Histopathological Evaluation of Cancer Tendency in the Buccal Mucosa of Aged Albino Rats (Histological and Immunohistochemical Study)

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Aim: the aim of this the study was to evaluate the histopathological changes of the buccal mucosa in the advanced age of albino rats and linking these changes with increased tissue cancer tendency.

Materials and methods: fourteen adult male albino rats were randomly divided into two equal groups as follow: Group 1: 6 months old group. Group 2: 24 months old group. all animals were euthanized and buccal mucosa specimen were processed for hematoxylin and eosin staining and immunohistochemically prepared for e-cadherin gene expression.

Results: histological examination of buccal mucosal tissues (epithelium and lamina propria) of group 2 showed significant atrophic changes and sort of loss of the tissue integrity as compared to group I.

Significant downregulation of e -cadherin gene expression was also noticed in the aged group.

Conclusion: the present study concluded that oral tissues demonstrate progressive decline in their functional properties as a result of changes related to aging.

Key words: buccal mucosa, e-cadherin, aged albino rat

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Introduction

Aging has always been a major area of concern and interest for a lot of researchers. It is generally a time-dependent functional decline affecting most living organisms (López-Otín et al., 2013), resulting from entropy on the cells, tissues, as well as organs of the animal. In order to increase life expectancy in humans, we must look into the cellular programs that are responsible for aging and how their dysregulation guides senescence and decline (DiLoreto & Murphy, 2015). Aging is characterized by a gradual loss of physiological integrity, leading to disrupted function and increased susceptibility to death. This decline is the major risk factor for human pathologies as cardiovascular disorders, diabetes, neurodegenerative diseases as well as cancer (López-Otín et al., 2013).

The epithelium has many vital roles in homeostasis as well as in creating and maintaining tissue barriers. These epithelial barriers have a crucial role in separating the internal from the external environment. In addition, they maintain fluid, electrolyte, as well as nutrient, and metabolic waste balance in multiple organs. The process of aging is linked to structural and functional changes occurring in these organs. This is in turn associated with increased fibrosis and subsequent inflammation. (Selman et al., 2016). The oral cavity lining protects against the masticatory forces as well as from other potentially toxic effects. The process of aging in the oral mucosa is characterized by changes occurring in the oral epithelial tissues including less prominent rete pegs, reduced mean thickness (Abu Eid et al., 2012), decreased cell density and lower mitotic activity. The underlying connective tissue also shows decreased blood supply. Subsequently, the oral mucosa will appear thinner, atrophic, friable and satin-like, with the gingiva losing its stippling feature. A decline in tissue regeneration eventually occurs as well as in the healing rates (Florina Andreescu et al., 2013).

The field of age-related changes of the connective tissue is one of the areas that have shown remarkable progress in the recent years. This is quite reasonable as connective tissue changes are strong indicators of aging as skin wrinkling, vascular disease as well as physical disabilities of joints (Schofield & Weightman, 1978). Age is the major predictor for increased risk of osteoarthritis. Almost 50% of all the cases of symptomatic knee osteoarthritis are diagnosed by the age of 55. By the age of 65, more than 75% of the cases are diagnosed. (Losina et al., 2013).

A key finding is that aging promotes inflammation of the joints by disrupting the activation/inhibition process of cellular inflammatory mediators, particularly in response to tissue damage and biomechanical stress (Matsuzaki et al., 2014). The incidence of temporomandibular joint (TMJ) osteoarthritis ranges from 28% to 38% and has also been found to increase with advancing age.

Musculoskeletal disorders are quite common among the elderly (Wolff et al., 2002), where the musculoskeletal tissues lose their ability to carry out their normal functions. This is related to an increase in bone fragility, as well as reduction in cartilage resilience and elasticity of the ligaments. In addition, musculoskeletal tissues also show overall loss of muscular strength and fat redistribution (Freemont & Hoyland, 2007).

Aging may have a notable effect on the periodontal ligament cells. It can induce a significant decrease in chemotaxis, motility, differentiation as well as proliferation rates. The chemotaxis and differentiation of osteoclasts is induced by devitalized osseous matrix which again might be influenced by age. (Milner et al., 2005).

