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Cadherin switching in oral squamous cell carcinoma: A clinicopathological study

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ABSTRACT

Background and aim: Oral squamous cell carcinoma (OSCC) is one of the most common malignancies worldwide as it represents the sixth most common cancer. Numerous molecular mechanisms have been explained to regulate OSCC progression, including epithelial-mesenchymal transition (EMT). Cadherin switching is the pivotal process that controls EMT in which E-cadherin reduces while N-cadherin elevates. This work aimed to clarify the role of cadherin switching in OSCC.

Material and methods: Thirty paraffin-embedded tissue blocks of OSCC including six cases with lymph node metastasis were subjected to immunohistochemical staining using antibodies against E&N-cadherins. Cell cultures were performed using OSCC cell lines (SCC-15/SCC-25) from the human tongue. F–12K medium (Kaighn's Modification of Ham's F12 Medium) was added as EMT inducing media. E&N-cadherin mRNA gene expression levels were detected by real time-polymerase chain reaction (RT-PCR).

Results: Cadherin switching through N-cadherin elevation and E-cadherin reduction was evaluated at the histopathologic level in primary and metastatic OSCC as well as at the genetic level within OSCC cell culture. Cadherin switching showed a significant correlation between E&N-cadherins at different histopathological grades of OSCC and in metastatic OSCC. Moreover, the level of mRNA gene expression of E&N-cadherins in human 15 SCC and 25 SCC cell lines with EMT-inducing media exhibited a significant correlation.

Conclusions: Cadherin switching is a crucial event in the EMT process. It may be used as a significant tool in the study of OSCC progression. Cadherin switching plays a significant role in the invasion and metastasis of OSCC.

1. Introduction

Oral squamous cell carcinoma (OSCC) is a predominant malignant disease worldwide that represents sixth cancer by incidence and eighth by death leading worldwide.[1–3] Globally, more than 350 000 oral cancer cases are diagnosed every year; moreover, the prevalence differs according to geographic distribution.[3] In Egypt, around 4500 cases were diagnosed every year while the 5-year survival rate was less than 65% over the world.[4,5] Additionally, despite educational measures for prevention and early detection, oral cancer patients are typically discovered in advanced stages, which is the most important prognostic factor for survival.[6]

Clinicopathological parameters such as age, clinical presentations, clinical staging, and differentiation of OSCC and molecular biomarkers were related to their association with local regional recurrence or death in OSCC patients.[7] However, the correlation between the clinicopathological characteristics and the recognition of high recurrence risk individuals hadn't been established as absence of definite clinicopathological parameters for identification of individual with high risk for local recurrence.[8] OSCC appears most commonly between the sixth and eighth decade's patients with different clinical presentations as endophytic ulcer or leukoplakia (white patches) or erythroplakia (red patches).[9,10] Broder's histological categorization of OSCC[11] is based mostly on the proportion of the tumor that resembles normal squamous epithelium. Tumors were assessed according to their degree of differentiation and tumor cell keratinization, and categorized as well,

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moderate, or poor. Previous researches highlighted the scope to understand the molecular mechanism that could regulate the poor prognosis of OSCC.[12,13]

The epithelial-mesenchymal transition (EMT) is a crucial molecular event that affects the behavior of cancer cells because it permits a polarized epithelial cell to undergo a series of biochemical changes that result in the acquisition of a mesenchymal cell phenotype. EMT is the process through which non-motile epithelial cells transform into motile cells able to proliferate, invade, and migrate to distant sites.[14–16]

Thus, the invasion and metastasis of OSCC cells depend mainly on EMT to be free and penetrate the basement membrane and also perform lymphnode metastasis.[17] One of the processes of EMT that has been studied and characterized is "cadherin switching" which refers to the detachment of epithelial cells' intercellular adhesion by suppression of E-cadherin (epithelial cadherin) expression, whereas cells undergoing EMT with abnormally N-cadherin (mesenchymal cadherin) expression at higher levels.[18]

The role of E and N Cadherin in EMT appears by breakdown of epithelial cell adhesion by limiting the production of E-cadherin while the cells express N-cadherin through which epithelial cells lose polarity and intercellular adhesion and gain migratory and invasive characteristics in order to transform into mesenchymal cells.

Subsequently, cadherin switching should be studied by evaluating the immune-reactivity of E&N-cadherin in OSCC and correlating their expression with clinical data such as age, sex, tumor site, clinical tumor stage (TNM), histological grade of the tumor and its metastasis if present. Therefore, this clinicopathological correlation may help to predict the early invasion, progression, or even distance metastasis of OSCC that will be useful as the future target of the therapeutic strategy of OSCC.[19,20] This study aimed to clarify the role of the cadherin switching mechanism in both histological differentiation and clinical behavior of oral squamous cell carcinoma.

2. Material and methods

The time period of the current study was nine months as followed:

Initially, in the first three months, the tissue samples with clinicopathological data were collected then the immunohistochemical staining of all cases and morphometric analysis were performed in the fourth, fifth and sixth months. In addition to three months had been taken for OSCC cell cultures and RT-PCR performing for E & N-cadherin gene expressions.

