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Highlights

- Pharyngeal Airway Space
- Improvement of Surgical Treatment
- Hyaluronic Acid Gel Injection
- Anti-Porphyromonas Gingivalis Levels

Discovering Thoughts, Inventing Future

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Lasing: Lighten Your Teeth Tone

By Sana Farooq & Nimra Iqbal
ITS Dental College

Abstract- Lightening tooth color is what a patient demands and can be successfully done by a wide variety of bleaching methods, including in-office (professionally administered), at-home (professionally dispensed) and over-the-counter (self-administered) techniques. This is a case report of treatment of 15 year old female patient with the chief complaint of discolored upper right front tooth region with laser bleaching with biolase.

GJMR-J Classification: *NLMC Code: WU 400*



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Lasing: Lighten Your Teeth Tone

Sana Farooq [α] & Nimra Iqbal [σ]

Abstract- Lightening tooth color is what a patient demands and can be successfully done by a wide variety of bleaching methods, including in-office (professionally administered), at-home (professionally dispensed) and over-the-counter (self-administered) techniques. This is a case report of treatment of 15 year old female patient with the chief complaint of discolored upper right front tooth region with laser bleaching with biolase.

I. Introduction

Laser-assisted bleaching is the best modality available in which laser accelerates release of free radicals within the bleaching gel to decrease time of whitening procedure.[1] In -office laser bleaching has an advantage of dentist control, avoidance of tissue exposure, minimal post bleaching hypersensitivity, reduced treatment time and enhanced patient satisfaction due to immediate results.[2] Laser whitening gels incorporate chromophores or activator specific to a particular wavelength which absorbs the laser light and quicken the bleaching process.[3]

II. Case Discussion

A 15 year-old female patient reported to the Department of Pedodontics and Pediatric Dentistry with the chief complaint of discolored tooth with respect to her right upper front tooth region since childhood. On examination, generalized grade 2 dental fluorosis was seen and Grade 4 Dental flourosis irt 12 and 14. The patient was explained about the various treatment modalities available, the procedures to be undertaken and an informed consent was obtained.

A thorough oral prophylaxis was done 3 weeks prior and polishing of the teeth was done. On the day of treatment removal of surface plaque and stain with pumice prior to administration of whitening agent.

Modified Dean's Fluorosis Index (1942) was recorded at baseline and the end of the first and second visit, respectively. Preoperative photographs were taken at baseline [Figure 1]. The patient was asked to wear protective eyewear. The teeth were isolated using cotton rolls. A separating media was applied and a liquid dam was applied along the gingival margins (Figure 2 a). The liquid dam was cured using standard curing light, holding the handpiece at least 2cm from tooth for 5 to 10 seconds.



Figure 1

Using an single tufted applicator tip apply 1 mm whitening gel 45% hydrogen peroxide (Figure 2 b) by mixing the two syringes containing activator and base till a uniform lavender colour(almost 20 times) is achieved according to manufacturer's recommend-dations (Figure 3). After an even mix was obtained, a thin layer of about 1 mm thickness was applied on dried teeth with the brush tip applicator. Apply a disposable sleeve over the handpiece's arch to disperse laser light over a broad area of teeth (Figure 2 c).

The tooth was irradiated using diode laser (BIOLASE™) with a power setting 7W power, continuous wave mode and 200 J energy output in contact mode for a time of about 30 second for each quadrant [Figure 4]. The gel was left on the teeth for an additional 5 minutes to allow the teeth to absorb the laser activated hydrogen peroxide which allows continued whitening after laser exposures. This procedure was repeated twice witin a gap of 1 week. At the end clean rubber dam with explorer and rinse. Apply desensitizer potassium nitrate for 15 to 20 minutes (Figure 2 d). In case of any discomfort the patient was asked to report back to the clinic. Indirect Composite veneering was done on follow up visit. The postoperative photographs were taken (Figure 5c) .The color change evaluation using 3D Vita shade guide was noted.

The patient was advised not to consume products that stain teeth for up to the next 48 hours such as coffee, tobacco, tea, tomato sauce, cold drink etc.

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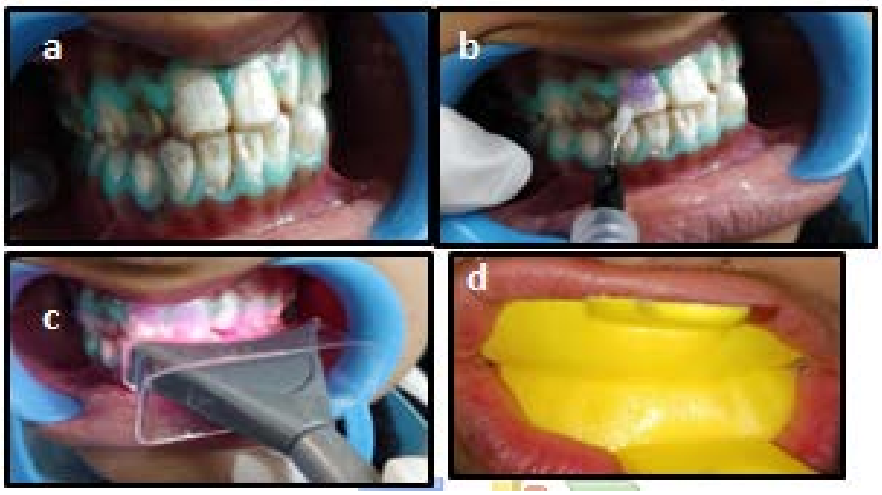


