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; 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. **Aims:** 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 while 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, 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. 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. **Conclusion:** 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

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 betel quid and this has been demonstrated as 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 the cancerous cells and the ability to invade tissue and/or metastasize to other sites, as well as acquiring the ability to

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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] Recently, color Doppler ultrasonography (CD-USG) has been used for detecting 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 indices (PIs) and resistivity indices (RIs). 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 compression Doppler ultrasonography in mapping of OSCC blood flow. The study was conducted in 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 the age range of 20–70 years. Out 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. After a detailed clinical history and clinical examination, CD-USG study was done and surgical intervention was carried out by incisional biopsy as indicated. The obtained biopsy specimens were submitted for histopathological examination and the final diagnosis was made and those which were OSCC as histopathologically confirmed diagnosis were included into the study and recorded in prescribed proforma. The clinical data thereafter were correlated with ultrasonographic findings. Color Doppler signals of diseased patients were compared with control group.

Inclusion criteria

- Clinically and histopathologically diagnosed cases with OSCC of buccal mucosa tongue and lip
- Patients between the age group of 20 and 70 years.

Exclusion criteria

- Squamous cell carcinoma of palate, alveolar mucosa, and gingival mucosa
- Recurrent cases of OSCC
- Patients suffering from 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.

CD-USG Examination: Ultrasonographic investigation of each mass was carried out using Philips EnVisors C Series of ultrasonogram [Figure 3] with linear transducer probe at a frequency of 7.5 MHz. Experienced and qualified sonologist of Department of Radiodiagnosis, who was unaware of clinical data and blinded about the cases performed ultrasonographic examination. During the ultrasonographic examination, 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 color Doppler 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, color Doppler mapping of the entire mass was done to detect 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°). Color Doppler gain was increased until 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 4-6]. 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 the flow signals in the tumor for spectral wave analysis. At least three vessels were sampled, and the measurements were repeated at least

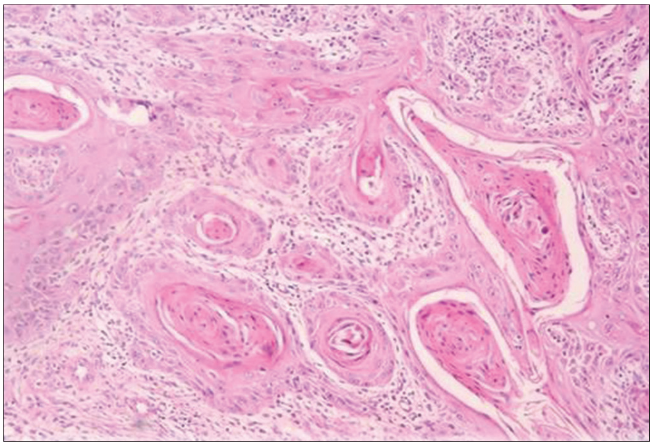


Figure 1: Photomicrograph showing well differentiated squamous cell carcinoma of a patient with malignancy

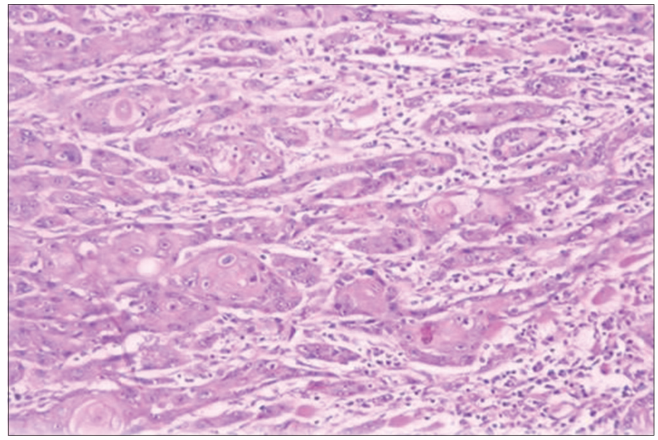


Figure 2: Photomicrograph showing moderately differentiated squamous cell carcinoma of patient with malignancy

