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RESEARCH ARTICLE

"THE RELATIONSHIP BETWEEN VASCULARITY WITHIN CERVICAL LYMPH NODES AND LYMPH NODE SIZE AMONG ORAL CANCER PATIENTS USING COLOUR DOPPLER ULTRASOUND"

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ABSTRACT

Background: Colour Doppler Ultrasonography (CDUS) has increased amount of information that can be obtained during examination of the cervical lymph nodes. Vascularity of the lymph node helps in diagnosing the cause of lymphadenopathy as vascularity is directly related to the actual pathology such as oral cancer. The aim of this study was to quantitatively evaluate the relationship between vascularity of lymph nodes and lymph node size on Color Doppler Ultrasound images of the patients with oral cancer. **Material and Methods:** 30 subjects taken from the cancer hospital with the age range of 20 to 75 years. CDUS evaluation of the cervical lymph nodes was performed on every patient. The vascular index defined as the number of pixels in the flow signals divided by the number of pixels in the whole lymph node, presented as a percentage. The number of isolated flow signal units in the lymph node parenchyma was counted by using Adobe Photoshop CS5 extended version. Isolated flow signal units of <3 pixels were excluded as noise signals. The scattering index defined as the number of isolated flow signal units in the lymph node parenchyma. The following lymph node size classification was considered for this study i.e. Group 1, 4-5.9 mm; Group 2, 6-7.9 mm; Group 3, 8-9.9 mm; and Group 4, ≥10 mm (median diameter, 12 mm; range, 10-27 mm). **Results:** Pair-wise comparison of mean Vascular Index between Lymph Node size classifications and mean Scattering Index between Lymph Node size classifications was done. There was a statistically significant difference seen in the values of Vascular Index between all Lymph Node (LN) classification ($p<0.05$) with the highest value with Group 1, followed by Group 2 then Group 3 and least with Group 4 and there was a statistically highly significant difference in the mean Scattering Index (SI) with various LN classification ($p<0.01$) with the highest value with Group 4, followed by Group 3 then Group 2 and least with Group 1. **Conclusions**: The metastatic lymph node size increases, the **Vascular Index (VI)** of metastatic lymph nodes decreases. Lymph node size of metastatic lymph nodes has an indirect relationship with the Vascular Index (VI). Thus, an increase in the vascular index was considered to be a useful Doppler ultrasound finding for detecting metastasis in small lymph nodes Thus, the CDUS could provide a potential means of visualizing and sampling suspicious carcinoma lesions in high-risk populations.

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INTRODUCTION

Oral Cancer is the commonest cancer worldwide. In the Asian subcontinent which comprises of India and Sri Lanka, oral cancer accounts for about 30 to 40% of all malignancies (Rao, 1998). It is the sixth most common neoplasm in the world (Stell and Maran's). The incidence of oral cancers, its anatomical complexity, esthetic outcomes in the basic functions of mastication, deglutition and speech makes it the most challenging of head and neck tumor management. Thus, to reduce the morbidity and mortality associated with advanced stages of oral cancer early detection is must.

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Lymphadenopathy refers to lymph nodes that are abnormal either in size or character of lymph nodes and is caused by invasion or propagation of either inflammatory cells or neoplastic cells into the node (Ferrer, 1998). One of the most common findings in head and neck disorders is Cervical Lymphadenopathy**.** Since the Cervical lymph nodes are frequently involved by inflammatory processes and from squamous cell carcinoma from head and neck cancer or primary lung cancer, they are considered to be different from other lymph nodes (Chang et al., 1994). Cervical Lymphadenopathy may be the only clinical finding or one of the several findings. They are common primary site for lymphoma and secondary site for metastasis from oral and extra oral malignancies. The analysis of the lymph node status is essential as it provides direction for treatment and prognosis

of the disease. There are numerous methods for diagnosing the etiology and source of lymphadenopathy. It is mandatory to determine presence or absence of cancer in neck nodes for cancer bearing patients because the prognosis and the therapeutic steps for patients depend on the staging of the cancer (Som, 1992; Carew, 2003). There are various methods to detect nodal involvement. Out of which, palpation to detect nodal involvement is considered to be inaccurate. The other characteristics such as site, size, growth patterns and angioinvasion can occur in a patient on whom palpation has been used as only tool in examining the lymph nodes in the neck for metastasis (Baatenburg, 1989). Imaging today plays an essential role in the evaluation of disease in the cervical lymph nodes and should be part of any thorough investigation of patients with head and neck cancers. Continuous advancement of imaging techniques lead to the increasing sensitivity of imaging modalities in the detection of lymph nodes.