The aging process also has a profuse effect on the stem cells. It is quite recognized that over the time, DNA mutations naturally

accumulate. To remove these damaged cells, our bodies have evolved highly specialized mechanisms. Although these repair mechanisms are quite beneficial, they lead to a reduction in the pool of available stem cells. Accordingly, our tissue homeostasis is dramatically affected, and our rate of healing is impaired. In short: less stem cells means a reduction in tissue function, and subsequently slower healing (Huang et al., 2016).

People do not die from "healthy" aging, they rather die from age-related illnesses. These include cardiovascular diseases as hypertension, heart failure and atherosclerosis, as well as other diseases; type II diabetes, osteoporosis and cancer (Blagosklonny, 2006) and (Tsang et al., 2007). These diseases are terminal manifestations of aging. With accelerated aging, age-related diseases also occur at an earlier stage in life. Healthy aging which is defined as late onset of diseases, is linked to longevity (Blagosklonny, 2010). For instance, centenarians who are 100 years old or older, demonstrate a delayed onset of age-related diseases as cancer, Alzheimer's disease, type 2 diabetes and cardiovascular diseases. That is to say, the slower the aging, the healthier the individual (Curtis et al., 2005).

Aging and cancer incidence:

The association of cancer incidence with age has been proven and thoroughly examined years ago as age-related declination of the immune defense mechanism has been proven in previous studies (Gardner, 1980). Accumulation of mutations and DNA methylation with age has been shown to cause carcinogenesis (Horvath, 2013), (Tomasetti et al., 2017), (Klutstein et al., 2017) and (Xie et al., 2018). General decline of the immune system, which is the front line of defense against cancer incidence, that occurs as individuals age has also been proven and studied previously (Aw et al.,

2007). Yet, the application of different immunotherapies on elderly patients requires critical studying of the effect of such alterations on the anti-tumor immune response.

The processes that transform adherent epithelial cells into separate migratory cells capable of causing the initial steps of carcinogenesis which include invasion of the surrounding extracellular matrix followed by distant metastasis, refers to as the epithelial-mesenchymal transition (EMT) as a whole. EMT is characterized biochemically and molecularly by modifications in the characteristics of the epithelial cells as cytokeratin loss, increase vimentin expression, cadherin switch and having collagen and spindle-like morphology as well as migration ability (Larue & Bellacosa, 2005). EMT is initiated by the occurrence of a series of changes in several gene expressions that cause epithelial cells to lose their epithelial properties and acquire mesenchymal cell traits. EMT plays the major role during tumor progression where the epithelial cells acquire enhanced motile and adhesive capacities that allow them to invade the extracellular matrix and metastasize (Thiery & Sleeman, 2006). These changes in the epithelial cells initiate the steps of carcinogenesis and these mesenchymal-like cancer cells are usually have an aggressive phenotype (Sarrió et al., 2008). The key changes that lead to EMT mainly include loss of the expression of E-cadherin gene and an increase in N-cadherin, SLUG (SNAI2), fibronectin, SNAI1, TWIST and vimentin. Many of these genes showed a down regulation in cancer (Thiery & Sleeman, 2006). Normal epithelial cells are characterized by a highly polarized morphology, with an intimate link of cell-to-cell junctions. A critical step during EMT is the loss of these intercellular connections allowing for physical detachment and migration of cancer cells from the primary

tumor. Thus, EMT can be monitored by the combined loss of epithelial cell to cell junction proteins, mainly E-cadherin, α-catenin and claudins. Also, the increase in expression of mesenchymal markers such as fibronectin, vimentin and N-cadherin. The cytoskeleton is also reorganized, which results in the loss of apical-basal cell polarity and the development of a spindle-shaped morphology (Huber et al., 2005) and (Trimboli et al., 2008). Loss of the cell-to-cell adhesion molecule's expression (E-cadherin) is one of the most important features of EMT in the development and growth of invasive and metastatic malignancies from epithelial tumors. In many cancer types, the loss of E-cadherin is typically shown to be accompanied by an increase in the expression of the mesenchymal cadherin, N-cadherin; this "cadherin switch" is thought to be required for tumor cells to acquire invasive qualities and is also a feature of EMT. (Cavallaro & Christofori, 2004).

