as the following forms: none, only during dressing, intermittent or continuous.

New healthy granulation tissue growth:

The depth and widest of the wound were measured every five days using a plastic measuring tab to evaluate the new granulation tissue formation and healing.

4.3 Procedure: Patient received either usual routine treatment (control group) or treatment of the wound with Hypochlorous acid (study group).

The study group: remove the old dressing away of the wound, then carefully wash the wound by using soaked gauze with normal saline (Nacl 0.9%) to remove and clean any debris or wound drainage, then irrigate the wound with Hypochlorous acid in a concentration ; sterile Nacl 0.9% to HOCL 50.5% at ratio 8:2 and leave for 5 minutes, repeat irrigating the wound with Hypochlorous acid in the same concentration and ratio once more and

left for 5 minutes before covered the wound with sterile dressing.

The control group: The same procedure was done for control group except, instead of Hypochlorous acid, diluted Hydrogen Peroxide 20% was used for cleaning the wound followed by washing with Nacl 0.9% then use of Povidine iodine solution as antiseptic solution and covered the wound with sterile dressing. Changing dressing was done twice or once daily, or once every other day according to the amount of exudates for both groups.

4.4 Follow up: The follow-up every other day of the two groups was done in plastic and vascular surgery clinic during each assessment, standard parameters of wound pain, odor, and amount and color of exudates were evaluated. After clinical improvement of the wound, the ulcer was operatively reconstructed by flap or graft.

5. Data Analysis:

The data obtained were reviewed, prepared for computer entry, coded, analyzed and tabulated. Descriptive statistics (i.e., frequencies, percentage, mean and stander deviation, etc…) was done using computer program SPSS version 17.Chi-square and T-test, test used to compare differences in the distribution of frequencies among different groups.

6. Results

6.1 This study was performed on 60 patients with infected diabetic foot ulcer divided equally to control and study group, their mean ages ranged between 33.10±10.91 and 33.10±10.91 respectively. There were 22 male and 8 female in study group and 20 male and 10 female in control group. The wound became clean in 70% and 100% of study patients at 10 days and 15 days respectively, compare to 3.33%, 13.33% and 53.33% of control group at 10, 15 and 30 days respectively.

The mean hemoglobin level was 10.33 ± 1.67 and 11.13 ± 1.77 for study and control patients respectively. While the mean White blood cells was 8.01 ± 0.55 and 12.52 ± 1.82 for study and control group respectively, Table (1).

HOCL was able to kill Candida, Proteus and Klebsella within 15 days and Psudomonas within 20 days while Methicillin resistant staphylococcus aureus (MARSA) after 25 days, while H2O2 can reduce the total numbers but could not able to kill any of 5 bacteria within 30 days, Fig (1).

56.7% and 66.7% of study and control groups respectively, were reported continuous pain at start of the study but that pain was disappeared after 5 days in study group, compared to 36.7%, 23.3%, 16.7%, and 13.3% wear reported continuous pain after 5, 10, 15 and 20 days respectively of control patients. P value 0.005, Table (2).

23 out of 30 patients of study patients had purulent discharges, which changed to non after 10 days, compared to 21 patients of control group had purulent discharges which changed to 10 patients still had purulent discharge during all time of the study Fig (2).

6.2 22 and 20 of study and control patients respectively started with high amount of exudates and decreased to nil in all study patients within 15 days and 9 patients of control group still had high amount of exudates after 30 days. Offensive odor was present at the beginning of all study and control patients, which completely disappeared within 10 days in study group, while 15 patients of control group still had offensive odor after 30 days. Fig (3).

There was highly statistical significance difference between study and control groups as regard decrease the depth and width of the ulcer. P value<0.001.(Tab 3).

