DOI: 10.7860/JCDR/2023/61707.17643

Review Article

Dentistry Section

Role of Animal Models in Periodontal Clinical Research and its Present-Day Status: A Narrative Review

MUSKAN BAHETI[1], KHUSHBOO DURGE[2], PAVAN BAJAJ[3], BHAIRAVI KALE[4], UNNATI SHIRBHATE[5]

ABSTRACT

The use of animal models have aided in the development of new information in periodontology research. Animal models enable legal acceptance of human welfare. Dogs, rats, ferrets, hamsters, mice and on rare occasions, rabbits and sheep have been used to study human periodontal diseases. Animal models were chosen because they have similar anatomical and physiological features of the oral cavity and periodontium, as well as the presence of causative agents that contribute to the occurrence of natural periodontal disease in humans. There has been a progression toward the development of a feasible and sufficiently accurate model that accurately reflects the true pathogenic mechanisms of living person periodontal disease. Non human primates have been used extensively in periodontal investigations as well as in medical technology to understand the origin of periodontal disease. Caries and calculus study is best accomplished through hamsters and rat. Periodontal disease and calculus formation in ferrets could be potential and encouraging in the research area. Thus, the structural and pathophysiology of the animal kingdom differs from that of human beings and seems sometimes troublesome with the latest therapies. Hamster stays an intriguing model for immunological studies. New possibilities in the periodontal analysis are now accessible, enabling broader cohorts that are easier to build. The goal of this review is to give an overview of the animal models that have been employed in the periodontal investigation. The purpose of this review is to identify the best animal model for periodontal research and also for the safety precautions for human beings. The use of fact-finding models used in periodontal disease is crucial to grasp the root source in the human being. Animal models are beneficial in periodontal surveys and an unavoidable step before accessing clinical testing with the latest biomaterials and therapies.

Keywords: Dogs, Hamsters, Host, Mice, Periodontal disease

INTRODUCTION

Periodontal disease is a highly persistent immunoinflammatory disease characterised by subgingival biofilms that comprise multiple potential periodontal pathogens all of which deteriorate periodontal structures [1]. Periodontitis is induced not only by microbial contamination but may also be linked to the host's vulnerability to the adverse effects of periodontal infection [2]. Individual differences in host responses play a notable role in the development of dental disease [3]. Although human cell growth is an effective method for duplicating certain components of the periodontal structure progression at the molecular level, there was a scarcity of information on the complex host immune response [4]. Periodontology research employs a variety of approaches, the most important of which is to utilise experimental periodontal models to investigate the origins of periodontal disorders. Numerous breeds were employed in the research of the development of periodontitis and to assess the disease's therapy alternatives [5].

In instances where tissue collection for histopathology is not widely accepted in humans, animal research is an excellent supplement to artificially inseminate the investigations prior to evaluating new clinical treatments. The most widely utilised species are monkeys, dogs, rodents, rabbits, ferrets, rats, pigs, and hamsters [5].

Criteria for Selecting Appropriate Animal Models

The study's objectives, along with lab constrictions such as keeping bigger or unusual animals, influence the selection of an experimentally induced model. Monkeys and dogs, which are massive species with moral and environmental concerns can only be used for the final phase of verification of prescribed therapies before they are used in human clinical care [5]. Animal models, such as rats as well as hamsters, are usually adequate for analysing the role of microbes, nutrition, or even other variables in periodontitis at the histopathologic level, which provides statistical validity and preclinical relevance [6]. For choosing appropriate animal models, the following criteria are mentioned in [Table/Fig-1] [7].

Appropriateness as a model
Data transferability
Microbes with hereditary homogeneity that can be used
Prior knowledge of biological properties
Price and availability
Ability to adapt the experimental intervention and ease of being used
Environmental effects
Moral consequences
Finding generalisation

[Table/Fig-1]: Criteria for selection of animal models [7].

Animal Models for the Analysis of Periodontal Pathogenicity

Animal models were chosen because they have similar anatomical and physiological features of the oral cavity and periodontium, as well as the presence of causative agents that contribute to the occurrence of natural periodontal disease in humans [5].

