Original Article

Histological Changes of Upper Lip from its Cutaneous to Mucosa Region among Males

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Abstract

Introduction: Morphometry of lip lining help 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 in Department of Anatomy the rectangle shaped skin specimen through upper lip which included skin, mucocutaneous junction and mucosa were stained with Haematoxylin and Eosin stain. Total of 30 slides were prepared. **Results:** Thickness of skin (epidermis +dermis) of lip ranged from 664.72 µm to 882.34 µm among males. Epidermal thickness increases on moving from cutaneous region to mucosa region of lip. Lowest contribution of stratum corneum in thickness of epidermis was observed in vermillion region while highest contribution was observed in skin region. It was found to be absent in mucosa region of lip. Rete pegs at dermoepidermal junction was found to be maximum in vermillion region and minimum in skin region .It's depth increased as we move from skin to mucosa region of lip. In cutaneous part of lip, rete pegs were shorter and blunt. In vermillion region, they were narrow, long and slender while they were longest with blunt end in mucosa region. Depth of dermis was found to be maximum in skin region while minimum in vermillion region. It ranged between 578 µm -825.10 µm. **Conclusion:** Care should be taken while using dermal fillers in lip augmentation surgeries especially in vermillion region due to its close proximity to musculature in core of lip.

Keywords: Vermillion, Mucocutaneous junction, Rete pegs, Dermatopharmacokinetics, Red line

Introduction

A mucocutaneous zone (MCZ) is of biological interest because of the nature of contact between skin and mucous membranes. This region of the body in which mucosa transitions to skin. It present in animals, at the orifices. In humans, MCZs are found at the lips, nostrils, eyelid, urethra, vagina, foreskin, and anus. At the mucocutaneous junction (MCJ) epithelium transitions to the epidermis, lamina propria to the dermis, and smooth muscle to skeletal muscle. It is 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.[1] The orifice of the mouth is surrounded by the lips (labia oris), which are two fleshy folds. Extention of the Upper lip (Labium superioris) from the base of the nose superiorly, to the nasolabial folds laterally, and the free edge of the vermilion border inferiorly. Cutaneo-vermillion junction line present between skin and vermillion called as "white skin roll" by Millard.[2] According to Ham, red-free margin of the lip is covered with a modified skin, which represents a transition from skin to the mucous membrane.[3] The junctional line between vermillion and mucosa also called as Mucovermillion border parallel the "white skin roll" described as "red line."[4] The lip can be divided into three zones depending on the characteristic of the epithelial lining. From the skin aspect, these are (i) orthokeratinized appendage have epidermis with a flat basement membrane with few, if any, rete ridges. (ii) Orthokeratinized vermillion with very thin stratum corneum. This region lacks salivary or sweat glands and is kept moist with the saliva of the tongue. Like skin epithelium, it contains melanocytes with melanin pigment. Connective tissue papilla of lamina propria is long, narrow and has capillary loops hence

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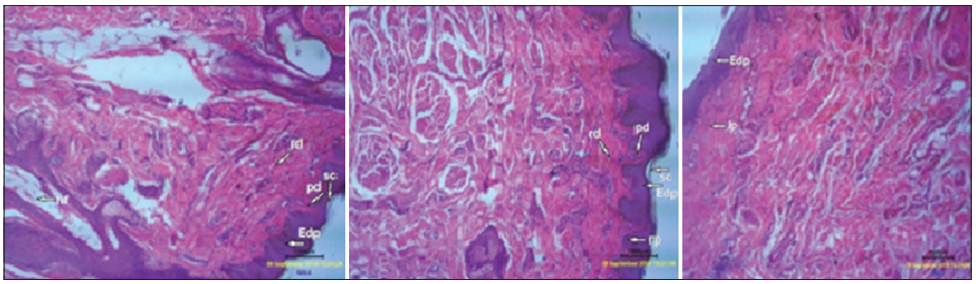

Figure 1: Microphotograph of skin ,vermillion and mucosa region respectively at 4X of upper lip in males showing Edp,pd (papillary dermis), rd(reticular dermis) sc,hf (hair follicle), rp(rete pegs)

red in color.[5] Also, red coloration is thought to result from translucency kept by constant hydration and decreased density of keratin.[6] (iii) labial mucosa (fold of mucosa on the inner surface of both lips is described as Median labial frenulum that connects the lip to the adjacent gum),[7] consisting of nonkeratinized or sometimes parakeratinized paler staining PAS positive mucosal stratified squamous epithelium, associated with mucous salivary glands.[8]

