Effect of intra-operative topical tetracycline versus 5-fluorouracil in prevention of post-mastectomy seroma in rabbit model

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Abstract: ***Background.*** Seroma is most common post-mastectomy and axillary dissection (AD) complication. This study aimed to evaluate and compare efficacy of tetracycline (TCN) or 5-fluorouracil (5-FU) intra-operative topical application in preventing post-mastectomy seroma development in rabbits. ***Material and methods.*** Forty adult female albino rabbits divided into 3 groups [control (n=12), TCN (n=14), 5-FU (n=14)] were used. Rabbits underwent unilateral thoracic mastectomy and AD. Immediately following operation, equal volumes of saline, TCN (5-10mg/kg b.wt), 5-FU (12mg/kg b.wt) were instilled under surgical skin flaps. On seventh post-operative day, seroma formation and wound healing processes were evaluated. Seroma fluid was aspirated and evaluated for sodium, potassium, chloride, total proteins levels. Histological examination of dissecting area was made. ***Results.*** Seroma developed in 22 rabbits (55.0%); 7 in saline (58.3%), 9 (64.3%) in TCN, 6 (42.9%) in 5-FU groups. Seroma fluid volume was lowest in 5-FU group. Seroma fluid nature was inflammatory exudate. Histopathological examination revealed lack of walling, inflammatory cellular infiltrate, blood extravasations, tissue necrosis in all groups with least changes in 5-FU group**.** ***Conclusions.*** Inflammatory cell exudate was common feature of rabbit with post-mastectomy seroma. 5-FU was best in decreasing seroma volume and minimizing histopathological changes in post-mastectomy wound healing.
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Key words: 5-Fluorouracil; Histopathology; Post-mastectomy; Rabbit; Seroma**;** Tetracycline.

1. Introduction

Seroma is most common problem occurring after mastectomy. It is accumulation of serous fluid under skin flaps following mastectomy or in axillary dead space immediate post-operative period. Seroma incidence varied from 10% to over 85% [1]. Most surgeons view it as necessary evil rather than complication, as it usually resolves within few weeks. However, excessive fluid accumulation will stretch skin and resulting in patient discomfort and prolonged hospital stay. Morbidities related to seroma include wound site infections, flap necrosis, wound dehiscence, and delay in adjuvant treatment [2].

Seroma formation prevention has become most important goal, due to lack of effective treatment. In all methods, the main preventive strategy is to reduce lymphatic leakage and obliterate dead space, including use of ultrasound scissors in performing lymphadenectomy, closed suction drainage, repeated aspirations, external compression on flaps, dead space closure by multiple-sutured flaps to underlying muscles, increasing collagen synthesis using several sclerosing agents at dissection areas, therefore fixing skin flaps to underlying muscles. Most of aforementioned methods have failed but some sclerosing agents, such as fibrin glue or fibrin sealant; have been found to be effective for seroma prevention. Nonetheless, agents have given rise to some side effects and cost-effective disadvantages [3]. Tetracycline (TCN) is antibiotic that proved to have potent sclerosing agent by enhancing fibroblast collagen synthesis. TCN was used for malignant pleural effusions [4], endometrial cysts [5], hepatic cysts [6], pericardial effusions [7], gallbladder lumen [8]. Two reports have suggested TCN efficacy, as topical sclerosing agent, in post-mastectomy seromas [9,10]. 5-Flourouracil (5-FU) is commonly used chemotherapeutic agent in breast cancer. Studies have shown that intra-peritoneal administration of 5-FU increases adhesion formation [11].

Based on these reports, an experimental trial was carried out to evaluate and compare preventive effects of intra-operative topical application of TCN and 5-FU, as adhesion-inducing agents in rabbit mastectomy model. The possible mechanisms of TCN and 5-FU actions were also investigated.

2. Material and Methods

The study protocol was approved by Medical Ethical Committee of King Fahed Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Forty female albino rabbits (3-4 months old); weighing between 2.50-4.50 kg were used. All animals were fed standard food and water (*Ad libitum* supplied by animal care unit in KFMRC). Rabbits were operated under Ketamin [Ketalar 50mg/ml, EspecIalidad USO Hospital Ario] and Seton 2% [EspecIalidad USO Veterinario]. Unilateral pectoral

modified mastectomy procedure was done as described previously [12]. Elliptical vertical incision around nipple of pectoral breast was made. Nipple with surrounding skin was discarded. Humeral attachment of pectorals muscle was ligated with 3/0 Polyglactin [Vicryl, Ethicon J&J]. The dissected breast together with small part of pectorals muscle, part of axillary fat, and associated lymph nodes were removed. The underneath of skin flap was scraped by surgical blade blunt side to traumatize subcutaneous lymph vasculature. Then mastectomy site was washed with normal saline and dried with sterile gauze.

