RESEARCH ARTICLE

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Effectiveness of Normal Saline Irrigation in Reducing Wound Contamination during Oral Cancer Surgery: A Cytological Analysis

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Abstract

Background: Tumour manipulation during surgery can lead to the dissemination of malignant cells and potential wound contamination. Despite the widespread practice of irrigating surgical sites with normal saline, the efficacy of this measure in reducing epithelial contamination in oral cancer surgery remains unclear. **Objectives:** This study aimed to assess the proportion of normal saline wash effluents contaminated by epithelial cells or debris during oral cavity cancer surgery, and to evaluate the effectiveness of saline irrigation in reducing contamination. **Methods:** A total of 132 patients with biopsy-proven, treatment-naïve squamous cell carcinoma of the oral cavity undergoing surgery were included. Wash effluents from the tumour bed and neck incisions were collected post-irrigation with normal saline and analysed using cell block cytology. Cytology smears from the tumour bed and incision edges were examined for cellular contaminants. Data were analysed using Chi-square tests and Mann-Whitney tests. **Results:** Epithelial or abnormal epithelial cells were detected in 24% of cell block samples, while 21% showed cellular debris. Following normal saline irrigation, the positivity rate for epithelial cells or debris in smears decreased from 55% to 7.6%, a statistically significant reduction ($p < 0.001$). Perineural invasion was significantly associated with the presence of exfoliated cells ($p = 0.037$). **Conclusions:** Irrigation with normal saline significantly reduces the presence of exfoliated epithelial cells and cellular debris in wound sites during oral cancer surgery. The results support the continued use of mechanical cleansing measures during surgery to minimize the risk of tumour cell implantation.

Keywords: Oral cancer surgery- Normal saline irrigation- Wound contamination- Exfoliated epithelial cells

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Introduction

Since 1885 it has been suspected that malignant cells can exfoliate due to tumour manipulation during surgery and implant at distant sites causing metastasis [1]. Multiple analytical and observation studies in the sixties have recorded the presence of malignant cell in saline wound washings and wound drainage fluids from patients undergoing surgery for Head and Neck cancers [2-5]. A more recent observation in 1996 demonstrated presence of malignant cells in washings collected from surgical instruments and the surgeon's gloves in fifteen patients of head and neck cancers [6].

Surgeons have traditionally tried to prevent implantation by washing the tumour bed and incision sites with normal saline and changing their gloves, instruments, drapes and surgical gauze after extirpation of the tumour. This was first mentioned in an editorial in 1937 [7]. Other authors have also recommended a change of instruments and drapes with copious washing of the operative site especially with hypotonic saline [8, 9].A recent survey questioned surgeons about their practices and beliefs regarding measures of wound protection [10]. It was found that forty-three percent surgeons used wound protectors and alternative barriers while fifteen percent used wound irrigation. When asked about the basis for such practices only four percent cited experimental evidence.

The practices of wound irrigation and change of gloves and instruments to prevent suspected wound contamination are prevalent in the Head and Neck Oncology unit of Mahavir Cancer Sansthan, Patna. But, as pointed out by Berger-Richardson et al. [10], there is no experimental or clinical evidence to support the practice. This raises two questions – are there epithelial cells or cellular debris in the normal saline wash after it has been used to irrigate the wound? Does this step lead to decrease in the proportion of contaminated wounds?

To answer the first question we collected the wash effluent and looked for cells after processing the sediment into cell blocks. To answer the second question we examined cytology smears taken from the wound after irrigation to look for cellular contaminants.

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The first objective of the study was to estimate the proportion of normal saline wash effluents contaminated by epithelial cell/ debris from the wound. The second objective was to determine the decrease in proportion of contaminated wounds after normal saline wash.

Materials and Methods

The study was carried out in the Head and Neck Oncology Department of Mahavir Cancer Sansthan on consecutive patients of biopsy proven, treatment naïve, oral cavity squamous cell cancers who gave consent for surgical excision of their tumour.