A subject of argument up to date is the significant linking of the laboratory rats' age with humans. Although, various researches have attempted to discover these links in several ways. However, no proper association was provided. Generally, rats have shown to rapidly grow early in their childhood where they mature sexually around six weeks old. However, they take 5-6 months later to acquire social maturity. Moreover, in adulthood, every day of the rat almost corresponds to 34.8 human days. In other words, one rat month is equivalent to three human years (Sengupta, 2011). In regards with the total life span of laboratory rats, it is about 2-3.5 years with an average of 3 years, as compared to humans with worldwide life expectancy of 80 years. (Sengupta, 2013). Thus, one human year almost equals two rat weeks (13.8 rat days) while correlating their entire life span. (Quinn, 2005) and (Sengupta, 2013). Thus,

these variations indicate the differences in their physiology, anatomy as well as developmental courses between rats and humans (Sengupta, 2011).

From the previously mentioned data, almost rare studies highlighted the correlation between age changes and cancer incidence in buccal mucosa. The aim of this the study was to evaluate the histopathological changes of the buccal mucosa in the advanced age of albino rats and linking these changes with increased tissue cancer tendency. These were achieved through:

a) Routine histological examination of specimens from rats stained by hematoxylin and eosin.

b) Immunohistochemical staining of E-cadherin gene expression in specimens of the buccal mucosa at different ages in the rats.

c) Statistical analysis of e-cadherin gene expression.

Material and Methods
I- Sample Collection:
1- Animal Model
In order to conduct this study, fourteen adult, male Wistar albino rats (Rattus albus) weighing 150-200 grams were obtained from the animal house of Ain Shams University.

2- Animal Housing and Living Conditions:
At the Medical Research Centre (MRC) - Animal House Unit, Ain Shams University, the rats were housed in stainless steel cages. The rats were allowed access to a regular diet of bread and tap water ad libitum while being supervised by a specialized veterinarian. In order to maintain a good and clean working environment, the correct feeding and ventilation conditions were observed. Research Ethics committee of the Faculty of Dentistry at Ain Shams University evaluated and approved the protocol.

3- Animal Grouping:

The rats were evenly split among 2 groups of 7 each. The grouping of the animals was as follows:

Group 1: The 6-month-old group (representing young-aged animals) is about equivalent to 18 years of human age.

Group 2: The 24-month-old group (representing elderly animals), which is about equivalent to 72 years of human age.

These based on the study done by **Sengupta, 2013** who reported that, when comparing the entire life span, one human year is approximately equal to two rat weeks (13.8 rat days).

II- Euthanization:

1- Rats were euthanized by intravenous administration of an 80 mg/kg sodium thiopental anesthetic overdose.

2- Rat cheeks were excised so that laboratory processing and immunohistochemistry and H&E staining could be done.

3- The wastes and carcasses of the used animals were incinerated to ensure hygienic disposal.

III- Examination by hematoxylin and eosin:

1. The cheeks of the rats were trimmed and placed in capped, labelled, leak proof plastic jars containing 10% neutral buffered formaldehyde (El Nasr Pharmaceutical Chemicals®, Egypt) for 48 hours to allow for fixation.

2. Specimens were washed under running water, dehydrated by ascending concentrations of 50% 60%, 80%, 90%, 96% and 100% alcohol (Carlo Erba Reagents ®, Italy), and transferred to xylol (El Nasr Pharmaceutical Chemicals®, Egypt) to remove the alcohol from the specimen.

3. Specimens were then infiltrated by paraffin wax and embedded in paraffin wax blocks.

4. Embedded specimens were then sectioned mesiodistally into 5 micron-thick sections in the first molar area using a microtome (Histo-Line ® MR2258, Italy).

5. Sections were left to dry at 37 degrees Celsius in an air circulation drying oven (Thermo Scientific Heraeus UT 6060 ®, Italy).

6. In order to stain the sections with Hematoxylin and Eosin (H&E) stain and examine them under a light microscope, the sections were transferred to glass slides in descending percentages of alcohol (96%, 70%, and distilled water).

