

Maxillary Labial Frenum Morphology and Midline Diastema Among Children Aged 3-12 Years- A Cross-Sectional Study

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ABSTRACT

Dentofacial aesthetics plays a major role in a child's development and affects the child's psychology. Therefore, early identification of Midline diastema is crucial. This study aims to evaluate the Maxillary labial frenum morphology and associate it with Midline diastema among children aged 3-12 years in Chennai. A total of 100 children were evaluated and grouped. The Morphology of frenum and Midline diastema was assessed according to sewerin's typology. Data was tabulated and exported to SPSS version 20.0, IL, Chicago, USA for analysis. Simple frenum (60%) was predominant in terms of morphology, followed by Persistent tectolabial frenum (28%). Male children were common (52%). Children aged 7-9 years were 48%, followed by children aged 3-6 years (32%). 36% of the children had Midline diastema. Frenum morphology and Midline diastema had a statistically significant association ($p=0.00$, $p<0.05$). Most children with a Persistent tectolabial frenum had Midline diastema (20%). Age groups and Midline diastema also showed significant association ($p=0.00$, $p<0.05$). 25% of the children aged 3-6 years had midline diastema, whereas 10-12 year olds had the least (2%). Therefore, it can be concluded that Morphology of the Maxillary labial frenum is associated with Midline diastema.

KEY WORDS: FRENUM; LABIAL FRENUM; MIDLINE DIASTEMA; MORPHOLOGY OF FRENUM; PERSISTENT TECTOLABIAL FRENUM; SIMPLE FRENUM.

INTRODUCTION

The lips and the cheeks are attached to the Gingiva or the underlying periosteum by the labial frenum which is a fold of mucous membrane (Marques et al., 2006). The main function of a frenum is to provide stability

to the lips and tongue to which it is attached (Newman and Takei, 2006). Various freni present in the oral cavity are namely, Maxillary labial frenum, Mandibular labial frenum, and Lingual frenum (Mintz, Siegel and Seider, 2005). The perception of people in society and the psychological well being of an individual is greatly influenced by dentofacial aesthetics (Jonathan et al., 2018). Any abnormality in frenum morphology has always led to orthodontic treatment complications and relapse of treatment (Edwards, 1977).

The types of maxillary labial frenum morphology was given and classified by Sewerin's typology which is as follows - (Sewerin, 1971)

- Simple frenum
- Simple frenum with appendix

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Persistent tectolabial frenum
Simple frenum with nodules
Frenum with nichum
Double frenum
Bifid frenum

Miller states that the frenum should be considered as pathogenic when it is unusually large and wide with little or no area of attached gingiva along the midline. It is also considered abnormal if there is a shift in interdental papilla when it is shifted (Miller, 1985).

Our department is passionate about child care, we have published numerous high quality articles in this domain over the past 3 years (Govindaraju, Jeevanandan and Subramanian, 2017a, 2017b; Panchal, Gurunathan and Shanmugaavel, 2017; Ravikumar, Jeevanandan and Subramanian, 2017; Jeevanandan and Govindaraju, 2018; Nair et al., 2018; Ravikumar et al., 2018, 2019; Ravindra et al., 2018, 2019; Subramanyam et al., 2018; Vishnu Prasad et al., 2018; Jeevanandan, Ganesh and Arthilakshmi, 2019; Ramadurai et al., 2019; Ramakrishnan, Dhanalakshmi and Subramanian, 2019; Veerale Panchal, Jeevanandan and Subramanian, 2019; Vignesh et al., 2019; V. Panchal, Jeevanandan and Subramanian, 2019; Samuel, Acharya and Rao, 2020). With this inspiration we planned to pursue research on Maxillary labial frenum morphology and its association with Midline diastema.

The main aim of the study are as follows :

1. To estimate the prevalence of different morphologic types of Maxillary labial frenum among children aged 3–12 years.
2. To assess the association between Frenum morphology and Midline diastema in children.
3. To assess the association between Age and Midline diastema in children.

