Original Article

Color Doppler-ultrasonography in oral squamous cell carcinoma: Making ultrasonography more meaningful

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Abstract

Background: Although color Doppler ultrasonography (CD-USG) is useful in the diagnosis of various diseases of the head and neck, flow signals in the malignant oral tumors are less studied. This study aimed to study the usefulness of CD-USG in mapping OSCC of buccal mucosa, tongue, and lip.

Materials and Methods: This was a case–control study, conducted among 60 subjects aged 20–70 years. Group A consisted of 30 cases of OSCC of buccal mucosa, tongue, and lip, whereas Group B consisted of 30 controls. CD-USG investigation of each mass was carried out. The spectral waveform (time velocity Doppler spectrum) of flow signal was analyzed for the pulsatility index (PI), resistivity index (RI), peak systolic velocity (PSV) (m/s), and end diastolic velocity (EDV) (m/s). All patients had real-time, gray-scale sonography and CD-USG with spectral wave analysis.

Results: In this study, the mean value for RI in patients with malignancy was 0.40 + 0.14, whereas for healthy subjects, it was 0.83 + 0.07. The mean value for PI in patients with malignancy was 0.86 + 0.20, whereas for healthy subjects, it was 2.61 + 0.77. In the present study, the mean PSV in malignant masses was 31.72 + 13.48, whereas for healthy subjects, it was 43.87 + 20.95, and the EDV in malignant masses was 10.33 + 5.21, whereas for healthy subjects, it was 7.07 + 3.44.

Conclusions: The said Doppler indices were shown to be sensitive as well as specific for the diagnosis of malignant oral tumors. Although CD-USG cannot replace histopathological procedures, it plays a definite role as an adjunct to the clinical evaluation of OSCC cases.

Key Words: Color Doppler ultrasonography, oral cancer, pulsatility index, resistivity index

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INTRODUCTION

Oral cancer is the sixth most common cancer worldwide and shows marked geographic variation in occurrence.[1] Oral cancer is of paramount importance to dental professionals and constitutes a major public health problem in India.[2] The disproportionately higher prevalence of oral cancer in India as one of the fifth leading cancer in either sex is related to the use of tobacco in various forms, consumption of alcohol and low socioeconomic status of the affected individuals apart from poor oral hygiene, poor diet, and infections of viral origin. The most widespread form of tobacco is chewing tobacco with or without

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betel quid and this has been demonstrated as a major risk factor for oral cancer.[3] Exposure to such toxic agents results in the alterations of genes that are important in the regulation of various cellular functions. Some of these important changes include the acquisition of immortality by the cancerous cells and the ability to invade tissue and/or metastasize to other sites, as well as acquiring the ability to induce angiogenesis.[4] Malignant tissue, as a consequence of abnormal morphogenesis, has a structurally abnormal blood supply. It was noted that each tumor type had a characteristic vascular pattern and that the blood vessels do not determine the growth of tumors, but the tumor determines the growth pattern of blood vessels.[4] In recent, color Doppler ultrasonography (CD-USG) has been used for detecting blood flow signals in the vessels of malignant tumors by means of continuous pulsed-wave Doppler and color flow mapping techniques.[5] Vessels with low-impedance flow have low pulsatility index (PI) and resistivity index (RI). Studies have also revealed that this low-impedance tumor flow is helpful in differentiating malignant from benign tumors, as also the changes in blood flow in malignant tumors have some value in predicting the tumor response to chemotherapy.[6] Although CD-USG is useful in the diagnosis of various diseases of the head and neck, flow signals in the oral malignant masses are less studied; hence, the present study was designed to study the usefulness of CD-USG in quantifying oral squamous cell carcinoma (OSCC) vascularization and in determining the hemodynamic parameters by spectral analysis obtained during CD-USG procedure.

