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Color Doppler Ultrasonography in Mapping Oral Squamous Cell Carcinoma

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Abstract

Background: Although Color Doppler Ultrasonography (CDUS) is useful in the diagnosis of various diseases of the head and neck, flow signals in oral malignant masses are less studied; hence the present study assesses the usefulness of Color Doppler Ultrasonography in quantifying oral squamous cell carcinoma (OSCC) vascularization. In addition, we determine the hemodynamic parameters by spectral analysis obtained during a Color Doppler Ultrasonography procedure. We have studied the usefulness of Color Doppler Ultrasonography in mapping oral squamous cell carcinoma of the buccal mucosa, tongue and lip.

Methods: This case-control study enrolled 60 subjects aged 20-70 years. Group A constituted 30 cases diagnosed with oral squamous cell carcinoma and Group B constituted 30 healthy controls. Ultrasonographic investigation of each mass was performed. The spectral waveform (time-velocity Doppler spectrum) of the flow signal was analyzed for the pulsatility index, resistive index, peak systolic velocity (m/sec), and end diastolic velocity (m/sec). All patients had real-time, gray-scale sonography and Color Doppler Ultrasonography with spectral wave analysis.

Results: The mean value for the resistive index in patients with oral squamous cell carcinoma was 0.40±0.14 whereas for healthy subjects, it was 0.83±0.07. The mean pulsatility index value in malignant patients was 0.86±0.20 while for healthy subjects, it came-out to be 2.61±0.77.

Conclusion: These Doppler indices have been shown to be sensitive and specific for the diagnosis of malignant oral tumors. Although Color Doppler Ultrasonography cannot replace histopathological procedures, it plays a definite role as an adjunct to clinical evaluation of oral squamous cell carcinoma patients.

Keywords: Oral cancer, Color Doppler Ultrasonography, Pulsatility index, Resistivity index

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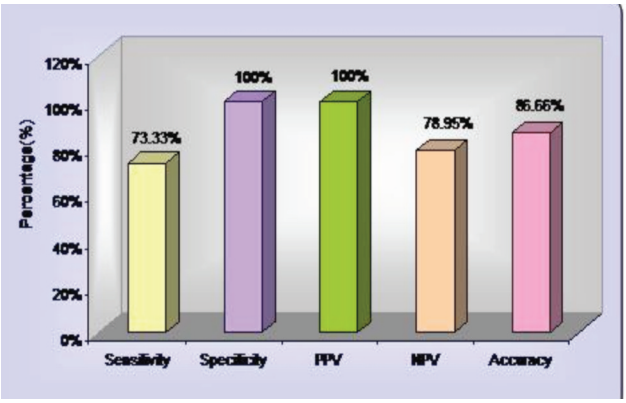
Introduction

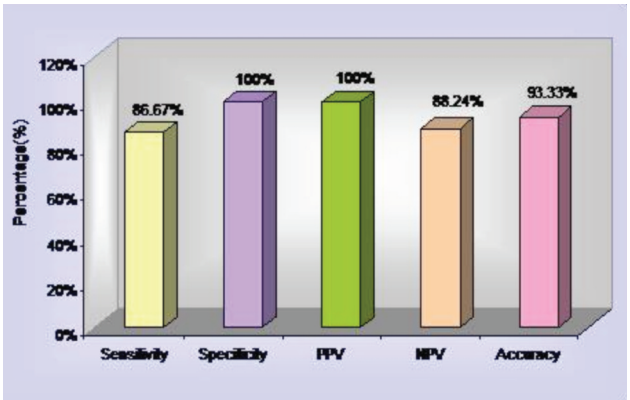
Oral cancer is the sixth most common cancer worldwide and shows marked geographic variation in occurrence.[1] Oral cancer is of paramount importance to dental professionals and constitutes a major public health problem in India.[2] The disproportionately higher prevalence of oral cancer in India as one of the fifth leading cancers of either sex is related to the use of various forms of tobacco, consumption of alcohol and low socioeconomic conditions related to poor oral hygiene, poor diet or viral infections. The most widespread form of tobacco is chewing tobacco with or without betel quid and this has been demonstrated to be a major risk factor for oral cancer.[3] Exposure to such toxic agents results in alterations of genes that are important in the regulation of various cellular functions. Some of these important changes include the acquisition of immortality by 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 tissues, as a consequence of abnormal morphogenesis, have structurally abnormal blood supplies. This is seen more in relation to the process of neoangiogenesis brought about to sustain cancerous growths depending upon their growth potential and rate. Of note, each tumor type has a characteristic vascular pattern.[4] Recently, Color Doppler Ultrasonography (CDUS) has been used to detect blood flow signals in 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 and resistivity indices. According to studies, this low-impedance tumor flow is helpful in differentiating malignant from benign tumors. Also, changes in blood flow in malignant tumors have also been of some significance in predicting tumor response to radio- and chemo-therapy.[6] Although CDUS is useful in the diagnosis of various diseases of the head and neck, flow signals in oral malignant masses are less studied. Hence, the present study was designed to assess the usefulness of CDUS in quantifying oral squamous cell carcinoma (OSCC) vascularization and in determining hemodynamic parameters by spectral analysis obtained during a CDUS procedure.