Operated animals were divided into three groups according to solution immediately added to post-mastectomy dead space prior to skin closure. Control group (n=12), rabbits underwent unilateral mastectomy and 3cc saline was added to post-mastectomy dead space. In TCN group (n=14), rabbits underwent unilateral mastectomy and 1.2cc TCN diluted in 1.8cc saline (5-10 mg/kg) was added to post-mastectomy dead space. In 5-FU group (n=14), rabbits underwent unilateral mastectomy and 0.8cc 5-FU diluted in 2.2cc saline (12 mg/kg) was added to post-mastectomy dead space. TCN and 5-FU doses for rabbits were calculated as **Paget and Barncs** [13].

All mastectomy wounds were vertically closed with 3/0 polyglactin [Vicryl, Ethicon J&J] continuous subcuticular stitches to prevent wound self dehiscence. Operated animals were observed post-operatively for 2 hours. Visual inspection was done daily for arm movements or wound complications.

On seventh day post-operatively, mastectomies area were inspected and palpated for swelling. Those suspected to having seroma were aspirated using (10 ml syringe) from most dependant axillary region. Dead space was opened and further aspiration of remaining fluid. Aspirated fluid was inspected for any turbidity, blood stain or suspended particles. Aspirated fluid characteristics were recorded. Biochemical analysis (total protein and electrolytes as sodium, potassium, chloride) of seroma fluid was done in biochemistry laboratory at King Abdulaziz University hospital.

Following seroma aspiration, animals were sacrificed with high-dose of ether anesthesia. Skin flaps and underlying tissues mastectomy region were dissected including axilla and thoracic wall, and fixed in 10% formalin. Formalin-fixed tissues were embedded in paraffin, cut into 5 µm thick slices and stained with hematoxylin and eosin for histopathological examination and Massion Trichrome staining of collagen fibers**.** Inflammatory cells in post-mastectomy seroma region were counted in five fields in each of five slides of serial sections using Image Pro-Express version 6 of studied photographs to confirm histological observations.

Statistical analysis. Data were analyzed by Statistical Package for Social Science (SPSS Inc., Chicago, IL) version 16.0. Quantitative data were expressed as mean +/- stander deviation (SD), and qualitative data as number & percentage. One Way ANOVA test was used for quantitative variable while Chi square test for qualitative variables. Significance (*P*) level was accepted at <0.05.

3. Results

Percentage of animals that developed seromas in saline, TCN and 5-FU groups were 58.3%, 64.3% and 42.9%, respectively with insignificance difference between them *(P* <0.727). The percentage of serous and serosanguineous seroma fluid were in saline (71.4%, 28.6%), in TCN (55.6%, 44.4%) and in 5-FU groups (54.5%, 45.5%), with insignificance difference between them *(P* <0.387). There was insignificance difference in amount of fluid aspirated from rabbits of saline, TCN, 5-FU groups (*P* >0.05). However, seroma development in 5-FU group was least in volume (Table 1).

There were insignificant difference in sodium, potassium, chloride and total proteins levels in seroma fluid of different studied groups (*P* >0.05) (Table 2).

Seroma of all groups showed marked inflammatory cellular aggregation. Dead space wall lacked fibrous sealing with dominating capillary dilation and vascular congestion in most seroma cases (Figs 1A, C, E). Extravasations of red and inflammatory cells in underlying healed tissue were observed. Inflammatory cells were mainly lymphocytes and eosinophils. All aforementioned findings were observed with lesser extent in 5-FU group (Fig 1F).

Non-seroma cases regardless of therapy type showed reduced dead space, free from cell debris or inflammatory cells. Floor was well delineated with either flat closely endothelial like cells or collagen deposit. Underlying floor tissue showed nearly mature fibrous scar with few inflammatory cells mainly lymphocytes (Figs 1B, D, F). Pro-image analysis of studied photographs confirmed histological observations where there was extremely significant decrease of inflammatory cells in post-mastectomy region of seroma rabbits treated by 5-FU when compared to saline and TCM groups (Table 3). Regards therapeutic regimen efficacy, it was found that 5-FU provided most satisfactory results.

