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Development of a biomarker of efficacy in second-line treatment for lymphangioma of the tongue: a pilot study

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Abstract

Lymphangioma of the tongue is a rare lymphatic malformation, and various authors have reported the successful use of sirolimus for its treatment. However, the safety of sirolimus in children needs further evaluation so that those who do not respond are not necessarily exposed to its potential adverse effects. We hypothesised that assessment of lymphangiogenesis can be used to predict whether the patient will respond to sirolimus, so we organised a prospective study after ethics committee approval had been given. After clinical and histological diagnoses of lymphangioma of the tongue had been confirmed, 16 patients were given sirolimus 0.8 mg/day in three divided doses. Clinical response was assessed and compared with lymphatic microvessel density (LMVD), which was calculated immunohistochemically using the monoclonal antibody D2-40 as the lymphatic endothelial marker. Nine patients responded well, five partially, and two failed to respond. Mean (SD) LVD among the good responders was 21.00 (3.74), whereas among non-responders it was 8.00 (4.24). There was a significant difference in mean LVD between good responders, partial responders, and non-responders ($p = 0.04$). Sirolimus is effective in treating children with lymphangioma of the tongue, and lymphangiogenesis is a useful therapeutic predictive marker.

Keywords: Lymphangioma tongue; vascular malformation; sirolimus; Lymphangiogenesis

Introduction

A lymphangioma is an anomaly of the lymphatic vessels. Oral lymphangiomas are usually located on the anterior two-thirds of the tongue, but can also involve the buccal mucosa, gingiva, palate, and lips.[1] Different treatments (which include operation, sclerotherapy, lasers, and steroids) have been used with variable results.[2] Lasers, intralesional bleomycin, and steroids are the most common first-line treatments, but most of the lesions are refractory to these.[1–3]

Following reports about the efficacy of sirolimus in complicated vascular anomalies in children,[3] various authors have reported its successful treatment of lymphangioma of the tongue.[4–7] We think that even though the evidence of its safety is growing, we need to know more before using the drug in children, as it should not be used as a blanket treatment for complicated vascular anomalies. Its use in children is further restricted by the fact that there is no effective biomarker that can predict its effect beforehand, and one of the ways of restricting it to children who will respond to the treatment is to develop an effective biomarker that can predict the response to treatment.

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We hypothesised that assessment of lymphangiogenesis could be used as a biomarker for response to treatment, and our aim was to assess the role of sirolimus as second-line treatment in patients with lymphangioma of the tongue and to evaluate the use of lymphangiogenesis as a biomarker.

Material and methods

The study was done in the Department of Paediatric Surgery and Oral Surgery between May 2016 and October 2017, and approved by the Institutional Review Board. All the patients with lymphangioma of tongue aged under 12 years during the study period were included, and written informed consent was obtained from a parent or guardian. The diagnosis was made by clinical examination and incisional biopsy. They were screened for infective foci by clinical examination, total blood counts, and radiographs of the chest. Patients with fever, cough, or contagious foci were initially excluded from the study, but were included when they had recovered. All the patients had received previous first line treatment with one or other treatment including intralesional bleomycin (n = 12), partial excision (n = 2), and intralesional triamcinolone (n = 2). Three patients showed a partial response to intralesional bleomycin, but none of the other treatments was effective. These patients were included in the study, as sirolimus was started at least six months after any previous intervention to remove any confounding response.

Sirolimus was given in a dose of 0.8 mg /day in three divided doses with trimethoprim-sulfamethoxazole as prophylaxis. Patients were followed regularly weekly with clinical examination and total blood count for the first four weeks and then monthly. All patients were photographed before the start of the treatment and then at each monthly visit.

The response was assessed clinically by two observers using serial photographs taken at an interval of four weeks. Response to treatment was graded as good (>75%), partial (75%-25%), or none (<25%). The two observers assessed the response according to the criteria without knowing which treatment the patient had. Each observer made their assessment twice. Both intrarater and inter-rater reliability were calculated as a measure of reproducibility. Observations were transformed into binary outcomes and Cohen's Kappa coefficient (k) was calculated for the first and second set of observations by the first observer (intrarater reliability) and also between the findings of two observers (inter-rater reliability).

The LMVD was calculated by immunohistochemical examination of the formalin-fixed blocks of the incision biopsies at the onset of the study (Appendix 1). Archival tonsillar tissue was used as a positive control. Lymphatic vessels were defined as the vessels that had endothelium that had immune positivity to D2-40, and had a lumen. The sections were scanned at low magnification (x 40), and LMVD was then calculated by counting all D2-40 immunostained vessels.[8] Two independent observers made LMVD counts that were later compared with the clinical response. Each rater made two readings, and the mean LMVD was calculated from these.

Table 1
Comparison of mean lymphatic microvascular density with clinical responses to sirolimus.