Materials and Methods

The present study was conducted in the Department of Oral Medicine and Radiology and Department of Radiodiagnosis from October, 2010 to March, 2012 to evaluate the efficacy of intra-oral CDUS in mapping OSCC blood flow. In this single blinded case-control study, cases between the ages of 20-70 years were chosen randomly. Of these 60 patients, Group A constituted 30 cases which were clinically



Graph 1. Comparison between percent of patients in terms of sensitivity, specificity, positive and negative predictive values, and accuracy in patients with malignancy and the control group Sensitivity=73.33%; Specificity=100%; Positive predictive; value=100%; Negative predictive value=78.95%; Accuracy=86.66%



Graph 2. Comparison between percent of patients in terms of sensitivity, specificity, positive and negative predictive values, and accuracy in patients with malignancy and the control group. Sensitivity=86.67%; Specificity=100%; Positive predictive value=100%; Negative predictive value=88.24%; Accuracy=93.33%.

Table 1. Comparison of resistivity index (RI) in patients with malignancy and the control group.

t	df	*P*-value	Mean difference	Std. error	95% Confidence interval of the difference	
					Lower	Upper
14.408	58	0.0001	-0.42	0.02	-0.48	-0.36

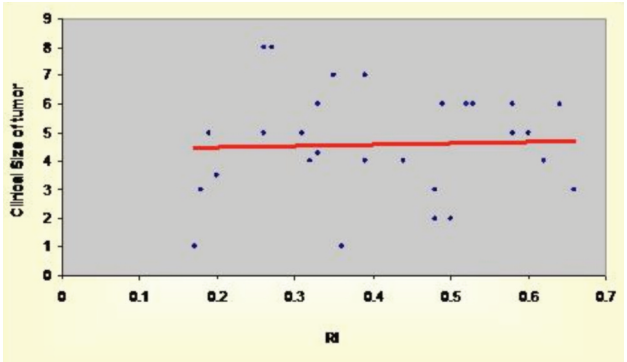
Significance: $P<0.05$

diagnosed with malignant ulcers of the oral mucosa and were then histopathologically diagnosed as squamous cell carcinoma of the buccal mucosa, tongue or lip of varying histopathological grades (Figures 1 and 2) and severity due to chronic tobacco use. Group B consisted of 30 age and sex matched individuals with clinically healthy buccal/oral mucosa. After a detailed history and clinical examination, a CDUS study was performed followed by an incisional biopsy, as indicated. The obtained biopsy specimens were submitted for histopathological examination and final diagnosis. Patients with histopathologically confirmed OSCC were enrolled in the study and were recorded in the prescribed proforma. Thereafter, the clinical data was correlated with ultrasonographic findings. Color Doppler signals from diseased patients were compared with the control group. Inclusion criteria included clinical and histopathologically diagnosed cases with OSCC of the buccal mucosa, tongue or lip. Patients were between the ages of 20-70 years. Patients were excluded if they had squamous cell carcinoma of the palate, alveolar mucosa or gingival mucosa, previously treated and recurrent cases of OSCC, and patients who were suffering from any systemic illness including diabetes, hypertension or endocrinal disorders. The study protocol was approved by the Institutional Ethics Committee. Each patient's detailed case history was taken and clinical findings were recorded in a structured proforma.