MATERIAL AND METHODS

For the study, 100 children belonging to 3–12 years were grouped into 3-6 years, 7-9 years and 10-12 years.

Inclusion criteria: Children in the age group of 3-12 years with normal dentition for their age group .

Exclusion criteria: Children with no systemic illness, no restorations done in the anterior teeth and children who haven't undergone orthodontic or orthopaedic treatment such as Rapid maxillary expansion or Milwaukee brace (Agarwal and Mathur, 2010).

Armamentarium: Mouth mirror, probe, tweezers, mouth masks, gloves, cotton roll, light source.

Study setting: General clinical examination for the children was done by the principal researcher. Instruments were sterilised. The type of morphology was evaluated under visual light according to Sewerin's typology. Data was tabulated.

Statistical analysis: Microsoft Excel 2016 data spreadsheet was used to collect data and later exported to the Statistical package for social science for windows (SPSS version 20.0, IL, Chicago, USA) for analysis. The distribution percentages were obtained following which associations were assessed using Chi square tests.

RESULTS AND DISCUSSION

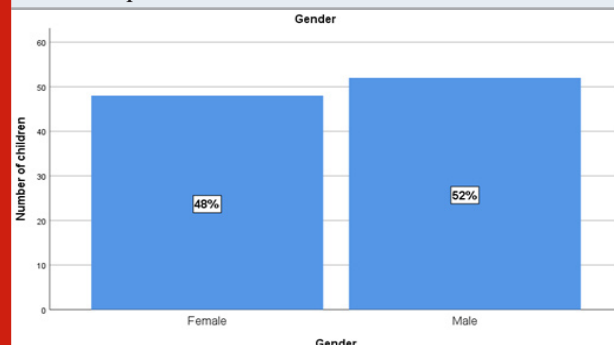
The final data set consisted of 100 children within the range of 3-12 years who were assessed for Maxillary labial frenum morphology and Midline diastema.

Children belonging to the age group of 7-9 years were 48%, followed by children aged 3-6 years (32%), whereas 10-12 year olds were the least among the study population (20%). (Table 1) Male children were more in number among the study population (52%). (Figure 1).

Table 1. Shows children within the age group of 7-9 years (48%), followed by children aged 3-6 years (32%), whereas 10-12 year olds were the least among the study population (20%).

Age groups (in years)	Number of Children (in percentages)
3-6	25.2
7-9	40.1
10-12	34.7

Figure 1: Shows the gender distribution of the study population (N=100). X axis represents the gender and Y axis represents the number of children. Male children were more compared to female children.



Simple frenum was predominant in terms of morphology (60%), followed by Persistent tectolabial frenum (28%). Bifid frenum and double frenum were not observed. (Figure 2) 36% of the children were diagnosed with Midline diastema. (Figure 3) Frenum morphology and Midline diastema showed a statistically significant association ($p=0.00$, $p<0.05$). Most children with a Persistent tectolabial frenum had Midline diastema (20%), followed by those with Simple frenum (12%), only 2% of children with a Simple frenum, either with appendix and nodules were observed to have midline diastema. (Figure 4).

Figure 2: bar chart depicts the morphology of the labial frenum among the study population (N=100). X axis represents the frenum morphology and Y axis represents the number of children. Most common type of morphology was the Simple frenum (Red), followed by Persistent tectolabial frenum (Blue), whereas Simple frenum with nodules (Orange) were the least common. There were no children with bifid and double frenum.