MATERIALS AND METHODS

The present study was conducted to evaluate the efficacy of intraoral CD-USG in mapping of OSCC blood flow. The study was conducted in the Department of Oral Medicine and Radiology, and Department of Radiodiagnosis during the period of October 2010 to March 2012. For this single-blinded case–control study cases were selected randomly with an age range of 20–70 years. Of the 60 subjects enrolled in the study, Group A consisted of 30 cases which were clinically diagnosed as malignant oral ulcers and histopathologically diagnosed as squamous cell carcinoma of buccal mucosa, tongue, or lip of varying histopathological grades [Figures 1 and 2] and severity due to chronic usage of tobacco, while Group B consisted of an equal number of age and sex matched 30 controls with clinically healthy buccal/oral mucosae and without any habits. There was no significant difference in the age and sex of the cases and controls included in the study. After a detailed clinical history and clinical examination, CD-USG procedure was carried out. The clinical data recorded previously was then correlated with CD-USG findings. CD signals of diseased patients were compared with the control group.

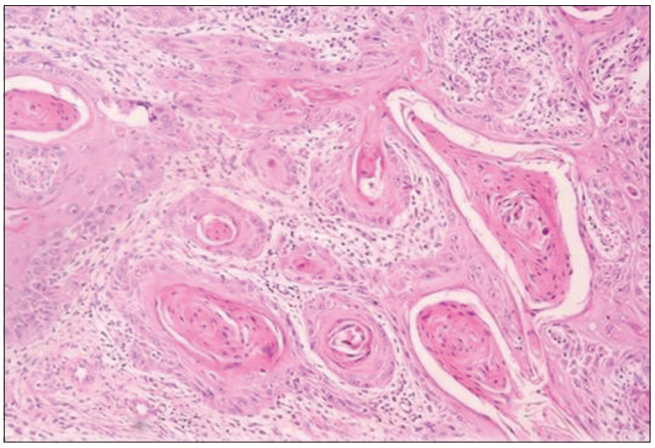

Figure 1: Photomicrograph showing well-differentiated squamous cell carcinoma of a patient with malignancy

Inclusion criteria

Clinically and histopathologically diagnosed cases with OSCC of buccal mucosa tongue and lip in the age range of 20–70 years.

Exclusion criteria

- Squamous cell carcinoma of palate, alveolar mucosa, and gingival mucosa;
- Recurrent cases of OSCC;
- Patients are suffering from systemic diseases including diabetes mellitus, hypertension, and endocrinal disorders.

The protocol of this study was approved by Institutional Ethics Committee. The patient's detailed case history was taken and clinical findings were recorded in structured proforma.

Color Doppler ultrasonography examination

USG investigation of each mass was carried out using Philips Envisors C Series of ultrasonogram [Figure 3] with the linear transducer probe at a frequency of 7.5 MHz. Experienced and qualified sonologist from Department of Radiodiagnosis, who was unaware of clinical data and blinded about the cases, performed the USG examination. During the USG examination, the patient was made to lie down on the examination table with the shoulders supported by a pillow and the operator seated on the right side of the examination table. The coupling gel was applied over the area of interest. The transducer was then moved in transverse or longitudinal direction whichever was more characteristic and informative. All patients had real-time, gray-scale sonography, and CD sonography

