E-ISSN: 2616-3470
P-ISSN: 2616-3462

www.surgeryscience.com
2019; 3(4): 151-156
Received: 16-08-2019
Accepted: 18-09-2019

Madhu CP
Department of General surgery, JSS Medical College, Mysuru, Karnataka, India

Deepak Naik P
Department of General surgery, JSS Medical College, Mysuru, Karnataka, India

Vishak Nair TU
Department of General surgery, JSS Medical College, Mysuru, Karnataka, India

Corresponding Author:
Madhu CP
Department of General surgery, JSS Medical College, Mysuru, Karnataka, India

Effect of topical hemocoagulase therapy in wound healing

Madhu CP, Deepak Naik P and Vishak Nair TU

DOI: https://doi.org/10.33545/surgery.2019.v3.i4c.234

Abstract
Aim: Objective of this study is to test the efficacy of topical hemocoagulase in bleeding control, wound reduction, epithelialization in non-healing and healing ulcers/wounds after debridement in comparison with conventional method of treatment.
Materials and Method: A prospective comparative study was conducted in JSS Hospital, Mysuru, Karnataka from August 2016 to October 2018. The source of data was patients attending the outpatient on a regular basis or those admitted as inpatients for the management of healing and non-healing ulcers. 60 patients were studied. 30 cases were randomly chosen for study with topical hemocoagulase and 30 cases received conventional dressing for healing/ non healing ulcers.
Results: Topical Hemocoagulase showed faster and better healing rates. The mean area reduction was statistically significant in the study group. The median baseline surface area of the wound at the beginning of the study was 40.00 among cases and 13.5 among the controls and there was no statistical difference between the cases and the control before initiation of the treatment. Post interventions the median surface area of the ulcer in cases were 13.5 and 16.0 among control. Decrease in surface area of the wound median value in cases were 20.50 and & among controls, which shows statistical significance. There were no adverse effects or reactions seen with the use of Topical Hemocoagulase among the study group.
Conclusion: This evaluation provides strong evidence that Topical Hemocoagulase dressings provide safe and cost effective method of enhancing the healing rates of non-healing wounds, reducing the overall hospital stay and morbidity.

Keywords: Topical hemocoagulase, healing and non-healing ulcer, hemostatic effect

Introduction
In the practice of general surgery a number of clinical situations are encountered where capillary oozing (tissue oozing) can be annoying problem. Capillary bleeds occur during reconstruction of deformed or damaged parts of the body. Control of tissue oozing and ensuring a cosmetically elegant scar is vital. In skin grafting capillary ooze occurs at both donor and receptor sites. Capillary bleeding implies breakdown in supply of nutrients and oxygen in the area, leading to impaired wound healing. Restoring the capillary tree ensures faster wound healing and hence reduced inflammation and infection. Control of capillary bleeds leads to decreased morbidity to the patient, improved healing and faster recovery.
Rapid and deliberate action is required to gain control of unanticipated surgical bleeding, and vigilance is required to recognize postoperative haemorrhage[1]. Controlling perioperative bleeding is of critical importance to minimizing haemorrhaging and limiting morbidity and mortality. There are several consequences of uncontrolled and excessive haemorrhaging: prolonged operative time, complication of surgical procedure, requirement of transfusions, a combination of hemodilution, hypothermia, consumption of clotting factors and development of acidosis which worsen the clotting process and cardiac arrest in the operating room [3]. Many human factors, transfusion practices, blood supply and anaesthetic management lead to potentially critical haemorrhaging [4]. Several factors can cause or exacerbate intraoperative bleeding and abnormal hemostasis [5].
Mild or moderate surgical bleeding may be straightforward to manage. However, bleeding may also be difficult to control, depending on factors such as bleeding severity, visibility and access to the bleeding source, anatomic location of the bleeding, patient coagulation status and surgical skill. Often, no single solution allows surgeons to rapidly stop bleeding. Such situations require the use of a combination of hemostatic products in addition to conventional methods,

which may be cumbersome, time-consuming and costly [2].
The critical requirements are mentioned below [6]:

1. Provide a localized conformal physical barrier to entrap red blood cells.
2. Provide a chemical platform that accelerates clotting regardless of the presence of heparin.
3. Stop bleeding in a rapid fashion (in <1 minute)
4. Prevent haemorrhaging during subsequent manipulation.