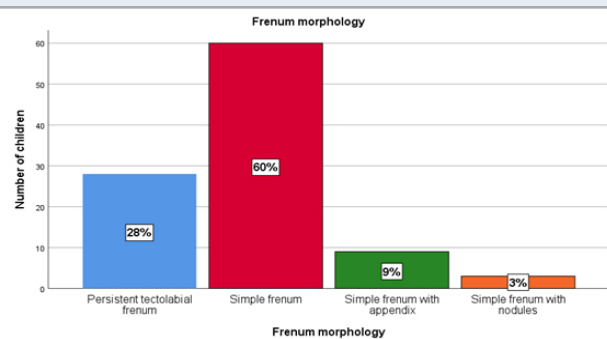
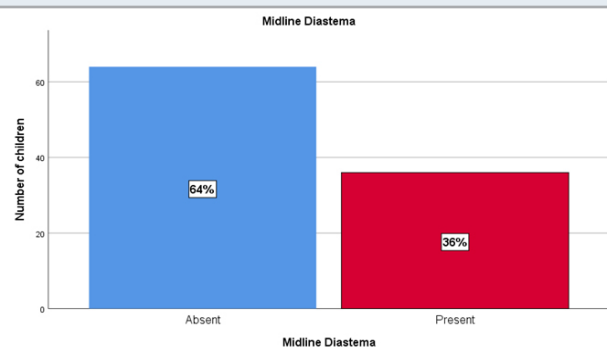


Figure 3: bar chart depicts Midline diastema among the study population (N=100). X axis represents the Midline diastema and Y axis represents the number of children. 36% of the children were diagnosed with Midline diastema.



Age of the children and Midline diastema showed a statistically significant association ($p=0.00$, $p<0.05$). 25% of the children aged 3-6 years had Midline diastema, whereas 10-12 year olds had the least occurrences (2%). (Figure 5).

Sewerin's typology was used in classifying the frenum (Figure 2), and it was found that the most prevalent morphologic type of maxillary labial frenum was the Simple type with a prevalence of 60%, followed by Persistent tectolabial frenum with a prevalence of 28%. Simple with appendix and simple with nodules were seen in 9% and 3% of children respectively. Similar studies by Díaz-Pizán et al and Braga et al (D'iaz-Piz'an, Lagrav'ere and Villena, 2006; Braga et al., 2007), also had similar results where the Simple type of upper labial frenum showed the highest prevalence followed by the Persistent tectolabial. Simple with nodule, Simple with appendix, Double, and frenum with nichum and Bifid were uncommon.

Figure 4: Bar graph depicting the association between Frenum morphology and Midline diastema. X-axis represents the type of morphology of the Maxillary labial frenum and y-axis represents the number of children. Blue depicts absence of Midline diastema, whereas red depicts the presence of Midline diastema. There was a statistically significant association between the Maxillary labial frenum morphology and the presence of Midline diastema (Pearson Chi-Square test, p value- 0.000, $p<0.05$). Most children with a Persistent tectolabial frenum had Midline diastema (20%), followed by those with simple frenum (12%), only 2% of children with a Simple frenum, either with an appendix or nodule were observed to have midline diastema.

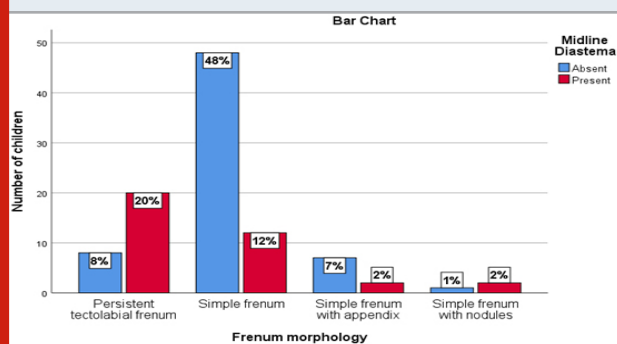
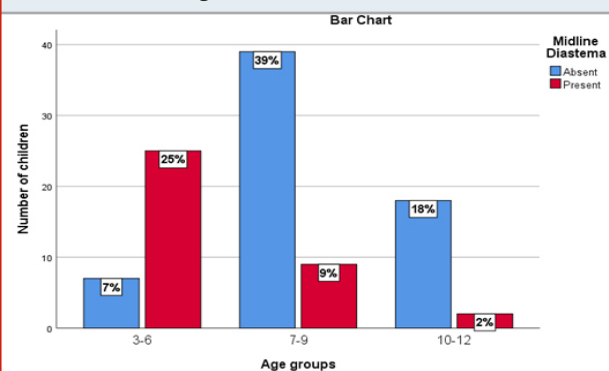


