

Histological changes at the commissure of the lip on approaching from its cutaneous to the mucosa region in males

ABSTRACT

Introduction: The morphology of the epithelium of the oral lips comprised keratinized external epithelium (anteriorly) and nonkeratinized or sometimes parakeratinized mucous membrane epithelium (posteriorly). Knowledge of morphometry of the lip lining helps in deciding the best site for choosing graft for its better uptake during several dermal grafting procedures following trauma or tumor excision following craniofacial cancers or cosmetic procedures.

Materials and Methods: Ten human male cadavers were procured at the Department of Anatomy King George's Medical University, Lucknow, Uttar Pradesh. The rectangle-shaped skin specimen through the right commissure of the lip which included the skin, mucocutaneous junction, and mucosa was stained with hematoxylin and eosin stain. A total of 30 slides were prepared. Thus, the readings were obtained for three regions, respectively, with the help of CAT-CAM E-series HD cameras which were installed in a light microscope.

Results: Thickness of skin (epidermis + dermis) of the lip ranged from 756 μm to 1068 μm among males. Epidermal thickness increases on moving from the cutaneous region to the mucosa region of the lip. The lowest contribution of the stratum corneum in thickness of the epidermis was observed in the vermillion region, while the highest contribution was observed in the skin region. It was found to be absent in the mucosa region of the lip. Rete pegs at the dermoepidermal junction was found to be maximum in the vermillion region and minimum in the skin region. Its depth increased as we move from the skin to the mucosa region of the lip. Depth of the dermis was found to be maximum in the skin region, while minimum in the vermillion region. It ranged between 291 μm and 693 μm .

Conclusion: Care should be taken while using dermal fillers in lip augmentation surgeries, especially in the vermillion region due to its close proximity to musculature in the core of the lip.

Keywords: Commissural lip, mucocutaneous junction, mucosa, rete pegs, skin, vermillion

INTRODUCTION

Epithelial surfaces meet and interact at a transition zone known as the mucocutaneous junction (MCJ). The morphology of the epithelium of the lips comprised keratinized external epithelium (anteriorly) and non-keratinized or sometimes parakeratinized mucous membrane epithelium (posteriorly). The MCJ has a critical function of separating two dissimilar epithelia while maintaining the structural parameters of the tissue, preventing mechanical damage from keratinized tissue, and limiting exposure to delicate mucosal surfaces. The MCJ is characterized by thickening of the epithelium, a marked relief of the superficial surface of the dermis due

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to the presence of papillae, and the absence of hair follicles and sebaceous glands.^[1] It is often the site of an arterial anastomosis, a watershed area of venous and lymphatic drainage, and sensory (but not motor) nerve overlap. This boundary is credited as an erogenous zone where nerve endings rise closer to the surface.^[2] Furthermore, red color is thought to result from translucency kept by constant hydration and decreased density of keratin.^[3] The vermilion border of the lips (lip for short) constitutes one of the most outstanding parts of the face because of its features that are distinct from the surrounding skin.

The lips (*labia oris*) are two fleshy folds that surround the orifice of the mouth. In youthful lips, the ideal ratio of the upper-to-lower lip is 1:1.6.^[4] The oral commissures are the angles where the upper and lower lips meet. Cutaneo-vermillion junction line presents between skin and vermilion called as “white skin roll” by Millard.^[5] According to Ham, red-free margin of the lip is covered with a modified skin which represents a transition from the skin to the mucous membrane.^[6] The junctional line between vermilion region and mucosa region of the lip is described as “red line.”^[7]

Four distinct zones are recognizable in the mucocutaneous junctional area of the oral commissure in the adult, skin, vermilion border, intermediate or vermilion zone, and oral mucosa. However, in the young age group, the intermediate zone is found at the base of the commissural pit. One side of the pit is lined by the oral mucosa and the other by the keratinized, thinner epithelium of the vermilion border. The commissural pit probably represents the site of fusion of the medial ends of the maxillary and mandibular embryonic processes.^[8] With age, the vermilion border increased significantly in length and thickness of its epithelium. However, the oral mucosa decreased progressively in thickness from infancy to old age.^[9]

In today's world, with the evolution of microsurgical technique, several dermal grafting procedures are done at the lip to restore tissue loss following trauma or tumor excision following craniofacial cancers or cosmetic procedures.^[10] Knowledge of quantitative and qualitative aspects of lip skin parameters allows for improved reconstructive outcomes in the field of plastic surgery while matching donor and recipient tissues for its proper uptake while grafting. This study also proves useful in dermatopharmacokinetics, in which we monitor the effect of drugs acting on connective tissue by translabial route and investigation of various metabolic and endocrine diseases of the lip. Stratum corneum is the principal barrier to drug absorption. Knowledge of its thickness is required to normalize drug penetration profiles during dermatopharmacokinetic studies. Data of drug

passage through this layer can be related to bioavailability in target tissue.^[11] Measurement of skin thickness, stratum corneum, and dermal thickness can also prove useful in esthetic surgery (lip augmentation surgery), as it is suggested that dermal fillers used in these surgeries should not be injected so deep because if it enters the muscle core of the lip, it can cause granuloma or necrosis due to being highly viscous in nature.^[12]