A) Non human primates: The smallest non human primate species, such as marmosets, weigh 300-355 gm, while the largest, such as chimps and gorillas, weigh upto 1,000 kilograms. These are all diphyodont genera. Teeth as well as roots have similar anatomy to humans, but they are smaller in size in marmosets. In most non human primates, canines are elongated and prehensile. Gorilla and baboon premolars have multiple roots. Monkeys have a distinct advantage in that they are phylogenetically similar to humans. When certain species of non human primates reach adulthood, they develop periodontal disease [8]. Spontaneous periodontal disease is highly hazardous to rhesus monkeys, cynomolgus monkeys, and baboons. The histological morphology of the periodontium appears to be similar to that of living things. An inflammatory reaction carried by periodontal disease is remarkably comparable to the inflammatory response experienced by people. Plasma cells, lymphocytes, as well as neutrophils invade ligaments and tendons. In periodontal studies, the marmoset seems to be the most widely employed non human primate [9].

B) Dogs: Gingival as well as periodontal disorders in dogs were the subject of numerous research. Due to its size and exceptionally cooperative attitude, the beagle is among the most widely used dogs. Overall periodontium structures and teeth sizes are remarkably identical to those seen in humans. Significant variations between humans and dogs include the absence of crevicular fluid and gingival sulcus well as a distinct composition of periodontal plaque and calculus [10]. All dogs have mixed dentition. All domestic dogs are predisposed to periodontal disease as adults, but they can be kept healthy with proper plaque control. Gingival recessions are seen in dogs with advanced periodontitis. Gram-positive cocci form the majority of supragingival plaque. Feeding a gentle, finely diced food that promotes the formation of supragingival plaque and calculus can hasten the progression of gingivitis in dogs [11]. In healthy dogs, the gingival sulcus is almost always absent. Gingivitis, which has already developed, causes inflamed gums in dogs. The premolar and molar teeth furcation sites may be affected by bone deficiency. Periodontitis affects bacterial colonies in dog models and is more severe than in domestic dogs [12]. The interproximal spaces degrade more commonly than the bifurcated regions. The premolars seem to be the most prevalently lost teeth. The scope and sites of periodontal diseases are not consistent with those of natural periodontal diseases which could be regarded as a dog model drawback. Though Dogs are served as animal models in a variety of procedures such as in mucogingival surgery, guided osseous regeneration, and even in implant surgery dogs appear to be the most utilised animal model for periodontal investigation because of their reproducible, critical-sized abnormalities [5].

C) Rats: In terms of periodontal disease causation, the rat seems to be the most thoroughly studied animal [13]. Wistar or Sprague-Dawley is the most widely utilised strain. The rat was chosen because it resembled a human. Rat's dental gingiva has numerous similarities with human dental gingiva in terms of structure such as having a thin gingival sulcus and junctional epithelium attached to the tooth surface. There are some variations such as:

- The keratinisation of the crevicular epithelium in rats [5].
- Relationship between the gingival and junctional epithelium with desmosomal contact between the most superficial cells of the gingival epithelium and the non keratinised cells of the junctional epithelium [5].

  Although there is a structure variation, the junctional epithelium appears to be a pathway for foreign substances, bacterial endotoxins, and inflammatory cell exudations, similar to that occurs in humans [14]. Rats' dental tissues change as they age, including continual tooth eruption and irreversible bone apposition in regards to the overall occlusal surface of the molar as well as cementum. All of these physical and age-related variations influence the analysis of periodontal disease [5]. Rats were utilised as mono contamination for various isolated gram-positive species of bacteria from the oral cavity of humans which led to periodontal disease in 84 days [15].

D) Rice rats: American species named swamp rice rat (*Oryzomys palustris*) is common throughout the southern United States [16]. They are prone to periodontal disease [12]. Rice rats were used to investigate nutritional impacts and treatment alternatives. Changes that occur throughout a rice rat are mentioned in [Table/Fig-2] [17-22].

E) Hamsters: Contagious periodontal disease due to plaque microbes has been demonstrated using hamsters. The most prevalent hamster is the golden Syrian hamster. The periodontium structure is histologically comparable to that of rats, though the interdental septum is thinner in this species due to its small size. To develop spontaneous periodontal disease, researchers employed an optimum diet heavy in carbohydrates, mainly sucrose [23]. A plaque was formed by formic acid-producing bacteria mixed with waste food in this diet, and it predominantly affected the palatal surfaces rather than the buccal surfaces [24]. The inflammatory process in hamsters is fairly limited, and it differs greatly from that in humans. The periodontal lesions and processes of alveolar bone resorption in rats affected by gram-positive bacteria are very similar to humans [5].