2.1. Clinico-pathological data and tissue samples

The study was carried as a retrospective study. Thirty formalin-fixed, paraffin-embedded tissue blocks of oral squamous cell carcinoma were included in this investigation. They were extracted from archival patient data at the Oral Pathology Department of the Faculty of Dentistry at Tanta University. Cases of OSCC were classified as well differentiated, moderately differentiated, or poorly differentiated OSCC (10 each). They included six cases of lymph node metastasis. Two cases were diagnosed as well differentiated OSCC, while four cases were diagnosed as poorly differentiated OSCC. The clinical data that included detailed case history including age, sex, site, and clinical tumor stage (TNM) of cases with lymph node metastasis were obtained via a retrospective review of the patients' medical files in addition to histopathological grades of tumor were gained from pathological reports. One tissue specimen obtained from pericoronal tissue from partialy impacted lower third molar during its extraction was used as normal tissue for E & N-cadherin expressions.

The current study was designed in accordance with the guidelines for the responsible use of archival patient data in research as a part of scientific research ethics recommendation of Ethical Committee at Faculty of Dentistry, Tanta University, Egypt.

2.2. Antibodies

Anti-E-cadherin antibody (Biogenex, USA, Clone 36, anti-rabbit monoclonal), Anti-N-cadherin antibody (Biogenex, USA, Clone EPR1792Y, anti-rabbit monoclonal), and An avidin–biotin-complex universal kit (ABC Elite Kit, Vector, Burlingame, CA, USA) were used in the current study.

2.3. Immunohistochemistry

Each paraffin block was sectioned by microtome. Each section was four-micron in thickness and obtained on glass slide. Three set of thirty cases (3 slide for each case) in addition to normal sections were prepared. One set was used for H&E staining for confirm the diagnosis and detect OSCC grades and the remaining two sets were used for immunohistochemical staining of E & N-cadherins. The slides were deparaffinized in xylene, rehydrated in graded alcohol series, and subjected to the following Streptavidin- Biotin Complex (ABC) staining procedure. To block the internal peroxidase activity, they were placed in hydrogen peroxide (0.3%) for 30 min at room temperature (RT).

Microwave heating was used to retrieve the antigens, initially for 3 min at 650 W and then twice for 3 min at 350 W. The sections were immersed in citrate buffer solution (pH 6.0). By incubating the sections for 30 min with diluted normal horse serum, non-specific binding was prevented. Separately, one to two drops of the primary antibody was applied to each region to thoroughly cover it. The E-cadherin and N-cadherin antibodies were diluted to 100 ml and the slides were treated with the primary antibodies overnight at 4 °C in a humidity chamber to keep the sections moist. Following that, a biotinylated secondary anti-mouse antibody was applied (for 30 min), followed by 30 min of incubation with an avidin-biotin complex, DAB (to create brown staining), and Mayer's hematoxylin (5 min for background staining). Following each process, the samples were put in phosphate buffer saline (PBS).

Negative control was performed by replacing the primary antibody by non-immune normal rabbit or mouse sera in one slide with each run, assisted as a negative control to evaluate the non-specific staining in order to approve the accuracy of the technique and exclude the false negative staining reaction.

3. Evaluation of immunostaining results

Two separate observers independently evaluated the immunohistochemical staining results by examining at least three high-power fields (HPFs) with an optical microscope (Olympus BX 50 light microscope, at x400); for each case, and the mean of these three fields was estimated and evaluated semi-quantitatively in all groups by calculating the immunoreactive score (IRS) as follows: IRS = percentage of immunopositive cells (A) x immunostaining intensity (B).

The immunoreactivity evaluation of E-cadherin (according to the following scale as modified from Kumar et al.[21]):

A- The percentage of E-cadherin immunopositive cells was measured and graded on a scale of 1–4.

1 <10%;
2 10–50%;
3 >50–80%;
4 >80% positive cells.

B - On a scale of 1–3 points, the intensity of E-cadherin immunostaining was categorized as follows.

1 Weak intensity of staining
2 Moderate intensity of staining
3 Strong intensity of staining

The total IRS varied from 1 to 12. Immunoreactivity is classified into

three groups depending on the final score:

low immunoreactivity with a total score between 1 and 4,
moderate immunoreactivity with a total score between 5 and 8, and
high immunoreactivity with a total score between 9 and 12.

The immunoreactivity evaluation of N-cadherin (according to the following scoring scale as modified from Harishchandra and Ahmed[22]):

A -The average proportion of positive cells per section was determined and evaluated across four categories:

score 0 (no positive cells).

1 is a low score (1–20% of positive cells);
2 is a moderate score (21–40% of protein-expressing cells); and
3 is a high score (>40% of protein-expressing cells).

B - On a scale of 1–3 points, the intensity of N-cadherin immunostaining was measured as follows.

1 Weak intensity of staining
2 Moderate intensity of staining
3 Strong intensity of staining

Total IRS immunoreactivity ranged from 0 to 9 and was divided into four groups based on the final score:

negative immunoreactivity with a total score of 0;
low immunoreactivity with a total score of 1–3;
moderate immunoreactivity with a total score of 4–6; and
high immunoreactivity with a total score of 7–9.

3.1. Morphometric study

For morphometric analysis, Olympus light microscope was used for imaging at the Oral Pathology Department, Faculty of Dentistry, Tanta University. Morphometric study and Image analysis were done on an IBM-compatible computer using the public domain, free software ImageJ program (National Institute of Health, Bethesda, Maryland, USA) (Public domain, image processing and analysis in Java), a Java application that runs in windows operating systems.

3.2. Cell lines and reverse transcription –polymerase chain reaction (RT-PCR)

Human tongue OSCC cell lines (SCC-15/SCC-25) were obtained from American Type Culture Collection (ATCC), USA. Vaccine Research Institute was able to cultivate cell lines (VACSERA, Agouza, Giza, Egypt). Culture media including Dulbecco's Modified Eagle's Medium (DMEM), Ham's F12 medium, and F–12K medium (Kaighn's Modification of Ham's F12 Medium) used as EMT inducing media were purchased from Sigma-Aldrich (St. Louis, MO, USA). OSCC cell lines were sub-cultured in two groups with the addition of F–12K medium and two groups without the addition of this inducing media for detection of genetic expression of E&N-cadherins.