The present study was carried out to formulate a basic background of regional differences which can set a standard to compare pathological and chronologically changes. Binnie and Lehner studied the histology of MCJ at the angle of the mouth of 30 postmortem subjects, ranging in age from neonates to 80 years. Sections were stained with hematoxylin and eosin (HE) and periodic acid–Schiff (PAS), for glycogen distribution. The study analyzed that the mean epithelial thickness at the skin, MCJ, and oral mucosa was 38 μ m, 63 μ m, 293 μ m, respectively, in 0–3 year age group, whereas in 20–50 year age group, it was 43 μ m, 85 μ m, 118 μ m, and 293 μ m, respectively, in the skin, vermilion, intermediate, and oral mucosa zone and in >50 year age group, it was 46 μ m, 97 μ m, 122 μ m, and 218 μ m, respectively, in the skin, vermilion, intermediate, and oral mucosa zone. They concluded that length and thickness of vermilion border increased significantly with age; however, the correlation between the age and thickness of vermilion border is almost entirely accounted for by the low values among infants. The thickness of the oral mucosa was significantly decreased with age. Skin increases slightly in thickness with age, but data were insufficient for the statistical analysis.^[8] Dimond and Montagna had done histological analysis of lips using hematoxyline, PAS, and acetylcholine esterase stains on scanning electron microscope. The study included four males (aged 20, 22, 25, and 65 years) and one female (aged 30 years). The study found the dermis of the vermilion border to be 0.5 mm thick due to its close proximity with musculature of lip. The study found that the epithelium of the labial mucosa measured 0.7 mm thick and its lamina propria was found similar in thickness to the dermis of cutaneous part of the lip.^[13] Satheer *et al.* (2011) concluded in their review article about the human lip that the mean epithelial thickness of the skin, vermilion zone, and oral mucosa was 46 μ m, 97 μ m, and 218 μ m, respectively. Likewise, epithelial glycogen also increases as we move from the skin to the mucosa.^[14]

MATERIALS AND METHODS

The present observational study was carried out at the Department of Anatomy King George's Medical University,

Lucknow, Uttar Pradesh from the period of September 2018 to August 2019. Ethical clearance was obtained from the Institutional Ethics Committee (Ref. code: 93rd ECM II B-Thesis/P7).

For the present study, ten human male cadavers were procured. Out of ten samples of the male lip, six samples were taken from fresh male donated body received in the department of anatomy 4–5 h after death; the next four samples were collected from forensic medicine 5–6 h after death. The age of male cadavers ranged between 5 and 75 years (mean age 45.5 years). The rectangle-shaped skin specimen measuring 1 cm long was taken through the right commissure of the lip which included the skin, MCJ, and mucosa. Thus, the readings from three fields per slide were obtained for three regions, respectively (skin, MCJ, or vermillion and mucosa region).

The cutaneous area presents with abrasions, breach, and infections such as boils, crusting, blackening of the skin; hemorrhaged sites were not taken for the study. Care was taken for the specimen not to have any kind of image artifacts from movement or orthodontic material.

For each lip specimen, three slides were prepared. Slides were stained with HE stain. Each of the slides contained horizontal sections of tissue covering the total skin thickness including skin, MCJ, or vermillion and mucosa region of the lip. Hence, a total of 30 slides were prepared.

Parameters such as epithelium thickness, depth, number and pattern of rete pegs at the dermoepidermal junction, and thickness of the dermis/lamina propria were taken in $\times 10$ magnification except thickness of the stratum corneum (Tsc) which was observed in $\times 40$ magnification. Each parameter was determined at two different locations in the same region per slide and then averaged to one data set to avoid the chance of error.

The above-mentioned parameters were measured with the help of CATCAM E-series HD cameras which were installed in a light microscope after removing eyepiece as the relay lens is built into the camera itself. Catyimage software was installed in personal laptop and scales were calibrated at $\times 4$, $\times 10$, $\times 40$, and $\times 100$. The camera was connected to the USB 2.0 port on laptop. USB 2.0 high-quality images were captured. Field of every zone, i.e., the skin, MCJ, and mucosa for every slide was read carefully, and various parameters (thickness) were measured using the scale.