Figure 3: Color Doppler ultrasound machine

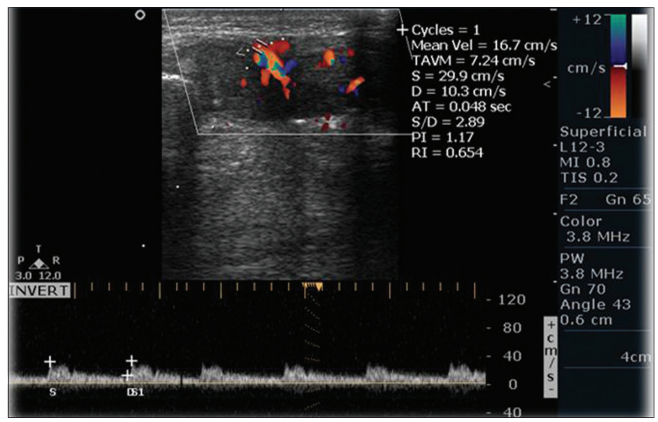


Figure 4: Photograph showing color Doppler signals of a patient with malignancy

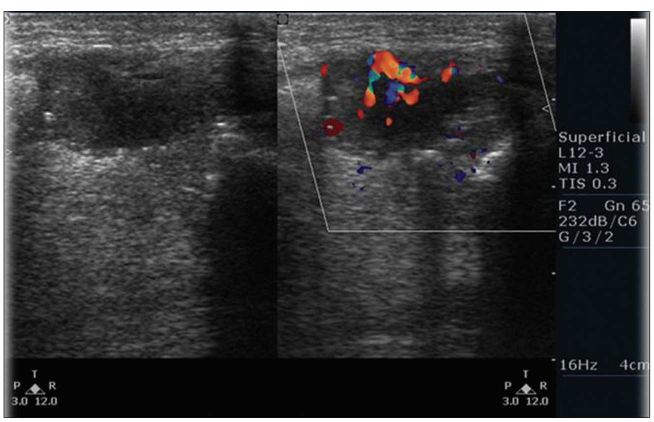


Figure 5: Photograph showing color Doppler signals of another patient with malignancy

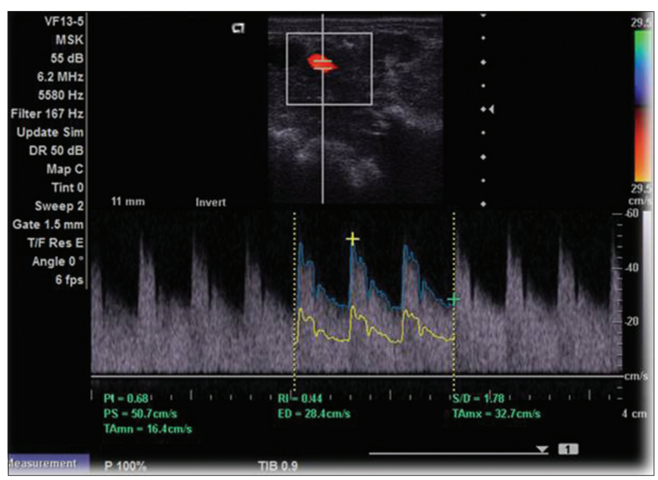


Figure 6: Photograph showing color Doppler signals of another patient with malignancy

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 7].

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 − ESV)/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°, by 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 were correlated with ultrasonographic findings. Both the clinical and CD-USG findings were correlated with final diagnosis and data obtained were subjected to statistical evaluation.

Statistical methods employed

Descriptive statistical analyses (i.e., mean and standard deviation [SD]) and Student's unpaired *t*-test were carried out for all the groups in this study. Analysis was done by using Windows GraphPad Prism 4 software and SPSS version 14.0 software.

