RESEARCH ARTICLE

Angiogenesis and Lymphangiogenesis in Oral Squamous Cell Carcinoma: Comparison of Japanese and Indian Cases

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Abstract

A comparative study between 17 Japanese and 19 Indian patients with oral squamous cell carcinomas (OSCCs) revealed that the tumour prognostic indicator mean vessel density (MVD) count for angiogenesis was relatively high at 57.1 in Indian as compared to 39.3 in Japanese (P=0.001) cases, whereas the lymph-vessel density (LVD) count for lymphangiogenesis was lower (12.8 vs 48.0, P=0.002). Both male and female Indians had higher MVD counts, but LVD counts were only slightly lower in females. MVD count was relatively high among the cases below 65 years old in both the countries (P=0.4). Japanese cases with Tongue cancer had higher MVD count, but the Indian cases had lower LVD counts. Size-wise, T2 and T3 had higher counts of MVD both in Indian and Japanese cases. MVD and LVD count was higher in grades II and III both in Japanese and Indian cases. There was insignificant difference of the MVD counts among smokers, but the tobacco chewers in Indian cases had higher counts of MVD and LVD (P value by Bartlett test 0.35, 0.57 respectively). The hot-spots of tumour sites had variable rates of lymphocyte infiltration showed higher MVD counts in all the cases. Although the clinical characteristics and demographic variables usually relate to MVD and LVD counts, the tendency of higher values, especially among tobacco chewers, identified as the highest risk group for occurrence of oral cancer needs to be investigated further.

Keywords: Oral squamous cell carcinoma - angiogenesis (MVD) - lymphangiogesis (LVD) - Japanese and Indian cases

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Introduction

Several studies showed that microvessel density could be a valid independent prognostic factor of tumour progression (Harrison, 1999; Carmeliet, 2000; Hasina, 2001). It has also been reported that there is an interrelation between higher microvessel density (MVD) and increased rate of tumour progression in oral squamous cell carcinoma (Folkman, 1990). Squamous cell carcinoma of oral cavity is identified as one of the ten most frequent tumours, and when larynx and pharynx are included it ranks 5th commonest malignancy, which generates nearly 37,200 new cases annually in the world (Weidner, 1998; Ahmedin et al., 2011). When we compare, we find that the incidence rate of OSSC is significantly low ie., 3-6% in Japan and most of the western countries comparing to India and South east Asian countries, ranges between 15% and 30% (Ascani et al., 2005). Oral Squamous cell carcinoma is strongly associated with Paan chewing, a long traditional habits in Southeast Asian population and consumption of Gutkha (a commercial brand of quid sachets), which is a popular personal habit especially among young age-group population of India (Keskar et al., 1998; Saman et al., 2002; Tanaka, 2005; Weitkunat et al., 2007). Until date, it has been remained unsatisfactory to find a suitable measure to prevent and control of oral cancer, despite several diagnostic and therapeutic applications are available. The prognosis is dependent on biological behaviour of tumours ie, its aggressiveness, which is assessed in terms of clinico-pathological characteristics, such as- TNM staging and histological grading (Gleich et al., 1996: 1997; Penfold et al., 1996; Folkman et al., 1998; Tae et al., 2000; Chitta, 2010). However, when a tumor grows, it demands more blood supply in order to provide oxygen and nutrients to rapidly growing uncontrolled cells. Therefore, new vessels are formed in a tumor mass, which is known as angiogenesis and lymphongenosis (Hasina et al., 2001).

Therefore, a rich vascular supply is required to keep growing of cancer tissues, and that helps develop metastasis. Hence, determination of level of angiogenesis and lympangiogenesis in terms of vessel density ie microvessel density-MVD and lymphovascular density -LVD is one of the prognostic indicators for a malignant growth (Gleich et al., 1996). It has been reported that MVD, and lympho vascular density (LVD), may be considered as an independent prognostic indicator in order to understand the rate of growth of a tumor, its size, and metastasis (Gleich et al., 1996; Penfold et al., 1996). However, there is no direct method of detection of angiogenesis and lymphangiogenesis, but an indirect method ie., counting of MVD and LVD by immunohistochemistry is available,

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which has been introduced by Weidner et al (Leedy et al., 1994).