Table (1): Comparison regarding seroma in different studied groups

Items	**Saline** (n=12)	**Tetracycline** (n=14)	**5-Fluorouracil** (n=14)	**Total** (n=40)	**Significance**
Seroma development	7 (58.3%)	9 (64.3%)	6 (42.9%)	6 (42.9%)	*P* <0.727
Aspirated fluid amount (ml)	6.64±8.06 (0.50-21.00)	6.61±7.58 (1.00-26.00)	2.17±1.51 (0.50-4.00)	-	**P* >0.993 ***P* >0.247 ****P* >0.226
Fluid type					
Serous	5 (71.4%)	5 (55.6%)	2 (33.3%)	12 (54.5%)	
Serosanguineous	2 (28.6%)	4 (44.4%)	4 (66.7%)	10 (45.5%)	*P* <0.387

Data was expressed as mean +/- stander deviation and range or number (%) as appropriate. *P:* significance between groups; **P*: significance between saline and tetracyclin; ***P*: significance between saline and 5-fluorouracil; ****P*: significance between tetracyclin and 5-fluorouracil.

Table (2): Comparison between seroma fluid components in different groups

Groups	**Sodium** (mmol/L)	**Potassium** (mmol/L)	**Chloride** (mmol/L)	**Total proteins** (g/L)
Saline (n=7)				
Mean±SD	139.57±2.88	5.11±0.95	105.71±3.77	44.86±6.82
Range	(134.00–142.00)	(4.10–6.90)	(102.00–113.00)	(36.00–53.00)
Tetracycline (n=9)				
	136.67±12.04	4.71±0.66	103.44±5.92	47.56 ±9.81
	(105.00–143.00)	(3.80–5.80)	(91.00–110.00)	(40.00–72.00)
	P >0.499	*P* >0.306	*P* >0.377	*P* >0.498
5-Fluorouracil (n=6)				
	138.17±4.91	5.03±0.64	102.50±4.59	47.50±4.28
	(129.00–141.00)	(4.40–6.20)	(94.00–106.00)	(44.00–55.00)
	P >0.766	*P* >0.850	*P* >0.260	*P* >0.547
	**P* >0.738	**P* >0.432	**P* >0.723	**P* >0.989

P: signifiance versus saline; **P*: signifiance versus tetracycline.

Table (3): Comparison between inflammatory cells numbers of seroma and non-seroma specimens in each group and between groups

Treatment	**Seroma**	**Non seroma**	**Significance**
Saline	35.00±3.00	14.00±4.00	*P* <0.001
Tetracycline	43.00±7.00	13.00±1.00	*P* <0.001 **P* <0.003
5-Fluorouracil	19.00±7.00	13.00±3.00	*P* <0.004 **P* <0.001 ***P* <0.001

P: Seroma versus non-seroma of same group.
**P*: Significance versus saline. ***P*: Significance versus tetracycline

