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The evaluation of correlation between Angiogenesis and invasion of Basal cell carcinoma

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ABSTRACT

Basal cell carcinoma (BCC) is the most common skin tumor. Although, it is recognized as a tumor with non-aggressive nature but cases with aggressive behaviour have been reported. None of the microscopic morphologic features can predict the aggressive behaviour of this tumor. Since angiogenesis as a known biological factor plays an important role in tumor growth and development, the present study was conducted to determine the role of angiogenesis in aggressiveness of basal cell carcinoma. Based on microscopic features, 96 H&E stained specimens of BCC were classified in 2 aggressive and non-aggressive groups. Then the microvascular density in both groups was determined by IHC staining technique using CD31 monoclonal antibody. Out of 96 examined samples, 63 were invasive and 33 were non-invasive samples. The mean vascular density in the invasive group was 37.5 and in the non-invasive group was 18.63 and there was a significant difference between the two groups (P <0.001). According to the ROC analysis, the sensitivity for vascular density was 92.1%, the specificity was 87.9%, positive predictive value 93.5% and negative predictive value was 85.3%. In tumors with more microscopic morphologic characteristics of aggressiveness, the microvascular density was higher. The microvascular density is higher in more morphologically aggressive tumors. Although none of the morphologic features can explain the aggressive behavior of the tumor alone, the number of microscopic characteristics of invasion and angiogenesis are important in this behavior. Therefore, skin tumors of this type should be investigated for vascular densities to better follow up of the patient

Keywords: Angiogenesis, Basal Cell Carcinoma, Tumor Invasion

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INTRODUCTION

Basal cell carcinoma is the most common skin cancer that its prevalence increases with age and exposure to sunlight (ultraviolet radiation). This tumor has a slow growth rate and rarely causes metastases and death; however local invasions and frequent recurrence are associated with increased morbidity in patients [1-5]. In terms of clinicopathology, there are two non invasive and invasive classic forms of this tumor [1]. In previous studies, the association of the type of

tumor and its histology with features such as the depth of the lesion and its location with the duration of the disease and its clinical behavior are investigated [6]. Reduced peripheral palisading and fibrotic stroma were associated with more invasive forms of the tumor, and a reduction in the incidence of syndecan 1 and Bcl2 and an increase in the incidence of P53 and AgNOR and the higher incidence of anuploidosis was demonstrated [3].

The role of angiogenesis as a biological agent involved in tumor invasion and its development is known [7-12]. This criteria was used to predict the invasive behavior of breast [8], ovaries [13], prostate [14], anal channel [15], larynx tumors [16], malignant melanoma [17], SCC of head and neck [18], stomach [19, 20] and lung tumors [21, 22, 23].

In some previous studies, such as Folkman [24, 25], Newell [26], Weninger [27], Loggini [28], Aslani [29], Yerebakan [6], Chin [7], Rathi [30], Winter [31], there is a significant relationship between vascular density and tumor invasion. Therefore, given the vague role of angiogenesis in basal cell carcinoma invasion and contradiction in previous studies, this study was conducted to investigate the association between microscopic invasion characteristics and angiogenesis in basal cell carcinoma.

MATERIALS AND METHODS

This is a cross-sectional study which was conducted on paraffin blocks with BCC diagnosis stored in the pathology department of Shahid Beheshti Hospital in Kashan during the years 1389-1392. At first, 120 samples with BCC diagnosis were selected by referring to the pathology department of Shahid Beheshti Hospital in Kashan. Then, the respective slides which were stained with hematoxylin-eosin in the archives section were reviewed by two pathologists and subsequently divided into two invasive and non-invasive groups based on histological criteria. Morphological criteria for the detection of invasive (BCC1) form noninvasive (BCC2) included nuclear pleomorphism, high mitotic index, lack of peripheral palisading pattern, stromal hyalinization and thin plate of neoplastic cells with invasive pattern. For a sample to be considered as an invasive, it should show more than two of the above criteria in at least 70% of the checked slides. In contrast, a non-invasive type, including cellular islets with sharp margin or organoid groups of basaloid cells with pale ovoid nucleus that show tipical peripheral palisading pattern and fibrosis in the stroma (Fig 1-4).