7. Discussion

Diabetic foot ulcer is more susceptible to infection than other wounds. Infection is one of the important factors that delay wound healing, thus good wound care is critical for normal wound healing (Niezgoda et al. 2010). The biggest challenge for the physicians and nurses is searching for a safe and effective topical antiseptic agent for treating infected diabetic foot ulcer. Many topical antiseptics are used nowadays for diabetic foot ulcer care, some of them are good in control of infection but their cytotoxic effect on epidermal and dermal cells limit their use (Sampson & Sampson 2008). Hydrogen peroxide is the most commonly antiseptic solution used for washing of diabetic foot ulcers but it is toxic to newly formed epithelium (Wilson et al. 2005). This is because hydrogen peroxide kills fibroblasts which are required for healing and epithelization and possibly destroys the normal cells that surrounding the wound (Wang et al. 2007). Also hydrogen peroxide was found to retard the healing and did not decrease bacterial load in human wounds contaminated with *Staphylococcus aureus (*Selkon et al. 2006). This is why many physicians are currently advice against using hydrogen peroxide to clean wounds (Wang et al. 2007).

Povidone-iodine has been demonstrated to be cytotoxic to the cellular components of wound healing (Khan et al. 2012). and thus it does not effectively promote good wound healing (Dumville et al. 2015).

The current study compare the effectiveness of Hypochlorous Acid versus Hydrogen Peroxide

followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers.

Diabetic foot ulcer that infected by Candida, Proteus, Klebsella, Pseudomonas, and MARSA are favorable niche for contamination, then colonization and biofilm formation (Sneader 2005). Biofilm is a complex structure of microorganisms that generate a protective shell, allowing bacteria to collect and proliferate. The biofilm prevent phagocytosis (Najafi et al. 2003), and increases resistance to antibiotics (Simmons et al. 2007; Ninnemann & Stein 2016).

Hypochlorous acid is highly active against all bacterial, viral, and fungal human pathogens (Zelic et al.2009) and a small amount of HOCL can kill spore-forming and non-spore bacteria in a short time period (Angelis et al 2012 ; Wolcott & Rhoads 2008).

Many studies reported that HOCL kills bacteria without cytotoxic effect to keratinocytes or fibroblasts and this enables body's natural healing process (Nelson & Bissell 2008), so it could be an alternative to H_2O_2 and Povidine iodine. The results of the current study supports the previous studies that, HOCL is highly effective antimicrobial agent against many pathogens such as Candida, Proteus, Klebsella, Pseudomonas, and MARSA compare to H_2O_2 and Povidine iodine in treating infected diabetic foot ulcers, and this is evidenced by rapid formation of healthy granulation tissue without further necrosis and quantitative micro- bacterial results.

The adverse effect of bacterial infection is the presence of tender painful wound (Eron et al. 2003; Elston 2005). HOCL is better than H2O2 and Povidine iodine as antimicrobial agent and thus reduce pain (King et al. 2006).

The results of the current study concomitant with the previous finding, where pain has been decreased and totally disappeared after using HOCL compared to the patients who were using H2O2 and Povidine iodine. Malodor can indicate the presence of a high number of micro-organisms beyond colonization.

The result of the current study reported that, malodors were completely eradicated in all patients of HOCL compared to 50% of control patients still have bad odor till the end of this study.

Mimi and Ahmed reported that HOCL softens the wound surface eschar, necrotic tissue and biofilm from the infected traumatic wound (Mekkawy & Kama 2014). And this concomitant with the current result where, the researchers observed that HOCL softens, cleans and removes the necrotic tissue and biofilm from infected diabetic foot ulcers.

Aratani et al. 2003) and Robson 2003) reported that, there is a significant reduction in exudates amount when using HOCL compare to use H_2O_2 and Povidine iodine as washing therapy. The current finding supports Aratani and Roboson reports where about 75% of the diabetic foot ulcers have high amount of purulent exudates at the beginning of the study in both groups which converted to nil after 10 days of using HOCL compared to 35% of control patients still have purulent discharges till the end of the study. HOCL demonstrates broad-spectrum antimicrobial activity against various microorganisms and achieves a marked reduction in the bacterial burden in septic wounds (Chopra 2003; Chopra et al. 2013). In the current study purulent exudates were found in the majority of control and study patients. The purulent exudates turned to non within 10days in all patients who using HOCL, but exudates still purulent even after 30 days in 8 patients who using H2O2 and Povidine iodine. This finding indicates that there was marked reduction in bacterial count in Hypochlorous acid group compared to H_2O_2 and Povidine iodine group.