Hemocoagulase

Certain snake venoms have been shown to aid in hemostasis [6]. Batroxobin from the venom of Bothrops atrox and B moojeni or B. Jararaca has been shown to induce defibrinogenation. The purified fractions of Batroxobin possess coagulant, proteolytic and esterolytic properties, with the primary mechanism of action being a proteolytic effect on circulating fibrinogen. Batroxobin cleaves both fibrinopeptides A and B [7]

Hemocoagulase has been classified amongst those substances whose action is predominantly thrombin like. The thrombin like action of the enzyme hemocoagulase is characterized primarily by the fact that blood coagulation takes place even when some clotting factors are absent, in particular calcium, prothrombin and various other plasmatic and platelet factors.

Mechanism of action of Hemocoagulase [7]

- The activity of hemocoagulase is attributed to batroxobin and factor activator X(FAX)
- Hemocoagulase can degrade an appropriate amount of fibrinogen on the Argl6-Glyl7 residues of the alpha chain, release fibrin peptides, and produce the unstable and soluble fibrin I monomers. On continuous effect of hemocoagulase, soluble fibrin I monomers form fibrin I polymers which help in platelet aggregation at the damage spot of the blood vessel, and help in formation of a thrombus. This facilitates preliminary hemostasis at the damage spot of blood vessel.
- Additionally, soluble fibrin I monomers rapidly degrade at the Argl6-Glyl7 residues of the beta chain, release fibrin peptide B and produce soluble fibrin II monomer which produces an insoluble fibrin thread after cross linking with the help of XIIIa and Ca^{2+}. The fibrin thread will form a net to rap plasma and blood cells on blood platelet thrombus and its vicinity, thus reinforcing the thrombus and potentiating hemostasis.
- The slight phospholipid- dependent FAX activity also indirectly aids at the site of damage of the blood vessel, thus facilitating the hemostatic effect.
- Batroxobin binds fibrinogen in a manner distinct from thrombin, which may contribute to its higher affinity interaction, selective fibrinopeptide A release and prothrombotic properties. The action of Hemocoagulase in this way is similar to that of thrombin, which converts fibrinogen to fibrin monomer without the intervention of any other factors.

However, there are some significant divergences between the mode of action of hemocoagulase and that of thrombin, the principal one being the following:

1. The action of thrombin may be inhibited by the Antithrombin normally existing in blood, whereas the action of hemocoagulase continues, even when Antithrombin is present.
2. Unlike thrombin, hemocoagulase is not absorbed by the fibrin clots and is therefore not neutralized by that mechanism, as occurs with thrombin.

The most important of these differences, from the practical point of view is the fact that hemocoagulase remains in the blood stream even when Antithrombin is present, and is not absorbed by the fibrin clots; this enables the action of hemocoagulase to be much more prolonged than is possible with thrombin, which is immediately neutralized by the fibrin clots.

In first place, it has been noticed that it is possible by means of electrophoresis, to establish the direct attack of hemocoagulase on fibrinogen, on which thrombin acts in a similar manner. As far as blood platelets are concerned, it has repeatedly been confirmed that when hemocoagulase comes into contact with platelets, it releases thromboplastinic factors from them. This effect may be demonstrated by means of the thromboplastin generation test.

Hemocoagulase is effective in various coagulopathies like haemophilia, Von Williebrand disease, thrombocytopenia, because of its unique mechanism of action that directly converts fibrinogen into fibrin.

Study suggest that hemocoagulase signals cell division, produces a fibrin bridge that encourages faster growth of collagen fibres beneath it, ensures growth of capillaries and migration of fibroblast in wound space, thus helps in cosmetically elegant faster wound healing.

Hemocoagulase is a pale yellow crystalline powder which is sparingly soluble in water but readily soluble in phenolated saline. It is active over a wide pH range of 4-8. A clotting enzyme of the venom Bothrops jararaca denoted FC-Bj was purified by gel chromatography on sepha-dex G-100 [8] The clotting factor coagulates fibrinogen to fibrin. The protein was of serine type.

Hemocoagulase induced fibrin deposition liberates structurally different degraded products. These products may have decisive role in improving the wound repair.

Hemocoagulase in wound healing [9, 10].