RESULTS

For this single blind cross-sectional study, 60 subjects were selected randomly within the age range of 20–70 years and having the mean age of 50.06 years ±13.08 SD for patients with malignancy to 41.03 years ±8.58 SD for the control group. There were 21 male and 9 female patients enrolled in the study group with a male to female ratio of 2.25:1, while in case of control group, 20 male and 10 female patients were included with the male to female ratio being 2:1. Out of the 30 patients with malignancy, 23 (76.7%) patients were having lesions irt buccal mucosa, 5 (16.7%) irt lip and 2 (6.7%) patients revealed malignancy of tongue. Among them, 14 (46.67%) patients were having a duration of lesion between 1 and 4 months, 10 (33.33%) patients between 5 and 8 months, 2 (6.67%) patients between 9 and 12 months, and 4 (13.33%) were having a frank lesion between 13–16 months. On history elicitation, 10 patients (33.33%) were having history of habit between 1–10 years, 6 (20%) were having between 11 and 20 years, 7 (23.33%) between 21 and 30 years, 5 (16.67%) for 31–40 years, and 2 (6.67%) were having habit since 41–50 years. According to the degree of differentiation noticed on histopathological examination, 19 (63.3%) lesions were well differentiated squamous cell carcinoma, 9 (30%) were moderately differentiated squamous cell carcinoma, while 2 (6.7%) lesions came out to be poorly differentiated squamous cell carcinoma. The role of color Doppler sonography 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 was installed in the computer of color Doppler machine for the calculation of PI and RI indices used in the formula as under:

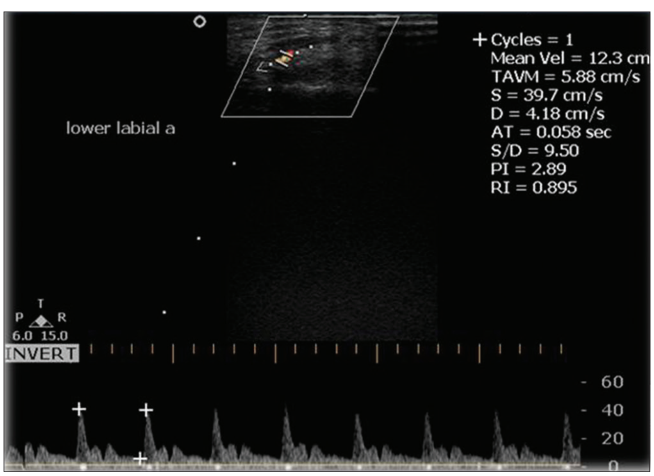


Figure 7: Photograph showing color Doppler signals of a subject selected from control group (right buccal mucosa)

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

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

Pourcelot' resistivity index

Table 1 shows a comparison of RI in patients with malignancy and the control group with cut-off value described. In this table, 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. The cut-off value was taken as 0.5. The *P* value came out to be 0.0001, which was highly significant.

Table 1: Comparison of RI in patients with malignancy and the control group with cut-off value

Group	*n*	Descriptive statistics Mean±SD				SEM
Malignant	30	0.40±0.14				0.02
Normal	30	0.83±0.07				0.01
Student's unpaired *t*-test						
t	df	*P*	Mean difference	SE difference	95% CI of the difference: Lower	Upper
14.408	58	0.000 significant, *P*<0.05	−0.42	0.02	−0.48	−0.36
Cut-off value	Patients with malignancy (%)	Control group (%)	χ^2	*P*		
≤0.50	22 (73.3)	0 (0)	25.71	0.0001 significant		
>0.50	8 (26.7)	30 (100)				
Total	30 (100)	30 (100)				

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

Graph 1 reveals comparison of RI in patients with malignancy and the control group.

Gosling's pulsatility index

Table 2 shows a comparison of PI in patients with malignancy and the control group with cut-off value described. In this table, 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. The cut-off value was taken as 1. The *P* value came out to be 0.0001, which was highly significant.

Graph 2 reveals comparison of PI in patients with malignancy and the control group.

Table 3 shows a comparison of PSV in m/s in patients with malignancy and the control group. The mean value in patients with malignancy came out to be 31.72 ± 13.82 whereas in the control group, it was 43.87 ± 20.95.