Wound healing is the end result of a series of interrelated cellular processes initiated by humoral factors such as growth factors. These cellular processes are inhibited by a large tissue bacterial bioburden (Robson 2014). Povidone-iodine has been demonstrated to be cytotoxic to the cellular components of wound healing (Aratani 2006). Hypochlorous acid controls the tissue bacterial bioburden without inhibiting the wound healing process (Serena et al. 2006). In the current study there is a significance difference in the wound healing between patients who using HOCL compare to patients who using H2O2 and Povidine iodine as washing therapy in infected diabetic foot ulcer.

8. Conclusions

Hypochlorous acid appears to be effective as a potent diabetic foot ulcer care agent against a wide range of microorganisms, inexpensive, easy to prepare, safe, and painless.

Hypochlorous acid controls the tissue bacterial bio-burden without inhibiting the wound healing process, rapidly relieves of pain, short hospital stay and the ulcer well prepared to natural healing or skin flap or graft.

Hypochlorous acid is an ideal wound care solution in cleansing infected diabetic foot ulcers.

HOCL as a cleanser agent appears to be effective on infected diabetic foot ulcers.

9. Recommendations

Farther studies to confirm the effect of HOCL with larger number of patients is needed.

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Table (1): Socio-demographic characteristics for control and study groups (n=60)

Variable	Group			
	Study(n=30)		Control (n=30)	
Age(years):				
▪ 22-32	9	30%	10	33.33%
▪ 33-43	9	30%	8	26.66%
▪ 44-55	12	40%	12	40%
Mean± SD	33.10± 10.91		32.89±10.21	
Gender:				
▪ Male	22	73.33%	20	66.66%
▪ Female	8	26.66%	10	33.33%
Wound bed clean/days				
▪ At 5 days	1	3.33%	0	0
▪ At 10 days	20	66.66%	1	3.33%
▪ At 15 days	9	30%	3	10%
▪ At 20 days	0	0	5	16.66%
▪ At 25 days	0	0	5	16.66
▪ At 30 days	0	0	16	53.33%
HGB(Mean± SD):	10.33± 1.67		11.13±1.77	
WBC(Mean± SD):	8.01±0.55		12.52±1.82	

Table (2): Frequency and percentage of wound pain among control and study groups

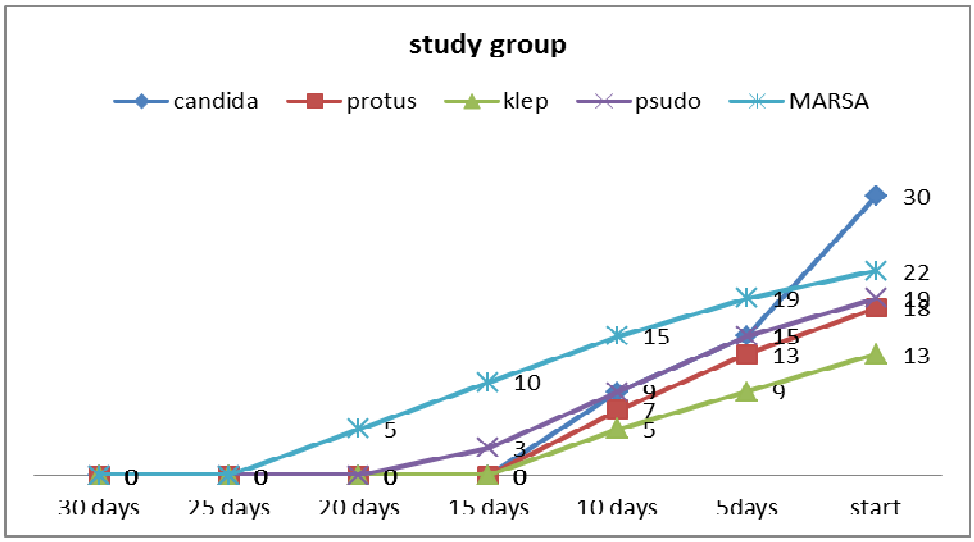
	Study(n=30)										Control (n=30)									
	At start		After5day		After10		After15		After20		At start		After5day		After10		After15		After20	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Wound-pain frequency:																				
▪None:	0	0	20	66.7	30	10	30	10	30	10	0	0	2	6.7	4	13.3	5	16.7	9	30
▪Only-at dressing:	4	13.3	6	20	0	0	0	0	0	0	2	6.7	6	20	8	26.7	10	33.3	11	36.7
▪Intermittent:	9	30	4	13.3	0	0	0	0	0	0	8	26.7	11	36.7	11	36.7	10	33.3	6	20
▪Continuous:	17	56.7	0	0	0	0	0	0	0	0	20	66.7	11	36.7	7	23.3	5	16.7	4	13.3
P. value	0.005 **																			