A 3 micron slice was prepared from each block for immunohistochemical staining with anti-CD31 antibody. The plates were placed on poly lysine slides and placed in an autoclave for 60 minutes and then kept at 37 degrees for one night. Then the slides were placed in xylol, 100 degrees alcohol and 96 degrees alcohol for 10 minutes sequentionally, to be dehydrated and deparafinated. It was then placed in 30% oxygenated water with methanol at ratio 9 to 1 for 15 minutes. Next, the slides were washed with distilled water for Antigen Retrieval process and then kept in citrate buffer at PH = 6 for 15 minutes. In the next step, in order to inhibit endogenous peroxidase and eliminate non-specific reactions the slides were placed in Protein blocking solution for 10 minutes followed by incubation with CD 31 ready to use antibody for one hour. The slides were then washed with a Tris buffer for 10 minutes. It was then incubated in EvisionTM + of Mouse / Rabbit as detection system for 30 minutes. The slides were then rinsed again with a Tris buffer for 10 minutes and at a later stage, DAB + solution was used as chromogen and the slides were incubated for 10 minutes in this chromogen. The slides were then washed again with fresh water to remove any unwanted material. For the background color, the slides were placed in Eosin for 10 minutes. At this stage, 96 and 100 degrees alcohol and xylol were used for hydration step. In the next step, lamel was pasted on it and tagged.

The resulting cross sections were initially examined with 40x magnification (lens number 4) for determining the hotspot vascular density points (Fig 5,6).

Non-quality slides in terms of staining and those which had granulation tissue or wound were excluded from the study. Finally, 96 good quality slides were evaluated, so that from each sample, 3 points with the highest vascular density were selected and examined by two pathologists with a magnification of 400 (High power field) (hpf).Fig7,8. The average number of vessels

counted in these 3 points was considered as microvascular density (MVD) of that sample. The mean vascular density was classified according to the number of counted vessels as follows:

1 Positive: The number of vessels count is <20 per hpf
2 Positive: The number of vessels count is 20 to 29 per hpf
3 Positive: The number of vessels count is 30-39 per hpf
4 Positive: The number of vessels count is 40 and > 40 per hpf
Finally, the data were entered into SPSS software and analyzed by Chi-square and T-test with significance level of 0.05.