- It hastens coagulation which is the precursor to wound healing by accelerating fibrinogen to fibrin conversion.
- It arrests capillary bleeding within 1 minute only at the site of haemorrhage.
- It signals faster cell division to promote wound healing.
- It promotes all phases of tissue repair.
- It produces a fibrin-fibrinonectin-collagen network that encourages growth of collagen fibres beneath it. It encourages growth of collagen formation by encouraging migration of fibroblasts into wound space and activation of platelet derived growth factor (PDGF)
- Increased fibrin deposition by hemocoagulase helps in faster connective tissue formation.
- It establishes capillaries in wound space which encourages wound healing.
- Perisurgical use of hemocoagulase induces angiogenesis and produces a wide variety of biological actions which is useful in wound healing.
- It enhances epithelialization to reduce healing time.
- It markedly reduces inflammation, infection and localized collection of blood at the site of injury.
- It keeps the edges glued up and maintains the proper approximity of suture line and seals the wound immediately. Thus prevents hypertrophy of scars.
- It avoids suppuration and keloid formation.

Objective

The Objective of our study was to assess the effect of topical hemocoagulase application in wound healing.

Methods

A prospective comparative study was conducted in JSS Hospital, Mysuru from August 2016 to October 2018. A total of 60 patients were selected for the purpose of the study based on the inclusion and exclusion criteria. Clinical examination of wound was carried out on alternate days. Healing was assessed and compared between the patients undergoing topical hemocoagulase therapy and conventional wound dressing.

Inclusion criteria

Patients aged between 15-75years admitted in general surgery and plastic surgery ward both male and female in JSS hospital for the treatment of non-healing ulcer between august 2016 to October 2018 were taken.

Exclusion criteria

Patients with known premorbid conditions like thromboembolic disorders, hypertension, hemophilia, diabetes mellitus, anticoagulant therapy, pregnant patients, hypersensitivity to hemocoagulase topical solution and other constituents of the formulation, HIV – positive patients and patients with mental illness.

Collection of data

On the first day of admission, after taking clinical examination and baseline investigations, Debridement of the ulcer under standard and sterile surgical technique was carried out and wound length and width was measured using a measuring tape and surface area of the wound were calculated and recorded.
Intraoperative The debrided site was then filled with sterile gauze soaked in hemocoagulase solution and time of application to complete stoppage of bleeding was recorded using a stop watch. In controls, saline soaked gauze was used instead of hemocoagulase.

Postoperative

Clinical examination of wound was carried out on alternate day by primary investigator and Healing was assessed and compared between the patients undergoing topical hemocoagulase therapy and conventional saline wound dressing.

Statistical methods

Data was entered into Microsoft Excel data sheets and was analysed using SPSS 21.0 version software. Categorical data was represented in the form of frequencies and proportions. Summary statistics was done by means of proportions for categorical/binary variables and mean, median, Standard deviation, Inter Quartile Range (IQR) for continuous variables. Inferential statistics was done by chi square test, independent t test/Mann Whitney test, and Paired t test/wilcoxan test.
$p<0.05$ will be considered statistically significant Chi square test/fisher exact test are used comparing two or more independent proportions. Fisher exact is used when the number of expected numbers in >25% cells is <5. Independent t test was used to compare means between independent groups/mutually exclusive groups. Mann Whitney test was used to compare the continuous variable which is not normally distributed/ ordinal variables between two independent groups. Wilcoxan test used for measuring paired difference of continuous variables at two point of time for the same group. Or difference in median of two paired groups.

Results

A total of sixty patients with non-healing ulcer who were treated in JSS hospital, Mysore, during 2016 and 2018 were included in the study. In our study the majority of our study subjects were between the ages of 30-60 years. 45.2% (14) of the study subjects were 51-70 years, 25.8% and 22.6% of the study subjects were in the, <30 years and 31-50 years respectively. The age was found to be statistically not significant between the groups.

Table 1: Age category

		Group			
		Case		Control	
		Count	Column N %	Count	Column N %
Age category	<30	8	25.8%	2	6.9%
	31-50	7	22.6%	9	31.0%
	51-70	14	45.2%	13	44.8%
	>71	2	6.5%	5	17.2%

In our study majority of the subjects affected from Non healing ulcers was Male patients (71%) and female (29%), the gender was found to be statistically not significant between the two.