*Significant (P< 0.05) ** highly significant p= 0.001

Table.(3): Distribution of the sample according to decrease the size of ulcer.

Variable	Group			
	Study(n=30)		Control (n=30)	
	Depth/decrease	Width/decreases	Depth/decrease	Width/decreases
At start	0 cm	0 cm	0 cm	0 cm
At 5days	1 cm	1.3 cm	0.2 cm	0 cm
At 10days	2.2 cm	2.5 cm	0.5cm	0.3 cm
At 15days	3.1	3.6 cm	0.9 cm	0.5 cm
At 20days	4.0 cm	4.6 cm	1.3 cm	0.9 cm
At 25days	5.7 cm	5.9 cm	1.5 cm	1.3 cm
At 30days	6.5 cm	6.6 cm	1.9 cm	2.00 cm
Mean± SD	2.00±0.00		0.52±0.03	
T-value	28.17			
P-Value	<0.001**			

*Significant (P< 0.05) ** highly significant p= 0.001



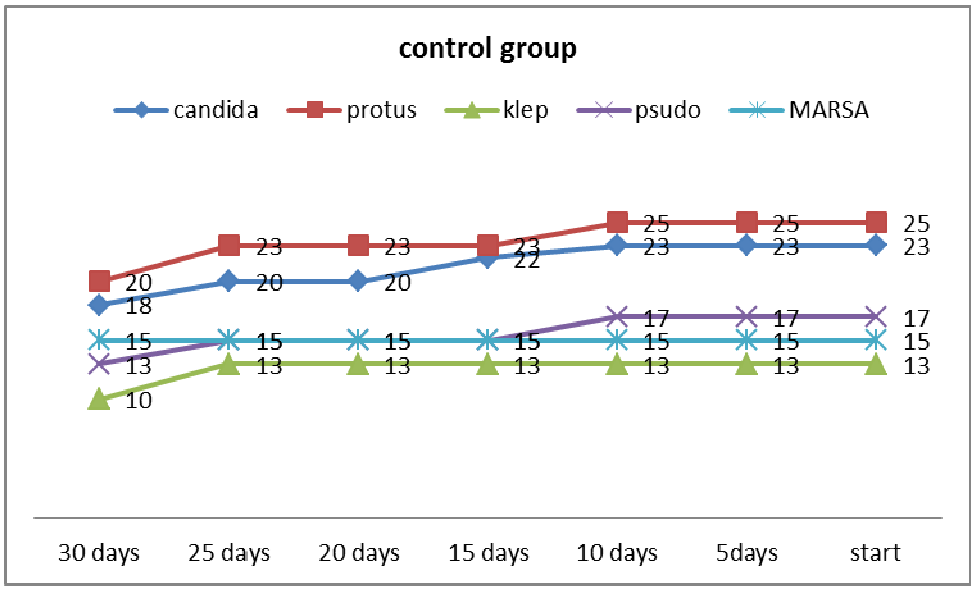
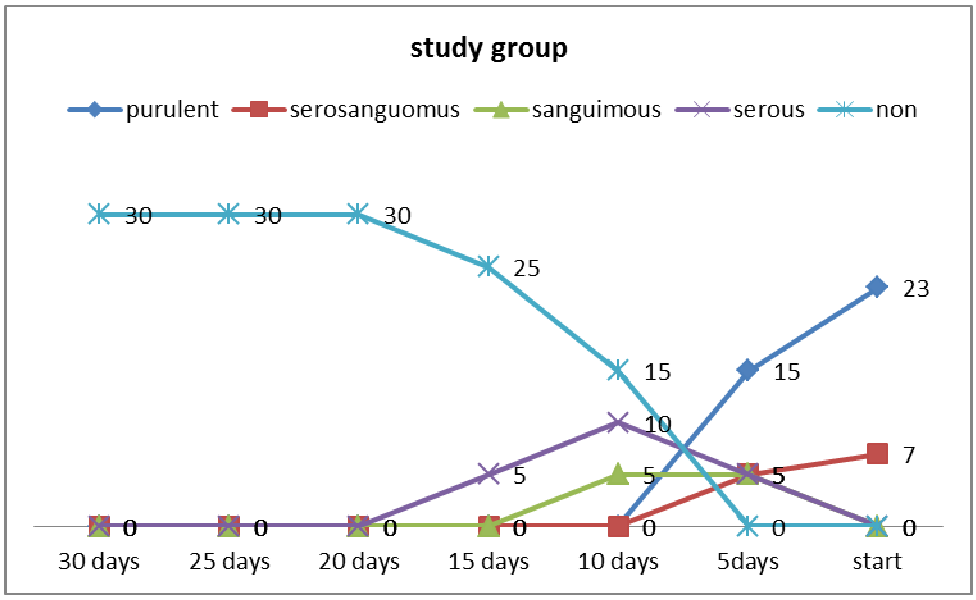