Total RNA was isolated from cells with the ISOGEN system (Nippon Gene Co., Ltd., Tokyo, Japan) and reverse transcribed with the Super-Script III First-Strand Synthesis System to generate cDNA (Life Technologies). Using particular primers for N- and E-cadherin and a MiniOpticon Real-Time PCR Detection System CFB-3120, a real-time reverse transcription polymerase chain reaction (RT-PCR) was carried out (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Using CFX Manager version 2.1, real-time monitoring of target gene amplification and measurement of gene expression levels were accomplished (Bio-Rad). To account for variance, GAPDH expression was used, and gene expression levels were represented as ratios to GAPDH from the same master reaction. For each gene, the following PCR primer pairs were used.

a. Human N-cadherin 5′-GACAATGCCCCTCAAGTGTT-3' (forward)
   5′-CCATTAAGCCGAGTGATGGT-3' (reverse)
b. Human E-cadherin 5′-TGCCCAGAAAATGAAAAAGG-3' (forward)
   5′-GGATGACAGCGTGAGAGA-3' (reverse)
c. Human GAPDH 5′-AATGCATCCTGCA CCACCA A-3' (forward)
   5′-GATGCCATAT TCATTGT CATA-3' (reverse)

3.3. Statistical analysis

The data were analyzed by carrying out one-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test to compare E&N-cadherins expression with the clinicopathological parameters using a statistical package for social sciences (IBM SPSS version 23.0 software for Windows, New York, USA). Spearman's correlation test was also used to detect the relation between E&N-cadherins in all cases of OSCC immunohistochemically and gene expression. All values were expressed as mean ± standard deviation (SD). The differences were regarded as statistically non-significant if the probability value (p) > 0.05, significant if $p < 0.05$, and highly significant if $p < 0.01$.

4. Results

4.1. Clinicopathological findings

The majority of OSCC affected were males in 20 cases (66.6%) and females were 10 cases (33.3%). Age ranged between 44 years and 85 years with a mean age of 64 years old. The tongue was the most common site involving 15 (50%), 8 cases (26%) appeared at the buccal mucosa, 3 cases (10%) at the retromolar area, 2 cases at the palate (6.6%), and the same in the lower lip (6.6%). Ulcers were the most represented clinical sign in 18 cases (60%), erythroplakic OSCC was in 6 cases (20%), and leukoplakic OSCC in 6 cases (20%). Metastatic OSCC cases were 3 cases at stage III and 3 cases at stage IV. The histopathological findings of OSCC were varied according to the patients 'age, sex, clinical presentation; location of the lesion; according to the WHO guidelines (Table 1).

4.2. Immuno-histopathological findings

Pericoronal tissue was obtained from partially impacted lower third molar (Figure (Fig.) 1A) during its extraction and was stained for E & N-cadherins (Fig. (1B, C)). It was noted that a positive mild reaction to E-cadherin expression was mainly localized at the cell membrane of basal and supra-basal cell layers (Fig. (1B)) but anti N-cadherin staining is not positive (Fig. (1C)).

The histopathological grades of OSCC lesions showed variation in expression of E &N-cadherins in the tissues as appeared in Fig. 2:

Well differentiated OSCC cases Fig. (2A) display intense E-cadherin membranous expression in malignant cells with deep staining Fig. (2D),

Table (1)
Illustrating the clinicopathological characters in patients with OSCC (Number (No.) = 30).

Variables	Category	No.
Age (years)	≥60	15
	< 60	15
Sex	Male	20
	Female	10
Location	Tongue	15
	Buccal Mucosa	8
	Retromolar area	3
	Lower lip	2
	Palate	2
Clinical Presentation	Ulcer	18
	Erythroplakic OSCC	6
	Leukoplakic OSCC	6
Histopathological Grades	Well	10
	Moderate	10
	Poorly	10
TNM Stages	III	3
	IV	3