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.[9] So, as plastic surgeons understanding thorough knowledge of quantitative and qualitative aspects of lip skin parameters allows for improved reconstructive outcomes while matching donor and recipient tissues for its proper uptake. 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.[10] The principal barrier of drug absorption is Stratum corneum. Knowledge of its thickness is required to normalize drug penetration profiles during dermatopharmacokinetic studies. Drug passage data through this layer can be related to bioavailability in the target tissue.[11] Measurement of skin thickness, stratum corneum, 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 lip, it can cause granuloma or necrosis due to being highly viscous in nature.[12]

Materials and Methods

The present observational study was done in the Department of Anatomy in collaboration with the Department of Forensic Medicine and Toxicology, King George's Medical University, Uttar Pradesh Lucknow, from September 2018 to August 2019. Ethical clearance was obtained from the Institutional Ethics Committee with IEC approval number-ECR/262/Ins/UP/2013/RR-16, King George's Medical University Lucknow. 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, next four samples were collected from forensic medicine 5–6 h after death. 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 upper lip, which included skin, MCJ and mucosa. Thus readings from three fields per slide were obtained for three regions, respectively (skin, MCJ or vermillion, and mucosa region).

Exclusion criteria

The cutaneous area present with abrasions, breech and infections such as boils, crusting, blackening of the skin, hemorrhaged sites were not taken for 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 Hematoxylin and Eosin 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 like epithelium thickness, depth, number, and pattern of Rete pegs at dermoepidermal junction, thickness of dermis/lamina propria were taken in ×10 except thickness of stratum corneum (Tsc) which was observed in ×40X 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 was installed in light microscope after removing eyepiece as the relay lens is built into the camera itself. Catymage software was installed in my personal laptop and scales were calibrated at ×4, ×10, ×40, ×100. Camera was connected to USB 2.0 port on my laptop. U S B 2.0 high-quality images were captured. Field of every zone, i.e., skin, MCJ, and mucosa for every slide was read carefully, and various parameters (thickness) were measured using the scale.

Value of each parameter in micrometer was entered into the word excel sheet. For each parameter, three values were taken from three slides stained with hematoxyline and eosin as stated above and the mean value was calculated in order to reduce the chance of error. The mean value of each parameter was entered in tabulated form. IBM Corp. Released 2012 .IBM SPSS Statistics for Windows,Version 21.0.Armonk, New York. was

used to analyze statistical changes. The microphotography was done with the help of device incorporated with software. The device was installed in a laptop and connected with microscope, and the photograph was clicked, which was focused on the screen.

Results

Refer to Figure 1, Among male subjects, thickness of epidermis increases from the skin to mucosa region of the lip. Its mean value ranged from 57.24 μm to 144.68 μm. On the other hand, Tsc decreases from skin to vermillion region, while found to be absent in the mucosa region. The mean value ranged between 14.21 μm to 14.58 μm. Thickness of dermis found least in the vermillion region (578.00 μm), while the maximum in skin region (825.10 μm).Total thickness of skin is calculated by adding epidermis and dermis. The number and depth of rete pegs were found to be maximum in the vermillion region. Total dermis of the upper lip was found to be comparable at skin, vermillion and mucosa region. Thickness of epithelium of Mucosa region was found to be significantly greater as compared to the skin and vermillion region. Number of rete pegs/field and depth of rete pegs was found to be significantly greater in vermillion region as compared to skin and mucosa region.

Discussion

Histological analysis of the topographical variations of the normal skin of lip is of relevance in dermatological research. Lip region of the face is of utmost significant value in the looks of an individual. Each lip has three distinct regions skin, mcz, and mucosa. The skin of lip consists of two distinct layers, an epidermis, and dermis. Each component varies according to age, gender, race, and anatomic location.[13] In the present study, regional variation was observed for various parameters of the lip in the different regions of the same site (i.e., skin, vermillion, and mucosa).

