IMMUNOHISTOCHEMICAL EVALUATION OF P-CADHERIN IN PERILESIONAL AREA OF OSCC

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Abstract

Background: New studies have been carried out in understanding the molecular mechanism of OSCC and identifying the molecular markers useful for diagnosing a tumor at an early stage. Diagnosis at an early stage, could be associated with the favorable prognosis and a 5-year survival rate of 60-80%.
Aims of the study: To assess P-cad expression at perilesional area of OSCC and correlate such expression to OSCC histopathological grading.
Materials and methods: Immunohistochemical evaluation of P-cad expression at perilesional area of 20 OSCC and 10 normal oral mucosa from archival paraffin blocks was done by using mouse monoclonal P-cad antibody. The sections were assessed according to the ability of surface epithelium at perilesional area of OSCC in showing normal expression pattern of P-cad in oral mucosa.
Results: The present study showed membranous basal and parabasal expression in all cases of normal oral mucosa while perilesional areas of OSCC revealed high multiple layers expression of P-cad in (45%) , this anomalous expression support the genetic alteration in perilesional area and gave a clue for a common cell clone for both tumor and the adjacent perilesional area. There were multiple layers staining of P-cad in (46.6%) of WDSCC, however, statistical analysis did not reach any significant level in relation to histopathological grading.
Conclusion: Increase P-cad expression at the perilesional area has an important initial role in early step of carcinogenesis, and the aberrant expression of P-cad at the perilesional area might have a role in the mode of the invasion and prognosis of oral cancer.

Keywords: P-cad, perilesional area, multiple layers staining

Introduction: Oral squamous cell carcinoma (OSCC) represents the sixth most diffused cancer in developed countries. In spite of new treatment modalities. The mortality rate remained high (~50%). New studies have been carried out in understanding the molecular mechanism of OSCC, and

identifying the molecular markers useful for diagnosing a tumor at an early stage. Diagnosis at an early stage, could be associated with the favorable prognosis and a 5-year survival rate of 60-80% (Aguzzi et al, 2009).

The tried clinical examination and heightened awareness remain the mainstay of early OSCC detection, however, technology available might help the development of useful techniques and devices for the detection of potentially pre-malignant and early malignant conditions (Kujan et al ,2009).

Clinical staging (TNM) have an important influence on the survival and prognosis of OSCCs patients. Unfortunately, approximately 60% to 65% of diagnosed patients are in late stage (stages III and IV). This delay could be related to either patient or professional cause (Gao and Guo , 2009; Akbulut et al , 2011).

The sequential genetic changes that drive a cell towards malignancy occur over several years. In view of this, the American Association for Cancer Research recognized the importance of targeting and treatment of early cancerous lesions to prevent or regress carcinogenesis (O'Shaughnessy et al, 2002). Significant efforts of identification of the biomarkers in normal tissues adjacent to tumors (peri-tumoral cancer fields), might be useful for primary chemoprevention studies (Tsao et al, 2004).

Individuals with one carcinoma of the head and neck region have an increased risk of developing a second malignancy; the frequency of that event varies from 16% to 36%. When a second malignancy occurs at the same time as the initial lesion, it is called a synchronous carcinoma (Schwartz et al, 1994). "Lateral cancerization" was subsequently used to indicate the lateral spread of tumors which was due to progressive transformation of cells adjacent to a tumor, rather than the spread and destruction of the adjacent epithelium by pre-existing cancer cells (Dakubo et al, 2007). It was observed that normal looking cells in close proximity to malignant cells were histologically abnormal and therefore were considered as a part of the transformed cells in a particular tumor field, and consequently were responsible for the occurrence of local tumor recurrences (Braakhuis et al, 2003). Second primary tumors are the chief cause of death in patients with an early stage diagnosis (Hong et al, 1990). The tendency to develop multiple carcinomas in the upper aerodigestive region is known as "field cancerization." (Dakubo et al ,2007; Slaughter et al ,1953) . Prolonged and diffuse exposure to local carcinogens, particularly tobacco combined with alcohol, appears to increase the malignant transformation potential of exposed epithelial cells (Fortuna and Mignogna, 2011).