Figure 5: Bar graph depicting the association between Age groups and Midline diastema. X-axis represents the age groups and y-axis represents the number of children. Blue depicts absence of Midline diastema, whereas red depicts the presence of Midline diastema. There was a statistically significant association between the presence of Midline diastema and age of the children (Pearson Chi-Square test, p value- 0.000, $p<0.05$). 25% of the children aged 3-6 years had Midline diastema, whereas 10-12 year olds had the least (2%). (Figure 5).



In previous studies conducted by Kakodkar et al and Thosar et al (Kakodkar, Patel and Patel, 2008; Thosar et al., 2017), the persistent tectolabial frenum type had the maximum potential to become a contributing factor in children with persistent midline diastema. The present study (Figure 4), also shows that most children with Midline diastema had this type of frenum (20%) There are studies which also prove otherwise, for example a study

performed by Bowsiya et al, concludes that the frenal attachment has an association with Midline diastema (Bowsiya and Arjunkumar, 2019) (21). Therefore, there are variations in association of level of insertion of frenum with midline diastema but persistent tectolabial frenum seen among children aged 3-6 years of age had a positive association with midline diastema .

On assessing the occurrence of Midline diastema among different age groups (Figure 3, Figure 5, 5), 36% of the children had Midline diastema, and the highest prevalence was observed among children aged 3-6 years (25%), whereas 10-12 years old had the least. These findings are consistent with the results of the study conducted by Rajani et al (Rajani, Biswas and Emmatty, 2018). Studies conducted by Bergström et al, Popovich et al, Taylor and Weyman, (Taylor, 1939; Weyman, 1967; Bergström, Jensen and Mårtensson, 1973; Popovich, Thompson and Main, 1977) also suggested that Midline diastema decreases with increasing age of the individual. Another study conducted by Richardson et al (Richardson et al., 1973), concluded that children aged 6 years had the highest prevalence of Midline diastema, which is also within the range of 3-6 years, proving that the prevalence observed in the present study is coherent with other studies. In a study conducted by Sagar et al, among children aged 15-25 years in the South Indian population, there were no incidences of double frenum which is similar to that of the present study (Sagar, Heraldsherlin and Moses, 2016).

Midline diastema occurring due to eruption of permanent canines are commonly seen as the ugly duckling stage among children aged 8-11 years, and is highly unlikely to occur in the age group of 3-6 years. Since 3-6 years of age is the predominant age group observed to have Midline diastema in this study and most children had a persistent tectolabial frenum attributing to their Midline diastema. It can be concluded that the persistent tectolabial frenum type occurring among 3-6 year old children can cause Midline spacing if left untreated.

Previous studies suggest that the level of frenal attachment migrates upwards with age. Current literature also suggests that orthodontists now consider frenectomy only after the orthodontic treatment is complete but persistent abnormal labial frenum causes midline diastema (Boutsis and Tatakis, 2011).

CONCLUSION

Within the limitations of the present study, Simple type of frenum was more prevalent. Out of children with Midline diastema in the sample (36%), 20% was found to be associated with Persistent tectolabial frenum. Frenum morphology and age of the children were both statistically associated with Midline diastema ($p=0.00$, $p<0.05$). Children aged 3-6 years of age had the highest occurrences of midline diastema (25%). Therefore, utmost care must be taken to eliminate persistent midline diastema at the earliest.