Table 2: Distribution of study subject according to gender.

		Group			
		Case		Control	
		Count	Column N %	Count	Column N %
Sex	Female	9	29.0%	5	17.2%
	Male	22	71.0%	24	82.8%

Table 3: Etiology of Ulcers included in the study sample

Etiology	Group			
	Case		Control	
	Count	Column N %	Count	Column N %
Carbuncle	2	6.5%	0	.0%
cellulitis	7	22.6%	4	13.8%
crush injury	1	3.2%	0	.0%
Degloving injury	1	3.2%	1	3.4%
fournier's gangrene	3	9.7%	1	3.4%
gangrene	4	12.9%	4	13.8%
healing ulcer left leg	0	0%	1	3.4%
infected ulcer	1	3.2%	0	.0%
lacerated wound	1	3.2%	0	.0%
necrotising fasciitis	3	9.7%	8	27.6%
non healing ulcer	2	6.5%	4	13.8%
Perianal abscess	0	0%	1	3.4%
PVD with Gangrene	0	0%	1	3.4%
right breast abscess	1	3.2%	0	.0%
Snake bite cellulitis	2	6.5%	0	.0%
soft tissue necrosis	0	0%	1	3.4%
spreading cellulitis	1	3.2%	0	.0%
traumatic laceration	2	6.5%	1	3.4%
trophic ulcer	0	0%	2	6.9%

Table 4: site of the wound

Site of the wound	Group			
	Case		Control	
	Count	Column N %	Count	Column N %
Breast	1	3.2%	0	.0%
Face	0	.0%	2	6.9%
Foot	8	25.8%	10	34.5%
Forearm	1	3.2%	0	.0%
Forehead	0	.0%	1	3.4%
gluteal region	1	3.2%	0	.0%
Leg	1	3.2%	4	13.8%
little toe	0	.0%	1	3.4%
lower limb	9	29.0%	8	27.6%
Perianal	0	.0%	1	3.4%
Right	1	3.2%	0	.0%
Scrotum	3	9.7%	0	.0%
Scrotum and perineum	0	.0%	1	3.4%
Thigh	4	12.9%	1	3.4%
upper limb	2	6.5%	0	.0%

Table 5: Comorbidities

	Group				
	Case		Control		
	Count	Column N %	Count	Column N %	p
DM	14	45.2%	18	62.1%	0.2
HTN	7	22.6%	1	3.4%	0.02
Any co morbidity	14	45.2%	11	37.9%	0.6

Table 6: Initial baseline wound area

	Group					
	Case			Control		
	Median	Q1	Q3	Median	Q1	Q3
Baseline area cm2	40.00	16.00	112.00	18.00	15.00	36.00

Table 7: Time required to stop bleeding

	Group			
	Case		Control	
	Mean	S D	Mean	S D
Time required to stop bleeding	51.13	15.15	51.38	11.17

Table 8: comparison of baseline and post intervention surface area of the ulcer.

	Group					
	Case			Control		
	Median	Q1	Q3	Median	Q1	Q3
Baseline area cm2	40.00	16.00	112.00	18.00	15.00	36.00
Post intervention Area cm2	13.5	6.0	60.0	16.0	8.0	20.0
p	<0.0001			<0.0001		

Table 9: Decrease in the surface area of the ulcer.

	Group					
	Case			Control		
	Median	Q1	Q3	Median	Q1	Q3
Decrease in Area in cm2	20.50	12.00	63.00	7.00	4.00	13.00

Table 10: No: of cases proceeded for split skin grafting in each group

		Group			
		Case		Control	
		Count	Column N %	Count	Column N %
Graft	NA	12	38.7%	15	51.7%
	Yes	19	61.3%	14	48.3%

22.6% in case group and 13.8% of control group consisted of cellulitis which was the major etiology. 45.2 % of the cases and 62.1% of the control were Diabetic, 22.6% of controls and 3.4% of the controls were hypertensive. The median baseline surface

area of the wound at the beginning of the study was 40.00 among cases and 13.5 among the controls and there was no statistical difference between the cases and the control before initiation of the treatment. Post interventions the median surface area of the ulcer in cases were 13.5 and 16.0 among control. Decrease in surface area of the wound median value in cases were 20.50 and & among controls, which shows statistical significance. Among test group 94.7 % of the grafts taken up and 50% of the grafts were taken up in control group, which is statistically significant difference for the present study. Mean percentage decrease in the surface area of the ulcer post intervention among cases is 55.01% with a standard deviation of 16.06 and mean percentage decrease in the surface area of the ulcer post intervention among controls is 34.58% with a standard deviation of 14.43%, which shows statistically significant difference between the cases and controls of the study group.