The value of each parameter in micrometer was entered into Word Excel Sheet. For each parameter, three values were

taken from three slides stained with HE as stated above, and the mean value was calculated to reduce the chance of error. The mean value of each parameter was entered in tabulated form. 21st version of SPSS software was used to analyze statistical changes.

The microphotography was done with the help of device incorporated within software. The device was installed in a laptop and connected with the microscope and the photograph was clicked which was focused on the screen.

TABLE SUMMARY

Among males, thickness of the depth of rete pegs, total dermis of commissure, and total skin thickness were found to be comparable at the skin, vermillion region, and mucosa region. Thickness of the epidermis was found to be significantly higher in the mucosa region as compared to the skin and vermillion region. Number of rete pegs/field was found to be significantly higher in the vermillion region, as compared to the skin and mucosa region.

DISCUSSION

Thickness of epithelium of the lip

Depth of the epithelium of the commissure of the lip, while moving from the skin to the mucosa, was found to be thickest at the mucosa. It exhibited a statistically significant difference at all regions (skin, vermillion, and mucosa) [Refer to Table 1 and Figure 1]. In the histological study by Binnie and Lehner in 1970 at the angle of the mouth of 30 cadavers analyzed, the mean epithelial thickness at the skin, MCJ, and oral mucosa was 38 μm , 63 μm , and 293 μm , respectively, in 0–3 year age group, whereas in 20–50 year age group, it was 43 μm , 85 μm , and 293 μm , respectively, in the skin, vermillion, and oral mucosa zone and in >50 year age group, it was 46 μm , 97 μm , and 218 μm , respectively, in the skin, vermillion, and oral mucosa zone. In the present study, we found mean epithelial thickness as 38 μm , 63 μm , and 293 μm , respectively, in 5–20 year age group. In 20–50 year age group, it was 77 μm , 92 μm , and 191 μm , respectively, in the skin, vermillion, and oral mucosa zone. In >50 year age group, 85 μm , 112 μm , and 273 μm , respectively, in the skin, vermillion, and oral mucosa zone. The values approximate the above study findings except in >50 year age group, which are marginally less, probably different ethnicity of subject and the mode of measurement might be responsible.^[8] The light microscopic study on 10 human lips in Argentina conducted by Lanfranchi and de Rey found thickness of the epithelium at the cutaneous part of the lip as 70 μm which is marginally higher than our findings. However, comparison

Table 1: Region-wise comparison of different parameters of commissure among male subjects

Parameters (μm)	Skin region			Vermillion region			Mucosa region			ANOVA	
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>F</i>	<i>P</i>
Thickness of Edp	10	81.28	26.20	10	135.30	53.23	10	199.08	112.89	6.413	0.005
Thickness of SC	10	25.48	10.64	10	20.36	9.23				1.321	0.265
Edp:Sc ratio	10	3.75	1.74	10	8.18	4.40				8.766	0.008
Number of rete pegs/field	10	3.60	0.70	10	7.40	1.07	10	3.90	0.74	61.173	<0.001
Depth of rete pegs	10	181.57	123.05	10	178.32	57.97	10	291.60	163.30	2.762	0.081
Total dermis (Pd+Rd)	10	986.70	589.19	10	672.90	285.84	10	556.90	270.82	2.954	0.069
Total skin thickness	10	1068.0	598.4	10	808.2	334.8	10	756.0	376.0	1.370	0.271

SD: Standard deviation, SC: Stratum corneum, Edp: Epidermis, Pd+Rd: Papillary dermis + reticular dermis

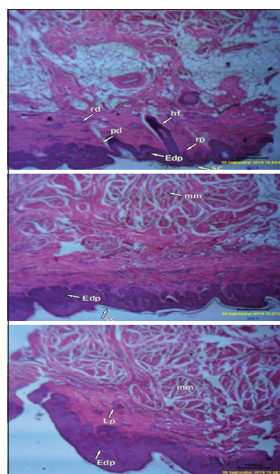


Figure 1: Microphotograph of commissural lip at 4X in skin, vermilion, and mucosa region respectively showing Edp, pd (papillary dermis), rd (reticular dermis), sc, hf (hair follicle), mm (muscularis mucosa), lp (lamina propria).

cannot be justified as sex, age, and site of the lip are not mentioned in the article.^[15] Jacobi *et al.* in an histological study of the lip epithelium of German subjects described its depth to be 74–148 μm . They did not mention the gender, age, or even site of the lip.^[16] Despite this, our observations at the vermilion region were coinciding. Although they did not mention the epithelial thickness of the mucosa of the lip, but our values were more than the range of depth they mentioned, suggesting that at the mucosa, the epithelial lining is thickest. Madhav Satheesh N.V *et al.* (2011) reported in their review article that the mean epithelial thickness of the skin, vermilion, and oral mucosa was 46 μm , 97 μm , and 218 μm , respectively.^[14] Our study findings are near with the above measurements despite of the fact that the article remains silent for age, gender, site, or even ethnicity.