At the age of two weeks	At three months of age	At five-nine weeks of age
Animals are quite vulnerable to dental problems [17-19].	Gingival tissues enlarge, leading to pocket development, debris deposition, and ulceration [20].	Gram (+) positive bacteria, Actinomyces, Lactobacilli, and Streptococci. sanguis were isolated from the oral cavity [21,22].

[Table/Fig-2]: Oral changes occurring on a rice rat.

F) Mice: It is widely used because of unique benefits like compact size, cheap, predictable age, genetic origin, regulated microbiota, including ease of operation in rodents. They, like mice, are members of the Gilres cohort and have now been frequently employed during periodontal investigations. In periodontal research, a variety of mice models were utilised, which are listed below in [Table/Fig-3] [25-38].

G) Ferrets: It is assumed that the domestic ferret (*Mustelaputoriusfuro*) is descended from the wild (European) polcat. The ferret is an ideal method for analysing calculus since its calculus is the same as human calculus and it is not dependent on the diet like that of the mouse and hamster. It's utilised to assess periodontal abnormalities [24,25]. Ferrets have mixed dentition [5].

Baker mouse model [32-38]	Murine back abscess model [25-31]
Alveolar bone resorption produced by oral biofilm inoculums has been measured by using this model [33].	The interplay between oral microflora and host reactions to a variety of oral diseases as monomicrobial illnesses can result in soft tissue damage can be studied by this model [25,27].
Mice around 10-week-old were orally infested by using microbes for example Porphyromanas gingivitis, and Aggregatibacter actinomycetemcomitans to test the pathogenicity of periodontal pathogens. To inhibit the oral microbes, mice were given medications such as Trimethoprim and sulfamethoxazole through their drinking water for about ten days before infection [32-35].	In comparison to a mono-infection, mixed infections such as Porphyromanas gingivalis or Aggregatibacter actinomycetemcomitans are used to demonstrate the production of bigger abscesses [28].
Periodontitis develops naturally in mice starting around nine-month-old and progresses with maturity same as like in human periodontal diseases [36,37].	The mice subcutaneous compartment paradigm investigates host-microbes interconnection and assesses variable differences within P. gingivalis strains that cause tissue injury and infiltration [29,30].
The bacteria utilised are one or two of at least 150 microbial kinds found in every dental plaque biofilm, hence this model may not fully replicate all elements of clinical periodontitis onset and progression [38].	The lesions which are not present in the mouth in this model can be used to investigate bacterially generated infections that cause soft tissue deterioration [26,31].

[Table/Fig-3]: Mice models utilised for periodontal research.

Merits and Demerits of Various Animal used for Study

The merits and demerits of various animals used for the study are shown in the given [Table/Fig-4] [13].

Animal Models used in the Periodontal Therapy

Various surgically produced periodontal abnormalities have been employed for 10 years to investigate the effects of Guided tissue regenaratin (GTR) and the utilisation of biomaterials such as enamelled extracellular matrix components. These surgical approaches are most widely used on Macaca fascicularis. Periodontal defects in experiments can be obtained in three ways shown in [Table/Fig-5] [5].

Animals	Merits	Demerits
Dogs	Develop periodontitis that is identical to human periodontitis in a natural or experimental setting.	Costly requires routine care. Tooth anatomy differs from that of human beings.
Non primates	Human-like dental structure, microbiota, and illness. Periodontitis can be either naturally or intentionally produced.	Extremely costly, with ethical and husbandry concerns.
Miniature pigs	Periodontitis and dental structure are similar to those of humans. Periodontitis can be caused naturally or artificially.	Costly; issues about animal welfare; and a scarcity of studies.
Rodents	A disease that has been induced experimentally. The molar structure is similar to that of humans. It is an inexpensive model.	Periodontitis resistance is built in. Microbiota that differs from the human microbiota. Because of its small size, there is a limited quantity of tissue available for the survey. Animals are required in wide quantities.
Ferrets	A disease that occurs naturally or is induced experimentally and is similar to a human disease.	Some issues with husbandry.

