Histological differences in wound healing in Maxillofacial region in patients with or without risk factors.

Jachmen Sultana, Motiur Rahman Molla, Mohammed Kamal, M. Shahidullah, Ferdousy Begum, Md. Abul Bashar

Abstract

Maxillofacial surgeons often deal with many cases of delayed wound healing, some are related to known risk factors and some are unknown. This study was aimed to assess the histological features of wound healing on day 0 and day 7 in postoperative cases of maxillofacial region in patients with or without risk factors. Microscopic examination of tissue specimen is a reliable and reasonably safe method to evaluate the histological differences. Six known risk factors for delayed wound healing were studied, which are commonly associated with maxillofacial pathology. Both clinical and histological examinations were performed for the evaluation of 32 postoperative cases. Out of 32 patients, 17 patients with risk factor were in study group and 15 apparently healthy persons without risk factor were in control group. Postoperative clinical evaluation of wound was done at 3rd, 5th, 7th, 9th, and 11th day and histological examination of tissue specimen was done on day 0 and day 7. Based on six histological parameters, microscopic examination of tissue specimen was done by both routine Haematoxylin and Eosin stain and Masson's Trichrome stain. In day 7, healing wound of study group having risk factors showed profound amount of granulation tissue, early collagen fibres, plenty inflammatory infiltrate, vertical orientation of reticular pattern of collagen and minimum amount of mature collagen in majority of patients which indicates delayed healing. On the other hand, in control group majority showed horizontally oriented mature collagen fibres in fascicle. Statistically, significant association was found between study and control subjects in terms of pattern of collagen tissue, amount of early and mature collagen tissue at the 7th day of follow up. Clinical evaluation also had strong association with histological state of healing.

Key words: *Wound healing, Postoperative, Maxillofacial region.*

Introduction

Impaired wound healing is a common clinical problem, which is a consequence of disordered collagen formation[1] and underlying predisposing conditions[2]. A weak scar may result from a large number of local or systemic factors[3]. Wound healing in the skin illustrates the general principles of healing that apply to all tissues and provides excellent models to study the factors that modulate the healing process. Whether a wound heals by primary or secondary intention is determined by the nature of the wound rather than by the healing process itself[3]. The only differences are quantitative not qualitative[4]. Collagen the most abundant protein in the body and plays a critical role in the successful completion of wound healing. Type I collagen is the major component of extra cellular matrix in skin. Type III, which is also normally present in skin, becomes more prominent and important during the repair process5. Individual changes that take place with regard to individual collagen fibrils include increased diameter, increased interfibril binding, and rearrangement of fibrils with time and become more organized in a manner that maximizes strength[1,6].

The orderly collagen formation at different stages of wound healing at different days can be seen microscopically by both routine Haematoxylin and Eosin and Masson's Trichrome stained sections[7]. In Masson's Trichrome stain, old collagen fibres take deep blue colour and the new collagen fibres stain light blue, represents mature or early collagen. Microscopically, collagen fibre orientation, its different pattern, variable amount of early and mature collagen, as well as presence or absence of inflammatory cells, granulation tissue and its amount are also observed. Based on these histological parameters actual healing state of a wound can be identified.

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Clinically wound healing of skin in Maxillofacial region occurs by 3-5 days[8] and in oral cavity, mucosal wound healing occurs by 5 to 7 days[9,10]. From day 3 to onwards the quantitative increase in collagen synthesis occurs and continue up to early 2nd week[3]. According to Cotran, Kumar and Collins quantitative increase in collagen synthesis ends in 10 days[11]. Adrian[5] mentioned it as 12 days but no occurs of quality of collagen fibril occurs[4]. And as in ninth[11] or tenth day the wound is moderately well healed and much greater proportion of tensile strength required and then become progressively stronger[12]. So, tissue specimen at 7th day of wound healing can provide a fair histological estimation of the process.

For these reason, intention of the present study is to evaluate the histological state of healing wound in postoperative cases of Maxillofacial pathology with or without risk factors after collecting the surgical tissue specimen at 7th day after the surgery.

Materials and Methods

This prospective study was undertaken in the department of Oral and Maxillofacial surgery and department of Pathology, BSMMU, Dhaka during the period of 1st July 2005 to 30th June 2007,based on an ethical approved protocol. The subjects in this study consisted of all surgical patients of Maxillofacial regions (age 10 - 65 years). Psychologically abnormal patient and medically unfit patient e.g. coagulopathies were excluded from the study.