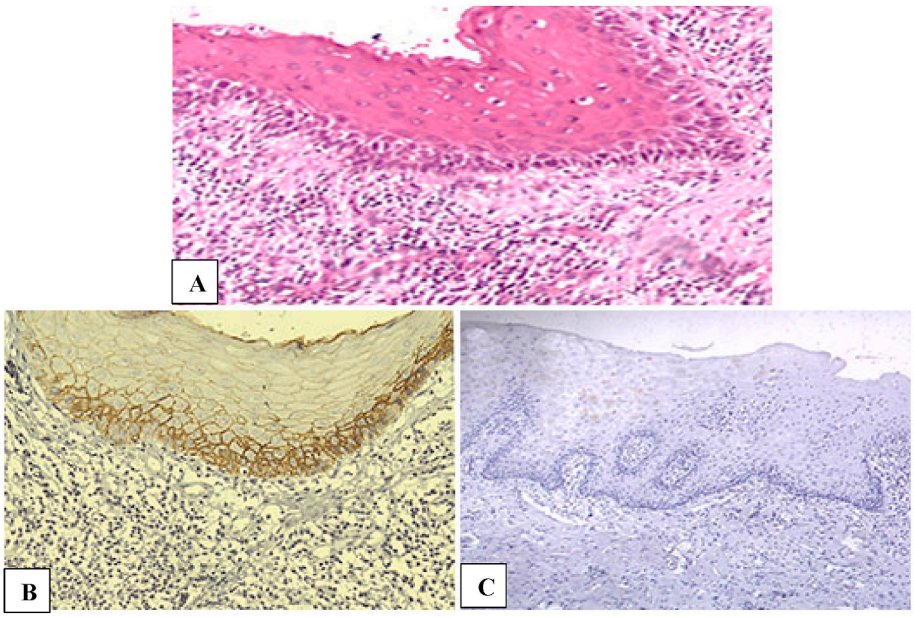

Fig. (1). Photomicrograph of peri-coronal normal epithelial tissue (A) H & E stained section x200. (B) anti- E cadherin stain reaction mainly localized at the basal and supra-basal cell layer. Avidin–biotin-complex (ABC) x200. (C) negative staining of normal epithelium by anti N-cadherin staining ABC x200.

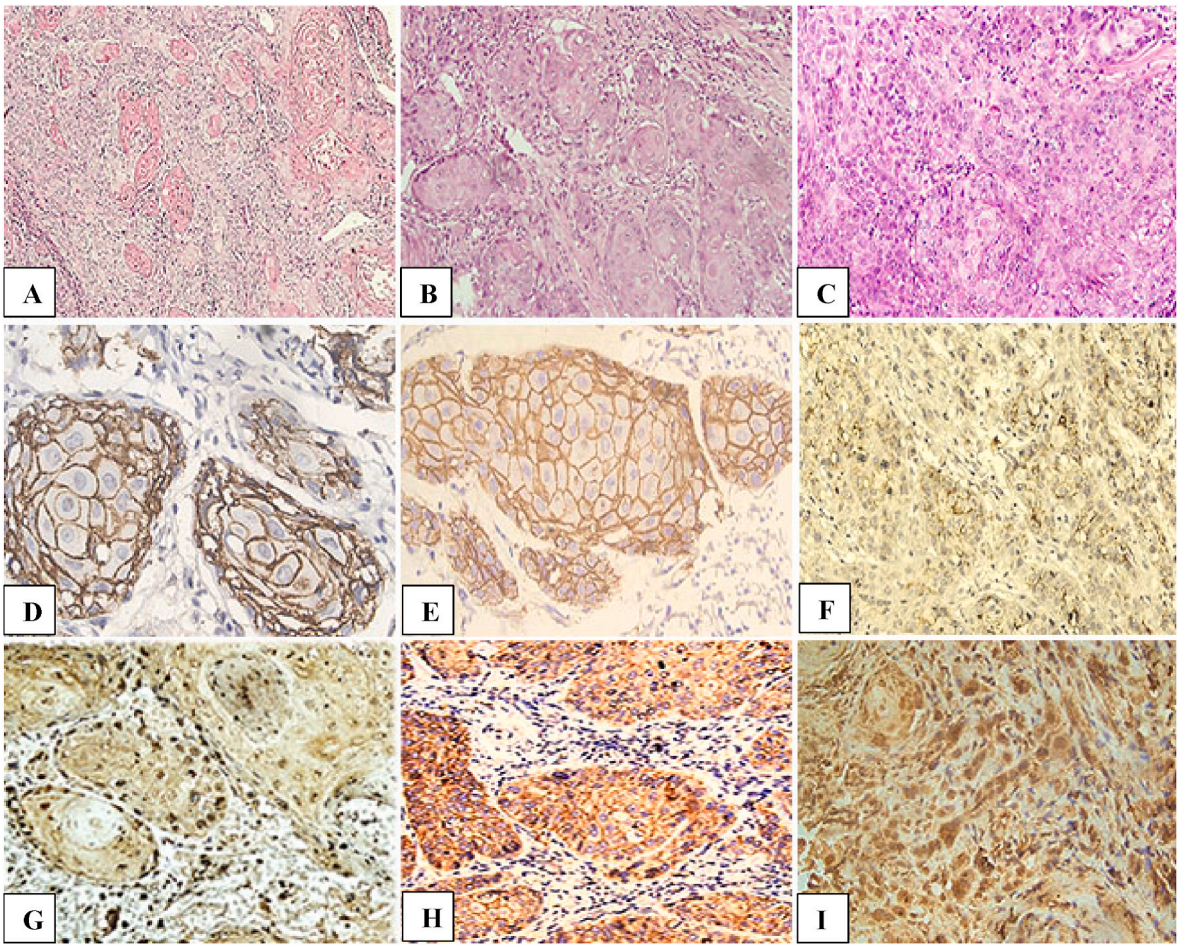

Fig. (2). photomicrograph of OSCC in different grades of OSCC: (A, D, G) well differentiated OSCC. (B, E, H) moderately differentiated OSCC. (C, F, I) poorly differentiated OSCC. (A, B, C) H&E staining x100. (D, E, F) E-cadherin staining ABC x200. (G, H, I) N-cadherin staining ABC x200.

while cytoplasmic N-cadherin presentation is faint in epithelial pearls of well differentiated OSCC Fig. (2G). Moderately differentiated OSCC cases Fig. (2B) demonstrate moderate E-cadherin expression in the epithelial nests Fig. (2E). However, N-cadherin expression is intense in the cytoplasm of epithelial cell nests of moderately differentiated OSCC cases Fig. (2H). Poorly differentiated OSCC cases Fig. (2C) shows faint membranous staining of E-cadherin Fig. (2F) despite intense N-cadherin staining in the cytoplasm of malignant OSCC invading cells Fig. (2I).