[Table/Fig-4]: Merits and demerits of animals used for study.

Acute model	Chronic model	Combined
Surgically induced deficiencies are created by eliminating the excess periodontal component. The investigational group has recurrent flaws [5].	Depending on the animal investors tumors are generated by wrapping orthodontic elastic bands, and silk sutures. Interproximal spaces have deeper defects than other surfaces [5].	Ligatures are used to assure calculus build-up and stop the flaws from spontaneously regenerating after being surgically produced [5].

[Table/Fig-5]: Various surgical approach model in animals for periodontal therapy.

Animal Models Utilised in the Development of Periodontal Vaccine

Humans have not yet been used as experimental subjects against bacteria in vaccine development studies, so for vaccine trials for vaccination safety and effectiveness testing, animal methods are designed. There is no ideal animal research model for vaccine trials against periodontitis with naturally occurring periodontitis in animals and humans based on the same aetiology, pathogenesis, and prevalence. Experimentally induced periodontitis models have been investigated as replacements. Some of these may not provide easy access to clinical conditions that can be easily evaluated for clinical efficacy. Dogs were not considered for periodontal vaccine research. It's worth noting that sheep (ovine) seem to develop naturally occurring periodontitis. There is homology to human strains of P.gingivalis in sheep. Non human primates have been considered for periodontal vaccine trials, including M.fascicularis, M.nemestrina, Marmosets, Baboons, and Chimpanzees. Periodontitis occurs naturally in less than 5% of M. fascicularis. Key pathogens associated with periodontitis have been identified in samples taken from adult M.fascicularis and M.nemestrina and identified by DNA probes aimed at studies of strains found in humans, according to research [37,39]. Page RC and Schroeder HE concluded that mice, rats, and hamsters are not suited for studies on the efficacy of periodontitis vaccines in humans due to continuous tooth eruption patterns and alveolar bone changes [9].

Drawbacks of Experimental Studies of Animals

The drawbacks of experimental studies of animals are infectious diseases are not always communicable to animals. Not all findings of animal studies are universally relevant to humans. Extrapolating findings from animal experiments to humans is difficult [38].

Ethical Considerations

The ethical and responsible use of animals in research is a constant source of growing concern. This problem can be resolved by creating the National Accreditation Board of Testing and Calibration Laboratories (NABL), which is a member of the International Laboratory. Planning should go into every aspect of medical research, including that which uses animals. Before approving each study, experts who assess a scientist's planned animal experiment weigh several factors. The most important thing is that the research must be relevant to human or animal health [39-41].

CONCLUSION(S)

The use of fact-finding models used in periodontal disease is crucial to grasp the root source in the human being. Animal models are beneficial in a periodontal survey and an unavoidable step before accessing clinical testing with the latest biomaterials and therapies. The miniature used for gum disease is almost identical to that of a human being. In terms of dental pathophysiology, monkeys appear to be the closest model to humans, but they are not considered due to financial constraints. More often than not, the dog is advantageous because of its reproducible decisive dimension, which allows the biomaterial to be tested for experiments. Germ-free rats utilise as a massive model in terms of cell biology investigation. Hamster stays an intriguing model for immunological studies. New possibilities in the periodontal analysis are now accessible, enabling broader cohorts that are easier to build. The comprehensive utility of these animal models plays an essential role in future studies, particularly in surgical settings.