Conflict Of Interests: No conflict of interests

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REFERENCES

- Agarwal, A. and Mathur, R. (2010) 'Maxillary expansion', *International journal of clinical pediatric dentistry*. Jaypee Brothers Medical Publishing (P) Ltd., 3(3), p. 139. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4993819/>.
- Bergström, K., Jensen, R. and Mårtensson, B. (1973) 'The effect of superior labial frenectomy in cases with midline diastema', *American journal of orthodontics*. Elsevier, 63(6), pp. 633-638. doi: 10.1016/0002-9416(73)90188-7.
- Boutsis, E. A. and Tatakis, D. N. (2011) 'Maxillary labial frenum attachment in children', *International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children*. Wiley Online Library, 21(4), pp. 284-288. doi: 10.1111/j.1365-263X.2011.01121.x.
- Bowsiya, S. and Arjunkumar, R. (2019) 'PREVALENCE OF LABIAL FRENUM ATTACHMENT AND FRENAL MORPHOLOGY AMONG PATIENTS REPORTING TO SAVEETHA DENTAL COLLEGE', *International Journal of Clinical Dentistry*. search.ebscohost.com, 12(3). Available at: <https://bit.ly/32RIGp3>
- Braga, A. T. et al. (2007) 'Descrição da morfologia dos frênulos labiais superiores em escolares de Teresina', *Rev Cir Traumatol Buco-Maxilo-Fac. revistacirurgiabmf.com*, 7(3), pp. 59-64. Available at: <http://www.revistacirurgiabmf.com/2007/v7n3/8.pdf>.
- D'iaz-Piz'an, M. E., Lagrav'ere, M. O. and Villena, R. (2006) 'Midline Diastema and Frenum Morphology in the Primary Dentition', *Journal of dentistry for children*. ingentaconnect.com, 73(1), pp. 11-14. Available at: <https://www.ingentaconnect.com/content/aapd/jodc/2006/00000073/00000001/art00003>.
- Edwards, J. G. (1977) 'The diastema, the frenum, the frenectomy: A clinical study', *American journal of orthodontics*. Elsevier, 71(5), pp. 489-508. doi: 10.1016/0002-9416(77)90001-X.
- Govindaraju, L., Jeevanandan, G. and Subramanian, E. M. G. (2017a) 'Comparison of quality of obturation and instrumentation time using hand files and two rotary file systems in primary molars: A single-blinded randomized controlled trial', *European journal of dentistry*, 11(3), pp. 376-379. doi: 10.4103/ejd.ejd_345_16.
- Govindaraju, L., Jeevanandan, G. and Subramanian, E. M. G. (2017b) 'Knowledge and practice of rotary instrumentation in primary teeth among indian dentists: A questionnaire survey', *Journal of International Oral Health*, 9(2), p. 45. doi: 10.4103/jioh.jioh_4_17.