4.2.1. *Morphometric analysis of E/N-cadherins in different grades of OSCC*

Well differentiated OSCC displayed high E-cadherin expression as 5 cases (50%) showed high IRS while 3 cases (30%) showed moderate IRS and 2 cases (20%) showed low IRS. Moderately differentiated OSCC exhibited moderate E-cadherin expression as 6 cases (60%) showed moderate IRS while 2 cases (20%) showed high IRS and 2 cases (20%) low IRS. Poorly differentiated OSCC displayed low E-cadherin expression as 8 cases (80%) showed low IRS while 2 cases (20%) showed moderate IRS.

Well differentiated OSCC displayed low N-cadherin expression as 6 cases (60%) showed low IRS and 2 cases (20%) showed negative IRS while 2 cases (20%) showed moderate IRS. Moderately differentiated OSCC exhibited moderate N-cadherin expression as 5 cases (50%) showed moderate IRS while 3 cases (30%) showed high IRS and 2 cases (20%) low IRS. Poorly differentiated OSCC displayed high N-cadherin expression as 8 cases (80%) showed high IRS while 2 cases (20%) showed moderate IRS.

The correlation between histopathological grades of OSCC and cadherin switching is discussed in Table 2.

There is **high statistical significance** between all histopathological grades of OSCC and cadherin switching as **p level = 0.000** (Fig. 3).

4.2.2. *Metastatic OSCC*

Metastatic OSCC appeared in cervical lymphnodes (Fig. (4A)). Malignant epithelial cells in lymphnode exhibited low E-cadherin expression (Fig. (4B)) as all the six cases (100%) showed low IRS with a mean of 3.33, whereas they exhibited high N-cadherin expression (Fig. (4C, D)) as 4 cases showed high IRS and 2 cases showed moderate IRS. The mean of N- cadherin in all cases was 7.3.

4.3. *Gene expression findings*

Enhanced expression of the N-cadherin in OSCC cells at mRNA level:

The expression of N-cadherin gene increased in cell culture with the addition of F–12K medium as this media induce EMT. However, the E-cadherin gene expression decreased in cell culture with the addition of F–12K medium as its EMT-inducing action. The mean of fold change (FLD) of N-cadherin was 2.84 in SCC 15 and 4.59 in SCC 25, while FLD's mean of E-cadherin was 0.65 in SCC 15 and 0.36 in SCC 25 (Table 3) (Fig. 5).

Table (2)
Relation between histopathological grades of OSCC and cadherin switching.

OSCC grades					
Cadherin switching		WD OSCC	MD OSCC	PD OSCC	ANOVA Test
E-cadherin IRS	No.	10	10	10	
	Min-Max	4–12	2–12	1–6	F test = 8.763
	Mean ± SD	8.2 ± 3.16	6.5 ± 2.72	3.4 ± 1.71	p value = 0.001**
N-cadherin IRS	No.	10	10	10	
	Min-Max	0–4	1–9	4–9	F test = 23.557
	Mean ± SD	1.7 ± 1.42	5.3 ± 2.63	7.9 ± 1.85	p value = 0.000**

IRS; immunoreactive scoring, WD OSCC; well differentiated oral squamous cell carcinoma, MD; moderately differentiated, PD; poorly differentiated, No.; number, Min.; minimum value, Max.; maximum value, SD; standard deviation, F test; ANOVA test, p value; probability value, **; high significance.

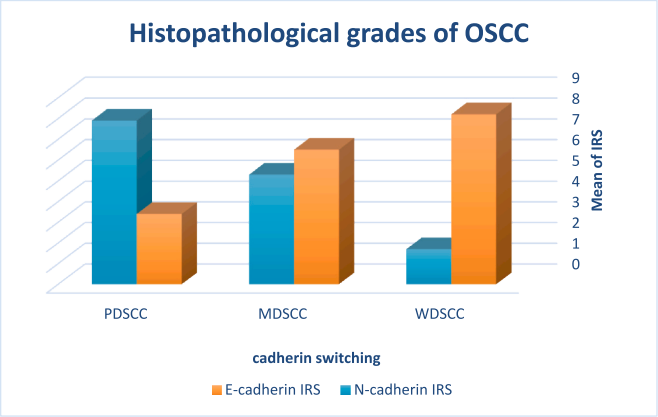

Fig. (3). Correlation between cadherin switching and histopathological grades of OSCC.

There was high statistical significance in E and N-cadherin gene expression as p value less than 0.01.

The inference of the current study is cadherin switching with significant statistical increasing of N-cadherin expression demonstrated in poorly differentiated OSCC and in cases of OSCC metastasis in cervical lymphnode and also significant increasing N-cadherin gene expression in EMT induced media could be considered as an important biomarker for development, progression and metastasis of OSCC.

