TYPE OF STROMAL COLLAGEN FIBERS IN THE ORAL SQUAMOUS CELL CARCINOMA… A HISTOCHEMICAL STUDY USING PICROSIRIUS RED STAIN

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ABSTRACT

Objective: Determination of the type of stromal collagen fibers in the Oral Squamous Cell Carcinoma.

Materials and Methods: Biopsies were taken from 71 patients of oral squamous cell carcinoma. Slides were stained with Picrosirius red (PSR) stain. All the samples were evaluated for the type of collagen fibers by examining under light microscope using polarizing lens.

Results: Type III collagen was the predominant type of collagen seen in the poorly differentiated oral squamous cell carcinoma cases, whereas type I collagen fibers were sparse. The percentage of type I collagen was highest in well differentiated oral squamous cell carcinoma cases and type III collagen fibers were infrequent. While the percentage of type III collagen fibers was more than type I collagen fibers in the moderately differentiated oral squamous cell carcinoma cases. In the present study, this variation in types of collagen fibers in the three histological grades of oral squamous cell carcinoma was found to be statistically significant (p= 0.08).

Conclusion: In the light of the results of the present study it is concluded the collagen profiling can be effectively used as a prognostic marker in oral squamous cell carcinoma cases. Abundance of type III collagen fibers in tumor stroma seems to be a poor prognostic indicator.

Key words: Oral Squamous cell carcinoma, Picrosirius red stain, Collagen

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is extremely invasive and because of its high metastatic potential, it is one of the most difficult tumors to treat [1]. A neoplasm consists of tumors cells and tumor-associated stromal cells. Fibroblasts, adipocytes, leukocytes, endothelial cells, nerve cells and macrophages are predominant tumor-associated stromal cells [2]. Stromal constituents not only provide nutrition for tumor growth but also prevent its metastasis by acting as a barrier.[3]. As the tumor progresses, the neoplastic cells interacts with stromal constituients [4,5]. Propagation of OSCC, breakdown of basal lamia and connective tissue (CT) modulation are closely interconnected.[6]. Tumor-associated stromal cells endorse inflammation, invasion, angiogenesis and the extracellular matrix (ECM) modeling via intercellular contact and growth factors, cytokines, hormones and enzyme proteinases such as matrix metaloproteinases (MMPs) release[7,8,9]. The neoplastic cells liberate an enzyme collagenase that assist in the breakdown of basal lamina[10]. This enzyme slits the peptide bonds in the collagen helical structure[11]. basal Basal lamina degradation leads to local invasion and metastasis[12]. MMPs are responsible for degradation of collagen that assist the neoplastic cells permeation, advancement and spread to distant sites. The degradation of ECM, a significant hallmark of tumor progression, permits tumor cells to invade the neighboring tissues[13,14].

The most abundant protein in the extracellular matrix (ECM) are Collagens, constituting almost 34% [1,15]. The collagen fibers acts as an efficient obstacle that the neoplastic cells must traverse for invasion and metastasis[16].

Histological evaluation of tissue biopsy is considered as a gold standard for diagnosing OSCC [17]. Various immunohistochemical markers are being used as prognostic indicators to determine tendency for invasion and metastasis of tumor. Thus helping in to plan the treatment accordingly. However these immunohistochemical staining procedures are costly[10].

Picrosirius red stain (PSR) is used for staining of type I and type III collagen fibers [18]. The distinctive feature of PSR staining technique is the attachment of

PSR dye molecules in a peculiar manner to collagen fibrils which enhances its polarizing birefringence. Collagen possesses a triple helical structure and its molecules are enriched with basic amino acids, consequently exhibit strong affinity for acidic dyes giving strong reaction with the these dyes [19,20]. Polarizing microscope is used to detect this property of collagen [20]. A conventional microscope can be used as a polarizing microscope by simply placing a polarizing lens in the illumination course [21].Closely packed thick fibrils collectively form a thick type I collagen fiber, that gives a strong birefringence of yellow to red colour. While loosely packed thin fibrils collectively form thin type III (reticular fibers) collagen fiber which exhibits a weak birefringence of green colour [22] .