Settings and Patients

Thirty two Maxillofacial surgical patients were included in the sample. They were enrolled in succession into a study group (n=17) who have one or more factors responsible for delayed wound healing. The factors are local malignancy, diabetes mellitus, local infection, poor nutritional status, local radiotherapy and immunosuppressive drugs. A control group (n=15) who were apparently healthy and without risk factors were also studied. They had no malignancy, nondiabetic, not infected, possesses good nutritional status and had no history of immunosuppressive drugs. Subjects operated for different pattern of benign maxillofacial pathology were included in the control group. These included patients of Benign tumors (Ameloblastoma, Giant cell granuloma, Cemento ossifying fibroma), Multiple fracture in Maxillofacial region, Temporomandibular joint ankylosis and Cystic lesions (Dentigerous cyst, Radicular cyst). In case of study group local malignancy (squamous cell carcinoma, verrucous carcinoma, chondrosar-coma), local pathology associated with local infection, local pathology associated with systemic effects, like diabetes mellitus, history of radiotherapy and chemotherapy, history of chemotherapy and malnutrition of different grades were included, Different pattern of incision were also used, these were Submandibular incision, Incision in Scalp region and Vestibular mucosal incision.

Study design

Design used in the study was as follows.

1. Preparation of wound healing assessment sheet.

a. Patients personal characteristics age, sex, diagnosis by history, physical examination, necessary records suggesting presence of certain risk factors of delayed wound healing.

b. Preoperative laboratory investigation - Fasting blood sugar or 2 hours after breakfast.

c. Patients Nutritional status[13,14] .

d. Written consent .

2. Postoperative clinical evaluation of wound at 3rd, 5th, 7th 9th, and 11th, days of surgery by observation of site of wound, length of incision, wound condition and swab for culture and sensitivity at 6th days[15].

3. Histological examination of tissue specimen both routine Haematoxylin and Eosin staining and Masson's Trichrome staining[16].

Surgical details

An elliptical incisional biopsy containing the tissue specimen of 5 mm in size and depth unto subcutis or up to submucosa was taken from the incisional edge of surgical wound of submandibular, facial and scalp region during initial surgery and at day 7. These specimens were preserved in 10% formalin and sent for histopathological examination. Peroperatively taken tissue specimen from the wound margin containing healthy tissue was used as a control for comparing postoperative day 7 healing.

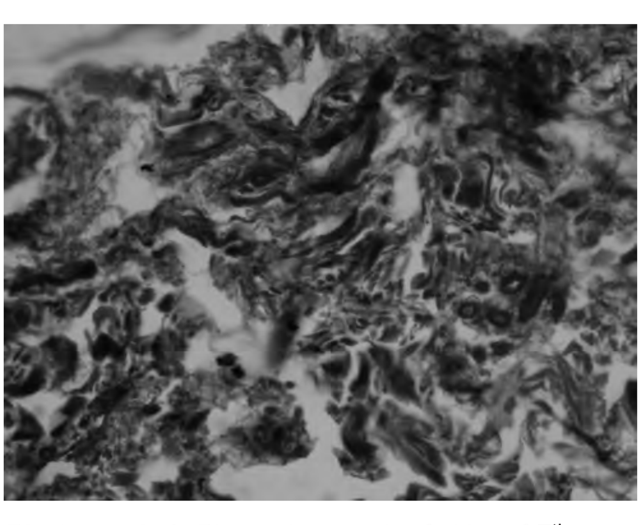
Clinical evaluation

In the present study local and general criteria of inadequate wound healing was observed by Haneya, Kawther and Olfat[17]. One score allotted for

each sign of redness, oedema, tenderness, discharge, dehiscence, stitch abscess, raised systemic temperature below 38^0C and 2 score for raised temperature above 38^0C. Scoring criteria: This was done by calculating fractions out of 9, with higher scores indicating poorer wound healing.