5. Discussion

Oral squamous cell carcinoma (OSCC) is considered the most prevalent oral cancer that appears in the oral mucosa's epithelial cells.[1,23] Oral epithelial cells adhere to one another by use of a calcium-dependent, developmentally regulated transmembrane protein called E-cadherin.[24] The absence of differentiation from the original cells implies that tumor cells are gaining a mesenchymal phenotype, which may be recognized more easily with a mesenchymal marker such as N-cadherin.[22] EMT, or the absence of epithelial morphology and gaining mesenchymal characteristics, occurs during tumor growth and correlates with the tumor's local invasiveness and metastatic potential.[25]

Consequently, EMT is a key process through which epithelial cells lose polarity and intercellular adhesion and gain migratory and invasive characteristics in order to transform into mesenchymal cells.[26] The breakdown of epithelial cell adhesion by limiting the production of E-cadherin (epithelial cadherin) while the cells express N-cadherin (mesenchymal cadherin) at a high level termed "cadherin switching" is one of the best processes that illustrate EMT.[11]

From this standpoint, it is critical to understand the molecular pathways regulating OSCC development, as this knowledge is required to improve patient prognosis.[27] During the initial phases of development, OSCC cells must dissociate from their original sites and penetrate both the basement membrane and the underlying connective tissues. This procedure requires the dissolution of a cell to cell adhesion. OSCCs with weak cell to cell adhesion invade in tiny subsets or as single cells, predisposing them to an advanced developmental stage. The nature of cancer cells and their interaction with their surroundings are critical for progression during the invasion.[28,29]

Subsequently, the purpose of the current investigation was to evaluate the immunological expression of E&N-cadherins in different histological grades of OSCC, as well as in lesions with regional lymph node metastasis. In addition, the genetic expression of E&N-cadherins in a variety of OSCC cell lines and cell cultures was investigated.

Normal pericoronal epithelium that removed during the extraction

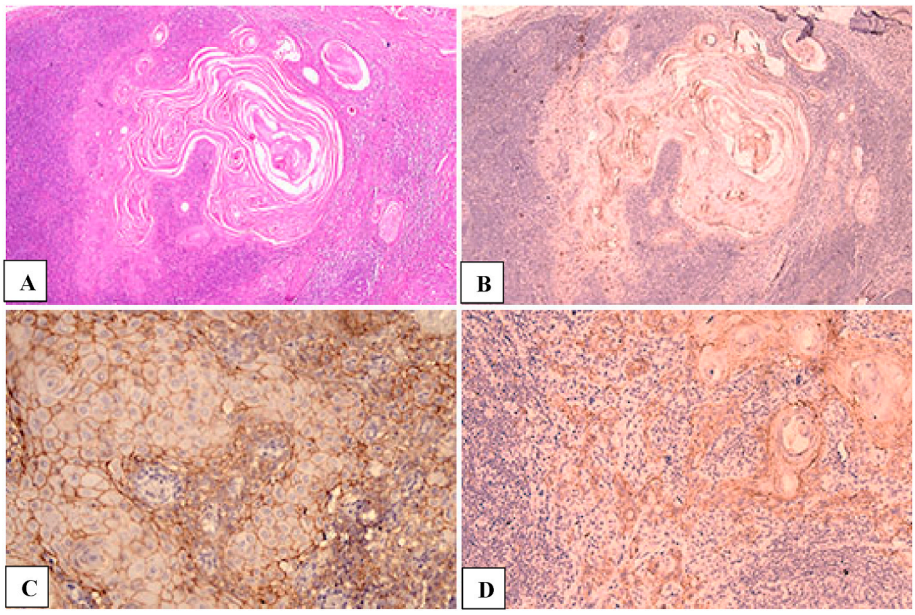

Fig. (4). Photomicrograph showing OSCC in lymphnodes (A) H & E staining x100. (B) E-cadherin staining ABC ×100 shows weak staining. (C, D) N-cadherin staining ABC ×100 shows intense staining.

Table 3
N and E-cadherin gene expression in different OSCC cell cultures.

Sample code	Gene expression		r (p-value)
	Fold Change		
	N-cadherin	E-cadherin	
SCC15/**F–12K**	2.84 ± 0.14	0.65 ± 0.03	−0.976 (0.000**)
SCC25/**F–12K**	4.59 ± 0.19	0.36 ± 0.02	
SCC15	1	1	
SCC25	1	1	

r; rank corr elation, scc15, 25 squamous cell carcinoma cell lines 15, 25, F–12K medium; Kaighn's Modification of Ham's F12 Medium.

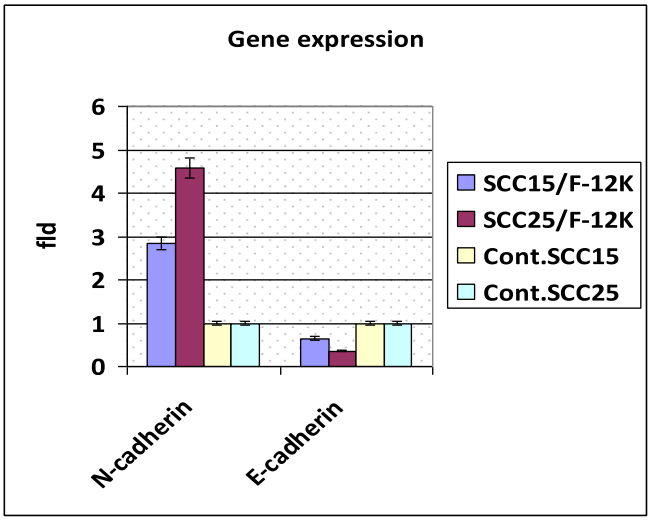

Fig. (5). E&N-cadherins gene expression in different OSCC cell cultures.