IV- Immunohistochemistry:

Tissue samples that had been embedded in paraffin wax were dewaxed and rehydrated using grade ethanol and distilled water. By allowing the substance to sit in 3% H_2O_2 in methanol for 10 minutes, endogenous peroxidase was inhibited. The antigen was recovered using a microwave for 10 minutes in a citrate buffer solution (2.1 g citric acid/L D.H2O, 0.37 g EDTA/L D.H2O, and 0.2 g trypsin) with a pH of 6.0, then washed in Tris-buffered saline (8 g NaCl, 0.605 g Tris) with a pH of 7.6. To block non-specific binding sites, the tissue segment was treated with power BlockTM reagent (BioGenex, San Ramon, CA, USA), an all-purpose proteinaceous blocking reagent, for 15 minutes at room temperature. The tissue sections were then incubated with the primary antibody (E-cadherin monoclonal antibody 4A2C7, Thermo Fisher, CA, USA) overnight at 4°C. The bound primary antibody was detected by incubation with the secondary antibody conjugated with horseradish peroxidase (BioGenex, San Ramon, CA, USA) for 30 minutes at room temperature.

After rinsing with Tris-buffered saline, the antigen-antibody complex was detected using 3, 3′-diamminobenzidine, the substrate of horseradish peroxidase. When acceptable color intensity was reached, the slides were washed, counter stained with hematoxylin

and covered with a mounting medium. Each slide was microscopically analyzed and enumerated the percentage of the positively stained cells semi-quantitatively **(Yoshida et al., 2001)**.

Statistical Analysis:

The statistical program for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used to analyze the data collected on the expression of e-cadherin. The mean ± standard deviation (SD) were used to express quantitative data. Frequency and percentage were used to express qualitative data.

The following tests were done:

- When comparing more than two means, use a one-way analysis of variance (ANOVA).
- Least Significant Difference (LSD) was used for multiple comparisons between various variables in the post-hoc test.
- The allowable margin of error was set at 5%, while the confidence interval was set at 95%. The p-value was thus considered significant as following:
- Probability (P-value)

– P-value ≤ 0.05 was considered significant.

– P-value ≤ 0.001 was considered as highly significant.

– P-value > 0.05 was considered insignificant.

Results

Histological Analysis

Examination of the histological sections obtained from the buccal mucosa of group 1 showed the normal histological features of both surface epithelium and underlying lamina propria. The epithelial covering of the buccal mucosa was para to ortho keratinized stratified squamous epithelium. It was composed of four layers: stratum basal, stratum spinosum, stratum granulosum, and stratum corneum. The epithelial rete pegs appeared short, wide and plenty.

The stratum basal or basal cell layer was composed of a single layer of columnar cells resting 90 degrees on a distinct basement membrane. The nuclei were oval, and deeply stained, occupying most of the cell body.

The stratum spinosum or prickle cell layer was composed of numerous rows. In the suprabasal layer, the cells appeared polyhedral in shape, with rounded, deeply stained nuclei. However, cells in the superficial layer became fainter with prominent nuclei. The stratum granulosum or granular cell layer appeared as two to three layers of wider and flatter cells. The nuclei looked dense, small and deeply stained. Abundant basophilic granules were also noted in the cytoplasm. The stratum corneum or the cornified cell layer covering the epithelium looked rather thick with flat horny scales.

The underlying lamina propria demonstrated dense bundles of connective tissue fibers, arranged vertically and longitudinally with many blood vessels in between (fig. 1A).

On the other hand, histological examination of group 2 sections revealed changes in histological structure. The total epithelial thickness was markedly reduced than that in group 1. The granular cell layer's thickness was noticeably decreased, with fewer amount of keratohyaline granules than in group 1. Also, the cornified layer looked thinner, and flatter, and was lacking the horny scales. In addition, the epithelial rete pegs appeared shorter and slenderer as compared to group 1. Disturbed integrity of the basal cell layer with partial discontinuity of the cell membrane were observed in some specimens.

Concerning the lamina propria, it looked thinner with loosely arranged fibers. It revealed a markedly decreased blood supply and cellularity as compared to group 1 (fig. 1 B).