Ultrasound is one of the useful imaging modality for evaluation of cervical lymphadenopathy. It is non invasive and inexpensive tool causing little to no discomfort to the patient. It is easily available and doesn't use radiation (Ishikawa, 1983). One of the advancement of Ultrasound is Colour Doppler ultrasound (CDUS). Colour Doppler Ultrasound can be used for any organ including lymph node to evaluate vascular status. Colour Doppler Ultrasonography (CDUS) has increased amount of information that can be obtained during examination of the cervical lymph nodes. The presence of intranodal vascularity, its distribution and estimation of the intravascular resistance and spectral Doppler analysis are evaluated with Colour Doppler. CDUS analyzes intranodal architecture by the assessment of visible vascular patterns or by Doppler spectral analysis of intranodal vessels. The intranodal vascular patterns using CDUS can be used to diagnose enlarged cervical lymph nodes. The pattern of vascular flow and presence of high intranodal vascular resistance have been used as key features to differentiate benign from malignant nodes (Ahuja et al., 2001). Vascularity of the lymph node helps in diagnosing the cause of lymphadenopathy as vascularity is directly related to the actual pathology present within the lymph node.[9] It was assumed that there was no relationship between the vascularity and lymph node size.

Aim and objectives: The aim of this study was to quantitatively evaluate the relationship between vascularity of lymph nodes and lymph node size on Color Doppler Ultrasound images of the patients with oral cancer.

MATERIALS AND METHODS

This cross-sectional study includes (n=30) subjects taken from the cancer hospital with the age range of 20 to 75 years. A written informed consent was obtained from all patients who participated in this study. A prior Institutional Ethical Committee approval was taken.

Inclusion criteria for patient's selection were:

- Patients with oral squamous cell carcinoma which was histopathologically confirmed.
- Patients with suspected metastatic cervical lymph nodes after clinical examination.

Exclusion criteria of the study were

- Those cases which showed cervical lymph node enlargement due to acute infection without any clinical evidence of oral or extraoral malignancy.
- Patients who had shown small lymph nodes with a short axis diameter of <3mm on ultrasound images were excluded from this study.

Patients were subjected to detailed history taking on the basis of customized case history proforma and clinical examination was done. Each patient was evaluated with CDUS for cervical lymphadenopathy. CDUS evaluation of the cervical lymph nodes was performed.

Machine specifications for CDUS evaluation: Corevision, Diagnostic ultrasound system, Philips model SSA-350, a linear transducer of L12-3 MHz and a cardia gel were used for CDUS procedure

Procedure for CDUS examination: All the examinations were conducted in the dark room with the patient lying in a desired position for the site of concerned cervical lymph nodes. Gel was applied over the neck area and transducer was placed parallel to cervical lymph nodes and was placed in both longitudinal and transverse plane. An adequate observation of the lymph node with the gray scale sonography was done then the Color Doppler examination was performed. The size, Color Doppler flow pattern and vascular resistance were studied. Data was acquired in digital imaging and communications in medicine (DICOM) and transferred to the server of a picture-archiving and communication system (PACS). The data was converted from DICOM to Joint Photographic Experts Group (JPEG) format and was transferred to a personal computer using a CD rom. The JPEG images were analyzed with application software (Adobe Photoshop CS5 extended version). Lymph node images of the maximum cut surface were analyzed with two indices, a vascular index and a scattering index. The vascular index indicated the abundance of blood flow on a Doppler ultrasound image. The scattering index indicated the degree of vessel discontinuity presented on a Doppler Ultrasound image. The methods for measuring these two indices are as follows:

- The boundary line between the lymph node and the background was drawn free-hand and the numbers of pixels in the extracted whole lymph node were counted.
- Flow signals within the lymph node were chosen by color assignment and the number of pixels for the chosen flow signals was counted.
- The vascular index defined as the number of pixels in the flow signals divided by the number of pixels in the whole lymph node, presented as a percentage. The number of isolated flow signal units in the lymph node parenchyma was counted. Isolated flow signal units of <3 pixels were excluded as noise signals.
- The scattering index defined as the number of isolated flow signal units in the lymph node parenchyma.

The following lymph node size classification was considered for this study i.e. Group 1, 4-5.9 mm; Group 2, 6-7.9 mm; Group 3, 8-9.9 mm; and Group 4, ≥10 mm (median diameter, 12 mm; range, 10-27 mm).[10] Different criteria considered in CDUS of the lymph nodes were the color flow signals or perfusion. The presence of peripheral flow suggested a

malignant nature. Histopathological diagnoses of the same lymph nodes were made from excisional biopsy to confirm the etiology of the lymph node enlargement, which were performed within a week of CDUS examination.