Discussion

The recent review lists 3 eligible studies showing efficacy of Hemocoagulase in wound healing after dental extractions in adults[10] and in oral maxillofacial surgery [11] and in abdominal incisions in general surgery [13].

A. Role of hemocoagulase as a local haemostatic agent after extraction of tooth - D. A. Solanki Nehal D. Modha

B. Vandana Shenoy K., Mohan Baliga, Sumitha Mahajan, Ramesh K. V. (2014) The effects of topical hemocoagulase solution on the healing process of post-extraction wounds: A split mouth design. J. Maxillofac. Oral Surg.

C. Role of local instillation of one percent feracrylum and haemocogulase on wound healing

Krishna Kumar Sing, Kamlesh Dhruv, D. R. Patel, U. S. Paikra.

In comparison with 3 of the clinical trials, which studies efficacy of wound healing, the age group of the cases and controls were largely in 60-70 year age group in Solanki study, 60.87% in Vandana Shenoy study and 48.7% in the present study. In the remaining studies including the present study, the difference in age between case and controls was not statistically significant.

In previous study of Dr D.A. Solanki, the mean time to stop the bleeding in the test group was 1.42 minutes and 2.18 in the control group and in our present study the mean time taken to stop the bleeding in test group after debridement was 51.13 sec with a standard deviation of 15.25 and for the control group is 51.38 with a standard deviation of 11.17, which showing no statistical significance, but relatively faster haemostasis was achieved in test group [10].

Dr Babu S Parmar, Dr Samir Mansuri in 2006 done a study and stated that, use of hemocoagulase after surgery not only provides faster hemostasis but enhances healing by rapid formation of healthy tissue and reducing the amount of infection which may alter the normal healing process [12].

There are studies which conducted on the effects of hemocoagulase on abdominal surgery and its effects on wound healing in abdominal surgery incisions, in urology procedures like TURP (transurethral resection of prostate) where they irrigate hemocoagulase after the resection, in paediatrics field which showing the effect of hemocoagulase on prevention of pulmonary haemorrhage in critical new-borns on mechanical ventilation, effects of hemocoagulase in conducting delivery, its effects on tissue biopsy with bronchoscopy, in ENT procedures like FESS and in dental extractions. Unlike the other studies, the present study which was conducted on its efficacy in wound healing after debridement of infected wound, non healing ulcers, necrotising fasciitis, diabetic foot and after that during daily dressings which was analysed using 3 components, i.e. stoppage time of bleeding after debridement, comparing the area of wound post debridement and at the termination of the treatment and duration of stay in the hospital which were compared with the control group (conventional dressing).

In our study, comparison between area of the wound before and after the intervention were measured in a systematic manner and daily examination was done by the principal investigator. The median baseline surface area of the wound at the beginning of the study was 40.00 among cases and 13.5 among the controls and there was no statistical difference between the cases and the control before initiation of the treatment. Post interventions the median surface area of the ulcer in cases was 13.5 and 16.0 among. Comparison of baseline and post intervention surface area of the ulcer which shows, statistically significant reduction of the surface area of the wound after initiation of the treatment.

Number of cases proceeded for split skin grafting in each group were recorded and was showing 61.29% of the cases proceeded for split skin grafting among test group and 48.28% of the controls proceeded for split skin grafting. In this study, 94.7% of the cases which has underwent Split skin graft in test group were fully taken up with no recording of any graft rejection and 5.3% of the grafts were partially taken up, which is statistically significant. Comparing with the test group, control group only have 50% of the grafts well taken up, with 21.4% rejection rate and 28.6% of the grafts partially taken up. Among the rejected grafts, 7% of the wound is healing with Hemocoagulase dressing.