P-cad is cell adhesion molecule, which is only expressed in the basal and suprabasal cell layers of the normal oral epithelium. During tumor progression, P-cad expression increases in the initial stage of tumor growth ,

whereas a reduced membranous and or an enhanced cytoplasmic expression of P-cad is observed at the invasion front of oral squamous cell carcinoma (OSCC) (Bauer et al , 2013).

Furthermore, studies revealed that the expression of an inappropriate cadherin in epithelial cells is yet another way that tumor cells can alter their adhesive function (Pyo et al,2007).

Understanding molecular mechanisms regulating oral carcinoma progression is a prerequisite for improving the patient prognosis. At the first step of progression, epithelial cells must sequester from their primary sites and invade into the basement membrane and underlying tissues. This step requires the dissociation of cell-cell adhesion. , later on invasion in a form of small subsets or individuals of cells, that predispose them to a more advanced state of progression (Hashimoto et al , 2012).

Aims of the study: To evaluate the P-cad expression and distribution whether single basal and multiple layers and its cellular localization (membranous or cytoplasmic and mix) in the oral epithelia at perilesional area of OSCC lesions and relate such expression to OSCC histopathological grading.

Materials and Methods

A total of 20 archival paraffin blocks previously diagnosed as OSCC with perilesional area were collected from Baghdad and Sulaimani histopathological centers. Ten blocks of mucoceal covered by normal oral mucosa were obtained from Oral Pathology Department at Sulaimani University and used as positive controls. The study was approved by both the scientific and ethical committee in the college.

Serial 4µm sections were cut, one section was stained with hematoxylin and eosin to identify the perilesional area of the lesion and histopathological grading of OSCC , the other section was mounted on positively charged slide and stained immunohistochemistry with P-cad . Sections were deparaffinized and rehydrated, then retrieved by antigen retrieval solutions (Citrate buffer, pH 6) by using the pressure cooker (for 15 min). Endogenous peroxidase activity was blocked by incubating the sections with 1% H2O2 in PBS (for 10 min). In order to prevent non – specific binding sections were incubated with 1.5% blocking serum (for 1 hour) . Then sections were incubated with the primary mouse monoclonal antibody of P-cad (U.S Biological; diluted at 1:20) for three quarters of an hour at 37°C in a humid chamber. Biotinylated secondary antibody was applied for 30 min at 37°C, then detection was performed by using avidin-biotin peroxidase technique for 30 min at 37°C . The reaction was visualized by incubation with diaminobendizine for 10 min, then sections were counter

stained with hematoxylin. Negative control slides were obtained by omitting the primary antibody.

Slides were assessed blindly by the author. The sections were evaluated according to the ability of surface epithelium in perilesional area adjacent to OSCC in showing normal pattern of membranous staining in basal and parabasal layer (Lo Muzio et al 2004 ; Lo Muzio et al 2005; Bauer et al, 2008) and the location of P-cad expression was determined (whether basal, multiple layers) and accordingly the surface epithelia in perilesional area , was evaluated by using the following score (Pyo et al 2007):

1. A increases expression: sections showed staining over suprabasal layer.
2. Unchanged expression, sections revealed immunoreactivity that was confined to limited layer of basal cells.
3. Decreased expression, sections showed faint staining.

SPSS statistical software was used to estimate Chi-square and Pearson's correlation coefficient. Probabilities of less than 0.05 were accepted as significant.

Results and Discussion

Several studies demonstrated the altered expression of P-cad and E-cad in OSCC. This work for the first time evaluate P-cad expression at perilesional area of OSCC.

Understanding of the molecular alteration of perilesional area could not only assist the diagnosis and prognosis of oral cancer but might also open up novel therapeutic approaches (Díez-Pérez et al, 2011).