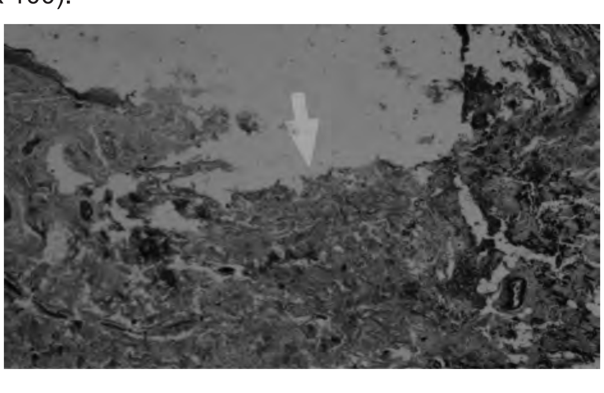
Histological evaluation

Based on six histological parameters of wound healing[7,18], biopsy obtained on peroperative day 0 and postoperative day 7 in both the groups were microscopically examined, The histological parameters were amount of granulation tissue, inflammatory infiltrate, collagen fibre orientation, pattern of collagen were seen after staining with routine stain and amount of early and amount of mature collagen were seen after staining with special stain: Masson`s Trichrome stain. Granulation tissue and early collagen are present in early stage of wound healing. Collagen fibres horizontally oriented and forms fascicle and all of these were mature collagen on day 7[4,5,7]. In comparison, in day 7 profound amount of granulation tissue, early collagen and plenty inflammatory infiltrate, vertical orientation of reticular collagen and minimum amount of mature collagen are the symbol of delayed healing[7,19]. The progression of healing was assessed histologically on the basis of individual criteria used for both initial surgery and at day[7].

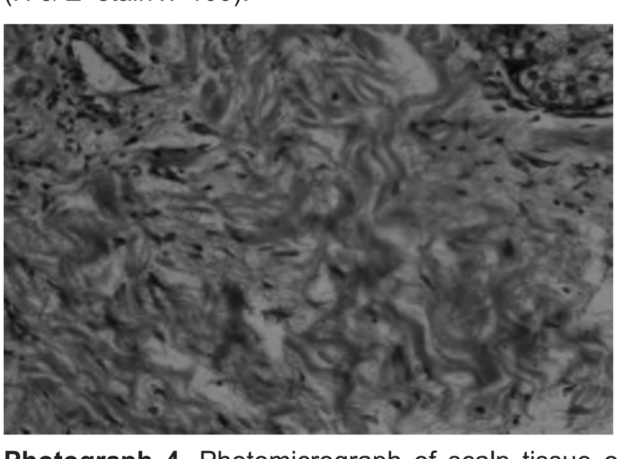


Photograph 1. Photomicrograph of skin of 7th post operative day showing reticular collagen, vertically oriented along the scar line inflammatory cells moderate. An example of delayed wound healing. (Masson's Trichrome stain x 200).

Photograph 2. Photomicrograph of skin of 7th post operative day showing horizontally oriented fascicle type of profound darkly stained collagen fibre. An example of good healing (Masson's Trichrome stain x 100).



Photograph 3 . Photomicrograph of mucosa of 7th postoperative day showing reticular collagen, vertically oriented . An example of delayed healing. (H & E stain x 100).



Photograph 4. Photomicrograph of scalp tissue of 7th postoperative day wound showing profound horizontally oriented fascicle type of collagen fibre. An example of good healing (H & E stain x 200).

Scoring criteria: The following scoring criterion was developed to compare healing status in both study and control groups in an ascending order for specific points. They were amount of granulation tissue (Profound - 1, Moderate - 2, scanty - 3, Absent - 4), inflammatory infiltrate (Plenty - 1, Moderate - 2, A few - 3), collagen fibre orientation (Vertical - 1, Mixed - 2, Horizontal - 3), pattern of collagen (Reticuler - 1, Mixed - 2, Fascicle - 3) , amount of early collagen (Profound - 1, Moderate - 2, Minimal - 3, Absent - 4) and amount of mature collagen (Profound - 1, Moderate - 2, Minimal - 3). Total healing score of each member was calculated by adding the score of individual criteria and with lower scores indicating poorer wound healing.Healing status was graded as good (16 - 19), fair (12 - 15) and poor (08 - 11).

Results

A total of 32 patients were evaluated. Among them 17 patients having risk factors for delayed healing were regarded as study patients and 15 patients were without risk factors. The mean age of the study group was 43.5 years and control was 45.9 years, t=0.618. No statistically significant mean age difference was detected between two groups of patients. According to distribution of patients by sex 22(68.8%) patients were male and the rest 10 (31.3%) were female with male and female ratio was 2.2:1 similarly analysis found. The male and female ratio was 2.2:1. Among the study group 58.8% were male and 41.2% female and among the control group highest percentage (80.0%) was male and 20% was female. However, analysis found no statistically significant sex difference between the two groups of patients.