REFERENCES

[1] Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809-20. Doi: 10.1016/S0140-6736(05)67728-8.
[2] Salvi GE, Lang NP. Host response modulation in the management of periodontal diseases. J Clin Periodontol. 2005;32(Suppl 6):108-29.
[3] Schenkein HA. Host responses in maintaining periodontal health and determining periodontal disease. Periodontol 2000. 2006;40:77-93. Doi: 10.1111/j.1600-0757.2005.00144.x. PMID: 16398686.
[4] Sfakianakis A, Barr CE, Kreutzer DL. Actinobacillus actinomycetemcomitans-induced expression of IL-1α and IL-1β in human gingival epithelial cells: Role in IL-8 expression. European Journal of Oral Sciences. 2001;109(6):393-401.
[5] Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: A review. The Open Dentistry Journal. 2010;4:37.
[6] Hajishengallis G, Lamont RJ, Graves DT. The enduring importance of animal models in understanding periodontal disease. Virulence. 2015;6(3):229-35.
[7] Davidson MK, Lindsey JR, Davis JK. Requirements and selection of an animal model. Israel Journal of Medical Sciences. 1987;23(6):551-55.
[8] Schou S, Holmstrup P, Kornman KS. Non human primates used in studies of periodontal disease pathogenesis: A review of the literature. J Periodontol. 1993;64:497-508
[9] Page RC, Schroeder HE. Periodontitis in man and other animals. A comparative review. S. Karger. 1982.
[10] Sorensen WP, Löe H, Ramfjord SP. Periodontal disease in the beagle dog. A cross sectional clinical study. J Periodontal Res. 1980;15(4):380-89. Doi: 10.1111/j.1600-0765.1980.tb00295.x. PMID: 6449573.
[11] Egelberg J. Local effect of diet on plaque formation and development of gingivitis in dogs. 3. Effect of frequency of meals and tube feeding. *Odontol Revy.* 1965;16:50-60.
[12] Page RC, Schroeder HE. Spontaneous chronic periodontitis in adult dogs: A clinical and histopathological survey. J Periodontol. 1981;52(2):60-73.
[13] Oz HS, Puleo DA. Animal models for periodontal disease. Journal of Biomedicine and Biotechnology. 2011;2011:754857.
[14] Listgarten MA. The similarity of epithelial relationships in the gingiva of rat and man. J Periodontol. 1975;46(11):677-80.
[15] Leonard EP. Periodontitis. Animal model: Periodontitis in the rice rat (Oryzomys palustris). Am J Pathol. 1979;96(2):643-46. PMID: 474713; PMCID: PMC2042444.
[16] Guvva S, Patil MB, Mehta DS. Rat as laboratory animal model in periodontology. International Journal of Oral Health Sciences. 2017;7(2):68. Doi: 10.4103/ijohs.ijohs_47_17.
[17] Gupta OP, Shaw JH. Periodontal disease in the rice rat: I. Anatomic and histopathologic findings. Oral Surgery, Oral Medicine, Oral Pathology. 1956;9(6):592-603.
[18] Shaw JH. Influence of alternating feedings of diets with high and low potentials to cause the periodontal syndrome in rice rats. Journal of Dental Research. 1969;48(3):486.
[19] Shaw JH, Griffiths D. Relation of protein, carbohydrate, and fat intake to the periodontal syndrome. Journal of Dental Research. 1961;40(3):614-21.
[20] Dick DS, Shaw JH. The infectious and transmissible nature of the periodontal syndrome of the rice rat. Archives of Oral Biology. 1966;11(11):1095-108.
[21] Toth A, Beck FM, Beck EX, Flaxman N, Rosen S. Effect of antimicrobial agents on root surface caries, alveolar bone loss, and microflora in rice rats. Journal of Dental Research. 1986;65(5):695-97.