Clinical response	Mean (SD) lymphatic microvascular density	p value
Good	21.50 (2.03)	
Partial	12.25 (4.78)	0.04
None	8.37 (4.77)	

Statistical analysis

Data were coded and summarised using IBM SPSS for Windows (version 23.0, IBM Corp). The Mann-Whitney U test was used to compare means and medians of quantitative data, and for qualitative data the chi squared test was used. Linear regression analysis was used to study the relation between the LVD and clinical response. Probabilities of <0.05 were accepted as significant.

Results

Sixteen patients were enrolled in the study. The most common complaint at presentation was difficulty in swallowing, followed by a burning sensation, and bleeding. All 16 patients were treated with sirolimus as the second line of treatment according to the protocol. Nine patients responded well (Figs. 1 and 2), five partially, and two did not respond. All patients had symptomatic relief in the form of less of a burning sensation and less bleeding. The Cohen's Kappa coefficient (k) for intrarater reliability was 0.58, indicating good agreement. For inter-rater reliability, the Cohen's Kappa coefficient was 0.75 indicating an excellent agreement between the first and second observer for clinical response. The median (range) follow up after completion of treatment was 17 (12-24) months.

The median (range) of the mean of two readings by each rater was 19.75 (10.87 - 21.62) for the first rater, and 18.25 (11.12 - 22.00) for the second. The distribution in the two groups was similar (Mann-Whitney U = 127, p = 0.97, two-tailed). The mean (SD) LMVD among good responders was 21.00 (3.74) with large, well-defined, lymphatic channels that stained uniformly for D2-40 (Fig. 3) whereas the mean LVD among those who did not respond was 8.00 (4.24) with ill-defined channels and patchy uptake of D2-40 (Fig. 4). The difference in mean LVD among the three groups was significant (p = 0.04) (Table 1). On linear regression analysis the coefficient of determination (R^2) was 0.71, suggesting that the clinical response and lymphangiogenesis are associated.

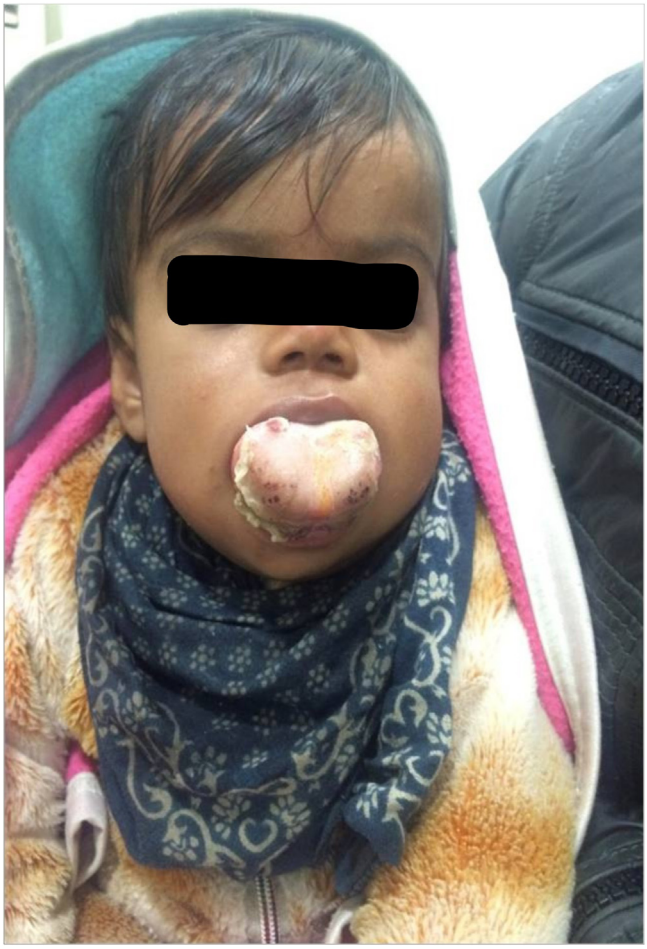


Fig. 1. A good responder to sirolimus before treatment. Published with the permission of the parents.

Discussion

Lymphangioma of the tongue is a rare lymphatic anomaly of the tongue that varies in presentation and severity. Histopathologically it has malformed dilated lymphatic channels (either solitary or grouped) that contain lymph or blood.[8,9] The International Society for the Study of Vascular Anomalies (ISSVA) classified these anomalies as lymphatic malformations, [10] which are categorised as microcystic, macrocystic, and mixed according to the size of the cyst based on the radiographic findings.[11]

Published studies have proved the efficacy and safety of sirolimus in the treatment of children with vascular malformations.[12,13] One of our patients developed superficial ulceration on the buccal mucosa after two weeks of treatment, so the sirolimus was discontinued for two weeks and later restarted with no recurrence. The response in bulky lesions (most patients) was more obvious than in flat lesions, but they also responded well in terms of symptoms such as burning sensation and bleeding.