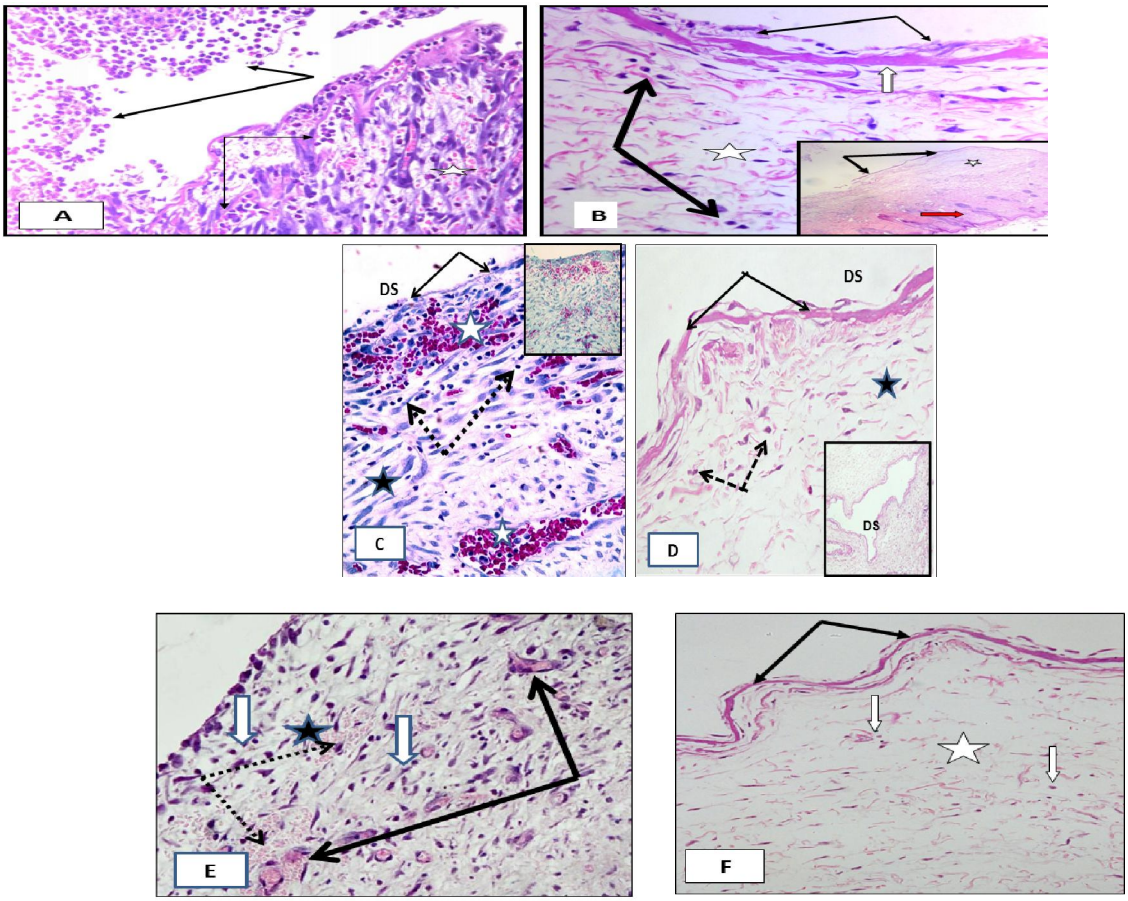

Figure (1): Histopathological changes in breast tissue
A: Saline treated seroma case showed marked cellular infiltration mainly eosinophils in dead space (thick arrows) and seroma floor (thin arrows).
B: Non-seroma case showed few cells, less granulation tissue (star) and attempt to walling (black arrows) in insert and collagen deposition (white arrow) (H&E X400).
C: Sections from tetracycline treated post-mastectomy seromas stained by Masson for collagen and H&E (insert) showed dominating of granulation tissue (black star), lymphocyte infiltrate (dotted arrows), dilated congested vessels and blood extravasations (white star).
D: Non-seromas case showed small dead space (insert), less granulation tissue (black star), few mononuclear leucocytes (dotted arrows), and collagen deposition delineates dead space (black arrows).
E: Dead space floor of post-mastectomy seroma from 5-fluorouracil group showed numerous congested capillaries (black arrows), high cellularity (star)
F: Non-seroma of same group showed fibrous sealing (black arrows), few lymphocytes (vertical white arrow) and absence of vascular congestion (H&E stain X200).

4. Discussion

Wound seromas represent significant cause of morbidity as retard wound healing, promotes wound infection and delays adjuvant therapy initiation. Management of these seroma in outpatient department causes additional burden on nursing staff and hospital resources. In this study, chemical and cellular examination of aspirated seroma fluid reveals its similarity to serum. Total protein, sodium; potassium; chloride levels were different from lymph but similar to inflammatory exudate suggesting its inflammatory origin. Similarly, **Watt-Boolsen *et al.*** [14] demonstrated that seroma is exudate resulting from acute inflammatory reaction, and concluded that seroma formation reflects increased intensity and prolongation of wound repair. **McCaul *et al.*** [15] demonstrated that drainage fluid similar to inflammatory exudate. Meanwhile, others [14] hypothesized that seroma is mostly originate from lymph.

Although several techniques have been reported for seroma formation prevention, there is no standard clinical approach in routine practice. Use of sclerosant in seroma management is not new. In humans,

