Angiopoietin-2 Immunohistochemical Expression in Oral Squamous cell Carcinoma

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ABSTRACT

Background: There are various secreted proteins affecting the prognosis of oral squamous cell carcinoma (OSCC) and one of them is Angiopoietin-2(Ang-2) which is thought to have an essential role in the development and progression of the tumor.

Aim of the study: This study was conducted to determine the expression of (Ang-2) in (OSCC) to assess its correlations with clinicopathological parameters of the tumor.

Material and Methods: 36 formalin- fixed, paraffin- embedded tissue blocks histologically diagnosed as OSCC were examined for Ang-2 immunohistochemical expression semi quantitively.

Results: The expression of Ang-2 was significantly associated with histopathological grade (P value=0.023), while there is no significant association with the clinical parameters analyzed in OSCC patients.

Conclusion: A significant association between Ang-2 expression and histopathological grade of OSCC may predict its biological behavior.

Key words: OSCC, angiogenesis, Ang-2. **(Received: 15/1/2018; Accepted: 19/2/2018)**

INTRODUCTION

Oral Squamous cell carcinoma (OSCC): is a malignant epithelial tumor of oral cavity that derived from the lining stratified squamous epithelium[1]. Both genetic and environmental factors in addition to the viral infections are incorporated in the pathogenesis of OSCC[2] An important number of patients developed OSCC at early stages, and with small sized tumor, they may develop poor prognosis ,so the level of histopathological differentiation can predict the biological specific and aggressive clinical behavior of the tumor[3;4] so when the tumor histopathologically appeared mature and look like the epithelial tissue which is originated from (quite resemblance to squamous cells, keratin pearls, less cells or nuclear pleomorphism) tend to grow slowly and not metastasized unless in latent stage which is called well differentiated, low grade, or grade I OSCC[5] In contrast, a tumor with marked pleomorphism and little or no keratin production may be so immature so it becomes difficult to identify the tissue of origin. graded as III and called poorly differentiated or high grade OSCC. The tumor appeared in between, graded II or so called moderately differentiated [6]

Angiopoietin-2 (Ang-2), a member of the angiopoietin family proteins functioned as ligands for the endothelial-specific tyrosine kinase receptor "Tie2" [7].In neoplasms of different histological origin (e.g., gastric, colon, prostate, breast, and brain carcinomas),the expression of Ang-2 is elevated and linked with poor prognosis[8].

Studies showed that Ang-2 may overexpressed in OSCC and it always associated with aggressive tumor behavior and poor prognosis [9,7]
The aim of present study is to evaluate the expression of Ang-2 in number of patients diagnosed as OSCC and its association with clinicopathological parameter of the disease.

MATERIAL AND METHOD

Tissue Sample

Thirty six retrospective formalin- fixed, paraffin-embedded tissue blocks diagnosed histopathologically as OSCC were enrolled in this study. The blocks were obtained from the archives of the Oral Maxillofacial Pathology Department / Dentistry Collage of Baghdad University. Demographic and clinical data : patients name, age, gender, clinical presentation, , and tumor site were obtained from the archive. Normal placental tissue of human for Ang-2 antibody immunohistochemical detection obtained from Al-Shaheed Ghazi Hospital, Teaching Laboratory Department/ Baghdad Medical City

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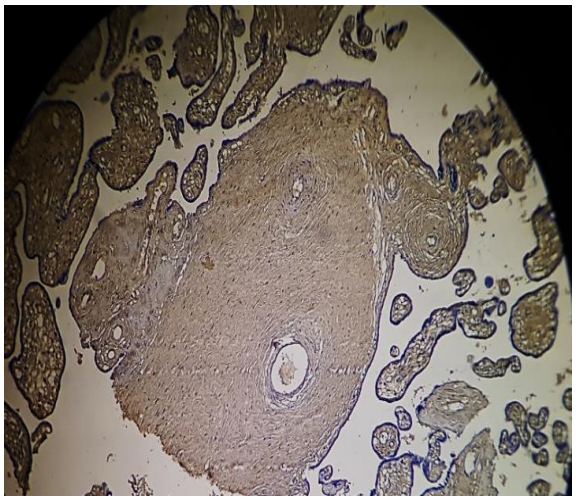

Figure 1: Immunohistochemical expression ofAng-2 in normal human placental tissues(X100).

Conventional Immunohistochemistry (IHC).