D2-40 is a specific lymphatic endothelial marker and has been used for assessment of lymphangiogenesis.[14] LVD has been shown to be a useful tool for the evaluation of lymphatic proliferation,[14] so we used it for specific staining of lymphatic endothelium and calculation of LVD. The more the proliferation of vessels, the better the response to sirolimus, so biomarkers like LMVD (which can predict the response to sirolimus) can be used to limit its use in children who respond to the treatment.

The main limitation of our study was the lack of an objective assessment of response to treatment. Ultrasound was not

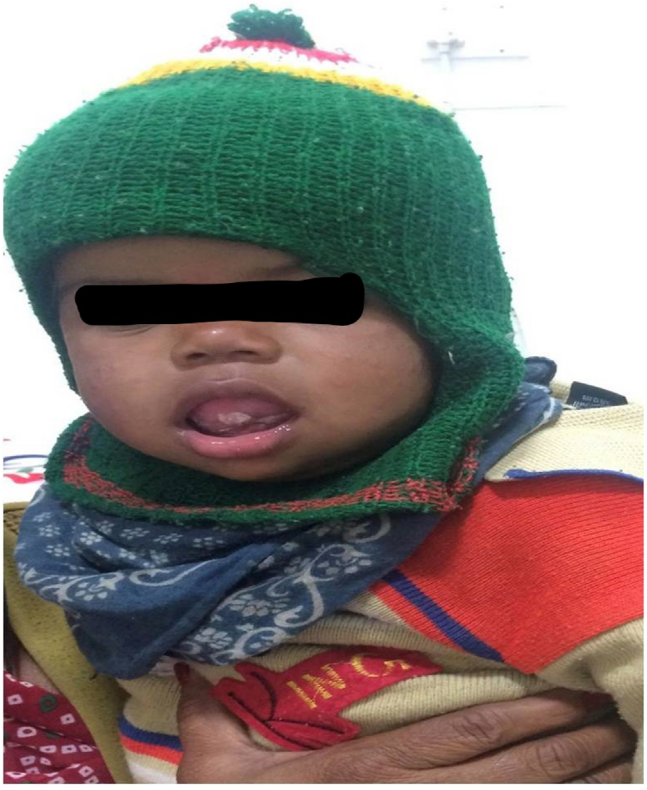


Fig. 2. A good responder to sirolimus after treatment. Published with the permission of the parents.

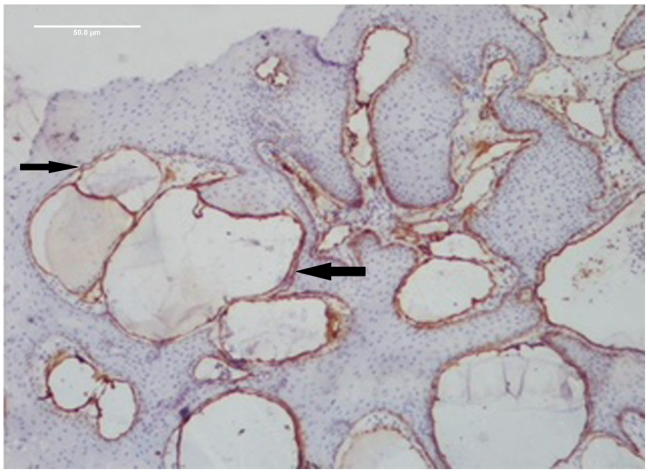


Fig. 3. Well-defined, D2-40-stained, lymphatic channels in a good responder to sirolimus.

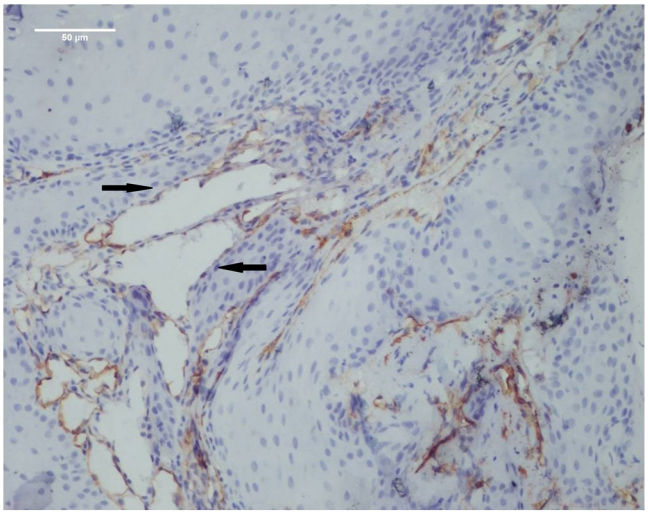


Fig. 4. Poorly-defined, D2-40 stained, lymphatic channels in a patient who did not respond to sirolimus.

possible because of the difficulty in using an ultrasound probe on the tongue. Magnetic resonance imaging was also difficult because of the requirement to sedate children with an already-compromised upper airway. We tried to take photographs in a standard manner, but it was not possible in all the cases, which was also a limitation.