Statistical procedures: Data obtained was compiled on a MS Office Excel Sheet (v 2010) and was subject to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Comparison of vascular index (VI) and scattering index (SI) with lymph nodes codes was done using Kruskall Wallis ANOVA. For the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

The distribution of the 30 subjects' according to the gender. Out of which, 20 (66.7%) were males and 10 (33.3%) were females [Table 1]. The number and percentage of the distribution of subjects as per lymph node size classification were analysed which suggested that the majority of lymph nodes belonged to Group 1 (36.7%) and least in Group 3 (10%)[Table 2].

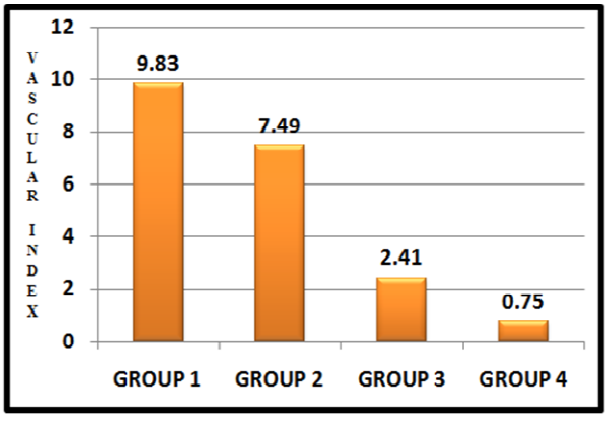
Table 1. Distribution of subjects by gender

GENDER	FREQUENCY	PERCENT
Males	20	66.7
Females	10	33.3
Total	30	100.0

Table 2. Distribution as per lymph node size classification

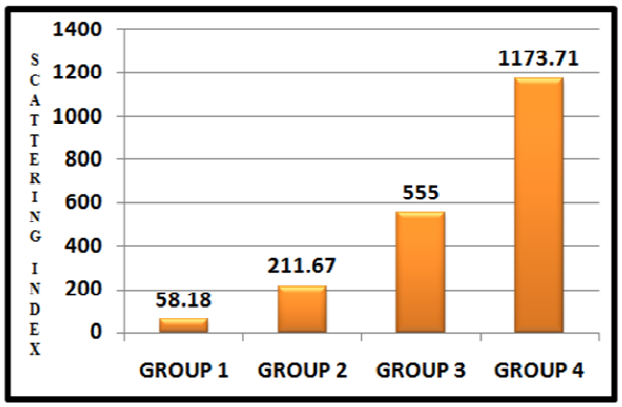
GROUPS	FREQUENCY	PERCENT
GROUP 1	11	36.7
GROUP 2	9	30.0
GROUP 3	3	10.0
GROUP 4	7	23.3
Total	30	100.0

GRAPH 3a: COMPARISON OF VASCULAR INDEX (VI) WITH LYMPH NODE (LN) CLASSIFICATION



Pair-wise comparison of mean Vascular Index between Lymph Node size classifications and mean Scattering Index between Lymph Node size classifications was done. There was a statistically significant difference seen in the values of Vascular Index between all Lymph Node (LN) classification ($p<0.05$) with the highest value with Group 1, followed by Group 2 then Group 3 and least with Group 4. [Table 3a and Graph 3a] and there was a statistically highly significant difference in the mean Scattering Index (SI) with various LN classification ($p<0.01$) with the highest value with Group 4,

GRAPH 3b: COMPARISON OF SCATTERING INDEX (SI) WITH LYMPH NODE (LN) CLASSIFICATION



followed by Group 3 then Group 2 and least with Group 1.[Table 3b and Graph 3b]

DISCUSSION

Recent advances in the field of radio diagnosis provide good opportunity for the evaluation of cervical lymphadenopathy. There are various imaging techniques like Ultrasound, Computed Tomography and Magnetic Resonance Imaging which have proved efficient in detection of enlarged nodes. Diagnostic imaging plays an additional important role in the evaluation of lymph nodes. Continuous advancement of techniques lend to increasing sensitivity of the imaging modalities. The Doppler ultrasound images have been used as **a** diagnostic modality to provide useful information about vascularity and blood flow distribution within lymph nodes (Kagawa, 2011). The present study was carried out to quantitatively evaluate the relationship between vascularity of lymph nodes and lymph node size on Colour Doppler Ultrasound images of the patients with oral cancer with age range of 20 to 75 years with mean age of 47.76 years. Table 1 showed distribution of subjects by gender. A total of 30 patients with cervical lymphadenopathy were included of which there were 20 (66.66%) male and 10 (33.33%) female**.**