Table 2: Comparison of PI in patients with malignancy and the control group with cut-off value

Group	*n*	Descriptive statistics Mean±SD	SEM
Malignant	30	0.86±0.20	0.03
Normal	30	2.61±0.77	0.14

Student's unpaired *t*-test

t	df	*P*	Mean difference	SE difference	95% CI of the difference Lower	95% CI of the difference Upper
11.95	58	0.000 significant, *P*<0.05	−1.74	0.14	−2.03	−0.36

Cut-off value	Patients with malignancy (%)	Control group (%)	χ^2	*P*
≤1.00	26 (86.67)	0 (0)	25.71	45.88
>1.00	4 (13.33)	30 (100)		*P*<0.0001
Total	30 (100)	30 (100)		significant

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

Table 3: Comparison of peak systolic velocity in patients with malignancy and the control group

Group	*n*	Descriptive statistics Mean±SD	SEM
Malignant	30	31.72±13.84	2.52
Normal	30	43.87±20.95	3.82

Student's unpaired *t*-test

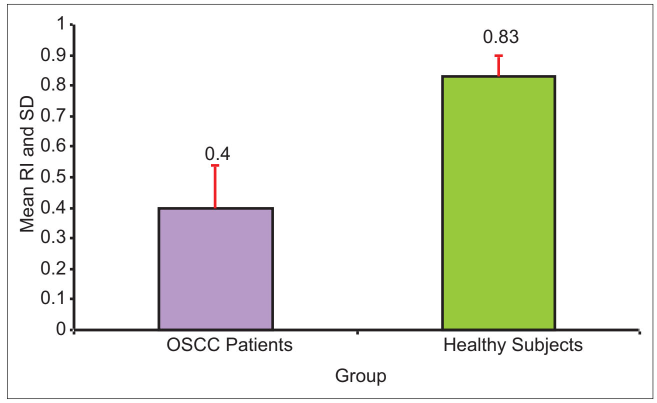
t	df	*P*	Mean difference	SE difference	95% CI of the difference Lower	95% CI of the difference Upper
2.65	58	0.010 significant, *P*<0.05	−12.15	4.58	−21.33	−2.97

SE: Standard error, CI: Confidence interval, SD: Standard deviation, SEM: Standard error mean

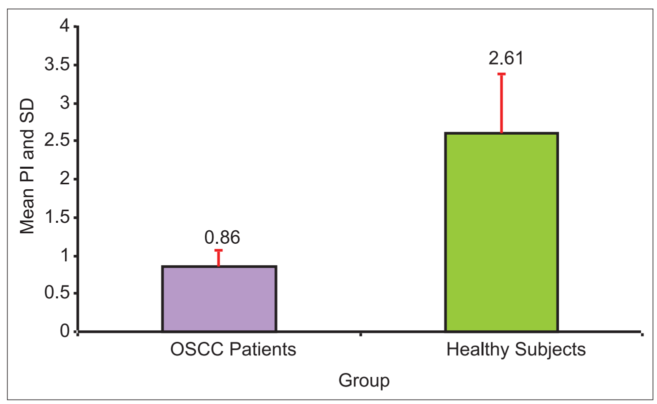
Graph 3 reveals comparison of PSV in patients with malignancy and the control group.

Similarly, Table 4 shows a comparison of EDV in m/s in patients with malignancy and the control group. The mean value in patients with malignancy came out to be 10.33 ± 5.21 whereas in the control group, it was 7.07 ± 3.44.

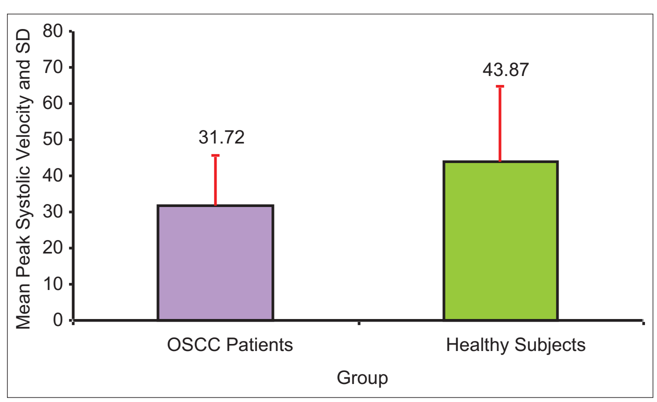
Graph 4 reveals comparison of EDV in patients with malignancy and the control group.