Conclusion

Sirolimus is an effective, safe, second-line treatment for children with lymphangioma of the tongue. Lymphangiogenesis is a valuable biomarker for the therapeutic response to sirolimus in these children. This study also opens up an area of research where other markers can be developed in patients with different complicated vascular anomalies.

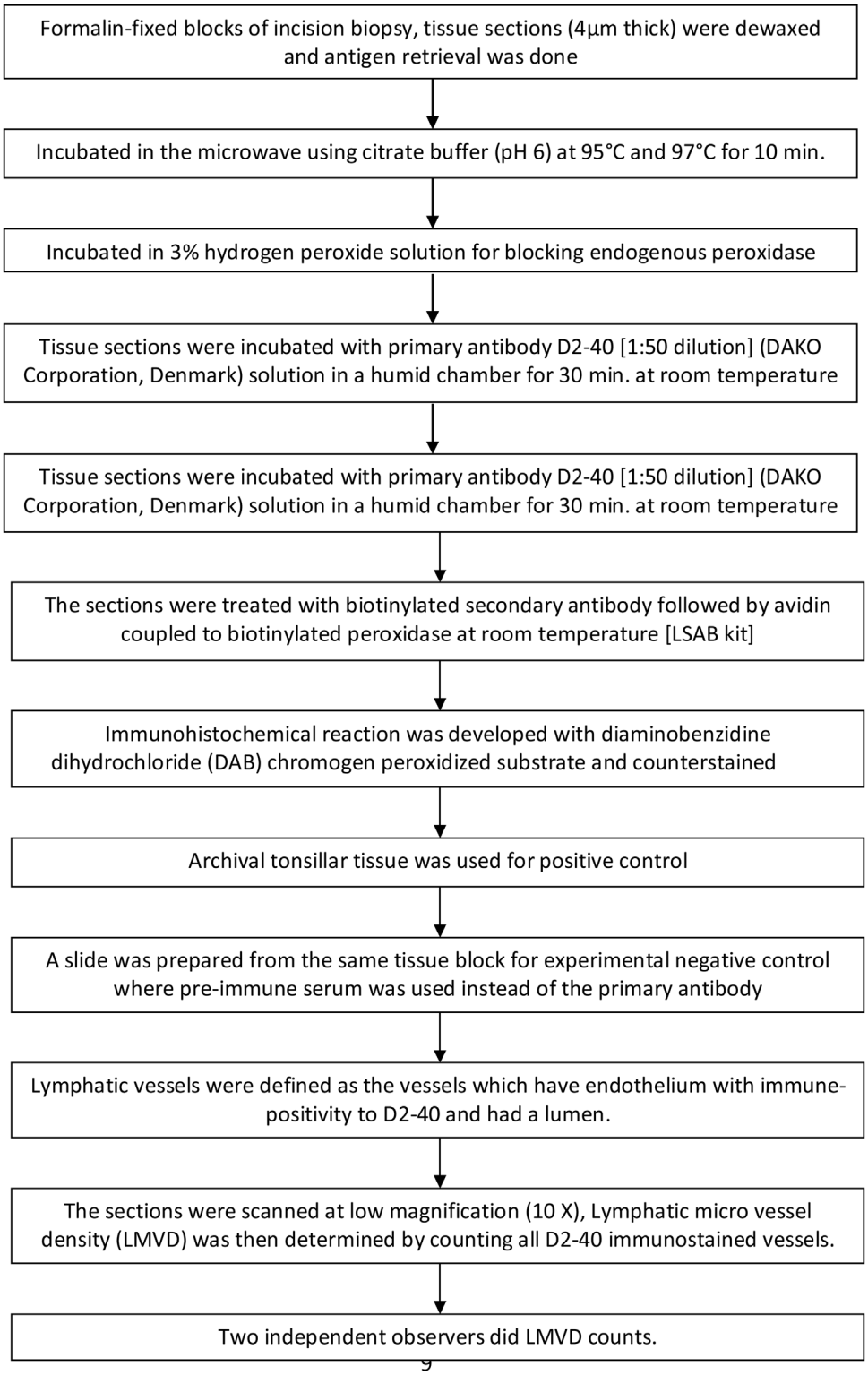
Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

The study was approved by the Institute Ethics Board, and the IRB approval number is N. Dean/2016-17/EC/023. The patients' parents or guardians gave their permission for publication.

Appendix 1 Immunohistochemistry



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