with spectral wave analysis. First, the mass was localized with real-time, gray-scale sonography, and the size (largest diameter) of the lesion was measured. Then, CD mapping of the entire mass was done to detect the blood flow. Sensitivity to low velocity (Doppler frequency shifts) was maximized by choosing a low-velocity scale (0.26 m/s for a Doppler angle of 0° or 180°). CD gain was increased until the background noise was apparent as a colored "snowstorm" across the image and was then decreased until only a few random specks remained visible. The mass was scanned slowly from margin to margin to detect blood flow which appeared as persistent areas of color with a curvilinear, tubular, or branching distribution on real-time images [Figures 4 and 5]. When the blood flow was detected on CD sonograms, pulsed-wave Doppler was used with the Doppler gate focused on the center of the flow signals and the transducer adjusted so that the Doppler angle Ø between the flow signals and the ultrasound beam was 60° or less. Pulsed-wave Doppler sonography was used to sample all the flow signals in the tumor for spectral wave analysis. At least three vessels were sampled, and the measurements were repeated at least 3 times. Spectral waveforms that were reproducibly similar over three consecutive cardiac cycles were regarded as satisfactory. Each spectral waveform was then recorded on a laser disk so that the Doppler indices and Doppler angle could be measured and calculated. The same procedure was performed for the subjects in the control group [Figure 6]. The spectral waveform (time-velocity Doppler spectrum) of flow signal was analyzed for the following Doppler indices: (1) PI: (Peak systolic velocity [PSV] – end diastolic velocity [EDV])/mean velocity, (2) RI: (PSV – EDV)/PSV, (3) PSV (m/s), and (4) EDV (m/s). PSV and EDV were corrected by the Doppler angle between the flow signals and the Doppler gate, if the angle was not 0° or 180°, using the microprocessing program in the sonographic unit. The average value of each Doppler index was used when multiple flow signals were detected in a tumor mass. Images were interpreted by comparing with the images of neighboring structures, and all the findings were recorded in the chart of prescribed proforma. The clinical data thereafter was correlated with USG findings. Both the clinical and CD-USG findings were

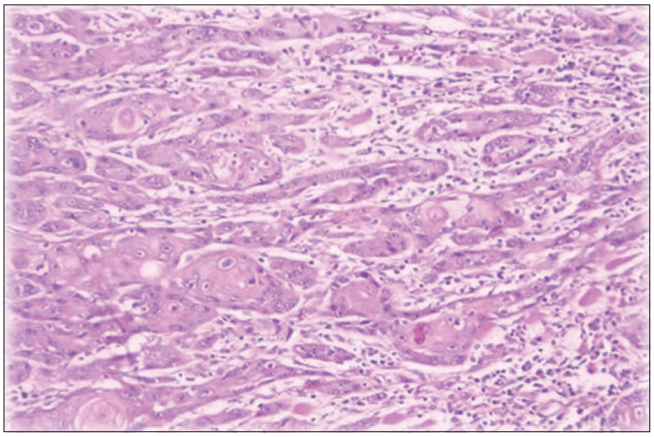

Figure 2: Photomicrograph showing moderately differentiated squamous cell carcinoma of patient with malignancy