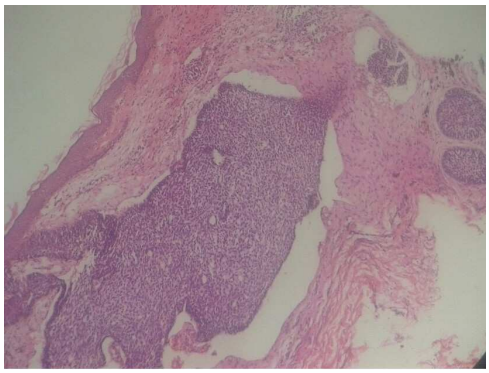

Fig1:BCC2,h&E stain,40x

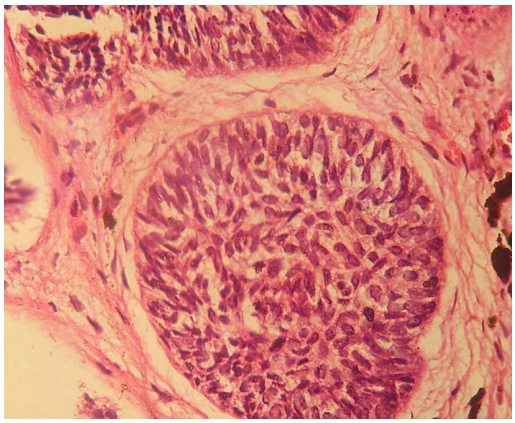

Fig2:BCC2,h&E stain,400x

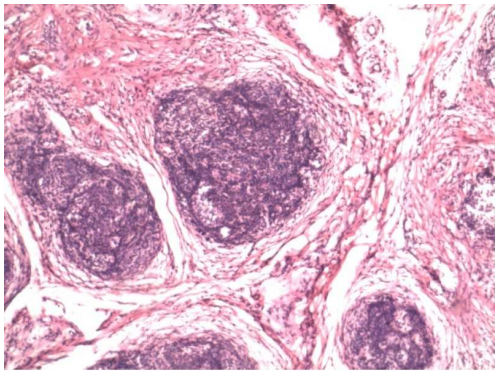

Fig3:BCC1,h&E stain,40x

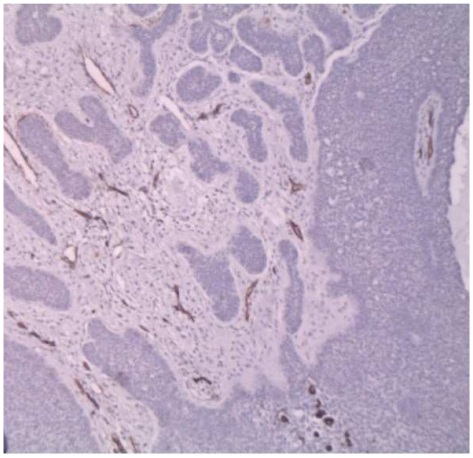

Fig4:BCC1,h&E stain,400x

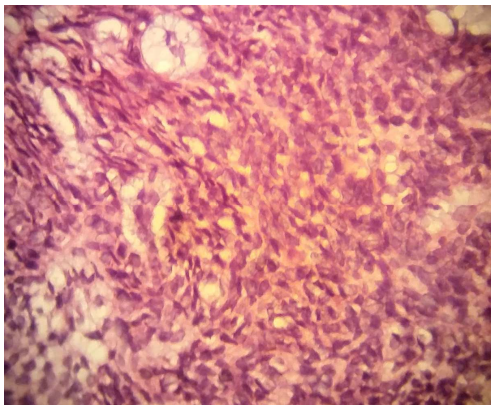

Fig5:BCC2,IHC stain,40X

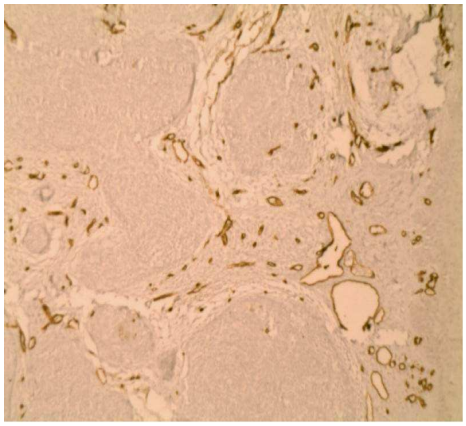

Fig6:BCC1,IHC stain,40X

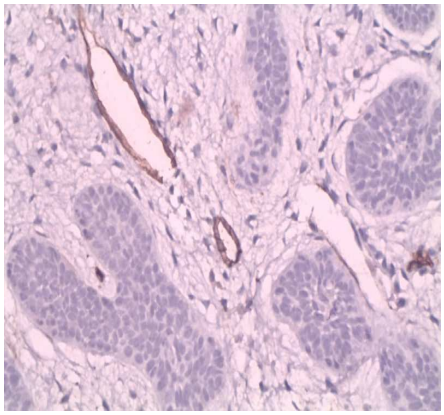

Fig7:BCC2,IHC stain,400X

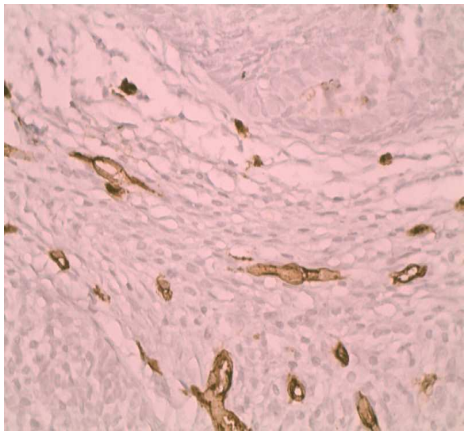

Fig8:BCC1,IHC stain,400x

RESULTS

Of the 96 examined samples, 33 had non-invasive patterns and 63 had invasive patterns. The vascular density has a normal distribution statistically and the mean of MVD was 31.02. The mean of MVD in BCC1 group was 37.50 and in BCC2 group were 18.63.