Table 1.

Distribution of patients by age and sex

Age in years	Sex Male No.	%	Female No.	%	Total No.	%	P Value
<25	2	9.1	1	10.0	3	9.4	
25-34	3	13.6	1	10.0	4	12.5	
35-44	4	18.2	1	10.0	5	15.6	
45-54	9	40.9	5	50.0	14	43.8	
>55	4	18.2	2	20.0	6	18.8	
Total	17	100.0	15	100.0	32	100.0	
Mean±SD (Range)	44.1±12.7 (10.60)		45.9±14.1 (10.60)		44.6±12.9 (10.60)		0.714

P value reached from unpaired student's test (p>0.05)

Among the study patients 11.8% patients found to have sign of infection. However among the control group no patients had sign of infection. Among the study patients 29.4% patients had no systemic disease and 70.6% had systemic diseases. The mean healing score of the patients having no systemic disease was 12.0± 3.5 and for the patients having systemic disease was 11.8 ± 2.2. However, analysis found no statistically significant mean difference between two groups p>0.05 .

Table 2

Mean distribution of healing score by systemic disease among the study group

	Study group: No systemic disease	Study group: Having systemic disease	p value
Mean healing score	12.0 ± 3.5	11.8 ± 2.2	0.879
Range	9.0 – 18.0	10.0 – 17.0	
N	5	12	

P value reached from non-parametric Mann Whitney U test (p < 0.05)

Correlation matrix between healing score and selected variables shows significant negative association between risk factors indicating that healing was better among the patients with low risk factors. The healing was better among the patients with lower age, male sex and minimum surgical incision. However, the correlation was not statistically significant.

Table 3

Correlation matrix between healing score and selected variables

Parameters	Healing score
Healing score at 7th day	-
Age in years	-0.082
Sex (0=female, 1=male)	0.198
Lengh of incision (cm)	-0346
Risk factors (0.none, 1=present)	-0.409*

* Correlation is significant at the 0.05 level (2-tailed)

On follow up clinical parameters of the control group showed the appearance of normal colour of postoperative wound at 5th day observation and became hundred percent healthy at 7th day observation which was early in comparison with study group where the normal colour appeared in all the patients at 9th day of observation. Other parameters also showed similar patterns. Percentage distribution of patients by histological findings showed initially all the study and control had similar pattern of microscopic feature but at the 7th day of follow up of the histological pattern was found to be different which was assessed on the basis of arbitrary score. There was statistically significant association between study and control subjects, in terms of pattern of collagen tissue and relative amount of early and mature collagen tissue (p<0.005) indicating the delayed wound healing among the study patients.

Figure 1. Histological pattern of collagen

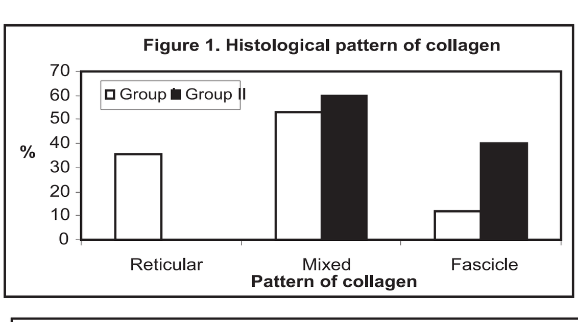


Figure 2. Amount of collagen (Early)