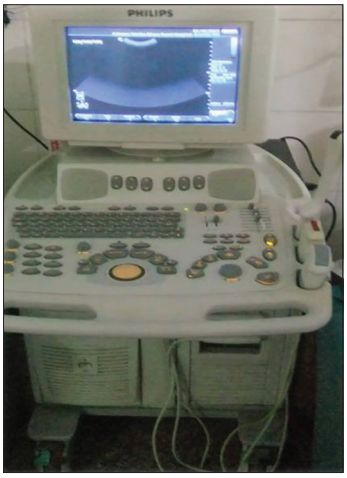

Figure 3: Color Doppler ultrasound machine

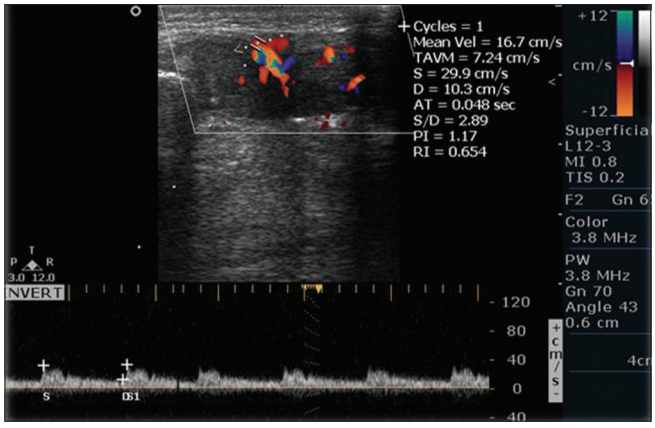

Figure 4: Photograph showing color Doppler signals of a patient with malignancy

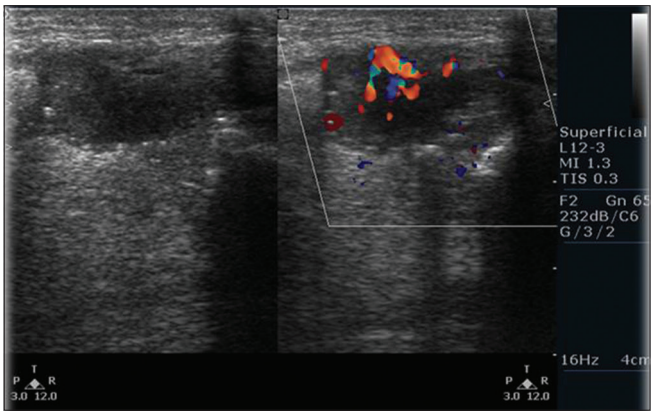

Figure 5: Photograph showing color Doppler signals of another patient with malignancy

correlated with final diagnosis, and data obtained was subjected to statistical evaluation.

Statistical methods employed

Descriptive statistical analyzes (i.e., mean and standard deviation) and student's unpaired *t*-test were carried out. The analysis was performed using Windows Graph pad, Prism 4 software, and SPSS version 14.0 software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

For this single blind cross-sectional study, 60 subjects were selected randomly within the age range of 20–70 years with clinical details as depicted in Table 1. The PI and RI indices used in the study were calculated from the formulae as under:

$$\text{Pourcelot's resistivity index} = \frac{\text{Peak systolic velocity} - \text{End diastolic velocity}}{\text{Peak systolic velocity}}$$

$$\text{Gosling's pulsatility index} = \frac{\text{Peak systolic velocity} - \text{End diastolic velocity}}{\text{Time averaged maximum velocity}}$$

The mean value for RI in patients with malignancy came out to be 0.40 + 0.14, whereas for the control group, it was 0.83 + 0.07 [Table 2]. The cut-off value was kept as 0.5, and a sensitivity of 73.33% was recorded with an accuracy of 86.66%. The mean value, again, for PI in patients with malignancy that came out to be 0.86 + 0.20 as against 2.61 + 0.77 for the control group was found to be statistically significant with a cut-off value kept at 1. A sensitivity of 86.67% was seen with an accuracy recorded of 93.33%. Tables 3 and 4 show the comparison of PSV in m/s in patients with malignancy and the control group. The mean value in patients with malignancy was 31.72 + 13.82 against the control group, where a mean value of 43.87 + 20.95 was recorded. Similarly, Table 5 shows the comparison of EDV in m/s in patients with malignancy and the control group with the mean value of EDV in patients with malignancy being 10.33 + 5.21 as against 7.07 + 3.44 in the control group. The results in this case two were found to be statistically significant.

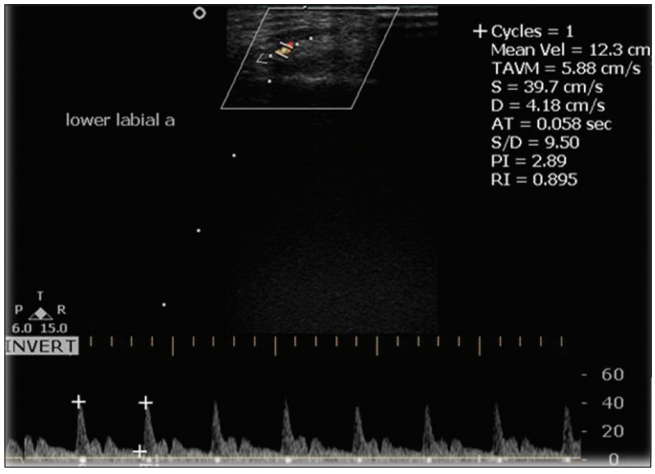

Figure 6: Photograph showing color Doppler signals of a subject selected from control group (right buccal mucosa)