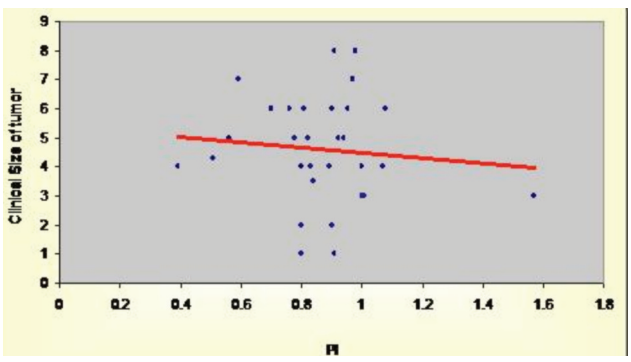
Color Doppler ultrasonography (CDUS) examination

Ultrasonographic investigation of each mass was carried out by using a Philips Envisors C Series ultrasonic equipment (Figure 3) with a linear transducer probe at a frequency of 7.5 MHz. An experienced, qualified Sonographer from the Department of Radiodiagnosis, who was unaware of the clinical data and blinded to the cases, performed the ultrasonographic examinations.

During the ultrasonographic examination, each patient was instructed to lie down on the examination table with the shoulders supported by a pillow and the operator seated by the right side of the examination table. Coupling gel was applied over the area of interest. The transducer was then moved in either a transverse or longitudinal direction- whichever was more characteristic and informative. All patients had real-time, gray-scale sonography and CDUS 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, Color Doppler mapping of the entire mass was performed to detect blood flow. Sensitivity to



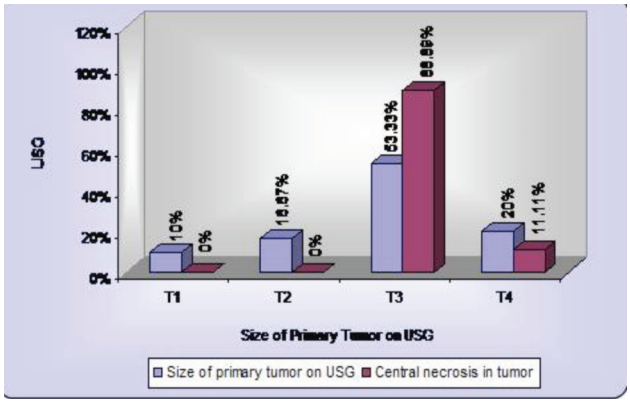
Graph 3. Correlation of clinical size of tumor with resistivity index (RI) in patients with malignancy.



Graph 4. Correlation of clinical size of tumor with pulsatility index (PI) in patients with malignancy.

low velocity (Doppler frequency shifts) was maximized by choosing a low-velocity scale (0.26 m/sec for a Doppler angle of 0° or 180°). Color Doppler gain was increased until background noise was apparent as a colored "snowstorm" across the image and then decreased until only a few random specks remained visible. The mass was scanned slowly from margin to margin to detect blood flow, which usually appeared as persistent areas of color with a curvilinear, tubular, or branching distribution on real-time images (Figures 4, 5 and 6). When blood flow was detected on Color Doppler 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 flow signals in the tumor for spectral wave analysis. At least three vessels were sampled and the measurements repeated at least three 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 subjects in the control group (Figure 7).

The spectral waveform (time-velocity Doppler spectrum) of the flow signal was analyzed for the following Doppler indices: (1) pulsatility index: [peak systolic velocity (PSV) - end diastolic velocity (EDV)]/mean velocity, (2) resistivity index: (PSV - EDV)/PSV, (3) PSV (m/sec), and (4) EDV (m/sec). Peak systolic velocity and end diastolic velocity were corrected by the Doppler angle between the flow signals and the Doppler gate, if the angle was not 0° or 180°, by using the micro-processing 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 comparison with images of neighboring structures, after which a diagnosis was made, and all findings were recorded in the prescribed proforma. Thereafter, the clinical data was correlated with ultrasonographic findings. Both the clinical and CDUS findings were eventually correlated with the final diagnosis. The resultant data was then subjected to statistical evaluation.



Graph 5. Distribution of patients according to size of primary tumor and their correlation with central necrosis of the primary tumor mass; USG- Ultrasonogram.