After deparaffinization and rehydration were done to the prepared histological sections, blocking of endogenous peroxidase was performed by incubation them with 3 % hydrogen peroxide for 10 min. then blocking for nonspecific antibodies binding by incubation them for 1 hour with normal goat serum. Sections were then incubated with primary antibody, an anti Ang-2 monoclonal antibody (1/100) dilution; (abcam company [MM0020-1F29] ab56301) overnight at 4° C. then washed with phosphate-buffered saline (PBS, pH = 7.0). By using the detection kit, the slides were incubated with secondary antibody (rabbit anti mouse antibody unconjugated then Gout anti –rabbit HRP conjugated) 10min for each followed by incubation with 3،-3،-diaminobenzidine chromogen (DAB)for 1min at 37°C.

All sections then stained with Haematoxylin as a counter stain, dehydrated through graded alcohols, and at last, mounted. For each IHC run a positive control tissue sample (normal placenta) and a negative control sample (consisted of sample in which the primary antibody were replaced by PBS) was included.

Immunohistochemical evaluation of Ang-2 antibody:

To assess the immunohistochemical expression of Ang-2, two pathologist who blinded to the any information presented in the case sheets of the patients two pathologists, who were blinded to the patients' information. Immuno-re activity of Ang-2 was primarily detected in the cytoplasm of tumor cells.

The expression was performed using a semi quantitative staining intensity score: no staining was scored as 0, faint staining as 1, moderate as 2, and high intensity staining as 3.[10]

Statistical analysis

Categorical variables were represented by number(n) and percentage (%) and the different percentages were tested using Chi-square test(x^2). Statistical significance was considered whenever the P value was less than 0.05.

RESULT

This study included thirty six of histopathologically confirmed OSCC cases, males were 20 (55.6%) while female were 16 (44.4%). Patients age ranged between 22-83 years, and the mean age was 52.4 years. The most predominant age group was (70-79) years which account 10 cases (27.78%). Clinically the study sample was presented most predominantly as an Ulcer (21 cases of 58.33%), while mass compromised 15 cases(41.67%). The most predominant affected site with tumor was tongue of 17 cases (47%) followed by floor of mouth account 7 cases (19%). Most predominant histopathological grade was moderately differentiated as 14 cases (38.89%), followed by well differentiated as 13 cases (36.11%), lasted with poorly differentiated 9 cases (25%).

Table (1) showed the association between gender and site, the association was found to be statistically non-significance (P value>0.05).

Table1: Distribution of study sample according to site and gender.

SITE	Gender					
	Male		Female		Total	
	N	%	n	%	n	%
Tongue	9	45.00	8	50.00	17	47.22
Floor of the mouth	2	10.00	5	31.25	7	19.44
Buccal mucosa	2	10.00	2	12.50	4	11.11
Mandible	3	15.00	0	.00	3	8.33
Soft palate	2	10.00	0	.00	2	5.56
Hard palate	1	5.00	1	6.25	2	5.56
Alveolar ridge	1	5.00	0	.00	1	2.78
Total	20	100.00	16	100.00	36	100.00

χ^2= 6.986 d f=6 p= 0.322

Table (2) showed that moderately differentiated OSCC was the most predominant histopathological grade account 14 cases.

Table 2: The distribution of study sample according to the histopathological grade of the Tumor.

Grade	n	%
well differentiated	13	36.11
Moderate differentiated	14	38.89
Poor differentiated	9	25.00
Total	36	100.00

Table (3) showed the distribution of study sample according to Ang-2. Half of cases with moderate staining, those with faint and strong staining represented (8,9 cases respectively), and one case only with no staining.

Table 3: SIS of Ang-2 antibody immunohistochemical expression in the study sample

	Score	n	%
SIS (Ang-2)	0	1	2.78
	1	8	22.22
	2	18	50
	3	9	25
	Total	36	100

Ang-2: Angiopoietin-2, SIS: staining intensity score. 0: No staining.1: faint staining.2: Moderate staining. 3: Strong staining.

Table(4,5, 6, 7) the association between Ang-2 expression with (age group, gender, clinical presentation, sites) were found to be statistically non significance (P value>0.05).