DISCUSSION

Angiogenesis has gained much attention in cancer growth and metastasis in the recent decades. Considering angiogenesis as a neoplastic marker for malignancy, CD-USG allowing a better insight into the biological behavior of the tumor makes the early diagnosis of cancer possible by detecting neo-vascularization in the tumor.[7,8] Many indices of waveform analysis have been devised to detect neo-vascularization in the tumor, but only two indices are in regular clinical use. Hence, in the present study, these two Doppler indices were chosen to assess the resistance of the vessels in malignant masses and healthy buccal mucosae. The malignant tumors, with their characteristic low-impedance flow, have a lower PI, RI, and PSV, while a higher EDV than do the healthy mucosa and benign growths. Although there are conflicting reports regarding this with some reports suggesting a higher vascular resistance in malignant tumors than as compared to the benign growths,[9-13] while the others suggesting either a lower, or atleast similar values as against the benign tumors.[14,15] Furthermore, it is generally argued that malignant tumors, usually being with the low-impedance flow, have lower PI and RI. Again, it is a common observation to have lower pulsatility

Table 1: Comparison of the clinical data in patients with malignancy and control group

Group	*n*	Mean age	SD	Male: Female	Site of lesion	Duration of lesion	Duration of habit	Degree of differentiation
Patients with malignancy	30	50.06	13.08	21:9	Buccal mucosa (23)	1–4 months (14)	1–10 years (10)	Well-differentiated OSCC (19)
Control group	30	41.03	8.58	20:10	Lip (5)	5–8 months (10)	11–20 years (6)	Moderately differentiated OSCC (9)
					Tongue (2)	9–12 months (2)	21–30 years (7)	Poorly differentiated OSCC (2)
						13–16 months (4)	31–40 years (40)	
							41–50 years (2)	

SD: Standard deviation, OSCC: Oral squamous cell carcinoma

Table 2: Comparison of RI in patients with malignancy and control group

Group	*n*	Mean	SD	SEM	*P*	*t*	χ^2
Patients with malignancy	30	0.40	0.14	0.02	0.0001 significant	14.408	25.71
Control group	30	0.83	0.07	0.01			

Sensitivity: 73.33%, Specificity: 100%, Positive predictive value: 100%, Negative predictive value: 78.95%, Accuracy: 86.66%. SD: Standard deviation, SEM: Standard error of mean, RI: Resistivity index

Table 3: Comparison of PI in patients with malignancy and control group

Group	*n*	Mean	SD	SEM	*P*	*t*	χ^2
Patients with malignancy	30	0.86	0.20	0.03	0.0001 significant	11.95	25.71
Control group	30	2.61	0.77	0.14			

Sensitivity: 86.67%, Specificity: 100%, Positive predictive value: 100%, Negative predictive value: 88.24%, Accuracy: 93.33%. SD: Standard deviation, SEM: Standard error of mean, PI: Pulsatility index

Table 4: Comparison of PSV in patients with malignancy and control group

Group	*n*	Mean	SD	SEM	*P*	*t*	χ^2
Patients with malignancy	30	31.72	13.84	2.52	0.010 significant	2.65	25.71
Control group	30	43.87	20.95	3.82			

SD: Standard deviation, SEM: Standard error of mean, PSV: Peak systolic velocity

Table 5: Comparison of EDV in patients with malignancy and control group

Group	*n*	Mean	SD	SEM	*P*	*t*	χ^2
Patients with malignancy	30	10.33	5.21	0.95	0.006 significant	2.86	25.71
Control group	30	7.07	3.44	0.62			