Thickness of the stratum corneum

Likewise, the epidermis, the stratum corneum, also exhibited regional variations as well as remarkable gender differences at all sites of the lip. In both the genders, the highest depth of The stratum corneum was observed in the skin region [Figure 1]. It was absent in the mucosa region of the lip. Jacobi *et al.* in their light microscopic study observed the Tsc of the

lip in eight human subjects native of Germany, as 13–28 μm thick.^[16] In our study, the values of stratum corneum thickness are falling in the above range [Refer to Table 1].

Epidermis / stratum corneum ratio

Edp:Sc ratio also exhibited regional and gender differences at all sites of the lip [Refer to Table 1 and Figure 1]. As the stratum corneum acts as a protection against frictional forces, it is assumed to be thicker at places where such forces are relatively more. This hypothesis was proved right in our study, as in males, the Tsc was found more at the skin region of the commissure lip, an area of moustache which is repeatedly shaved, hence subjected to greater frictional forces.

Rete pegs: Number and depth

Number of rete pegs per field showed remarkable regional differences. They were found maximum in the vermilion region, while minimum in the skin region of the lip. Depth of rete pegs was found maximum in the vermilion region, while minimum in the skin region of the commissural lip. [Refer to Table 1 and Figure 1]. The number and depth of rete pegs ensure stronger adherence between the dermis and epidermis. A greater number of rete pegs ensures an increase in the number of basal germinative cells and greater surface area for superficial dermal capillary plexus for the exchange of nutrients. Jacobi *et al.* observed the thickness of rete ridges of lip in eight human native of Germany using a light microscope, approximated 150–300 μm thick^[16] which were found to be in consensus with our values; however, comparison cannot be justified as the site of the lip and sex of subjects are not mentioned in the article.

Dermis

The total dermis was found to be more in the skin region of the commissural lip [Refer to Table 1 and Figure 1]. In the microscopic study of Dimond and Montagna on 4 males (aged 20, 22, 25, and 65 years) and 1 female (aged 30 years) found the dermis of the vermilion border to be 0.5 mm thick, and the lamina propria of the mucosa was found to be similar in thickness to the dermis of its cutaneous part.^[13] Data

were found to be approximated to our findings. However, the thickness of the lamina propria was not as thick as the dermis of the skin in our observations. We found significant differences in its depth. Explanations are not plausible for the above discrepancies, though age, gender, and ethnic variations may play some role.

It is very clear from the ongoing discussion that factors such as method, criteria of measurement of the depth of the layer, ethnicity, age, gender, site, and region play an important role in morphometrical analysis of the lip. These variables have to be considered before any surgical or dermatological procedures are to be performed.

CONCLUSION

The present observational study was undertaken to assess region-wise differences in lip histology on approaching MCJ at the commissure of the lip in males. The measurement of the epidermis and dermis was considered for evaluating skin thickness. The following conclusions were drawn for various parameters.

Thickness of the skin (epidermis + dermis) of the lip ranged from 756 μm to 1068 μm among males. Epidermal thickness increases on moving from the cutaneous region to the mucosa region of the lip. The lowest contribution of the stratum corneum in thickness of the epidermis was observed in the vermilion region, while the highest contribution was observed in the skin region. It was found to be absent in the mucosa region of the lip; therefore, the mucosa region of the lip can be considered as the best site for giving drugs via translabial route as the stratum corneum is the principal barrier in percutaneous absorption. Rete pegs at the dermoepidermal junction was found to be maximum in the vermilion region and minimum in the skin region. Its depth increased as we move from the skin to the mucosa region of the lip. A pattern of rete pegs also showed a characteristic feature in every region of the lip. In the cutaneous part of the lip, rete pegs were shorter and blunt. In the vermilion region, they were narrow, long, and slender, while they were longest with blunt end in the mucosa region. Depth of the dermis was found to be maximum in the skin region, while it was minimum in the vermilion region. It ranged between 291 μm and 693 μm . Care should be taken while using dermal fillers in lip augmentation surgeries, especially in the vermilion

region due to its close proximity to musculature in core of the lip.

This study tried to create a baseline comparison to establish the presence of regional variation in morphometry of lip lining, which will help in deciding the best site for choosing graft for its better uptake. *In vivo* measurements should be performed to assess the depth of the dermis and epidermis or corresponding areas of the mucosa for the proper match of donor and recipient tissue during several dermal grafting procedures following trauma or tumor excision following craniofacial cancers or cosmetic procedures.

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Conflicts of interest

There are no conflicts of interest.

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