Although MVD and LVD count is strongly interrelated with clinic-pathological status of malignant condition, the values may vary based on demographic pattern and life style factors, such as- personal habit (s) etc. of a patient/ patient group. In this context, oral cancer cases of Japan and India were studied to compare the counts for MVD and LVD, because, the Indian patients had chewing tobacco habit, on the other hand, Japanese patients had no such habit, but alcohol drinking and smoking were recorded in them – and those factors may have different influence on tumour progression. In India, a study was carried out to investigate MVD and Mast Cell Dentisty (MCD) in OSCC to compare the normal tissues (Bhushan, 2010). No study has been undertaken to investigate angiogenesis and Lymphangiogesis among quid-chewing associated oral cancer, neither comparison was reported among the cases that do not have chewing quid habit, such as, Japanese oral cancer patients. Therefore, this study aimed to elucidate any correlation between immunohistochemically stained vessel ie., MVD and LVD and the demographic variables including clinic-pathological parameters (ie., age, sex, site and size of the tumor, TNM staging and histological grading). This study was carried out in two groups of oral cancer patients ie, the Indian patients associated with chewing tobacco habit, and Japanese patients who were not associated with such habit.

Materials and Methods

Biopsy proven 17 Japanese cases (average age 64.5 year) of oral squamous cell carcinomas (OSCCs), and those of 19 Indian cases (average age 55.1 years) were included in this study, matched with age (combined average age 59.5 years) and sex. The micro-vessel density (MVD) and lympho-vessel density (LVD) were measured on anti-

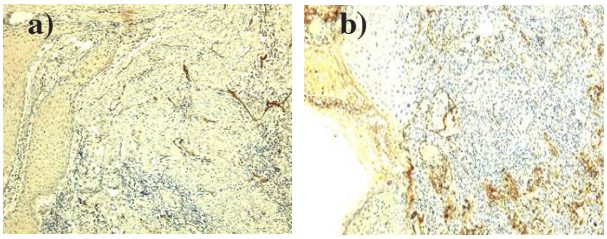

Figure 1. Relation of MVD and LVD. a) Tumour vasculature (angiogenesis) was examined by Factor-8. b) lymph vessels (lymphangiogenesis) were examined by D2-40 immunostaining

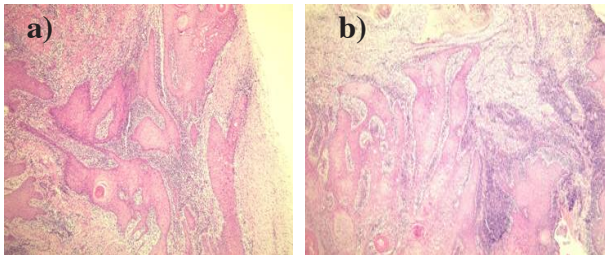

Figure 2. Types of Infiltration. a) C-type infiltration of lymphocytes in peri-tumoral and intra-tumoral zones. b) Yamamoto-Kohama-3 (YK-3) stromal invasion with irregular and scattered cell nests

Table 1. Average MVD and LVD Counts in Japanese and Indian Oral Squamous Cell Carcinoma Cases

Cases	Angiogenesis (MVD)	Lymphangiogenesis (LVD)
Japanese(n=17)	39.3*	48.0**
Indian (n= 19)	57.1	12.8

*P= 0.001, ** P=0.002 by unpaired Student's t-test

Factor-8 antibody and Anti-D2-40 (anti-factor VIII related von Willebrand factor antibody (BioGenex, San Ramon, CA) immuno-positive specimens, respectively. In order to quantify MVD and LVD, 5 maximum vessel density fields were selected from around the tumour cells (the 'hot-spot') and examined under 200-fold magnification and averaged (Figure 1a, 1b). The level of lymphocytes at peri-tumoral and intra-tumoural zone were also examined by estimating the pattern of lymphocyte infiltration, such as- scattered, moderate and marked under 200-fold magnification and scored (Figure 2). The pattern of stromal invasion was estimated in and around cancer-nest areas and assessed according to the grading of the Yamamoto-Kohama (YK) classification (Oda et al., 2004) (Figure 2b).