Results

This single blind, cross-sectional study

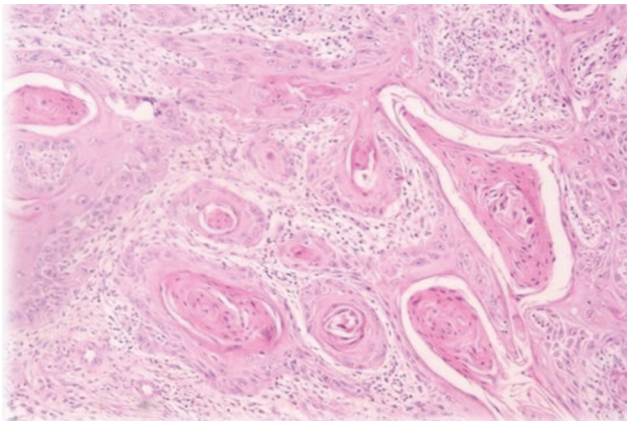


Figure 1. Photomicrograph showing well-differentiated squamous cell carcinoma in a study patient.

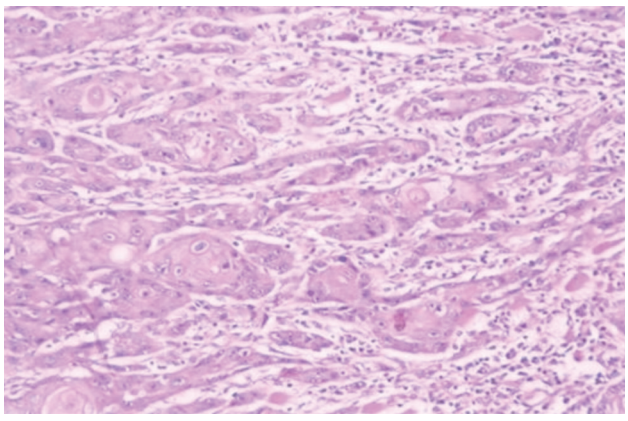


Figure 2. Photomicrograph showing moderately differentiated squamous cell carcinoma in a study patient.

Table 2. Comparison of pulsatility index (PI) in patients with malignancy and the control group.

t	df	*P*-value	Mean difference	Std. error	95% Confidence interval of the difference	
					Lower	Upper
11.95	58	0.0001	-1.74	0.14	-2.03	-0.36

Significance: $P<0.05$

randomly selected 60 cases between the ages of 20-70 years. All 60 cases had a mean age of 50.06±13.08 years. Group A malignant cases had a mean age of 48.33±13.04 years and Group B control cases had a mean age of 38.95±8.25 years. There were 21 male and 9 female patients enrolled in the study group with a male to female ratio of 2.25:1, whereas in the control group there were 20 male and 10 female patients with a male to female ratio of 2:1.

Out of the 30 patients in Group A, 23 (76.7%) had lesions of the buccal mucosa,[5] (16.7%) of the lip, and 2 (6.7%) had lesions of the tongue. Duration of lesions was as follows: 14 (46.67%) had a lesion between 1-4 months, 10 (33.33%) from 5-8 months, 2 (6.67%) from 9-12 months and 4 (13.33%) from 13-16 months. On history elicitation, 10 (33.33%) patients had a history of tobacco use of 1-10 years, 6 (20%) reported use of 11-20 years, 7 (23.33%) between 21-30 years, 5 (16.67%) for 31-40 years and 1 (3.33%) had this habit for 41-50 years. According to the degree of differentiation on histopathological examination, there were 19 (63.3%) with well-differentiated squamous cell carcinoma, 9 (30%) had moderately differentiated squamous cell carcinoma, and 2 (6.7%) cases had evidence of poorly differentiated squamous cell carcinoma.

The role of CDUS as a mean of differentiating benign from malignant diseases is based on detection of intra-tumor vessels that exhibit low impedance or high systolic flow. The software installed in the computer of the Color Doppler machine for the calculation of pulsatility index (PI) and resistivity index (RI) is used in the following formula:

$$\text{Pourcelot's RI} = \frac{\text{PSV - EDV}}{\text{PSV}}$$

$$\text{Gosling's PI} = \frac{\text{PSV - EDV}}{\text{Time averaged maximum velocity}}$$

The mean value for RI in patients with malignancy was 0.40±0.14, whereas for the control group, it was 0.83±0.07, which was statistically significant ($P<0.0001$) (Table 1). Graph 1 shows the sensitivity, specificity, positive and negative predictive values, and accuracy of the test. The results were as follows: sensitivity (73.33%), specificity (100%), positive predictive value (100%), and negative predictive value (78.95%). Hence, the accuracy of this test was