Interestingly, authors detected 72.73% methylation of p16 in healthy peritumoral tissue, suggesting that methylation of this marker might be an early event in carcinogenesis and could therefore serve as a prognostic and diagnostic marker (Kato et al,2006). On the other hand, a higher level (up to 4-fold) of methylation was found in histologically healthy samples from patients previously treated for OSCC, indicating a greater predisposition to tumor recurrence (Ruesga et al, 2007). Furthermore, it was reported that E-cad methylation was found in 33.3% of the perilesional area, with 12.5% of these showing the same degree of methylation as the cancerous lesion. (Díez-Pérez et al, 2011; Yeh et al, 2002).

This study showed normal basal and parabasal expression in all cases of normal oral mucosa Fig 1-A. All the perilesional cases were positive for P-cad. Multiple layers expression of P-cad was found in 45% of perilesional area of OSCC , as shown in Table 1, Fig 1-B. Pyo et al, 2007 found 53% of P- cad multiple layer expression in the OSCC islands, this evidence support molecular findings shown that a tumor could be surrounded by a mucosal field of genetically altered cells. Furthermore, these cells could share some

or even all genetic markers with the tumor, indicating that both have arisen from a common cell clone (Braakhuis et al, 2002). In addition this over expression of P-cad at perilesional area of oral cancer might give an idea that this marker could play an important role in early stage of carcinogenesis.

7/15 (46.6%) of WDSCC showed multiple layer expression Table 2, Fig1-B. This anomalous or over expression of P-cad in perilesional area could be a biological marker for the initial phase of tumor growth (Lo Muzio et al, 2005). On the other hand, 5/15, 33.3% of WDSCC revealed faint cytoplasmic staining Table -2, Fig 1-C, this finding could be attributed to the size of perilesional area as 4/5 of these cases revealed small perilesional area. Furthermore 3/15, 20% of WDSCC demonstrated anomalous expression of P-cad that was limited to basal layer (cytoplasmic or mixed membranous and cytoplasmic) Table 2, Fig 1-D. As the loss of P-cad expression probably comes after cytoplasmic relocalization and considered as a late event prior to invasion (Williams et al, 1998). Thus this expression might have a role in the mode and path of invasion (whether the cancer cells invasion occur in sheets or strands or single separating cells) and later on prognosis of OSCC.

Finally the single PDSCC case showed membranous expression of P-cad in its perilesional area that is limited to basal layer, Table 2, Fig1-E , although this expression normally detected in normal oral mucosa , this result probably might be due partially to its small perilesional area and the limitation in the collected sample in the present study in general. However, statistical findings did not show any significant difference with histopathological grading as $p > 0.05$.

Conclusion

The over expression of P-cad in perilesional area of OSCC declare the important role of this marker in early step of carcinogenesis and support the field of cancerization. Furthermore, the anomalous or over expression of this marker could have a role in subsequent method of invasion and prognosis of OSCC.

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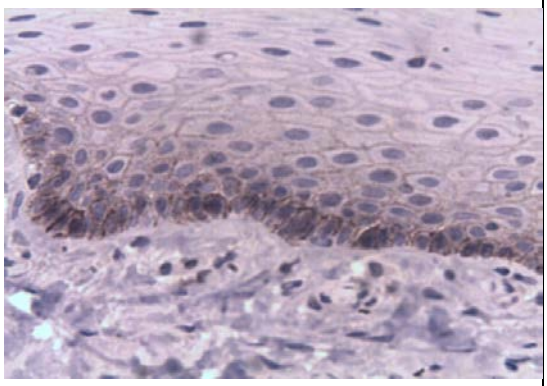
Table -1: P-cad expression in perilesional area of OSCC

Positive cases 20	Staining limited to basal layer		Multiple layers staining		Faint cytoplasmic staining	
	No	%	No	%	No	%
	4	20	9	45	7	35