Immunohistochemical Analysis

In this study, immunohistochemical analysis of e-cadherin gene expression was performed

on 2 groups, an old and a young group, each consisting of 21 specimens retrieved from a total of 14 albino rats, 7 rats each.

Immunohistochemical examination of the young group showed a generalized immunopositive reaction of the epithelial as well as the connective tissue cells (fig. 2 A). The positive gene expression was pronounced in all the specimens of this group, with a clear reaction in all the layers of the epithelium as well as most of the cells of the connective tissue, including the fibroblasts, endothelial cells and a sporadic reaction in the inflammatory cells. The reaction was generally cytoplasmic rather than nuclear in all specimens.

On the other hand, immunohistochemical results of the rat specimens from old group of rats showed positive cases with variable degrees of gene expression. Mild immunopositivity was seen in the epithelium as well as a few connective tissue cells. The expression was generally weak and the positive cells were mostly the endothelial cells lining the blood vessels rather than fibroblasts (fig. 2 B).

The mean and standard deviation of both groups are presented in (table 1). The histogram of the collected data for the old and young groups are shown in (fig. 3) respectively.
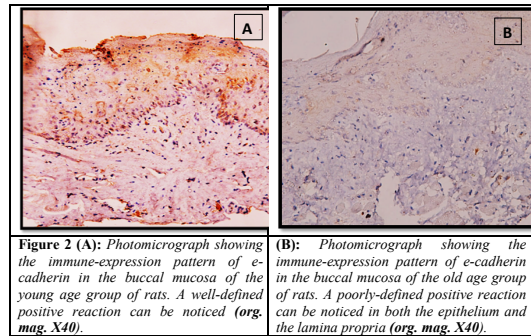

Figure 2 (A): *Photomicrograph showing the immune-expression pattern of e-cadherin in the buccal mucosa of the young age group of rats. A well-defined positive reaction can be noticed (**org. mag. X40**).* **(B):** *Photomicrograph showing the immune-expression pattern of e-cadherin in the buccal mucosa of the old age group of rats. A poorly-defined positive reaction can be noticed in both the epithelium and the lamina propria (**org. mag. X40**).*
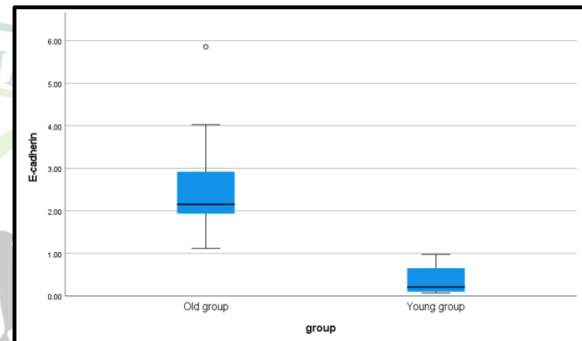

Figure 3: Histogram representing the collected data regarding e-cadherin expression for the both groups, the young and the old groups respectively. T-test showed statistical significant difference between the two groups (The significance level was set at $P \leq 0.05$).

Table 1: The mean of the area fraction of gene expression in both groups (**Group 1:** young group, **Group 2:** old group).

	Group 1			Group 2			
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	P value
E-cadherin	2.15	1.93	2.92	0.21	0.10	0.65	<0.001

Discussion

Over the past decades, theories of aging have progressed from early hypotheses of activity and detachment to theoretical approaches with a more direct focus (Martin et al., 2015) . Connective tissue alterations in the composition, as well as in the structural and mechanical integrity have been a characteristic feature of the natural aging process and various pathologies as well. Collagens, which are the most abundant matrix proteins account for the overall rigidity and elasticity of tissues (Panwar et al., 2018). In this study we outlined age changes occurring in buccal mucosa monitoring this histologically and immunohistologically.
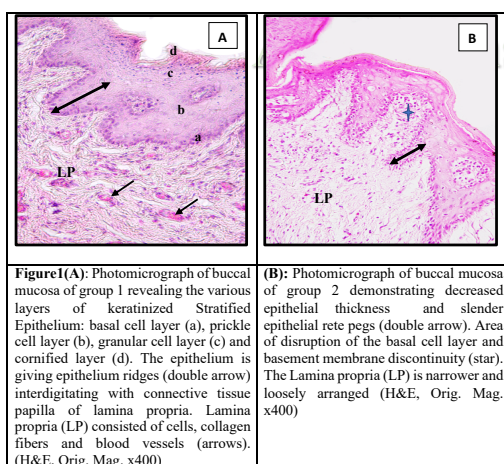