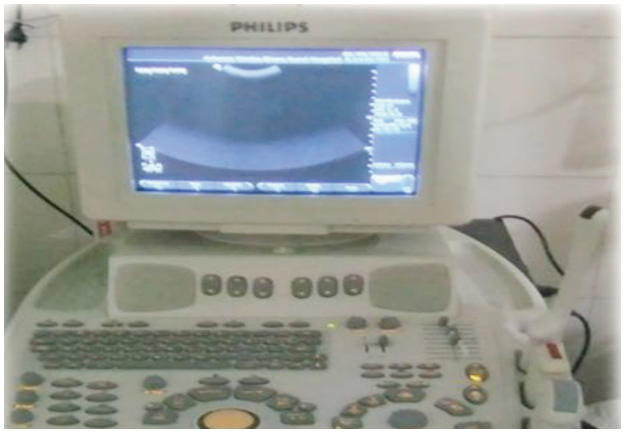


Figure 3. Color Doppler Ultrasound (CDUS) machine.

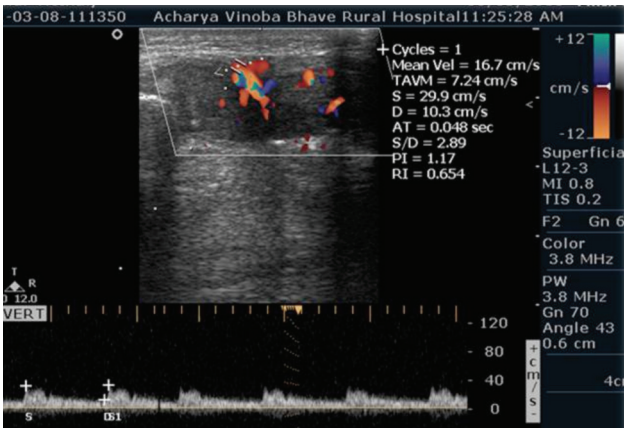


Figure 4. Photograph showing Color Doppler signals of a patient with malignancy.

Table 3. Comparison of peak systolic velocity (PSV) in patients with malignancy and the control group.

t	df	*P*-value	Mean difference	Std. error	95% Confidence interval of the difference	
					Lower	Upper
2.65	58	0.010	-12.15	4.58	-21.33	-2.97

Significance: $P<0.05$

86.66%.

The mean value for PI in patients with malignancy was 0.86±0.20, whereas for the control group, it was 2.61±0.77, which was statistically significant ($P<0.0001$) (Table 2). Graph 2 shows the sensitivity, specificity, positive and negative predictive value, and accuracy of the test as follows: sensitivity (86.67%), specificity (100%), positive predictive value (100%) and negative predictive value (88.24%). Hence, the accuracy of the test was 93.33%.

The mean PSV (m/sec) in patients with malignancy was 31.72±13.82 m/sec, whereas in the control group, it came-out to be 43.87±20.95 m/sec. The *P*-value was statistically significant (Table 3).

Similarly, Table 4 shows statistically significant values of a comparison of EDV (m/sec) in patients with malignancy and the control group. The mean EDV (m/sec) in patients with malignancy was 10.33±5.21 m/sec, whereas in the control group, it came-out to be 7.07±3.44 m/sec.

Table 5 and Graph 3 show the correlation between clinical tumor size and RI in patients with malignancy. The *P*-value was statistically insignificant as the value obtained was 0.87, being more than 0.05.

Table 6 and Graph 4 show the correlation of clinical tumor size and PI in patients with malignancy. The *P*-value was statistically insignificant as the value obtained was more than 0.05.

Also, CDUS correctly identified lymph nodes in the neck areas of 30 patients. While performing CDUS of 44 palpable lymph nodes, an additional 12 lymph nodes were discovered. These nodes were located in a clinically inaccessible region or were deep-seated. After CDUS evaluation of these lymph nodes, the total lymph nodes included in the study were 56 (44+12). Out of 56 lymph nodes, 42 (75%) had an intact hilum, whereas 14 (25%) showed loss of hilum architecture.

Table 7 and Graph 5 show the distribution of patients according to primary tumor size and their correlation with central necrosis of the tumor mass. This result was significant in patients with T3 and T4 tumors ($P<0.0$).