- Jeevanandan, G., Ganesh, S. and Arthilakshmi (2019) 'Kedo file system for root canal preparation in primary teeth', *Indian journal of dental research: official publication of Indian Society for Dental Research*, 30(4), pp. 622–624. doi: 10.4103/ijdr.IJDR_238_18.
- Jeevanandan, G. and Govindaraju, L. (2018) 'Clinical comparison of Kedo-S paediatric rotary files vs manual instrumentation for root canal preparation in primary molars: a double blinded randomised clinical trial', *European archives of paediatric dentistry: official journal of the European Academy of Paediatric Dentistry*, 19(4), pp. 273–278. doi: 10.1007/s40368-018-0356-6.
- Jonathan, P. T. et al. (2018) 'Maxillary labial frenum morphology and midline diastema among 3 to 12-year-old schoolgoing children in Sri Ganganagar city: A cross-sectional study', *Journal of the Indian Society of Pedodontics and Preventive Dentistry*. Medknow Publications and Media Pvt. Ltd., 36(3), p. 234. doi: 10.4103/JISPPD.JISPPD_51_18.
- Kakodkar, P., Patel, T. and Patel, S. (2008) 'Clinical assessment of diverse frenum morphology in', *Internet Journal of Dental Science*. pdfs.semanticscholar.org, 7(2). Available at: <https://pdfs.semanticscholar.org/178c/56aa668595a7e39dedfd6dcfd9c5f84b88e9.pdf>.
- Marques, L. S. et al. (2006) 'Malocclusion: Esthetic impact and quality of life among Brazilian schoolchildren', *American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics*. Elsevier, 129(3), pp. 424–427. doi: 10.1016/j.ajodo.2005.11.003.
- Miller, P. D., Jr. (1985) 'The Frenectomy Combined with a Laterally Positioned Pedicle Graft: Functional and Esthetic Considerations', *Journal of periodontology*. Wiley Online Library, 56(2), pp. 102–106. doi: 10.1902/jop.1985.56.2.102.
- Mintz, S. M., Siegel, M. A. and Seider, P. J. (2005) 'An overview of oral frena and their association with multiple syndromic and nonsyndromic conditions', *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. Elsevier, 99(3), pp. 321–324. doi: 10.1016/j.tripleo.2004.08.008.
- Nair, M. et al. (2018) 'Comparative evaluation of post-operative pain after pulpectomy with k-files, kedo-s files and mtwo files in deciduous molars -a randomized clinical trial', *Brazilian Dental Science*, 21(4), p. 411. doi: 10.14295/bds.2018.v21i4.1617.
- Newman, M. G. and Takei, H. H. (2006) 'Periodontal plastic and esthetic surgery. Carranza's Clinical Periodontology'. Philadelphia: Saunders.
- Panchal, V., Gurunathan, D. and Shanmugaavel, A. K. (2017) 'Smartphone application as an aid in determination of caries risk and prevention: A pilot study', *European journal of dentistry*, 11(4), pp. 469–474. doi: 10.4103/ejd.ejd_190_17.
- Panchal, V., Jeevanandan, G. and Subramanian, E. (2019) 'Comparison of instrumentation time and obturation quality between hand K-file, H-files, and rotary Kedo-S in root canal treatment of primary teeth: A randomized controlled trial', *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, 37(1), pp. 75–79. doi: 10.4103/JISPPD.JISPPD_72_18.
- Panchal, V., Jeevanandan, G. and Subramanian, E. M. G. (2019) 'Comparison of post-operative pain after root canal instrumentation with hand K-files, H-files and rotary Kedo-S files in primary teeth: a randomised clinical trial', *European archives of paediatric dentistry: official journal of the European Academy of Paediatric Dentistry*, 20(5), pp. 467–472. doi: 10.1007/s40368-019-00429-5.
- Popovich, F., Thompson, G. W. and Main, P. A. (1977) 'The maxillary interincisal diastema and its relationship to the superior labial frenum and intermaxillary suture', *The Angle orthodontist*. europepmc.org, 47(4), pp. 265–271. Available at: <https://europepmc.org/article/med/335923>.
- Rajani, E. R., Biswas, P. P. and Emmatty, R. (2018) 'Prevalence of variations in morphology and attachment of maxillary labial frenum in various skeletal patterns-A cross-sectional study', *Journal of Indian Society of Periodontology*. Wolters Kluwer--Medknow Publications, 22(3), p. 257. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6009156/>.
- Ramadurai, N. et al. (2019) 'Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial', *Clinical oral investigations*, 23(9), pp. 3543–3550. doi: 10.1007/s00784-018-2775-5.
- Ramakrishnan, M., Dhanalakshmi, R. and Subramanian, E. M. G. (2019) 'Survival rate of different fixed posterior space maintainers used in Paediatric Dentistry - A systematic review', *The Saudi dental journal*, 31(2), pp. 165–172. doi: 10.1016/j.sdentj.2019.02.037.
- Ravikumar, D. et al. (2018) 'DNA profiling of Streptococcus mutans in children with and without black tooth stains: A polymerase chain reaction analysis', *Dental research journal*, 15(5), p. 334. doi: 10.4103/1735-3327.240472.
- Ravikumar, D. et al. (2019) 'Evaluation of McNamara's analysis in South Indian (Tamil Nadu) children between 8-12 years of age using lateral cephalograms', *Journal of oral biology and craniofacial research*, 9(2), pp. 193–197. doi: 10.1016/j.jobcr.2018.06.001.
- Ravikumar, D., Jeevanandan, G. and Subramanian, E. M. G. (2017) 'Evaluation of knowledge among general dentists in treatment of traumatic injuries in primary teeth: A cross-sectional questionnaire study', *European journal of dentistry*, 11(2), pp. 232–237. doi: 10.4103/ejd.ejd_357_16.
- Ravindra, V. et al. (2018) 'A comparative evaluation between dermatoglyphic patterns and different terminal planes in primary dentition', *Journal of clinical and experimental dentistry*, 10(12), pp. e1149–e1154. doi: 10.4317/jced.55259.