The time taken per dressing among test group was same as that of conventional dressing. No cost was incurred by the patient as all material required were available as hospital supply. No adverse effects were seen with hemocoagulase dressing as studies demonstrated the safety and efficacy of hemocoagulase in human healthy volunteers. It is an aqueous sterile solution of hemocoagulase enzyme isolated from the venom of Bothrops atrox or Bothrops Jararaca, so it may present the risk of immunogenic reactions, but there is no human to human disease transmission like HIV or Hepatitis B, thus making a safe modality of treatment

Limitations

The study was conducted maximum for a month, so a conclusion on the long term healing of the wounds could not be drawn.

The time required for complete epithelialisation of the wounds and long term compliance of the patient for repeated hemocoagulase dressings was not studied

Observer and patient were not blinded increasing the risk of bias.

The wound was studied in only 2 dimensions wound perimeter plotted on graph. In certain instances, however filling up of the wound bed was not as significant as wound perimeter reduction. In retrospect, wound volume measurement rather than area would have been a more accurate approach of judging results.

The study in spite of its shortcomings does indicate that topical hemocoagulase is more effective than standard conventional therapy in helping an ulcer to heal and that it has the potential to be useful, safe and cost effective adjunct to wound healing.

Conclusion

With the use of Topical hemocoagulase dressing in comparison with the control group for the treatment of ulcers after debridement of ulcers after debridement, the following conclusions were derived,

- Hemocoagulase showed faster and better healing rates among the study group.
- Area reduction was statistically significant in the test group.
- Relative faster hemocoagulase were achieved in the study group even though the median baseline surface area in the study group was larger than the control group.
- There were no adverse effects or reactions seen when topical hemocoagulase were applied over the ulcer.
- It is a cost effective procedure, helps in early and effective skin grafting with higher percentage of success compared to the control group and reduced the stay in hospital and giving a good healthy scar.

References

1. Hammond KL, Margolin DA. Surgical haemorrhage, damage control and the abdominal compartment syndrome. Clin Colon Rectal Surg. 2006; 19(4):188-194.
2. Corral M, Ferko N, Hollmann S *et al*. Health and economic outcomes associated with uncontrolled surgical bleeding: A retrospective analysis of the Premier Perspectives Database. Clinicoecon outcomes Res. 2015, 409-421.
3. Marieta M, Facchini L, Pedrazzi P *et al*. Pathophysiology of bleeding in surgery. Transplant Proc. 2006; 38(3):812-814
4. Zhou CG, Shi HB, Liu S *et al*. Transarterial embolization for massive gastrointestinal haemorrhage following abdominal surgery. World J Gastroenterol. 2013; 19(40):6869-6875.
5. Irita K. Present status of critical haemorrhage and its management room. Rinsho Byori. 2014; 62(12):1275-1279.
6. Kumar VA, Wickremasinghe NC, Shi S *et al*. Nainofibrous snake venom hemostat. ACS Biomater Sci Eng. 2015; 1(12):1300-1305
7. Shenoy AK, Ramesh KV, Chowta MN *et al*. Effects of Botropase on clotting factors in healthy human volunteers. Perspect Clin Res. 2014; 5(2):71-74.
8. Stocker I (Meier J, Snake Venom proteins in Hemostasis: New result *Folia haematol* int mag klin morpho blutforsch, l989; l6(6):95-953
9. Udupa SL, Shaila HP, Udupa AL *et al*. Effects of botropase on wound healing. Biochem Arch. 1991; 7:207-212
10. Vandana Shenoy K, Mohan Baliga, Sumitha Mahajan, Ramesh KV. The effects of topical hemocoagulase solution on the healing process of post-extraction wounds: A split mouth design. J. Maxillofac. Oral Surg, 2014
11. Role of hemo-coagulase as a local haemostatic agent after extraction of tooth. Solanki DA, Modha ND. Int J Res Med. 2015; 4:14-17.
12. Levy JH. Haemostatic Agents. Transfusion. 2004; 44:58S-62S.
13. Mingwei ZHU, Jinduo CAO, Zhengeng JIA, Zhiquan DUAN, Guojin LIU, Junmin WEI. Department of General Surgery, Beijing Hospital, Beijing 100730, China; Hemocoagulase in abdominal operation and its effect on hemoagglunation [J]: Chinese Journal of Surgery, 2002-08.