Figure1(A): Photomicrograph of buccal mucosa of group 1 revealing the various layers of keratinized Stratified Epithelium: basal cell layer (a), prickle cell layer (b), granular cell layer (c) and cornified layer (d). The epithelium is giving epithelium ridges (double arrow) interdigitating with connective tissue papilla of lamina propria. Lamina propria (LP) consisted of cells, collagen fibers and blood vessels (arrows). (H&E, Orig. Mag. x400) **(B):** Photomicrograph of buccal mucosa of group 2 demonstrating decreased epithelial thickness and slender epithelial rete pegs (double arrow). Area of disruption of the basal cell layer and basement membrane discontinuity (star). The Lamina propria (LP) is narrower and loosely arranged (H&E, Orig. Mag. x400)

In this study, fourteen albino rats were chosen, mainly because of their histological similarities to humans. In addition, they were easy to handle and were relatively of low cost. (Bryda, 2013).

Adult male albino rats were selected rather than female ones to avoid the interference of hormonal disturbances (Lightfoot, 2008).

The Hematoxylin and Eosin results showed obvious reduction in epithelial thickness in old group compared to young one. This was in accordance with the results of Carrard et al., 2008 who reported a gradual decrease in the epithelial thickness of rats' tongue from adulthood into senescence. This may be due to decreased cellularity with aging. Gutierrez-Aranda et al., 2010 reported that a decrease in adult stem cell numbers, which are necessary for maintaining tissue homeostasis and regenerative capacity, is the cause of diminished cellularity. As a result, stem cell exhaustion, also known as the quantitative and qualitative reduction in stem cell function during life, has been hypothesized as one of the causes of ageing. In vitro cell migration speed was also found to decrease with ageing in studies involving rats and mice (Svensson et al., 2016). Therefore, a decrease in cell proliferation and stem cell quantity and quality could be the cause of the second group's buccal mucosa's reduced cellularity.

In our study, the cornified layer seemed flatter and narrower than that of the buccal mucosa in group 1. The epithelial ridges also looked shorter and slimmer as compared to group 1. All these results were in accordance with the results of Elias, 2018 who studied the effect of aging on rats' oral mucosa and reported that the cornified layer of gingiva and buccal mucosa reduced in thickness and the epithelial ridges became more regular and shorter in aged group compared to adult group. These could be also related to what have been mentioned before by Gutierrez-Aranda et al., 2010 and Svensson et al., 2016.

As for the lamina propria, we observed that collagen content decreased in group 2 compared to group 1. These results agreed with Demaria et al., 2015 who discovered that the dermis of aged skin had sparse, broken-up collagen. This reduction maybe contributed to the reduction of ascorbic acid levels by age. Zanoni et al., 2013 performed a study to examine the effects of ascorbic acid on elderly rats. This vitamin is crucial for maintaining type I collagen and is likely to cause a decrease in the proportion of fibers. The fact that older people have lower levels of ascorbic acid and, as a result, proline residues that have not been hydroxylated, supports this. As a result, tropocollagen's alpha chains are unable to form stable helices, and these helices are unable to form fibrils. In addition, the lamina propria of group 2 showed decrease in cellularity and vascularity. All these results was in agreement with Florina Andreescu et al., 2013. In the current study, there was an apparent reduction in blood vessels in aged rats' lamina propria. This came in accordance with Gunin et al., 2015 as they showed that numbers of blood vessels were gradually decreased by age. This could be due to the toxic cytokines produced in chronic inflammation caused by aging process, which probably induce death of some preexisting endothelial cells and impair the regeneration ability of the others (Chung & Eun, 2007) and (Seo et al., 2010).

Concerning the immunohistochemical analysis, our results showed a marked decrease in e-cadherin gene expression in the old age group. A clear positive cytoplasmic reaction was observed in specimens of both age groups with a noticeable increase in the epithelium as well as the connective tissue cells of the buccal mucosa of the young age group.