Discussion

The mean age of patients in the present study was 50.06±13.08 years. The reason for a higher proportion of patients that used tobacco was

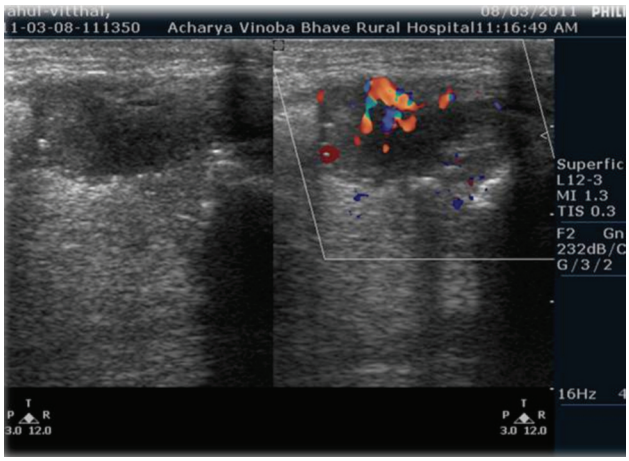


Figure 5. Photograph showing Color Doppler signals of another patient with malignancy.

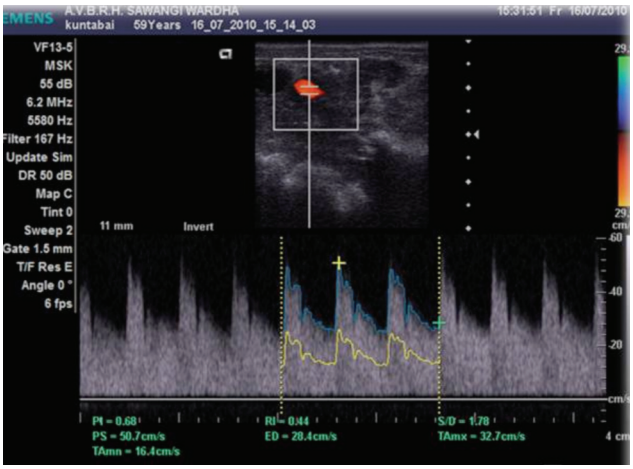


Figure 6. Photograph showing Color Doppler signals of another patient with malignancy.

Table 4. Comparison of end diastolic velocity (EDV) in patients with malignancy and the control group.

t	df	*P*-value	Mean difference	Std. error	95% Confidence interval of the difference	
					Lower	Upper
2.86	58	0.006	3.26	1.14	0.97	5.54

Significance: $P<0.05$

probably related to ease of availability, low cost and socio-cultural acceptance. The present study consisted of 30 patients diagnosed with malignancies, of which there were 21 males and 9 females. The male to female ratio was 2.25:1. In a study conducted by Ascani et al in 2005,[7] there were 46 males and 15 females with OSCC, with a male to female ratio of 3.06:1. The most common site for OSCC in the present study was the buccal mucosa. In the current study, 23 (76.7%) patients had malignancies on their buccal mucosae, 5 (16.7%) were on the lip area and only 2 (6.7%) were located on the tongue. The reason for increased incidence of OSSC of the buccal mucosa in India might be attributed to the habit of placement of tobacco + betel nut + lime quid/pan with tobacco/panmasala/gutka in the buccal vestibule.[8] Similar results were obtained in study conducted by Rengaswamy et al on oral cancer patients.[9] In their study, the buccal mucosa was the most common site that accounted for more than 60% of OSCC patients.

For decades, angiogenesis has gained much attention in cancer growth and metastasis. Considering angiogenesis as a neoplastic marker for malignancy, CDUS has allowed better insight in the biological behavior of the tumor. Early diagnosis of cancer could become possible by the detection of neovascularization in the tumor.[10, 11] Many indices of waveform analysis have been devised but only two 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 normal mucosa. The malignant tumors, with their characteristic low-impedance flow, had a lower PI, RI, and PSV, along with a higher EDV compared to healthy masses. Given that neovascularization is an obligate event in malignant change, this recognition may enable us to observe the earliest stages of oncongenesis.