seromadesis has been reported with erythromycin [16], povidone iodine [17], talc [18] and hypertonic saline [19]. Tetracycline sclerotherapy can administrate in two ways, first topically to chest wall and skin flaps prior to skin closure [20], while second is to aspirate seroma postoperatively and instill tetracycline after its dilution in 0.9% NaCL [9]. The present study revealed that at 7th day post-mastectomy, seroma developed in all studied groups (saline 58.3%, TCN 64.3%, 5-FU 42.9%). Meanwhile, volume of aspirated seroma fluid was least in 5-FU group. Wide dead space, fibrous walling lack, vascular congestion and inflammatory cellular infiltrate mainly by lymphocytes and eosinophils were most histopathological features in post-mastectomy seroma developed in TCN group. In 1986; **McCarthy *et al.*** [21] designed controlled trial to study tetracycline effects on patients with prolonged drainage after mastectomy. Trial was aborted as tetracycline was painful and not effective. In 2000, researchers at Mayo Clinic [20] used tetracycline (1g TCN in 100 cc 0.9% NaCL) during surgery (intra-operative) as prophylaxis; however, this trial was stopped because 2 weeks postoperatively, tetracycline group had more seromas than saline group (53% vs. 22%, P <0.01). Although **Sitzmann *et al.*** [9] reported good results of sclerotherapy after mastectomy; their study was retrospective and anecdotal in only 5 patients. In **Hokkam *et al.*** study [22], treatment of 49 patients with post-mastectomy seromas by aspiration and sclerosant solution instillation containing tetracycline (2g tetracycline dissolved in 100ml NaCL 0.9% +10ml lidocaine). They reported that thirty six patients (73.4%) were successfully treated with one sclerotherapy session while nine patients (18.4%) needed two sessions and four patients (8.2%) needed three sessions. Majority of patients (85.7%) had no complications after technique.

In this study, intra-operative application of 5-FU leads to reduction in number of rabbit models that developed post-mastectomy seroma compared to saline and TCN groups (42.9% versus 58.3%, 64.3%). Also, amount of aspirated seroma fluid was insignificantly lower in 5-FU compared to other groups. Histological examination of seroma area in 5-FU group reveals decrease dead space, vascular congestion, inflammatory cells infiltration, enhances healing and normal scaring process. Similarly, **Kocdor *et al.*** [23] reported that 5-FU decreased angiogenesis, vascular congestion and inflammatory cells in seroma of rat model. It has been suggested that vasodilatation is obvious during inflammation period of wound healing and event for cellular efflux and fluid extravasation [24]. Eosinophils predominance observed in present samples was mostly had role in vascular dilatation and fluid efflux. Allergic reaction to iodine was reported to be among etiological factors [25]. The decrease in cellular infiltrate and fibroblast proliferation observed in present study may be attributed to tumoricidal effect of 5-FU [2].The mechanisms underlying seroma prevention by 5-FU remain unclear. But possible explanation can be due to decrease in inflammatory cells may underline decrease in seroma volume via decreasing cytokines. **McCaul *et al.*** [15] reported that seromas contain significantly more granulocytes and monocytes than lymphocytes. It has been shown that seroma contains IgG, granulocytes, leukocytes, which are normally present in acute inflammatory exudates [26]. Another important finding of this study is decreased angiogenesis and vascular congestion in 5-FU rabbits. Decreased angiogenesis and vasodilation may reduce fluid efflux from capillary bed towards dissection surfaces. Cytokines, prostaglandins, vasoactive amines and angiogenic factors released from acute inflammatory cells play important role in vasodilation and angiogenesis [24]. Angiogenesis role on seroma formation were investigated by **Wu *et al.*** [25] who examined vascular endothelial growth factor (VEGF) and endostatin levels in wound fluid and plasma of patients mastectomy patients. Especially in early postoperative period, significantly higher VEGF and lower endostatin levels were detected in seroma fluid compared with plasma which suggested that angiogenesis is initiated immediately after surgery and wound fluid is more informative than blood. On contrary, 5-FU, as antimetabolite, is able to inhibit VEGF-mediated angiogenesis [27]. Therefore, in present study, decreased angiogenesis or vascular congestion may be contributing factor for seroma prevention. 5-FU may significantly affect some microvessel network patterns such as interconnective loop formation or microvessel branching in dose dependent fashion [28]. In present study, 5-FU was applied directly to healing tissue that may produce significant vascular cell toxicity and flap ischemia and necrosis. Thus, applications of diluted 5-FU diminish ischemic complications at wound sites.

5. Conclusion.

Post-mastectomy seroma is common complication. Seroma fluid nature was inflammatory exudate. Intra-operative application of 5-FU was superior to TCN in decreasing incidence and the amount of post-mastectomy seroma fluid. The mechanism of action could be via decreasing inflammatory cells infiltrate or decreasing vasodilatation and angiogenesis or promoting fibrous walling. Further study is going on to investigate cytokines role and vasoactive mediators in seroma formation so an appropriate antidote could be applied for its prevention.

6. References

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