An attempt has been made in this research to study the stromal collagen as a prognostic indicator in OSCC.

MATERIALS AND METHODS

Sections from 71 oral squamous cell carcinoma cases of different histological grades were used to conduct this study. Samples were collected from Myo Hospital/King Edward Medical University (KEMU), Nawaz sharif Hospital/ De'montmorency college of dentistry and Post Graduate Medical institute (PGMI), Lahore.

METHODOLOGY

Tissue sections of 3-5 µm thickness were cut from tumor tissue blocks by rotary microtome. Slides of the tissue sections were made and Picrosirius red staining of the sections was done as per protocol [23- 26] .Following steps were carried out:

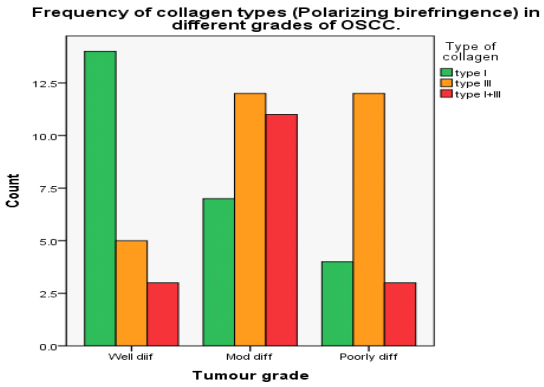
1. After dewaxing and hydrating the paraffin sections, nuclei were stained for 8 minutes with Weigert's haematoxylin, and then washed in running tap water for 10 minutes.
2. Each slide was stained with Picrosirius red for one hour to give near-equilibrium staining. (Staining will not improve with longer times and even if the color looks fine, shorter times of staining should be avoided to get best results).
3. Washing of each slide in two changes of acidified water was carried out followed by vigorous shaking for physical removal of most of the water from the slides.
4. Three changes of 100% ethanol was used for dehydration of slides.
5. Xylene was used for clearing and DPX (resinous media) for mounting of the slides.

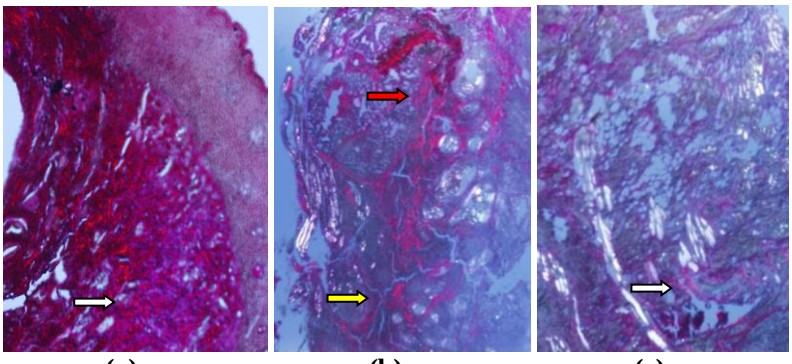
Microscopic Interpretation:

Picrosirius red stained tissue sections were observed under the light microscope with the help of a polarizer. Type of the collagen fibers was determined by observing their polarizing birefringence. It was seen that type I collagen gave reddish orange polarizing birefringence and type III gave greenish yellow polarizing birefringence.

RESULTS

In the present study tissue sections from 71 OSCC cases were examined. Histopathologically, there were 22 well differentiated OSCC cases, 31 moderately differentiated OSCC and 18 poorly differentiated OSCC cases.