In this study, the mean RI value in malignant patients was 0.40±0.14, whereas for healthy subjects, it was 0.83±0.07. The cut-off value was 0.5. Mean PI in malignant patients came-out to be 0.86±0.20, while for healthy subjects, it was 2.61±0.77 with a cut-off value of 1. These findings agreed with the previous reports that a low impedance Doppler flow signal was associated with malignant tumors in other organs.[12-15] This difference in the distal impedance between the neovascularized tumor vessels and the supposedly normal structured vessels in normal mucosa made it possible to differentiate malignant oral lesions from normal buccal/oral mucosae with color and pulsed wave Doppler sonography. When a cut-off value was used, these Doppler indices were shown to be sensitive and specific for the diagnosis of malignant oral tumors. The high sensitivity and specificity of these Doppler variables implied a potential role, CDUS might have, in determining oral malignancies. For an undiagnosed lesion in the oral cavity, the low impedance flow signal seen on Color Doppler sonograms suggested a high probability of the lesion being malignant.[12-15] In the present study, the mean PSV in malignant masses was 31.72±13.84 m/sec, whereas for healthy subjects, it was 43.87±20.95 m/sec The

Table 5. Correlation of clinical size of tumor with resistirity index (RI) in patients with malignancy according to Spearman's rank correlation coefficient

	N	Mean	Std. deviation	Rho (p)	*P*-value
RI	30	0.40	0.14		
Clinical size of tumor	30	4.59	1.83	0.049	0.797

P-value not significant (NS), >0.05

Table 6. Correlation of clinical size of tumor with pulsatility index (PI) in patients with malignancy according to Spearman's rank correlation coefficient.

	N	Mean	Std. deviation	Rho (p)	*P*-value
PI	30	0.86	0.20		
Clinical size of tumor	30	4.59	1.83	-0.102	0.592

P-value not significant (NS), >0.05

EDV in malignant masses was 10.33±5.21 m/sec, whereas for healthy subjects, it came-out to be 7.07±3.44 m/sec. Both PSV and EDV are supposed to be influenced by the Doppler angle between the flow signals and the ultrasound beam.

From the present study, it can be summarized that after clinical examination, CDUS should be the first modality used for investigation as it is readily available and does not involve ionizing radiation.

Despite its acceptance as an adjunct to clinical evaluation, there were certain limitations such as the limited sample size. Another limitation was the capability to detect color flow pattern and Doppler spectral evaluation which depended on the efficacy of the transducer, CDUS machine and sonographer's skill. This limitation however could be overcome by improvising CDUS technology. The present study thus further paves the way for a larger, multi-institutional study to investigate the multiple vascular assessments in order to determine the role of color flow Doppler in the pre-operative prediction of oral tumor masses. In addition, more work is required to determine whether the use of CDUS will permit earlier detection and staging of oral cancer, therefore improving the dismal prognosis of such patients.

To conclude, Color Doppler flow imaging provides information on blood flow that supplements the information gained by the routine sonography and therefore, is useful in the diagnosis OSCC. Color Doppler Ultrasonography is useful for showing vascularity in oral masses and in differentiating malignant from benign growths. The variations seen in the blood flow in a malignant tumor after radio-chemo-therapy might also be of use for predicting response of a tumor to treatment.

Conflicts of interest

No conflict of interest is declared.

Acknowledgement

We express our appreciation to all patients who participated in this study without whom it would not have been feasible.

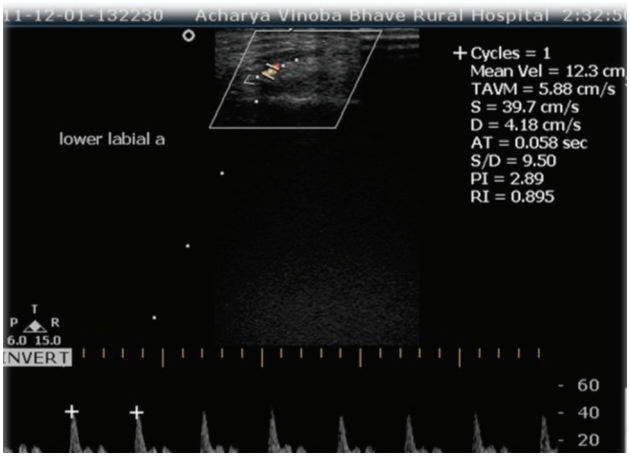


Figure 7. Photograph showing Color Doppler signals of a control group subject (right buccal mucosa)

Table 7. Distribution of patients according to size of primary tumor and their correlation with central necrosis of the tumor mass.

	Size of primary tumor on USG	Central necrosis in tumor	ꭓ2-value 38.74
T1	3 (10.00%)	0 (0.00%)	
T2	5 (16.67%)	0 (0.00%)	
T3	16 (53.33%)	8 (88.89%)	*P*<0.0001
T4	6 (20.00%)	1 (11.11%)	
Total	30 (100%)	9 (100%)	

P-value significant (S), <0.0001; USG: Ultrasonogram.

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