Figure 1. Status of 5 microorganisms in study and control group from the start to the end of the study (n=30).



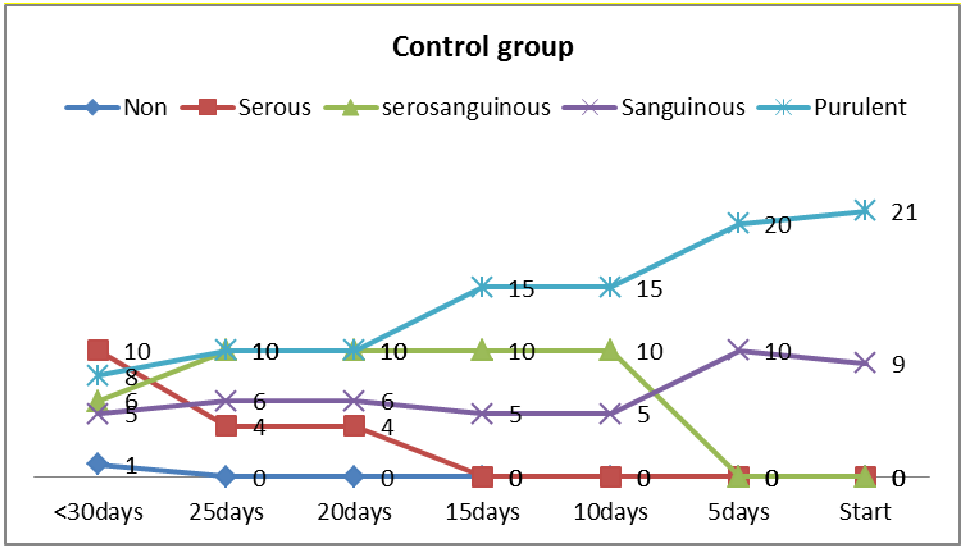
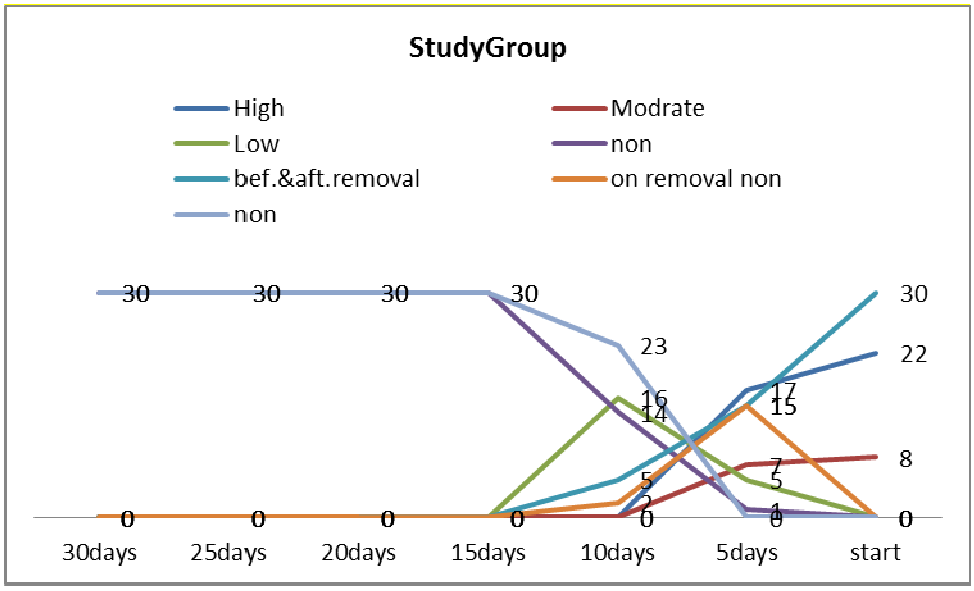