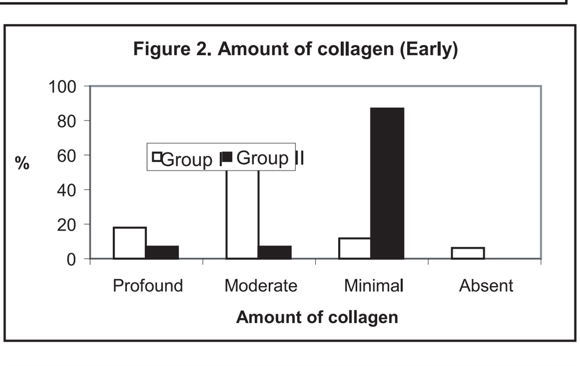
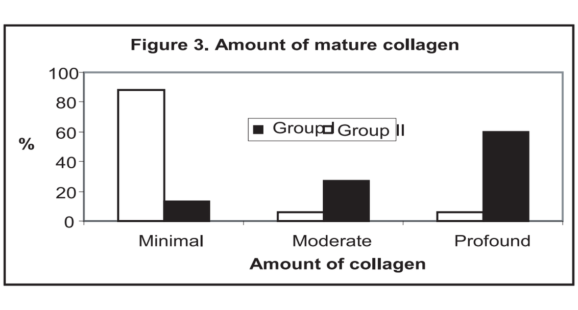
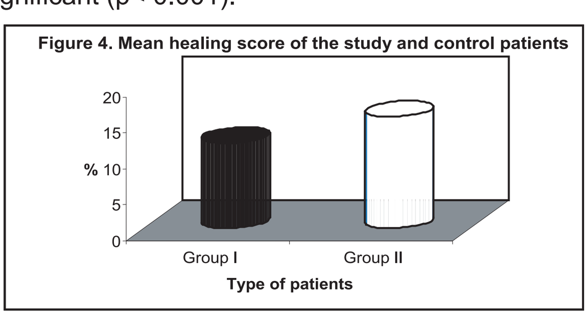


Figure 3. Amount of mature collagen



Analysis also indicates that the mean score of histological pattern was significantly low among the study patients (11.9±2.5) compared to control (15.5±3) and the mean difference was statistically significant (p< 0.001).

Figure 4. Mean healing score of the study and control patients



Discussion

The assessment of histological state of healing wound in with risk factors or without risk factors associated patients is an important step in clinical practice and further beneficial to postoperative management of surgical patients. The orderly collagen formation at different stages of wound healing at different days have been recognized as histologic hallmark of wound healing. Based on six established histological parameters of wound healing at the 7th day of follow up histological pattern was found to be different in between study and control subjects. Results of this study demonstrate that risk factors of the study group showed delayed wound healing in comparison with control group. The mean age of experimental subjects was 44.6 ranging from 10 - 65 years. As we know wound healing is faster in the young age and it is normal in old age unless associated with some debilitating disease. The multifactorrial nature of healing in the elderly persons makes it difficult to determine whether impaired healing is due to aging process or to other factors. So, different age groups were taken both for study and control group, age and sex were matched. This study finding was compared with previous study results[17]. Though statistically not significant, in this study healing was found better among male which is different from other studies[19]. As it is well documented that sex hormones modulate healing and males are vulnerable to delayed healing. There gender related trends warrant further investigation. The mean length of incision is 17.9 cm for study group and 12.3 cm for control group. The mean difference is statistically significant. But to find out any relationship of healing status with selected variables like length of incision, correlation matrix between healing score and selected variables was done. Though it was found statistically not significant it also warrants further investigation. It also compares with Dermarchez et al. 1986, Cavani et al. 1993, cited in Escamez et al.[18], where shows minimal incision heals early. Subjects containing different pattern of Maxillofacial pathology was included in this study. Among the study patients, 70.6% had systemic diseases. The most frequent systemic anomalies were malnutrition of different grades (47%) and two patients had diabetes mellitus. But the mean healing score among the study group by presence or absence of associated systemic disease had no significant statistical difference, as was observed by Funda et al.[20], except significant difference with control group. It was found that on histological evaluation of wound different microscopic features displayed the definitive histologic state of healing wound. Out of 32 patients, 17 patients were with risk factor in study group from that 14 patients showed poor healing, 2 patients showed fair healing and only one patient showed good healing in comparison with 15 control group patients without risk factor, where good healing was observed in 10 patients, fair healing

showed in 4 patients and poor healing only in one patient. It should be mentioned that the above result with poor healing in study group was observed only on 7th day after surgery. Subsequent healing condition after 7 days of study needs further follow up to comment about late healing condition of study group. Though all risk factors of wound healing like, operative factor, severity of surgical trauma etc. could not be evaluated in the present study it is distinctly pointed out that risk factors in the study group had significantly weaker wounds than the control group without risk factors. It was also found that on clinical assessment normal colour appeared early in control group patients without risk factors than with risk factors in study group. Similar pattern of hotness, swelling and tenderness were found during follow up period in both study and control group, indicating that there was strong association with histological state of healing.

Conclusions

Based on the results of the study, it can be concluded that delayed wound healing occurs in risk factors associated with conditions like local malignancy, local infections, and associated systemic diseases like diabetes mellitus, history of radiotherapy and chemotherapy.

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