Graph 1: Comparison of resistivity index in patients with malignancy and the control group



Graph 2: Comparison of pulsatility index in patients with malignancy and the control group



Graph 3: Comparison of peak systolic velocity in patients with malignancy and the control group

Graph 5 reveals sensitivity, specificity, positive, and negative predictive value and accuracy of the test. With sensitivity being 73.33%, specificity being 100%, positive predictive value being 100% and the negative predictive value being 78.95%, the accuracy of the test came out to be 86.66%.

Graph 6 reveals sensitivity, specificity, positive, and negative predictive value and accuracy of the test. With sensitivity being 86.67%, specificity being 100%, positive predictive value being 100%, and the negative predictive value being 88.24%, the accuracy of the test came out to be 93.33%.

Table 5 and Graph 7 show the correlation of clinical size of the tumor with RI in the patients with malignancy. The *P* value in this case came out to be 0.87.

Table 6 and Graph 8 show the correlation of clinical size of the tumor with PI in the patients with malignancy. The *P* value in this case came out to be more than 0.05.

USG correctly identified lymph nodes in 30 patients' neck. While performing USG of palpable 44 lymph nodes, additional 12 lymph nodes were discovered as they were located in a clinically inaccessible region or were deep-seated. After USG evaluation of these lymph

Table 4: Comparison of end diastolic velocity in patients with malignancy and the control group

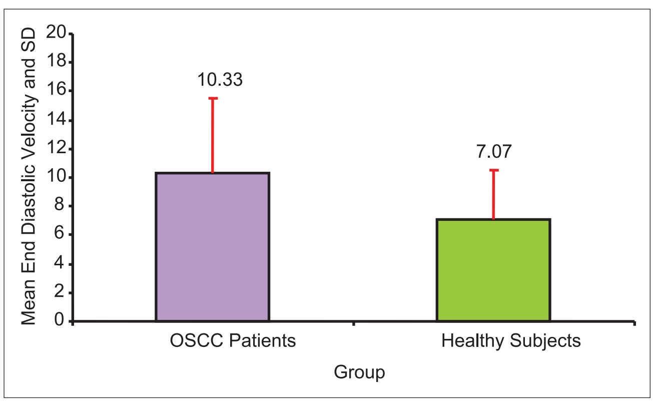
Group	n	Descriptive statistics Mean±SD	SEM			
Malignant	30	10.33±5.21	0.95			
Normal	30	7.07±3.44	0.62			
Student's unpaired *t*-test						
t	df	*P*	Mean difference	SE difference	95% CI of the difference	
					Lower	Upper
2.86	58	0.006 significant, $P<0.05$	3.26	1.14	0.97	5.54

SE: Standard error, CI: Confidence interval, SD: Standard deviation, SEM: Standard error mean

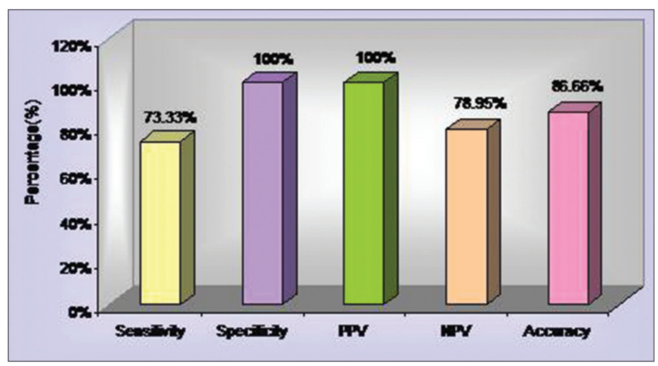
Table 5: Correlation of clinical size of tumor with RI in patients with malignancy

	n	Spearman's rank correlation coefficient Mean±SD	ρ	P
RI	30	0.40±0.14	0.049	0.797 NS, $P>0.05$
Clinical size of tumor	30	4.59±1.83		