(a) **(b)** **(c)**

Photomicrograph(a)Reddish orange polarizing birefringence showing type I collagen in well differentiated OSCC (PSR;200x); Photomicrograph(b)Moderately differentiated OSCC(PSR;100x),Yellowish orange polarizing birefringence showing collagen Type I & collagen Type III ; Photomicrograph(c) Poorly differentiated OSCC(PSR;200x), Greenish yellow polarizing birefringence showing collagen type III

Picrosirius red staining of the sections showed a variation in type of collagen fibers in different histological grades of OSCC. Type I collagen fibers were predominant in well differentiated OSCC (63.6%) cases, 22.7% cases have type III fibers and both type I and III fibers were seen in remaining 13.6% cases. In moderately differentiated OSCC, 22.5% have type I collagen, 41.9% have type III and 35.4% have both type I and III collagen fibers. While in poorly differentiated OSCC, only 27.7% have type I fibers, 61.1% cases have type III fibers and 11.1% have both type I, III fibers.

DISCUSSION

Oral cancer is a huge health dilemma wordwide [27]. Almost 50% increase in incidence of OSCC is seen in the last few years [28]. Unfortunately, most of the OSCC patients are at an advanced stage (III-IV) at the initial presentation and nearly one third of them are positive for lymph nodes metastasis [29]. At an advance stage (III, IV), five year survival rate is less than 25% while at early stages (I ,II) is nearly 80% [30].

Cancers are intricate tissues comprising tumor cells and neighboring stroma [31]. Carcinogens induce stromal alterations which favor the expression as well as propagation of pre-neoplastic phenotypes[32]. Hence when the cell becomes neoplastic, the neighboring microenvironment will be affected through constant tumor stromal communications [31]. The reactive alterations in the neoplastic stroma may modify the biological aggressiveness of oral carcinoma[33]. Extracellular matrix changes are now considered among the prognostic indicators which point out the susceptibility of neoplastic cells to penetration and spread to distant sites[34].

Various recent studies conducted on tumor stroma indicated protective role of collagen against metastasis [16].

In the current study, we observed that Type III (greenish yellow polarizing birefringence) collagen was the most abundant type of collagen seen in poorly differentiated OSCC and the least common type seen in well differentiated OSCC cases. Results of the studies conducted by Rakheja etal. (2014) ; Kumari etal.(2016); Kardam etal. (2016); John etal. (2016) support present study results where poorly differentiated OSCC cases predominantly showed type III (greenish yellow polarizing birefringence) collagen[22,35-37].

Collagen Type I (Reddish orange polarizing birefringence) was the most abundant type seen in well differentiated OSCC cases and the least abundant type in poorly differentiated OSCC cases. Rakheja etal. (2014) ; Kardam etal.,(2016); Alrani etal. (2016); Kumari etal. (2016) ; Manjunatha etal.(2016); John etal. (2016) showed similar results in their studies[22,34-37]. The percentage of collagen type III was more than collagen type I in moderately differentiated OSCC cases of the present study. Studies conducted by of Alrani etal. (2016); John etal. (2016) support our results. DeWever et al., (2008); Kalele et al., (2015) also supported these findings and declared that stroma containing fibrillar collagens (type III) as a poor prognostic indicator[1,20,37,38]. A study conducted by Allon et al.,(2006) on stromal differences in salivary gland tumors also stated that as compared to pleomorphic adenoma, percentage of collagen type III was high in adenoid cystic carcinomas and polymorphous lowgrade adeocarcinomas [19]. Koren etal., (2001) in their research conducted on follicular thyroid carcinomas also reported abundance of Type III collagen at the sites of invasion [39]. Junqueira etal., (1986) also mentioned in their study on human osteosarcomas that collagen Type III was seen in the anaplastic areas of osteosarcomas. [40]. Lubkin and Jackson.,(2002) stated in their research that capsule and a dense extracellular matrix slows down tumor growth or progression [41].

CONCLUSION

In the light of the results of the present study it is concluded the collagen profiling can be effectively used to correlate the qualitative nature of the collagen to the progression of OSCC. The abundance of type III collagen in tumor stroma seems to be a poor prognostic indicator. This can guide the clinicians to plan the treatment or follow-up accordingly. However cohort studies are required to establish association of type of collagen with clinical behavior and progression of OSCC.

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