Patients of oral cavity cancers with skin ulceration or those with histology other than squamous cell cancers were excluded. Patients with significant co-morbidities making them unfit for surgery or those who refused surgery were also excluded. Patients who took any part of their treatment (primary, adjuvant or neoadjuvant) outside the institute or did not complete their therapy were excluded.

After neck dissection and excision of the primary tumour, the tumour bed and neck were washed with five to fifteen hundred millilitres of Normal saline. This was collected and immediately sent to the pathologist for preparation of cell block. Thereafter, smears were taken from the tumour bed and the neck incision edges.

Processing of the wash fluid: The complete wash water was centrifuged at one thousand rpm for ten minutes and the supernatant discarded. The sediment was added to Lysis buffer(1:10n dilution) containing 0.37 gm. of EDTA,0.84 gm. of Sodium Bicarbonate and 8.02 gm. of Ammonium Chloride in hundred ml distilled water. The resultant solution was centrifuged at one thousand rpm for ten minutes and the supernatant with Lysis buffer was discarded. The sediment was added to 0.5 ml plasma followed by 0.5 ml Thrombin to form a clot. A MICROM STP120 machine was used to process the clot using 10% formalin. The processed section was embedded in wax and 3µm sections were cut using a microtome. The sections were stained using H & E stain and examined using light microscopy with 40X and 100X magnification by two consultant Onco-pathologists. Whenever there was a difference of opinion the sections were examined by both under a penta-head microscope and a consensus opinion was recorded as the final result.

Processing of the smears: The smears were air-dried, fixed with methanol and stained with Gimsa stain. They were examined by light microscopy under 40X and 100X magnifications by the same two pathologists.

The results of cell block examination and smear were classified as:

1. Positive – presence of epithelial cells or abnormal/ malignant cells

2. Suspicious – presence of cellular debris other than blood cells

3. Negative – acellular slides or slides with only blood cells

For the purposes of further data analysis both the 'positive' and 'suspicious' categories were treated as 'positive'.

Other predictor variables included age, gender, site of tumour, morphological pattern of tumour, pathological T and N status, Depth of Invasion, Lymphovascular invasion, perineural metastasis, distance of shortest margin and Worst pattern of invasion. Of these age, depth of invasion and distance of shortest margin were continuous variables. The rest were recorded as nominal or ordinal variables.

Categorical data (nominal and ordinal) were summarized as counts and proportions and analysed using Chi-square tests. Continuous data were summarized as Mean ± SD and compared using Student T / Mann-Whitney test. Software used included Microsoft Excel and Jamovi [11]. The study was approved the ICE-RMRIMS Ethics committee vide letter No. RMRI/EC/53/2022.

Results

One hundred thirty-two consecutive treatment – naïve patients of biopsy – proven squamous cell cancers of the oral cavity presenting to the Head and Neck Oncology unit of Mahavir Cancer Sansthan who were all fit and consenting for surgery were recruited. All patients with skin ulceration by tumour extension were excluded.

All tumours were excised with negative margins of more than five mm with an elective neck dissection. Following the resection the tumour bed and neck incisions were washed with five to fifteen hundred ml of normal saline. The wash effluent was processed as described above. Smears were also taken from the tumour bed and neck incision site and processed as described above.

We collected one hundred thirty – two wash effluent samples – one from each patient, and two hundred and sixty-four cytology smears from the tumour bed and neck incision wound of each patient. Since the cytology smears were taken in pairs from the tumour bed and neck incision of the same patient, each such pair was combined into one result. If either the smears showed any epithelial cells or cellular debris it was labelled positive. Therefore we now had one hundred and thirty two cell block cytology samples and one hundred and thirty two smear cytology. Samples of twenty-eight patients were omitted from the analysis due to incomplete data i.e. either one of the smears was missing.

Most of the tumours were present in the gingivo-buccal sulcus followed by the tongue and most were either ulceroproliferative or ulceroinfiltrative morphologically. Two-thirds of the tumours were moderately differentiated while commonest T and N status were T2 and N0.Lymphovascular invasion was found in twenty-one percent (22/104) patients and perineural involvement was present in only five patients. While none of the patients showed presence of malignant cells on the cut margins, the margins were close (less than 5 mm) in twenty-five percent (26/104). The demographic details are given in Table 1.