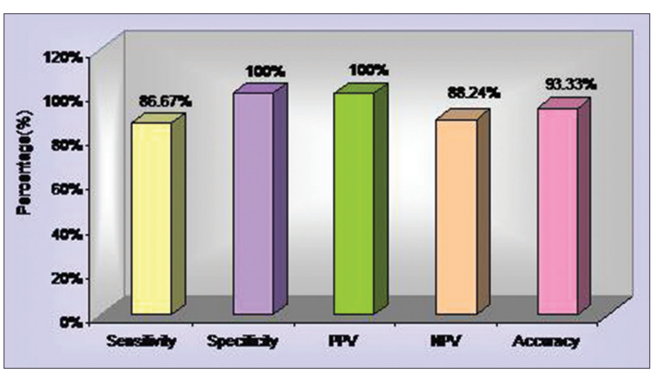
SD: Standard deviation, RI: Resistivity index, NS: Not significant



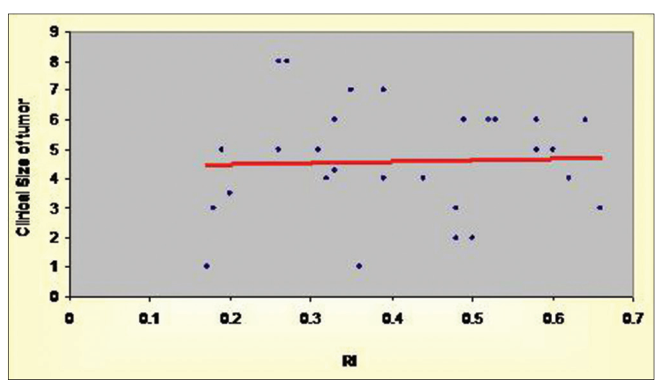
Graph 4: Comparison of end diastolic velocity in patients with malignancy and the control group



Graph 5: Comparison of percentage of patients in terms of sensitivity, specificity, positive and negative predictive values, and accuracy in patients with malignancy and the control group



Graph 6: Comparison of percentage of patients in terms of sensitivity, specificity, positive and negative predictive values, and accuracy in patients with malignancy and the control group



Graph 7: Correlation of clinical size of tumor with resistivity index in patients with malignancy

nodes, the total lymph nodes included in the study were 56 (44 ± 12). Out of 56 lymph nodes, 42 lymph nodes (75%) were having intact hilum whereas 14 showed loss of hilum architecture (25%).

Table 7 and Graph 9 show the distribution of patients according to the size of primary tumor and their correlation with central necrosis of their tumor mass. It was significant with T3 and T4 size as *P* value came out to be <0.0.

DISCUSSION

The mean age of patients in the present study was 50.06 ± 13.08. The reason for a higher proportion of patients with habits is probably related to the easy availability, low cost, and sociocultural acceptance. The present study consisted of 30 cases diagnosed with malignant oral ulcers, of which 21 were male and 9 were female patients, with a male to female ratio being 2.25:1. In a study conducted by Ascani *et al*. in 2005,[7] there were 46 males and 15 females with OSCC with male to female ratio being 3.06:1. The most common site for the occurrence of OSCC in the present study was buccal mucosa. Twenty-three (76.7%) patients were having malignancy irt buccal mucosa, five irt lip, and two irt tongue (6.7%). The reason for increased incidence of OSCC of buccal mucosa in India may be attributed to the habit of placement of tobacco + betel nut + lime quid/pan with tobacco/Pan masala/Gutka in buccal vestibule.[8] Similar results were obtained in the study conducted by Sankaranarayanan *et al*. on oral cancer patients.[9] In their study, buccal mucosa was the most common site and accounted for more than 60% of the OSCC patients. 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 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 thus chosen to assess the resistance of the vessels in malignant masses and healthy buccal mucosae. The malignant tumors, with their characteristic low-impedance flow, had a lower PI, RI, and PSV, and a higher EDV than did healthy masses. Given that neovascularization is an obligate event in malignant change, this recognition may enable us to observe the earliest stages in oncongenesis. 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. The cut-off value was taken as 0.5. Mean value for PI in patients diagnosed with malignant ulcers was 0.86 ± 0.20 whereas for healthy subjects, it was 2.61 ± 0.77. The cut-off value was taken as 1. These findings are in agreement with the previous reports that a low-impedance Doppler flow signal is associated with malignant tumors in other organs.[12-15] This difference in the distal impedance between the neo-vascularized tumor vessels