Figure 2. Relative number of patients with different wound exudates among study and control group.



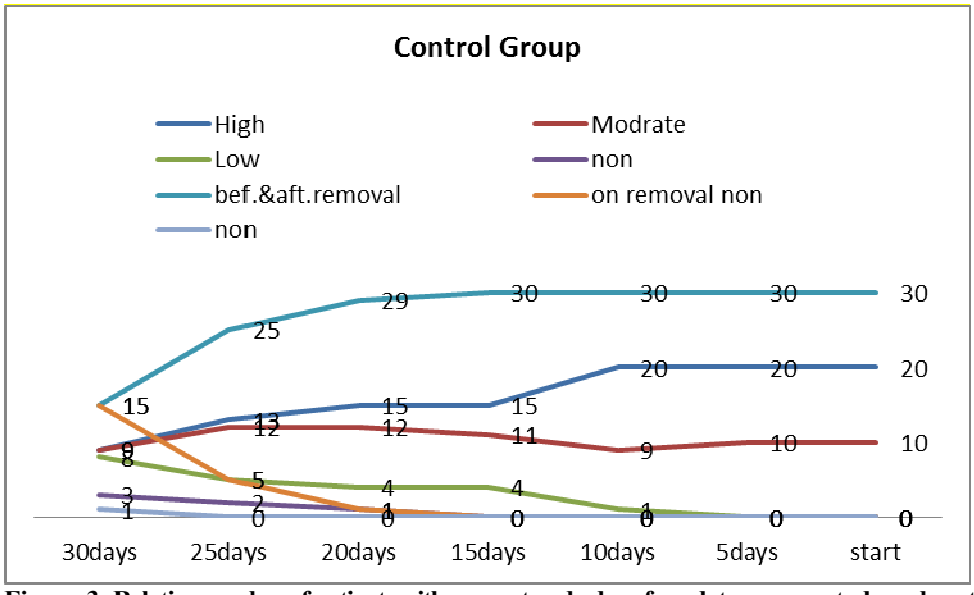


Figure 3. Relative number of patients with amount and odor of exudates among study and control group.

Figure 4. Male patient has infected Lt. diabetic foot ulcer after amputation big toe, **A-** at start, **B-** after 5 days of using HOCL, **C-** after 10 days **D-** after 15 days **E-** after 20 days **F-** after 25 days **G-** Complete ulcer healing after 30 days of using HOCL.

Figure 5. Female patient has infected diabetic foot ulcer after amputation, A- at start there is necrotic tissue, B- after 5 days of using HOCL, C- after 10 days D- after 15 days E- healthy granulation tissue after 20 days F- after skin graft.


A


B


C


D


E


F

Figure 6. male patient has infected diabetic Rt. foot ulcer. A- at start, B- after 5 days of using HOCL, C- after 10 days D- after 20 days E- after 25 days F-compete ulcer healing after 30 days of using HOCL.