of an impacted lower third molar had intense positive E-cadherin staining at the basal, supra-basal layers whereas it showed mild E-cadherin staining at the superficial layer of epithelium. These findings were in a line with Mehendiratta et al.[30] who also observed that the positive reactivity with anti-E-cadherin gradually diminished in the superficial layers or lost in the cells that had undergone keratinization. They suggested that the decreasing of E-cadherin expression may play a role in the normal desquamation of epithelium and believed that the processes regulating E-cadherin expression in the superficial layers of epithelium were not well known.[30] E-cadherin was suggested to have a role in maintaining cellular polarity and proper epithelial tissue architecture.[31] In addition, the current investigation revealed that N-cadherin did not express within the normal oral epithelial cells. This finding was in agreement with Hashimoto et al.[27] and Angadi et al.[32] who observed that N-cadherin was absent from normal oral epithelial cells. These results confirmed Pyo et al.'s study as they attributed the cause of the absence of N-cadherin expression in normal squamous epithelium to the fact that N-cadherin is typically expressed by neural cells, whereas these cells did not present within normal squamous epithelium.[33]

The differentiation between E−cadherin staining that located in the cellular membrane and N-cadherin staining in the cellular cytoplasm in addition to the cellular membrane appeared clearly in the immune-stained sections of the present study. Angadi et al. also reported the same noticeable change of staining from membranous staining of E-cadherin to the cytoplasmic staining of N-cadherin. They documented that this shift was essential for OSCC cells to enter the underlying connective tissue and during lymph node metastasis.[32] Angadi et al. also proved that there was a significant negative correlation between the localization of E&N-cadherins (−0.019), therefore they concluded that membranous E-cadherin was shifting to the cytoplasm and being replaced by N-cadherin, resulting in EMT. Accordingly, they considered this shift of the studied proteins as an important aspect of EMT.[32]

In prior research, Kaur et al. and Gonzalez-Moles et al. established that the activity of E-adhesive cadherin is essential for its interaction with the catenin considered as a cytoplasmic adaptor protein and binds the E−cadherin terminal tail to the actin cytoskeleton. Without this cadherin-catenin complex, cells are more likely to undergo a cytoplasmic shift, resulting in loss of cellular adhesion. Consequentially, this cytoplasmic shift was associated with an increase in malignant invasive with high histological grade, and lymph node metastasis.[34,35]

The clinicopathological correlation identified in clinical and histopathological data by analysis of the clinical presentation, clinical staging, and differentiation of OSCC in the current study seemed to be correlated with the expression of OSCC biomarkers of E & N-cadherins

known as cadherin switching. These results were in line with Liu et al. who approved that the importance of clinicopathological parameters and molecular biomarkers was related to their association with local regional recurrence or death in OSCC patients.[7] Furthermore, Ginos et al. proposed that clinicopathological factors and tumor-specific molecular markers capable of identifying individuals at increased risk of local recurrence had not been established.[8] In addition, Pyo et al. stated that current treatment choices for OSCC and pharyngeal SCCs should be determined by clinicopathological factors such as age, sex, race, lymph node metastatic stage, and histopathological grade.[33]

In the current study, the OSCC histopathological grade demonstrated a statistically significant correlation with E&N-cadherin expression as in WDOSCC, increasing expression of E-cadherin and decreasing expression of N-cadherin, whereas in MDOSCC, moderate expression E&N-cadherin. PDOSCC, on the other hand, had low E-cadherin expression and high expression of N-cadherin.

Furthermore, Pyo et al. Kamikihara et al., and Han et al. demonstrated cadherin switching. They hypothesized that cadherin switching altered the stroma-oriented adhesion profile of malignant cells, improving their motility and invasiveness.[33,36,37] The current findings corroborate those of Islam et al. who demonstrated that N-cadherin overexpression seems to restrict E-cadherin expression, a process recognized as cadherin switching and that N-cadherin expressing epithelial cells exhibit greater motility and further invasion.[38] Bryan et al. observed an increase in N-cadherin expression and a reduction in E-cadherin expression during the cadherin switching process in bladder cancer.[39]

Nevertheless, the current study's findings were in contrast with Hashimoto et al. who hypothesized that cadherin switching was not involved in the progression of OSCC and demonstrated that OSCC progression was associated with a reduction in expression of E-cadherin but not with cadherin switching.[27] Similar to Hashimoto et al. Ukpo et al. discovered that cadherin expression in oropharyngeal squamous cell carcinoma is unrelated to histopathologic grade, metastasis, or prognosis.[40] In addition, Costa et al. were in contrast to the current study as they observed that there is no cadherin switching in OSCC samples that examined in their study.[41] Additionally, Nieman et al. established that E-cadherin replacement in malignant cells didn't affect the aggressive phenotype.[42] They concluded that N-cadherin enhances breast cancer cells' motility along with a reduced E-cadherin expression.[42]

Furthermore, E-cadherin expression declined gradually from well differentiated OSCC to moderately differentiated OSCC to poorly differentiated OSCC in the current research. The previous findings corroborated Kaur et al. who observed a reduction in E-cadherin immunoreactivity as OSCC progressed from well to poorly differentiated OSCC, concluding that the expression of E-cadherin was inversely associated with cell differentiation loss and reduced significantly in OSCC cases that presented in advanced stages.[34] These results supported Bagutti et al. who observed a link between E-cadherin expression and degree of differentiation: tumors with the lowest degree of differentiation had the lowest E-cadherin expression.[43] In the present investigation, the development and invasion of OSCC are related with decreased E expression, as determined by immunostaining. Yang et al.[44] and Fan et al.[45] demonstrated that the reduced expression of E-cadherin was considered an OSCC prognostic factor.