Statistical significance was calculated by using unpaired Student's t-test and the Bartlett test.

Results

Angiogenesis (MVD count) was significantly lower in Japanese cases compared to Indian cases (P= 0.002),

Table 2. Relationship of Factors with MVD or LVD

Variable	Japanese			Indian		
	N	MVD	LVD	N	MVD	LVD
Gender:						
Male	14	42.1	41.0	12	54.8	11.7
Female	3	26.3	80.8	7	61.2	14.6
Age:						
<65	9	46.6	55.2	14	57.3	13.4
>65	8	32.1	41.2	5	60.3	11.2
Clinicopathological Site:						
Tongue	12	40.7	39.3	7	55.2	2.7
Others	5	36.2	64.0	12	58.3	12.8
T grade (Size of the tumour):						
I	2	32.2	44.8	6	52.9	13.9
II, III	15	40.3	50.9	13	59.1[g]	12.3[h]
Histological Grade:						
I	10	39.6	38.9	12	54.4	11.2
II, III	7	39.0	61.1	7	61.9	15.5
Smoking habit:						
No	10	53.9	52.7	11	57.0	13.2
Yes	7	39.6	55.5	8	57.8	11.3
Chewing-tobacco habit:						
No	0	0	0	6	53.1	14.4
Yes	0	0	0	7*	62.8	11.4
				6**	55.8	12.9
Alcohol drinking habit:						
No	7	37.3	63.5	17	58.1	12.5
Yes	10	43.6	37.2	2	48.2	15.2
Lymphocyte infiltration:						
A (Scattered)	3	44.3	38.5	7	61.6	18.0
B (Moderate)	4	40.8	28.4	8	52.4	9.3
C (Marked)	10	37.3	58.8	4	58.9	10.7

*<15 yrs; **>15 yrs;

but the Lymphangiogenesis (LVD count) was higher in Japanese cases (P= 0.001) (Table 1).

Relationship between angiogenesis or lymphangiogenesis and clinicopathological characteristics was summarized (Table 2). LVD counts in Japanese female cases tend to be higher compared to Japanese males in general and Indian females in particular (Table 2). On the contrary, the Japanese males tend to have higher MVD, and the count is higher in Indian OSCC cases, who had grade-I, but the LVD count is also higher in Grade II and III in Japanese cases (Table 2). The Indian cases had fewer number of LVD counts irrespective of their primary site of occurrence of OSCC. On the other-hand, the MVD count in the Indian cases tends to be higher in other sites except tongue, where the Indian cases had an MVD count of 56.3, compared to 36.2 in Japanese patients (Table 2).

Personal habits (tobacco smoking, chewing and alcohol drinking) and Angiogenesis is tended to be higher among Indian patients, most of them had chewing tobacco habit, compared to that in Japanese cases (Table 2), who did not have such chewing habit. The lymphocyte infiltration is also tends to be higher in Indian OSCC cases, where the MVD count is 58.9 in Indian cases compared to 37.3 in the Japanese cases (Table 2). However statistically, although there was an overall difference of values, but the level of significance was not strong between angiogenesis or lymphangiogenesis and the clinicopathological parameters recorded, including chewing tobacco habit, which had relatively higher counts of MVD and LVD (P value by Bartlett test 0.35, 0.57 respectively).