Thickness of epidermis

Depth of epithelium of upper lip, while moving from skin to mucosa ,was found to be thickest at the mucosa (refer to Figure 1).The upper lip of males exhibited a statistically significant difference in all regions (skin, vermillion, and mucosa). In the light microscopic study on ten human lips in Argentina conducted by Lanfranchi and De Rey found the thickness of epithelium at cutaneous part of the lip as 70 μm which is marginally higher than our findings. Howsoever, comparison cannot be justified as sex, age, and site of the lip are not mentioned in the article.[14] Jacobi *et al.* in a histological study of 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.[15] Despite this, our observations at the vermilion region and mucosa were coinciding. In histological study conducted by Paes *et al.* at Neetherland, found epidermal thickness at philtrum in males as 271 ± 164 μm in old subjects.[16] In the present study, we found a relatively lower depth of epidermis (57.24 ± 10.24 μm in males). Probably, the difference may be accounted for due to variation in reference points for measuring epidermis. In our study, depth was measured from the surface to dermal papillae, whereas in the above study, reference point could have been the dermo-epidermal junction at rete pegs. Furthermore, the discrepancy of values may be due to differences in the mean age of subjects of the two studies. Satheesh M *et al.* reported in their review article, mean epithelial thickness of skin, vermillion, and oral mucosa as 46 μm, 97 μm, and 218 μm respectively.[17] Our study findings of the upper lip are in consensus with the above measurements despite the fact that the article remains silent for age, gender, site or even ethnicity. Chopra *et al.* observed epidermal thickness of upper lip vermillion and philtrum in 3 males and 7 females cadavers (81.6 ± 11.3 years) of American origin by using triplicate computerized method as 62.62 ± 57.79 μm and 48.07 ± 13.85 μm, respectively.[18] In our study, mean epidermal thickness at vermillion and philtrum in males was 86.72 ± 31.49 μm and 57.24 ± 10.24 μm, respectively, which are almost near to the above study findings. The differences in observations can be clearly explained by the fact that in these study measurements from both genders were averaged.

Thickness of stratum corneum

Likewise epidermis, stratum corneum also exhibited regional variations in the upper lip among males. At all the sites of the lip, the highest depth of stratum corneum was observed in the skin region(refer to 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.[15] In our study, the values of stratum corneum thickness were falling in the above range. In another histological study conducted by Paes *et al.* in 30 males and females cadavers (age ranged 75–93 years) of Neetherland, found Tsc at philtrum in males as 19 ± 9 μm.[16] These values were coinciding with our observations. Edp: Sc ratio also exhibited regional differences at lip. The highest contribution of stratum corneum in the epidermis was seen in skin region of lip, while lowest contribution of stratum corneum in the vermillion region. As stratum corneum act as a protection against frictional forces, is assumed to be thicker at places where such forces are relatively more. This hypothesis was proved right in our study. Because, in males the Tsc was found more at skin region of upper 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 vermillion region while minimum in skin region of lip (refer to Figure 1). Depth of rete pegs was found maximum in the vermillion region of the upper lip of males, while minimum in skin region of the lip. Number and depth of rete pegs ensure stronger adherence between 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 the lip in eight human natives of Germany by using a light microscope, approximated 150–300 μm thick.[15] Which were found to be in consensus with the values in the vermillion region of the upper lip; however, values at the mucosa region were relatively in the higher range. Comparison cannot be justified as the site of lip and sex of subjects is not mentioned in the article.

Dermis

Total dermis was found to be more in skin region of the upper lip in males(refer to Figure 1). In the microscopic study of Dimond and Montagna on four male subjects (aged 20, 22, 25, and 65 years) and 1 female subject (aged 30 years) found dermis of the vermillion border to be 0.5 mm thick and lamina propria of mucosa was found to be similar in thickness to the dermis of its cutaneous part.[19] Data were found to be approximated to our findings at the upper lip. However, the thickness of lamina propria was not as thick as the dermis of 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 like method, criteria of measurement of the depth of layer, ethnicity, age, gender, site, region, etc. play an important role in the morphometrical analysis of 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 the region-specific differences in lip histology on approaching MCJ at the upper lip in males. The measurement of epidermis and dermis were considered for evaluating skin thickness. The following conclusions were drawn for various parameters. Thickness of skin (epidermis + dermis) of upper lip ranged from 664.72 μm to 882.34 μm among males. Epidermal thickness increases on moving from cutaneous region to mucosa region of lip. The lowest contribution of stratum corneum in the thickness of epidermis was observed in the vermillion region while the highest contribution was observed in the skin region. It is absent in the mucosa region of the lip; therefore, it can be considered as the best site for giving drugs through translabial route as the stratum corneum is the main barrier in percutaneous absorption. Rete pegs at dermoepidermal junction were found to be maximum in the vermillion region and minimum in skin region per field. Its depth increases as we move from skin to mucosa region of the lip. Pattern of rete pegs also shows a characteristic feature in every region of the lip. In the cutaneous part of lip, rete pegs were shorter and blunt. In vermillion region, they were narrow, long and slender while they were longest with blunt end in mucosa region. Depth of dermis was found to be maximum in skin region while minimum in vermillion region showing its close proximity to musculature in core of lip. It ranged between 578 μm and 825.10 μm. 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 lip. This study tried to create a baseline comparison to establish the presence of regional variation in lip morphometry, 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 dermis and epidermis or corresponding areas of mucosa for the proper match of donor and recipient tissue.

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Conflicts of interest

There are no conflicts of interest.

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