N-cadherin expression also increased gradually from well differentiated OSCC to moderately differentiated OSCC to poorly differentiated OSCC in the current study. These findings were in line with Harishchandra and Ahmed's who demonstrated high statistical significance in progressive elevation of N-cadherin expression with less differentiated tumor cells.[22] Lawson et al. confirmed these findings as they observed the increased invasiveness in OSCC cells expressing N-cadherin.[46] Liu et al. similarly observed a progressive increase in N-cadherin expression in cancer prostate with increasing histological grade.[47] These findings were corroborated by Kamikihara et al. who showed increased N-cadherin expression in patients with advanced gastric cancer.[36] Consequently, the results of the current study accepted that N-cadherin expression in OSCC can be regarded as a reliable marker of EMT. Ozaki-Honda et al. agreed with these findings and concluded that N-cadherin expression was a valid marker for prognostic prediction in OSCC patients.[48]

Furthermore, in the current study, the metastatic OSCC lesions within lymphnodes showed a negative correlation between E&N-cadherins as they demonstrated reduced E-cadherin expression with elevated N-cadherin expression. Nguyen et al. also in previous studies was confirmed by the present results as they concluded that cadherin switching may be a critical process in OSCC metastatic potential.[49] Tanaka et al. also conducted clinical research on E-cadherin in OSCC patients and suggested that reduced E-cadherin expressing cases had a higher number of regional and distant metastases.[50]

In contrast, Balasundaram et al. argued in their study that E cadherin was likely not the primary predictor of regional metastasis in OSCC, contradicting the current findings.[51] In addition, Mehendiratta et al. found no association between E-cadherin and lymph node status in their analysis.[30] Cheng et al. also showed that there is no statistically significant relationship between the expression of E-cadherin and lymph node metastasis in OSCC.[52] Ukpo et al. also found no link between cadherin expression and oropharyngeal squamous cell carcinoma metastasis.[40] Guo et al. found an increase in E-cadherin expression in OSCC lymph node metastasis in both human and animal samples.[53] Using a systemic review and meta-analysis, Zhao et al. documented that the expression of E-cadherin has a prognostic value for the OSCC patients but couldn't judge whether there is EMT in the process of OSCC development or not.[54]

In the current study, it was planned to use RT - qPCR in addition to immunohistochemistry was recommended to reduce subjectivity in the visual evaluation and immunohistochemically stained slide scoring, and to get more repeatable staining intensity findings through digital analysis. Sinn et al. agreed with this work by comparing immunohistochemistry with polymerase chain reaction (PCR) for the evaluation of several markers in breast cancer and concluding that RT-qPCR was much more specific.[55] Sathish et al. had also demonstrated that qPCR has greater sensitivity and specificity than other known techniques such as immunohistochemistry.[56]

In the current investigation, the gene expression of E&N-cadherins in human 15 SCC and 25 SCC cell lines in various culture media were statistically significant. The addition of F–12K medium, which induces EMT, enhanced the expression of the N-cadherin gene in cell culture, but the expression of the E-cadherin gene decreased. These findings corroborate those of Nguyen et al. who discovered cadherin switching in HOC313 and HOC719-NE cell lines displaying EMT features such as spindle form and strong invasive capacity.[49] The current findings are in a line with Schipper et al. who reported that epithelioid phenotype cancer cell lines expressed E-cadherin and were non-invasive while those with a fibroblastoid phenotype lacked E-cadherin expression and showed high invasion.[57] In a previous study, Islam et al. confirmed these findings by examining human squamous cell carcinoma cultures UM–SCC–1 (SCC1), UMSCC-11A (11A), and UM–SCC–B (11B) and demonstrating that ectopic expression of N-cadherin in epithelial cells led to the down-regulation of E-cadherin and a motile, scattered fibroblastic phenotype. They concluded that N-cadherin-expressing squamous cell carcinomas were more invasive.[38] Consequently, this significant reduction in E-cadherin expression during the OSCC invasion demonstrates "E cadherin's role as an invasion suppressor".[31]

Nevertheless, Maeda et al. who studied gene expression in Mouse NMuMG cells (CRL-1636) and human MCF10A cells (CRL-10317), found that cadherin switching is not necessary for the morphological changes associated with EMT.[58] The current study is also in opposite to Hashimoto et al. as they examined human OSCC cell lines (TSU, HSC2, KOSC2, KOSC3, SCCKN, OSC19, and Ca9.22) and assessed cadherin gene expression using real-time PCR. They discovered that the expression of N-cadherin was negligible. They also concluded that further research on

cadherin regulation by cellular environments would advance our knowledge of how OSCC progresses.[27]

The reasons for contrast in the current study and other studies were that using of diverse cell lines of OSCC and inducing growth media in the current study weren't included in other studies. Also, by means of RT - qPCR in addition to immunohistochemistry was recommended in the present study to reduce subjectivity in the visual evaluation and immunohistochemically stained slide scoring, and to get more repeatable staining intensity findings through digital analysis.

Many factors in the current study lead to the limitation of reporting a definitive conclusion like the smaller sample size, the wide range of patients' age, the different sites of OSCC lesions, and the smaller number of metastasized OSCC to the lymph nodes. Therefore, future recommendations were considered as further studies having more cases and more specific antibodies, various cell lines of OSCC, different inducing growth media, and a consistent application of interpretation will be needed to provide further insight and confirm the results of the current study.

6. Conclusions

In conclusion, cadherin switching is a critical event in EMT and appears clearly in EMT inducing environment. Cadherin switching with clinicopathological parameters has a critical role in the development, invasion, and metastasis of OSCC. Both E&N-cadherins are considered accurate progressive and metastatic markers for OSCC. Cadherin switching biomarkers may have significant impact in developing targeted therapy with further useful modalities of treatment to interfere with OSCCs growth and reduce their aggressiveness and metastasis.

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