[22] Lallam-Laroye C, Escartin Q, Zlowodzki AS, Barritault D, Caruelle JP, Baroukh B, et al. Periodontitis destructions are restored by synthetic glycosaminoglycan mimetics. Journal of Biomedical Materials Research Part A: An official Journal of the Society for Biomaterials, The Japanese Society for Biomaterials, The Australian Society for Biomaterials, and the Korean Society for Biomaterials. 2006;79(3):675-83.
[23] King JD. Experimental investigations of periodontal disease. British Medical Journal. 1947;2(4537):987.
[24] Weinberg MA, Bral M. Laboratory animal models in periodontology. J Clin Periodontol. 1999;26(6):335-40.
[25] Kesavalu L, Ebersole JL, Machen RL, Holt SC. Porphyromonas gingivalis virulence in mice: Induction of immunity to bacterial components. Infection and Immunity. 1992;60(4):1455-64.
[26] Feuille F, Ebersole JL, Kesavalu L, Stepfen MJ, Holt SC. Mixed infection with Porphyromonas gingivalis and Fusobacterium nucleatum in a murine lesion model: Potential synergistic effects on virulence. Infection and Immunity. 1996;64(6):2094-100.
[27] Kesavalu L, Holt SC, Ebersole JL. Trypsin-like protease activity of Porphyromonas gingivalisasis a potential virulence factor in a murine lesion model. Microbial Pathogenesis. 1996;20(1):1-0.
[28] Kesavalu L, Holt SC, Ebersole JL. Porphyromonas gingivalisvirulence in a murine lesion model: Effects of immune alterations. Microbial Pathogenesis. 1997;23(6):317-26.
[29] Kesavalu L, Holt SC, Ebersole JL. Virulence of a polymicrobic complex, Treponema denticola and Porphyromonas gingivalis, in a murine model. Oral Microbiology and Immunology. 1998;13(6):373-77.
[30] Chen PB, Davern LB, Katz J, Eldridge JH, Michalek SM. Host responses induced by co-infection with Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans in a murine model. Oral Microbiology and Immunology. 1996;11(4):274-81.
[31] Baker PJ, Evans RT, Roopenian DC. Oral infection with Porphyromonas gingivalis and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. Archives of Oral Biology. 1994;39(12):1035-40.
[32] Pierce DL, Nishiyama SI, Liang S, Wang M, Triantafilou M, Triantafilou K, et al. Host adhesive activities and virulence of novel fimbrial proteins of Porphyromonas gingivalis. Infection and Immunity. 2009;77(8):3294-301.
[33] Polak D, Wilensky A, Shapira L, Halabi A, Goldstein D, Weiss EI, et al. Mouse model of experimental periodontitis induced by Porphyromonas gingivalis/ Fusobacterium nucleatum infection: Bone loss and host response. J Clin Periodontol. 2009;36(5):406-10.
[34] Kinane DF, Hajishengallis G. Polymicrobial infections, biofilms, and beyond. J Clin Periodontol. 2009;36(5):404-05.
[35] Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. J Clin Periodontol. 2008;35(2):89-105.
[36] Beertsen W, Willenborg M, Everts V, Zirogianni A, Podschun R, Schröder B, et al. Impaired phagosomal maturation in neutrophils leads to periodontitis in lysosomal-associated membrane protein-2 knockout mice. The Journal of Immunology. 2008;180(1):475-82.
[37] Novak EA, Shao H, Daep CA, Demuth DR. Autoinducer-2 and QseC control biofilm formation and in vivo virulence of Aggregatibacter actinomycetemcomitans. Infection and Immunity. 2010;78(7):2919-26.
[38] Caton J, Mota L, Gandini L, Laskaris B. Non-human primate models for testing the efficacy and safety of periodontal regeneration procedures. J Periodontol. 1994;65(12):1143-50.
[39] Persson GR. Immune responses and vaccination against periodontal infections. J Clin Periodontol. 2005;32:39-53.
[40] Muthanandam S, Muthu J, Mony V, Parvathy RI, Prem LK. Animal models in dental research- A review. International Dental Journal of Student Research. 2020;8(2):44-47.
[41] Nokhbatolfoghahaei H, Paknejad Z, Bohlouli M, Rad MR, Khojasteh A. Animal models in dental research. In Applications of Biomedical Engineering in Dentistry. 2020 (pp. 377-442). Springer, Cham.

PARTICULARS OF CONTRIBUTORS:
1. Intern, Department of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi, Wardha, Maharashtra, India.
2. Assistant Professor, Department of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi, Wardha, Maharashtra, India.
3. Associate Professor, Department of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi, Wardha, Maharashtra, India.
4. Assistant Professor, Department of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi, Wardha, Maharashtra, India.
5. Postgraduate Student, Department of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi, Amravati, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Muskan Baheti,
Intern, Department of Periodontics and Implantology, Sharad Pawar Dental College and Hospital, DMIMS, Sawangi, Wardha-442001, Maharashtra, India.
E-mail: bahetimuskan31@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 01, 2022
- Manual Googling: Jan 24, 2023
- iThenticate Software: Feb 02, 2023 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Nov 25, 2022**
Date of Peer Review: **Dec 22, 2022**
Date of Acceptance: **Feb 09, 2023**
Date of Publishing: **Mar 01, 2023**