Table 6: Correlation of clinical size of tumor with PI in patients with malignancy

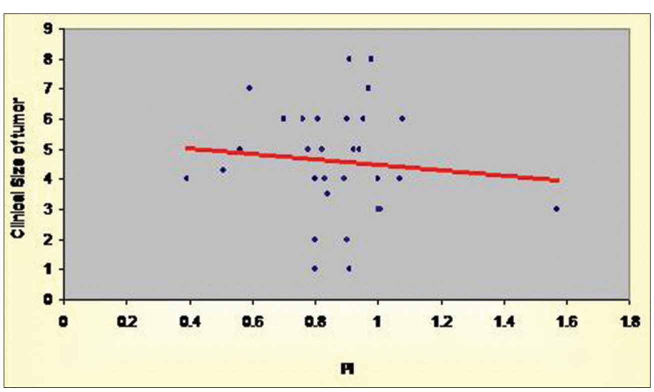
	n	Spearman's rank correlation coefficient Mean±SD	*ρ*	*P*
PI	30	0.86±0.20	−0.102	0.592 NS,
Clinical size of tumor	30	4.59±1.83		*P*>0.05

SD: Standard deviation, PI: Pulsatility index, NS: Not significant

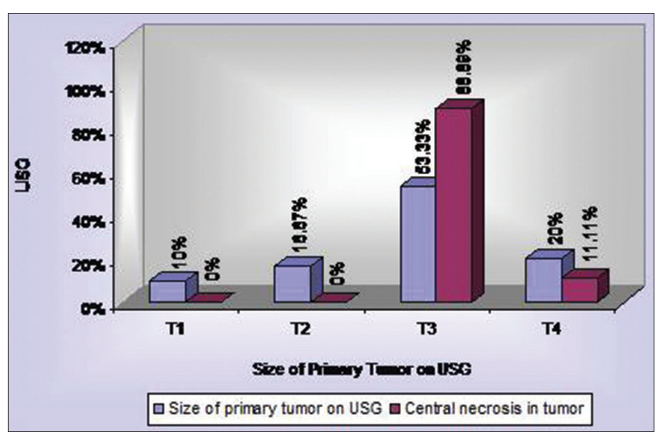
Table 7: Distribution of patients according to size of primary tumor and their correlation with central necrosis of their tumor mass

	Size of primary tumor on USG (%)	Central necrosis in tumor (%)	χ^2
T1	3 (10.00)	0 (0.00)	38.74
T2	5 (16.67)	0 (0.00)	*P*<0.0001
T3	16 (53.33)	8 (88.89)	significant
T4	6 (20.00)	1 (11.11)	
Total	30 (100)	9 (100)	

USG: Ultrasonography



Graph 8: Correlation of clinical size of tumor with pulsatility index in patients with malignancy



Graph 9: Distribution of patients according to size of primary tumor and their correlation with central necrosis of primary tumor mass

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. 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 imply a potential role CD-USG in determining oral malignancies. For an undiagnosed lesion in the oral cavity, the low impedance flow signal seen on color Doppler sonograms suggests a high probability of the lesion being malignant.[12-15] 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. 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 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 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. We recommend a multi-institutional study to investigate the multiple vascular assessments to determine the role of CD-USG in the preoperative prediction of the 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.

Color Doppler flow imaging provides 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 decrease in blood flow in a malignant tumor after chemotherapy or irradiation may also be useful for predicting the response of a tumor to the treatment.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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