Results of cell block cytology: twenty – four percent (25/104) cell block cytology samples showed epithelial or abnormal epithelial cells and twenty – one percent (22/104) samples showed cell debris. On the other hand, fifty-eight percent (56/104) were acellular or had only blood cells. Since the samples with either epithelial cells

Table 1. Clinical and Pathological Details of Patients Stratified According to the Presence of Epithelial Cells in Lavage

	Lavage positive	Lavage negative	P value
Site of tumor			0.466
Buccal mucosa	7	5	
Gingivo-buccal complex	21	27	
Retromolar trigone	2	2	
Tongue	17	23	
Morphology			0.466
Infiltrative	0	1	
Proliferative	0	1	
Ulcerative	1	2	
Ulcero-infiltrative	25	21	
Ulcero-proliferative	19	28	
Verrucous	0	1	
Polypoidal	0	1	
Thickening	1	0	
T stage			0.856
T1	12	12	
T2	18	21	
T3	10	12	
T4a	7	12	
N stage			0.205
N0	31	34	
N1	6	10	
N2a	2	0	
N2b	1	8	
N2c	2	3	
N3a	1	0	
N3b	3	2	
Grade of differentiation			0.336
Grade 1	18	14	
Grade 2	28	41	
Grade 3	1	1	
Lymphovascular invasion			0.844
Absent	33	39	
Present	14	18	
Perineural Invasion			0.037
Absent	47	52	
Present	0	5	
Shortest Margin			
Mean ± SD (in millimeters)	6.53 ± 3.54	7.09 ± 2.73	0.226
Depth of Invasion			
Mean ± SD (in millimeters)	8.77 ± 4.14	10.0 ± 5.7	0.348
Worst pattern of Invasion			0.119
1	0	3	
2	3	4	
3	27	20	
4	7	16	
5	7	9	

and/or cellular debris were all considered positive this resulted in a positivity rate of fifty-five percent (57/104).

Results of smear cytology: Following washing of the tumour bed and neck incision with normal saline none of the smears showed any epithelial or abnormal epithelial cells. Only eight (8/104) samples showed cellular debris, but since these were also considered 'positive' the positivity rate was 7.6%.

The normal saline wash resulted in a decrease in positivity rate from fifty-five percent to 7.6%. This decrease in positivity rate was statistically significant (chi-square = 29.8, df = 1, $p < 0.001$).

The clinico-pathological predictors variables were analysed for their association with presence of epithelial cells/abnormal epithelial cells/cellular debris in the wash effluent and the results are given in Table 2. The only predictor with significant association was presence of perineural involvement ($p = 0.037$).

Discussion

This study was done to estimate the proportion of tumour beds and neck incision sites contaminated with exfoliated epithelial or abnormal epithelial cells. To estimate this we examined the wash effluents collected after washing the tumour bed and neck incision sites with normal saline. Since normal saline is isotonic, the wash will result in only mechanical cleansing of the wound without any cytotoxic or osmotic action.

We found that twenty-four percent of wash samples contained epithelial/abnormal epithelial/ malignant epithelial cells. Furthermore, another twenty-one percent of samples showed cell debris but no epithelial/abnormal epithelial cells. The former were considered 'positive' while the latter were considered 'suspicious'. This answers our first question – epithelial/abnormal epithelial cells do exfoliate during radical surgery of Oral cavity tumours and can be found on the tumour bed and incision sites if not washed away.

Other authors [2-5] who have examined wound washings of patients undergoing radical resection for head and neck cancers have found presence of abnormal/ malignant epithelial cells in 7.5 – twenty-six percent and suspicious in thirteen to nineteen percent of wash samples. These authors have taken wash samples from cancers of all sites of the upper aerodigestive mucosa whereas we took samples from the oral cavity only. In spite of this difference in sample selection, comparison of these results show insignificant differences between them (chi^2 = 3.35, df = 6; $p = 0.764$).