Out of 33 samples with non invasive pattern, 23 cases (69.69%) had a MVD of 1 positive, 7 cases (21.21%) had a MVD of 2 positive and 3 cases (9.09%) had a MVD of 3 positive. Of 63 samples with invasive pattern, 2 cases (3.7%) had a MVD of 1 positive, 13 cases (20.63%) had a MVD of 2 positive, 25 cases (39.6%) had a MVD of 3 positive and 23 cases (36.5%) had a MVD of 4 positive. Independent t-test showed that the mean microvascular density in invasive groups was significantly higher than non-invasive ones ($P < 0.001$).

So, probably, microvascular density can be a good predictor for the invasive nature of BCC specimens. Therefore, the ROC curve was used to estimate its predictive value. Given that the area under the curve was 95%, it can be concluded that microvascular density could be a good predictor. Using the ROC curve (diagram 1), the cut of point of the microvascular density was obtained 25, with a sensitivity of 92.1%, specificity of 87.9%, positive predictive value of 93.5%, and a negative predictive value of 85.3%.

As shown in the Table 1, the lack of peripheral palisading, irregular cellular plates and stromal hyalinisation are the most important invasive criterias and MVD of more than 25, respectively. Based on the findings in Table 2, with an increase in the number of invasive criterias reported in BCC tumors, MVD also increases. $P < 0.001$ (Fisher's Exact Test).

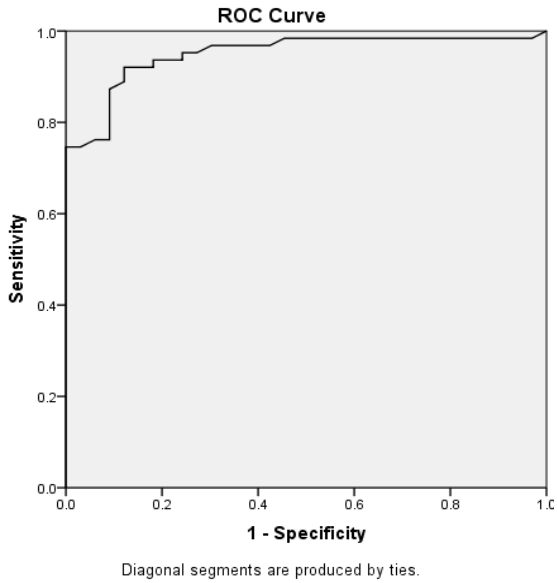

Diagram 1: ROC curve to predict invasive pattern using microscope density

Table 1: Comparative Study to determine the Importance of different histological invasion criterias in Invasive pattern and Microvascular Density

Invasion criteria	Number of positive cases	The number of positive cases in BCC1 group	The percentage of positive cases in BCC1 group	The number of positive cases has MVD> 25	The number of positive cases has MVD> 25
Lack of peripheral palisading	44	44	100%	41	93.2%
Irregular cellular plate patterns	53	51	96.2%	48	90.6%
Stromal hyalinisation	21	20	95.2%	19	90.5%
Nuclear pleomorphism	28	25	89.3%	24	85.7%
High mitotic index	16	15	93.8%	13	81.2%

DISCUSSION

BCC is categorized into invasive (BCC1) and non-invasive (BCC2) groups, based on clinicopathologic criteria. Among the microscopic morphological factors that play an important role in this classification are invasive criterias and the most significant of them are peripheral palisading of tumor cell islands, stromal hyalinisation changes, nuclear pleomorphism, high mitotic index, and irregular cellular plate patterns.

Angiogenesis is one of the biological indicators that determines the prognosis of various tumoral lesions [7-12]. In recent years, due to the unknown nature of invasive BCC, several studies have examined the association between the MVD and invasive pattern of the tumor. Yet, to determine the MVD, Several endothelial cell index have been used in immunohistochemical staining methods,such as platelet factor VIII in the study by Sari Aslani [29], CD34 and VEGF in the study by Loggini [28]. The specificity and sensitivity of these indices are less than CD31 [32].

Yerebakan, using CD31 and Ki67, examined the relationship between vascular density of recurrent and non-recurrent lesions with mitosis [6]. Chin, using CD31, compared vascular density in the trunk and the stroma of BCC, Scc, and TE [7]. Abbas Rathi examined the vascular density of Scc and BCC using this index [30].