Discussion

Table 1 shows that the expression of MVD for angiogenesis is significantly lower in Japanese cases compared to Indian cases, but the expression of LVD for lymphangiogenesis is higher in Japanese cases (P=0.001). Japanese female cases had higher expression of LVD compared to Japanese males in general, and Indian females in particular. As we aimed to investigate a correlation or difference of the parameters among chewing associated OCCC (Indian patients) and that among non-chewers (Japanese patients), we have seen that although the MVD count tend to be higher among Indian cases, but LVD is significantly low. The reason we do not know. Other studies showed that, when a malignant growth shows higher rate of angiogenesis, the rate of Lymphangengesis is also increased simultaneously (Tae et al., 2000). but in this study we find only higher MVD (for angiogenesis) among Indian cases and low LVD, which is different from Japanese cases.

The difference between MVD and LVD in male and female neither reported nor interpreted elsewhere, at the same time, any racial difference. If we look through the gender, there was a difference of counts; Japanese female cases had higher expression of LVD compared to Japanese males in general. But, other studies in Japan showed that there is no significant difference of the counts by gender. In this study why Japanese male cases had higher counts of MVD and LVD needs, to get an answer, we need to investigate with more number of samples. There is no significant difference of expression of MVD by gender among Indian cases , the reason we also do not know.

It has been seen that, MVD count is more in grade-I of Indian OSCC cases, but LVD count is relatively higher in Grade II and III among Japanese cases (Table 2). Although, there was no significant difference between MVD and LVD counts and the primary site of occurrence, the Indian cases had fewer number of LVD counts irrespective of site of occurrence (Table 2). The results of this study differs from other results elsewhere, where there is no significant difference in terms of clinicopathological parameters (Folkman et al., 1998; Zetter et al., 1998). The site -wise, the patients who had tongue cancer, showed slightly higher count of MVD, and again that was insignificant. But Indian cases had relatively higher counts of MVD in all the primary sites of occurrence, at the same time, the other studies elsewhere explained that there is no such significant difference of MVD and LVD counts (Gleich, 1996).

In this study, we found that, MVD count in Indian population is relatively higher compared to other sites except tongue, where the Indian population had an MVD count of 56.3 compared to Japanese cases, which were 36.2. Again, when we compare the counts in terms of their personal habits, we found that, MVD count is relatively higher among Indian cases compared to Japanese cases, but there is a difference between the groups with and without the habit of chewing tobacco (Table 2), will need to be investigated further. There were no OSCC cases in Japan who have tobacco chewing habit like in South East Asian population. Again, the lymphocyte infiltration is markedly higher in Indian cases ie., 58.9 compared to Japanese cases which was 37.3 (Table 2), which is reported elsewhere too (Zetter et al., 1998).

In this study we could not find a strong correlations between microvessel density, Lymph vessel density (MVD, LVD) and clinico-pathological conditions, such as: age, sex, tumour localization and size, TNM stage and histological grading. Although Statistical analysis had shown, and microvessel density differs in the 3 histological groups (Grade I, II, III) (p =0.9, 0.8, 0.1), no statistical correlation was observed between microvessel density and other clinical parameters such as age, sex, tumour site and size as well. However, clinical and experimental evidence explains that, new vessel formation is an essential condition for tumour growth and progression (Williams, 1994; Alcade et al., 1995; Shpitzer et al., 1996; Den et al., 1997).

Until date, several studies revealed that MVD and LVD represent a valid independent prognostic factor for overall survival and disease-free survival in primary tumors, which shows a significant correlation between high intra-tumoural micro-vascularization, the presence of metastasis and poor prognosis, not only in oral cancer but also in other types of solid tumours, such as breast cancer (Gasparini et al., 1994; Zetter et al., 1998). In this study we did not have an extensive investigation with a good number of samples. But in future it will require to examine OSCC interrelated with development of micro-vessel and rate of metastasis especially among the cases who have tobacco chewing habit- a cause of high death

toll each year in India and regional countries.

In conclusion, there is a significant difference of the lymphangenosis (LMV counts) which is higher in Japanese samples (P=0.001), but the angiogenesis (MVD counts) is significantly higher (P=0.002) in Indian cases. It needs further investigation in order to elucidate any functional and biological significance of difference on angiogenesis and lympahangiogenesis between Japanese and Indian cases, having different demographic pattern and personal habits especially focussing chewing tobacco associated OSSC.

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