Table 4: Association between Ang-2 expression and Age group in the study sample

Age groups	Ang-2									
	No staining		Faint		Moderate		Strong		Total	
	N	%	N	%	N	%	N	%	N	%
<0r=39	0	.0	1	12.5	6	33.3	0	.00	7	19.4
40-49	0	.0	1	12.5	3	16.7	1	11.12	5	13.9
50-59	1	100	2	25.	0	.00	2	22.22	5	13.9
60-69	0	.0	2	25.	4	22.2	3	33.33	9	25.0
>0r=70	0	.0	2	25.	5	27.8	3	33.33	10	27.8
Total	1	100	8	100	18	100	9	100	36	100

$x^{2=}$**14.184 d f =12 P=0.289**

Table 5: Association between Ang-2 expression and Gender of the study group

Gender	Ang-2									
	No staining		Faint		Moderate		Strong		Total	
	N	%	N	%	N	%	N	%	N	%
Male	1	100.	5	62.5	11	61.11	3	33.33	20	55.56
Female	0	.00	3	37.5	7	38.89	6	66.67	16	44.44
Total	1	100.	8	100.	18	100.	9	100.	36	100.

$x^{2=}$ **2.981 df-3 P=0.395**

Table 6: The association between Ang-2 expression and Clinical presentation of OSCC.

Clinical Presentation	Ang-2									
	No staining		Faint		Moderate		Strong		Total	
	N	%	N	%	N	%	N	%	N	%
Ulcer	1	100.	6	75.	8	44.4	6	66.7	21	58.3
Mass	0	.00	2	25.	10	55.6	3	33.3	15	41.7
Total	1	100.	8	100.	18	100.	9	100.	36	100.

x^{2} **=3.314 d f=3 P=0.346**

Table 7: Association of Ang-2 expression and Site of distribution of study sample.

SITE	Ang-2									
	No staining		Faint		Moderate		Strong		Total	
	N	%	N	%	N	%	N	%	N	%
Tongue	0	.00	5	62.5	8	44.4	4	44.5	17	47.2
Floor of mouth	0	.00	0	.00	6	33.2	1	11.1	7	19.4
Buccal mucosa	1	100	0	.00	1	5.6	2	22.2	4	11.1
mandible	0	.00	2	25.	0	.00	1	11.1	3	8.3
Soft palate	0	.00	1	12.5	1	5.6	0	.00	2	5.6
hard palate	0	.00	0	.00	1	5.6	1	11.1	2	5.6
Alveolar ridge	0	.00	0	.00	1	5.6	0	.00	1	2.8
Total	1	100	8	100	18	100	9	100.	36	100

x^{2}**=21.852 d f=18 P=0.239**

Table (8) showed the association between Ang-2 expression and histopathological grade of OSCC of the study sample, the association was found to be statistically significant(P value=0.023).

Table 8: The association between Ang-2 expression and the grade of the study sample.

Grade	Ang-2									
	No Stain		Faint		Moderate		Strong		Total	
	N	%	N	%	N	%	N	%	N	%
W.D	0	.00	7	87.5	4	22.2	2	22.2	13	36.1
M.D	0	.00	1	12.5	9	50.	4	44.4	14	38.9
P.D	1	100	0	.00	5	27.8	3	33.3	9	25.
Total	1	100	8	100.	18	100.	9	100.	36	100.

X^2=14.674 d f=6 P=0.023*

Ang-2: Angiopoietin-2. W.D: Well Differentiated. M.D: Moderately Differentiated. P.D: Poorly Differentiated. *: P Value<0.05.

Figure 1: No staining (Score0 in SIS) of Ang-2 expression of the studied samples. (X100).

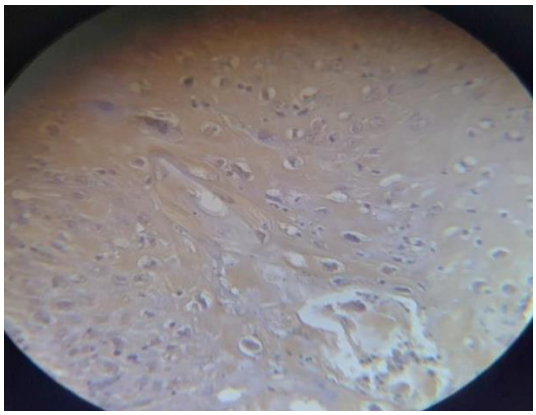

Figure 2: Faint staining (score1 in SIS) of Ang-2 expression in the studied samples (X100).