In the present study, the relationship between invasive morphological criterias and vascular density was investigated. Of the 96 BCC skin samples studied, 33 had noninvasive patterns and 63 had invasive patterns. This result is similar to the result of the Yerebakan study [6]. Therefore invasive lesions are more common than non-invasive lesions, which itself adds importance to the attention of patients with BCC. Other studies have not indicated the prevalence of invasive types of this tumor [7-28, 31].

The mean of microvascular density in the non-invasive group was lower than the invasive group [18.63 vs 37.3] (Pvalue <0.001). This result is similar to the results of study by Sari Aslani [29], Loggini [28], Yerebakan [6], Chin [7], Rathi [30] and Winter [31] and due to the role of angiogenesis in the growth and expansion of tumors [1, 7, 24-28], it is expected.

The most similar study to the present study is the study by Sari Aslani et al., which investigated the vascular density in two invasive and non-invasive BCC groups, using platelet factor VIII. He investigated the type of histology, size and location of the tumor, age, sex, rate of recurrence and being multi-focal lesions;he did not find any significant relationship between age, sex, and location with microvascular density index [29]. Because the specificity and sensitivity of platelet factor VIII in detecting endothelial cells is lower than CD31 [32]; and according to the suggestion of Sari Aslani, the present study completes his study, using the CD31 index.

Results showed that 69.69% of non-invasive lesions had MVD 1 positive. In invasive lesions, 1 positive MVD was observed in only 2 samples (3. 17%) and most of the invasive lesions had the vascular density 3 positive (39.70%) and 4 positive (36.6%).

This review method has not been carried out in analyzing the results of other studies so far [6-28, 7-31]. Based on the results of this study, the higher number of positive invasive criterias in a lesion, the higher microvascular density is in that,this relationship is significant based on fisher's exact test analysis (P <0.001). Other microscopic invasive indicators investigated in this study have not been survied in any of the previous studies [28, 7-31].

Investigation of invasive microscopic criterias and their association with vascular density was rarely seen in previous studies. Yerebakan, using the CD31 and Ki67 index, simultaneously evaluated the vascular density and mitosis in recurrent and non-recurrent BCC lesions [6]. In his study, the increase in mitosis has a significant relation with the mean vascular density of the tumor in recurrent tumors and corresponds to the results of our study [6].

Other microscopic invasive criterias investigated in our study have not been studied in any of the previous studies yet [28, 7-31]. Also, in none of the previous studies, the Cut of point of microvascular density was not been determined, and the comparison of the importance of morphological criterias in angiogenesis has not been investigated.

So the determination of the relationship between these morphological indices and the biological index of angiogenesis is significant in this study.

Also, based on the findings of this study, it can be concluded that the higher the number of invasive microscopic criterias, especially the lack of peripheral palisading and irregular cell plates and stromal hyalinisation in the sample, is associated with a higher vascular density and aggressive behavior and this should be taken into consideration in follow up of the patients.

It is worth noting that, despite the high sensitivity and specificity of the CD31 index in determining vascular density, due to the fact that this index is positive in tumor stromal cells, including neutrophils, monocytes, platelets and histocytes, it is possible that small vessels with these cells will be confused [32]. To reduce this error, the specimens were examined by two pathologists, and only those with a clear lumen and a typical vascular building were counted as vessels, and counting of suspected and bad-colored instances was avoided.

Table 2: Comparison of the number of positive invasive criterias in different BCC groups based on vascular density

			Microscopic Parameter						
			0	1	2	3	4	5	Total
MVD Group	<20	Count	19	4	1	1	0	0	25
		% within MVDGroup	76.0%	16.0%	4.0%	4.0%	.0%	.0%	100.0%
	20-29	Count	5	2	9	3	1	0	20
		% within MVDGroup	25.0%	10.0%	45.0%	15.0%	5.0%	.0%	100.0%
	30-39	Count	2	1	15	9	0	1	28
		% within MVDGroup	7.1%	3.6%	53.6%	32.1%	.0%	3.6%	100.0%
	40 & more	Count	0	0	15	5	3	0	23
		% within MVDGroup	.0%	.0%	65.2%	21.7%	13.0%	.0%	100.0%
Total		Count	26	7	40	18	4	1	96
		% within MVDGroup	27.1%	7.3%	41.7%	18.8%	4.2%	1.0%	100.0%

P<0.001(Fisher's Exact Test)

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