SD: Standard deviation, SEM: Standard error of mean, EDV: End diastolic velocity

and resistivity values in relation to the inflammatory lesions including cervical abscesses which are more common than OSCC. Usually, there is decreased vascularity as seen in the cases of cervical abscesses, although in case of OSCC, central necrosis displaces vascular networks toward the periphery, thereby having high vascularity in the periphery and relatively absent vascularity in the central regions; in fact, the presence of peripheral vascularity is a feature, that is, strongly suggestive of malignancy. The change in the values of PI and RI are, therefore, obtained accordingly and hence, the difference can be made out between the inflammatory/reactive growths and the malignant masses, especially on CDs if not, plain USG.[16-20] The vascular pattern of a tumor is an accurate method of distinguishing benign from malignant growths. Again, it is said that the vascular resistance in malignant growths in the early enough stages is said to be low because of the phenomenon of neo-angiogenesis. The RI and PI values are then later found to increase in the late stages because of the compression of the intra-tumoral vessels by the tumor cells. In this study, the mean value for RI in patients diagnosed with malignant ulcers was 0.40 + 0.14, whereas for healthy subjects, it was 0.83 + 0.07 with a cut-off value of 0.5, while mean value for PI in patients diagnosed with malignant ulcers came out to be 0.86 + 0.20, whereas for healthy subjects, it was 2.61 + 0.77 with a cut-off value of 1. These findings were in agreement with the previous reports that a low-impedance Doppler flow signal is associated with malignant tumors in other organs.[21-24] This difference in the distal impedance between the neo-vascularized tumor vessels and the supposedly normal structured vessels in normal mucosa makes it possible to differentiate malignant oral lesions from the normal buccal/oral mucosae with color and pulsed-wave Doppler sonography. Different cut-off values for RI (0.6, 0.7, and 0.8) and PI (1.1, 1.5, and 1.6) have been reported with different sensitivities and specificities for RI being 47–81% and 81–100% and for PI being 55–94% and 97–100%, respectively.[9-11,14] With a cut-off value taken as 0.5 for RI values and 1 for PI values, these Doppler indices were shown to be sensitive and specific for the diagnosis of malignant oral tumors to the extent of 73.33% and 100% for RI values and 86.67% and 100% for PI values in our study. The high sensitivity and specificity of these Doppler variables imply a potential role CD-USG might have in determining oral malignancies and possibly, in the early diagnosis of malignant oral tumors by detecting neo-angiogenesis at an early enough stage. For an undiagnosed lesion in the oral cavity, the low-impedance flow signals seen on CD sonograms suggest a high probability of the lesion being malignant.[21-24] In the present study, the mean PSV in malignant masses was 31.72 + 13.48, whereas for healthy subjects, it was 43.87 + 20.95 while the EDV in malignant masses came out to be 10.33 + 5.21m, whereas for healthy subjects, it was 7.07 + 3.44. The higher PSV and low values for EDV are also explained on the basis of the compression of the intra-tumoral vessels by the tumor cells. However, both PSV and EDV are influenced by the Doppler angle between the flow signals and the ultrasound beam. From the present study, it can be summarized that after the clinical examination, CD-USG should be the first modality used for the investigation as it is readily available and does not involve ionizing radiation. In spite of its acceptance as an adjunct to clinical evaluation, it carries certain limitation, such as sample size was limited and the ability to detect the color flow pattern and Doppler spectral evaluation dependent on efficacy of the transducer, CD-USG machine, and sonologist's skill. This limitation can be overcome with the advent in improvisation in CD-USG technology. Moreso, much work has not been done in relation to the observations of the various Doppler indices in relation to OSCC. We recommend

a multi-institutional study to investigate the multiple vascular assessment parameters to determine the role of CD-USG in the preoperative prediction of oral tumor mass. In addition, more work is required to determine whether the use of CD-USG will permit earlier detection and staging of oral cancer and therefore, improve the dismal prognosis of such patients.

CD flow imaging provides the information on blood flow that supplements the information gained by the routine sonography, and thus is useful in the diagnosis OSCC. CD-USG is useful for showing vascularity in oral masses and very useful in differentiating malignant from the benign ones. The eventual decrease in blood flow in a malignant tumor after treatment/radio-chemotherapy might also be useful for predicting the response of a tumor to the treatment based on the characteristic low-impedance flow shown by the malignant tumors.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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