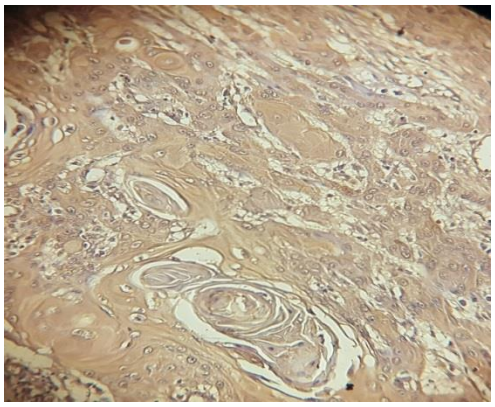

Figure3: Moderate staining (score2 at SIS) of Ang-2 expression in studied samples (X100).

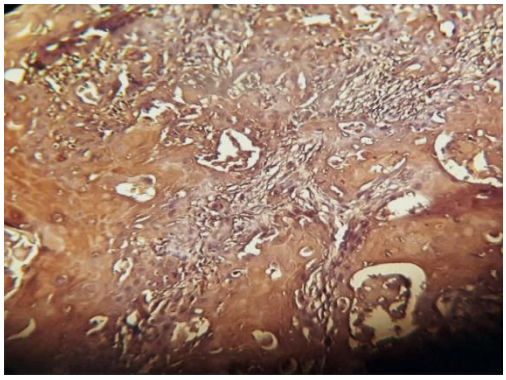

Figure4: Strong staining(Score3 in SIS) of Ang-2 expression of the studied samples (X100).

DISCUSSION

There are numerous molecules overexpressed in OSCC, associated with aggressive behavior of the tumor and may affect its prognosis.

According to the current study; Ang-2 was expressed in 75% of the cases (ranged between moderately stained as 50%, to strong stained as 25% of the cases). Although the association between the Ang-2 expression and clinicopathological parameters of the studied tumor was found to be statically non significance(P value>0.05).

There was significance association between Ang-2 expression and the histological grade of the tumor (P value=0.023) which agreed with previous studies [11,7] but disagreed with [9,12,13] While tumor progressed, there is a great need for oxygen and the microenvironment suffered from hypoxic condition that s stimulate ECs, tumors cells to secret different cytokines which enhanced angiogenesis, and one of them is Ang-2[14,15] Overexpression of Ang-2 could lead to decrease cell apoptosis so promotes tumorgenesis, on the other hand, Ang-2 thought to have an essential role in the angiogenesis process by enhanced epithelial-mesenchymal transition "[16, 17, 18]

Previous studies showed that Ang-2 induces transformation of noncancerous liver to hepatocellular carcinoma [19]. In addition, that Ang-2 mostly associated with disease progression, metastasis, and poor prognosis[20,8] Recent studies on Ang-2-VEGF-A CrossMab showed The efficient and less harm effects on animals proposed that it characterized a new and active therapeutic chance for patients with malignancy with the probability to replace Bevacizumab as a

CONCLUSION

Ang-2 was overexpressed in OSCC and significantly associated with histopathological grade of the tumor.

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الخلاصة

الظهور الكنمائى النسجى المناعى لمعلم Angiopoietin-2 و تقدم علاقته بالنواحى السريرية والمرضية لسرطان الفم الحرشفى

الخلفية:هناك الكثر من البروبنات التي تفرز والتي لها تاثيرفي توقع نتائج مرض سرطان الفم الحرشفي واحد هؤلاء هو المعلم(Ang-2)Angiopoietin-2 ويعتقد ان له دور اساسي في نشوء وتفاقم هزا النوع من السرطان.

اهداف الدراسة: ان هذه الدراسة تشير الى تحديد الظهور المناعي لمعلمAng-2 في سرطان الفم الحرشفي وتقييم ارتباطه بالنواحي السريرية والمرضية والتمايز النسيجي لهذا النوع من الورم.

الطرق و الادوات:تضمنت الدراسة ست وثلاثون عينة مسترجعة استخرجت من المقاطع النسيجية المطمورة بشمع البرافين ثم اجريت عليها الفحوصات النسيجية والمناعية النسيجية الكيميائية لمعلمAng-2 وقرات النتائج باستخدام طريقة حساب شدة التصبيغ شبه الكمية.

النتائج: بينت النتائج ظهور المعلم المناعيAng-2وارتباطه مع الدرجة النسيجية المرضية والعلاقة ذو دلالة معنوية (P value=0.023)

كما اوضحت هذه الدراسة عدم وجود دلالة معنوية مع النواحي السريرية والمرضية لسرطان الفم الحرشفي.

الاستنتاجات: كشفت هده الدراسة مستويات مرتفعة من التعبير المناعي لمعلمAng-2 وهذا يشير لاهميته في تقييم السلوك البيولوجي وتخمين نتائج المرض.