Table- 2: P-cad expression and localization in relation to histopathological grading at the perilesional area of OSCC*
No significant difference was found as P >0.05

Histo-pathological grading of OSCC	Staining limited to the basal layer (4)						Multiple layers staining (9)						Faint cytoplasmic staining (7)			
	Memb		Cyto		Mix		Memb		Cyto		Mix		Basal		Basal and parabasal	
Positive cases	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Well 15			1	6.6	2	13.3	1	6.6			6	40	3	20	2	13.3
Moderate 4											2	50	2	50		
Poor 1	1	100														

A-



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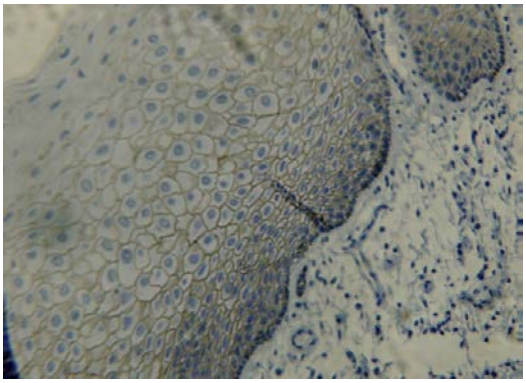

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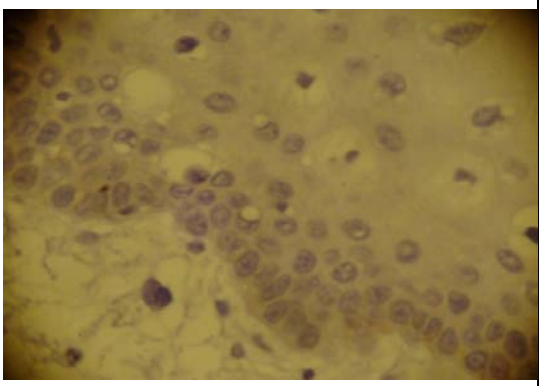
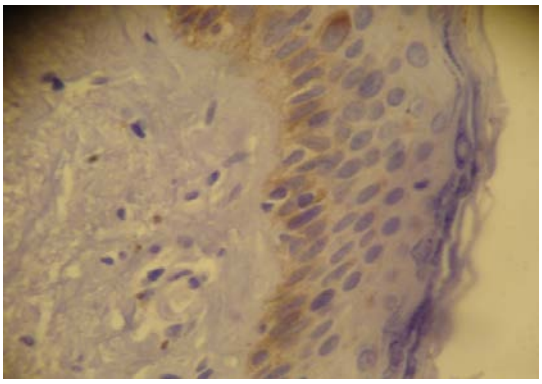


D-



E-

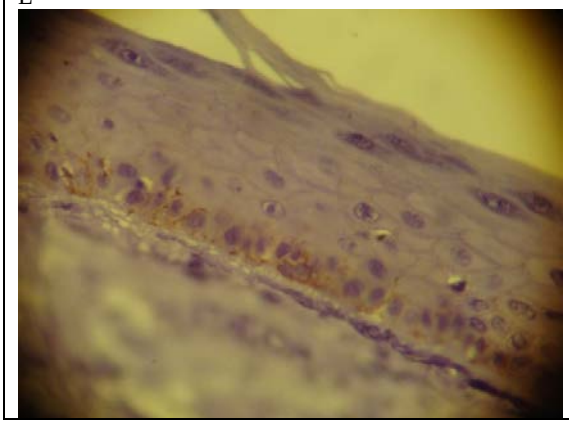


Figure1- P-cad expression in normal oral mucosa and perilesional areas of OSCCC X40 A- Positive expression of P-cad in normal oral mucosa shows membranous basal and parabasal expression B- WDSCC with membranous expression of multiple layers C – WDSCC with faint cytoplasmic expression D-WDSCC with membranous and cytoplasmic expression limited to basal layer X40 E-. PDSCC with membranous expression limited to basal layer