Our results showed downregulation of the E-cadherin expression in the cell membrane in the old age group in comparison to the young

aged one. Former researchers have revealed significant reduction in the expression of E-cadherin molecule in dysplastic oral mucosa as well as in OCSCC (Chaw et al., 2012), (Kaur et al., 2013) and (Kyrodimou et al., 2014) noting that loss of cell-cell contacts is one of the main features of dysplasia. In addition, the conclusions of Freitas Silva et al. backed up our findings where downregulation of E-cadherin consequently resulted in phenotypic changes that occur in initial stages of oral carcinogenesis (de Freitas Silva et al., 2014). It is strongly proposed that the downregulation of E-cadherin is the key event of EMT, leading to loss of cell cohesion. This is viewed as a central step in the process of cancer initiation, progression and metastasis, where the tumor cells are able to move through the extracellular matrix. During tumor progression, several genetic or epigenetic mechanisms are responsible for the loss of E-cadherin function, most of which occur at the transcriptional level (Cavallaro & Christofori, 2004). This could explain what we have been noticed in group 2, regarding the loss of integrity of the basal cell layer.

Our results showed that the loss of the expression of the E-cadherin protein in the cell membrane was related to reduction of nuclear as well as cytoplasmic expression of this molecule by the aging cells. Several studies have also demonstrated similar outcomes (Fan et al., 2013). However, the frequency and the intensity of E-cadherin expression in the cytoplasm of aging or neoplastic cells remain debatable (Chan et al., 2014).

Loss of function of E-cadherin molecule promotes active signals supporting migration of neoplastic cells, invasion as well as infiltration. It is also related to involvement of lymph nodes which is a hallmark for neoplastic transformation. Moreover, altered expression of E-cadherin has been associated with metastatic dissemination, eliciting survival, cell locomotion, proliferation and proteolysis in primary and distant sites (Nijkamp et al., 2011). Nevertheless, authors up to date have not found an association between the staining pattern and the histological grade of the aging cells nor did they find a correlation between the loss of E-cadherin and malignant transformation. Thus, more studies are required to focus on the E-cadherin role in modifying the behavior of aging cells and associating this behavior with the risk of neoplastic transformation.

In numerous carcinomas, neoplastic transformation has been accompanied by loss of differentiation of the epithelium, as well as a gain in mesenchymal phenotype. These have also been shown in events linked to embryogenesis, healing and metastasis (Huber et al., 2005), where one of the E-cadherin roles seems to be cell motility control. During embryogenesis or tissue healing, E-cadherin downregulation allows for migration of regenerating epithelium over the ulcerated area (Halbleib & Nelson, 2006). It is this specific function of cell motility control that has proposed E-cadherin as an 'invasion suppressor' molecule and that in the process of cancer progression, loss of E-cadherin allows for the infiltration of adjacent normal tissues (Cavallaro & Christofori, 2004) and (Zhang et al., 2006).

Several researchers have proposed that EMT may be an indicator of progression and prognosis of OCSCC cases (Chaw et al., 2012) and (Theveneau & Mayor, 2012) where increased expression of mesenchymal genes has been observed with carcinoma progression. This expression is usually associated with loss of epithelial features, as decreased intercellular adhesion and a loss of epithelial cell polarity as well as increase in cell motility. These changes have been observed early in the development of OCSCC, as well as in oral dysplasia. Thus, the identification of genes and their proteins which could play a role in the transitional

process may be possible indicators of neoplastic transformation (Smith et al., 2009) and (Chaw et al., 2012).

Loss of E-cadherin disrupts not only cell–cell junctions but also allows for loss of the normal organ architecture. The cells are now free to move both horizontally and vertically within the epithelial layer as they are no longer constrained within a functional syncytium (Zhang et al., 2006) and (Boccaccio & Comoglio, 2006). This is a histopathological hallmark of neoplastic transformation.

Conclusions

We conclude that loss of E-cadherin with aging may be an early indicator and a possible biomarker to identify the increased risk of malignant transformation in the oral mucosa, thus providing an opportunity for early intervention or prevention of neoplasia in high-risk patients.

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