- Ravindra, V. et al. (2019) 'A comparative evaluation between cheiloscopy patterns and the permanent molar relationships to predict the future malocclusions', *Journal of clinical and experimental dentistry*, 11(6), pp. e553–e557. doi: 10.4317/jced.55776.
- Richardson, E. R. et al. (1973) 'Biracial Study of the Maxillary Midline Diastema', *The Angle orthodontist*. Allen Press, 43(4), pp. 438–443. doi: 10.1043/0003-3219(1973)043<0438:BSOTMM>2.0.CO;2.
- Sagar, S., Heraldsherlin, J. and Moses, S. (2016) 'Morphological variation of abnormal maxillary labial frenum in South Indian population', *International Journal of Pharmaceutical Sciences and Research*. *International Journal of Pharmaceutical Sciences and Research*, 7(5), p. 2142.
- Samuel, S. R., Acharya, S. and Rao, J. C. (2020) 'School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial', *Journal of public health dentistry*, 80(1), pp. 51–60. doi: 10.1111/jphd.12348.
- Sewerin, I. (1971) 'Prevalence of Variations and Anomalies of the Upper Labial Frenum', *Acta odontologica Scandinavica*. Taylor & Francis, 29(4), pp. 487–496. doi: 10.3109/00016357109026535.
- Subramanyam, D. et al. (2018) 'Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries', *European journal of dentistry*, 12(1), pp. 67–70. doi: 10.4103/ejd.ejd_266_17.
- Taylor, J. E. (1939) 'Clinical observations relating to the normal and abnormal frenum labii superioris', *American journal of orthodontics and oral surgery*. Elsevier, 25(7), pp. 646–650. doi: 10.1016/S0096-6347(39)90066-9.
- Thosar, N. et al. (2017) 'Assessment of maxillary labial frenum morphology in primary, mixed, and permanent dentitions in Wardha district', *European Journal of General Dentistry*. Medknow Publications and Media Pvt. Ltd., 6(1), p. 14. doi: 10.4103/2278-9626.198601.
- Vignesh, R. et al. (2019) 'Management of Complicated Crown-Root Fracture by Extra-Oral Fragment Reattachment and Intentional Reimplantation with 2 Years Review', *Contemporary clinical dentistry*, 10(2), pp. 397–401. doi: 10.4103/ccd.ccd_671_18.
- Vishnu Prasad, S. et al. (2018) 'Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India', *Special care in dentistry: official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry*, 38(1), pp. 58–59. doi: 10.1111/scd.12267.
- Weyman, J. (1967) 'The incidence of median diastemata during the eruption of the permanent teeth', *The Dental practitioner and dental record*. ncbi.nlm.nih.gov, 17(8), p. 276. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/5228690>.