The results were in accordance to the study conducted by Asthana et al. (2016) Table 2 showed distribution as per lymph node size classification. Out of 30 patients, maximum patients, 11 (36.7%) were in Group I with the diameter between 4-5.9 mm lymph node; 9 patients (30%) were in Group 2 with the diameter of 6-7.9mm of lymph nodes, followed by Group 4 which showed 7 patients (23.3%) with diameter of ≥10 mm of lymph nodes and lastly only 3 patients (10 %) in Group 3 with the diameter of 8-9.9 mm of lymph nodes. In our study, comparison of Vascular Index (VI) and Scattering Index (SI) with lymph nodes (LN) size classification was carried out (Table 3). There was a statistically significant difference ($p<0.05$) seen in the values of (VI) between all (LN) size classification with the highest value with Group 1, followed by Group 2 then Group 3 and least with Group 4. The vascular index of metastatic lymph nodes was highest in Group 1 where the size of the lymph node was smallest. As the lymph node size increased the vascular index of metastatic lymph nodes decreased. The vascular index of metastatic lymph nodes was lowest in Group 4 where the size of the lymph node is largest. There was a statistically highly significant difference when the comparison of mean (VI) with (LN) size classification was done with the p value <0.05.

Table 3a. Pair-wise comparison of mean Vascular Index between Lymph Node size classifications

LYMPH NODE SIZE CLASSIFICATION	N	VASCULAR INDEX MEAN	Std Deviation	Median	Mean rank	Chi square	P value of KW ANOVA
GROUP 1	11	9.83	6.363	12.73	14.10	6.352	<0.05*
GROUP 2	9	7.49	4.269	9.42	10.76		
GROUP 3	3	2.41	1.042	2.33	3.55		
GROUP 4	7	0.75	0.688	0.79	1.54		
TOTAL	30	20.48	12.362				

Table 3b. Comparison of mean Scattering Index between Lymph Node size classifications

LYMPH NODE SIZE CLASSIFICATION	N	Scattering index mean	Std. Deviation	Median	Mean rank	Chi square	p value of KW ANOVA
GROUP 1	11	58.18	39.686	69	7.73		
GROUP 2	9	211.67	124.355	246	13.89		
GROUP 3	3	555.00	19.975	277	22.00	22.558	0.000**
GROUP 4	7	1173.71	137.380	1012	27.00		
Total	30	414.20	459.176				

* = statistically significant difference ($p<0.05$)

** = statistically highly significant difference ($p<0.01$)

= non significant difference ($p>0.05$)

The result was similar to studies conducted by Herman PG and Giovagnorio F [13] et al who stated that; there is hypervascularity within the metastatic lymph nodes. Also, there was a statistically highly significant difference in the mean Scattering Index (SI) with various Lymph Node (LN) size classification ($p<0.01$) with the highest value with Group 4, followed by 3 then Group 2 and least with Group 1. The results were in accordance to the study conducted by Kagawa T et al [10] which stated that as there was increase in metastatic lymph node size, the vascular index decreased and scattering index increased. The scattering was seen more in larger metastatic lymph nodes than smaller metastatic lymph nodes because the increased number of blood vessels produced by angiogenesis is displaced by the growth of tumor cells, forcing the blood vessels to move around or through tumor cells.

Limitations and Scope

Present study analyzed the relationship between vascularity within cervical lymph nodes and lymph node size among oral cancer patients using Colour Doppler ultrasound. However, the limitation of the study is that the sample size was small. Thus, for further evaluation and future scope, more studies with a larger sample size needs to be carried out.

Conclusion

From the above study, it can be concluded that **as** the metastatic lymph node size increases, the Vascular Index (VI) of metastatic lymph nodes decreases. Lymph node size of metastatic lymph nodes has an indirect relationship with the Vascular Index (VI). Thus, an increase in the vascular index was considered to be a useful Doppler ultrasound finding for detecting metastasis in small lymph nodes and as the metastatic lymph node size increases, the Scattering Index (SI) of metastatic lymph nodes also increases. Lymph node size of metastatic lymph nodes has a direct relationship with the Scattering Index (SI). Thus, the CDUS could provide a potential means of visualizing and sampling suspicious carcinoma lesions in high-risk populations.

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