Similar results have been found in wash water after resection of malignant tumours in other organs. Thirty – three percent of pleural washings from patients undergoing surgery for lung cancers were found to contain cancer cells [12]. Similarly, seven out of eight pelvic washes have been found to contain residual cancer cells following robot-assisted radical cystectomy [13]. Viable colo-rectal cancer cells have been found in fourteen out of nineteen colorectal lavage specimen, seventeen out of thirty fluid specimen after irrigation of proximal cut end of the

Table 2. Results of Univariable Analysis of Clinical and Pathological Predictors with the Presence of Epithelial/ Abnormal Epithelial Cells and Cell Debris – the two together are labelled 'positive'.

Variable	Lavage positive	Lavage negative	Test Statistic*	Degrees of freedom	P value
Site	47	57	Chi^2 = 2.4	4	0.663
Morphology	46	55	Chi^2 = 6.66	7	0.466
T – stage	47	57	Chi^2 = 0.774	3	0.856
N – stage	46	57	Chi^2 = 9.72	7	0.205
Pathological stage			Chi^2 = 3.68	4	0.423
Grade	47	56	Chi^2 = 2.18	2	0.336
LVI	47	57	Chi^2 = 0.0388	1	0.844
PNI	47	57	Chi^2 = 4.33	1	0.037
WPOI	44	52	Chi^2 = 7.57	5	0.182
Margin (less than/ greater than or equal to 5 mm	6.53 ± 3.54	7.09 ± 2.73	Chi^2 = 2.08	1	0.149
DOI	8.77 ± 4.14	10.0 ± 5.7	U = 1097		0.348

resected specimen and twenty one out of twenty five fluid specimen after irrigation of the distal cut end of the resected bowel [14]. Please note that both the pleural and peritoneal spaces are closed spaces therefore increasing the risk of implantation metastasis. In comparison the tumour bed and neck incision sites are open tissue sites and the wash water flows over them to be collected.

Many agents have been examined to determine their efficacy in prevention of implantation of malignant cells, ranging from formaldehyde to local cytotoxic agents [2, 5, 15–17) We have found that, following mechanical cleansing with normal saline, zero smears showed epithelial cells and only eight smears showed cellular debris. Therefore, proportion of positive smears decreased from twenty four to zero percent and suspicious smears from twenty one to 7.6%. Umpleby et al. [18]found povidone-iodine solution achieved near-total cell kill at all concentrations and Fumito Ito et al. [19]found that fifteen minutes of exposure to hypotonic water results in cell lysis. In contrast our results suggest a significant decrease in epithelial cells due to just the mechanical cleansing action of isotonic normal saline.

Limitations The methodology used by us has not included the use of any form of immunohistochemical staining to identify epithelial cells because literature records an accuracy rate of 85 – 90% for the cell block cytology method used [20].

In conclusions, exfoliated epithelial cells and cellular debris were found in fifty-five percent of normal saline wash water following radical resection of Oral cavity cancers. This is significant proportion and raises the spectre of implantation metastasis. A simple wash with isotonic normal saline results in substantial decrease in presence of epithelial cells and cellular debris from fifty-five percent to about eight percent. The mechanical cleansing by normal saline is responsible for this reduction.

Therefore, it is suggested that the precautions taken by surgeons to change instruments, gloves and drapes after resection of tumour and mechanical cleansing of the surgical field with normal saline should be continued.

Author Contribution Statement

Dr.Pankhuri Shandilya-conceptualization of study and collection of data. Dr.Ajay Vidyarthi--conceptualization of study and revision of manuscript. Dr.Hiralal Ash- revision of manuscript. Dr.Amit Kumar-sample processing. Dr Varun Ketan-sample processing.

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If it was approved by any scientific Body/ if it is part of an approved student thesis

ICMR Ethics Committee Rmrims Patna

How the ethical issue was handled (name the ethical committee that approved the research)

ICMR Ethics Committee Letter NO.RMRI/EC/53/2022

Any conflict of interest

None.

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