Figure 2: a): Placement of gingival barrier liquid dam
b) Application of bleaching agent
c) Applying biolase laser system d)Apply desensitizer



Figure 3

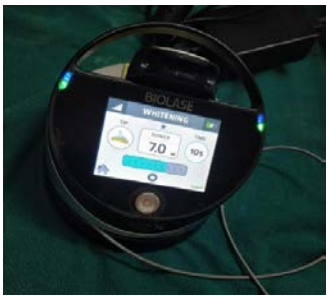


Figure 4



Figure 5: a) Preoperative photograph b) Post laser bleaching c) Composite veneering

III. Discussion

Initially bleaching was primarily performed with 35%–37% carbamide peroxide or 30%–40% hydrogen peroxide and the use of hydrogen peroxide (H_2O_2) for conventional bleaching was introduced way back in 1884. [4,5,6] Light sources were marketed with the idea that light plays a significant role in tooth bleaching as catalyst for the ionization of Hydrogen peroxide in the bleaching gel and increasing the bleaching effect. [7]

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Anaraki *et al* showed that the damage caused by bleaching with a 810 nm diode laser was less than the one caused by conventional bleaching without any laser activation.[4,5] Chromophores in the laser-activated gels absorb the narrow wavelength of diode lasers thereby increasing the efficacy of bleaching.[5]

Its seen that the rate of the chemical reaction increases double fold with a rise of 10°C. Also the thickness of the bleaching gel layer is an important factor to ensure that the laser light can pass through this layer. The distance between the handpiece or fibre end and the gel is important criteria while considering the energy and impact of bleaching. [3]

When Biolase Laserwhite20™ is used in combination with a bleaching gel containing a chromophore (sulphorhodamine) photodynamic reactions occur (photochemical activation of the gel with limited photothermal activation). This combination of wavelength and specifically dyed bleaching gel also allows for safe bleaching (no damage of the enamel, no heating of the pulp) when the manufacturers instructions are followed.[3]

IV. Conclusion

Vital bleaching is the cosmetic dental procedures asked by patients to seek a more pleasing smile. Laser bleaching causes profound quicker whitening with little or no surface alterations. The degree of whitening varies from patient to patient depends on type of stain, enamel thickness, tooth structure and age.

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Effect of 0.12% Chlorhexidine Gel and Tooth Brushing on *Porphyromonas Gingivalis* Levels in Subgingival Plaque and Matrix Metalloproteinase-8 (MMP-8) Levels in Gingival Crevicular Fluid of Children and Adolescents with Down Syndrome

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Abstract- *Background:* To find out innovative and user-friendly methods that can be practiced daily to improve the gingival and periodontal health of Down syndrome adolescents and to improve their quality of life.

Aim: This study aims to determine if chlorhexidine gel (0.12%) and tooth brushing had any effect on the levels of MMP-8 in GCF and the levels of Porphyromonas gingivalis in subgingival plaque of DS children and healthy children.

Keywords: *down syndrome, prevention, chlorhexidine, periodontal health, gingival health.*

GJMR-J Classification: *NLMC Code: WU 240*



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Abstract- Background: To find out innovative and user-friendly methods that can be practiced daily to improve the gingival and periodontal health of Down syndrome adolescents and to improve their quality of life.

Aim: This study aims to determine if chlorhexidine gel (0.12%) and tooth brushing had any effect on the levels of MMP-8 in GCF and the levels of Porphyromonas gingivalis in subgingival plaque of DS children and healthy children.

Methodology: Twenty Down syndrome and twenty healthy children formed Group I and Group II, respectively. Following the collection of baselines GCF samples and plaque samples, oral prophylaxis was carried out by the same examiner in both groups. In Group I, chlorhexidine (0.12%) gel was applied over the gingiva once in two weeks for 3 months. Parents were given oral hygiene counseling on the method of tooth brushing for their children. A second sample of GCF and plaque was obtained from Group I at the end of 3 months. Oral hygiene reinforcement was given for the children once in two weeks for the next three months and the third sample of GCF and plaque was obtained from Group I at the end of the 6th month.

Results: A significant difference was observed in mean MMP-8 levels and mean *Porphyromonas gingivalis* levels between Down syndrome and healthy children ($p<0.001$).

Conclusion: The levels of MMP-8 and Pg. in Down syndrome children showed improvement following the use of 0.12% chlorhexidine gel.

Keywords: down syndrome, prevention, chlorhexidine, periodontal health, gingival health.

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I. Introduction

Down syndrome is a genetic disorder caused by the presence of all or part of an extra 21st chromosome and is also known as trisomy 21. Intellectual disability, cardiac anomalies and an altered immune system in individuals with Down syndrome can have a profound effect on their oral health.[1] Children with Down syndrome (DS) experience a high incidence of rapid, destructive periodontal disease, which may be related to local factors such as tooth morphology, bruxism, malocclusion, and poor oral hygiene as well as systemic factors such as altered immune/inflammatory responses. Early colonization of periodontal pathogens is another important contributing factor to their increased susceptibility to periodontitis. 2-4 Among the pathogens, *Porphyromonas gingivalis* is the predominant pathogen seen in subgingival dental plaque of DS adolescents.[4]

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which have been considered as mediators of extracellular matrix degradation and remodeling during periodontal diseases. The host response thought to be caused by interactions between dental plaque, calculus, and oral bacteria, leads to inflammatory cascades involving increased activities of proteolytic enzymes in gingival tissue, gingival crevicular fluid, and saliva. The expression of MMPs and their levels in the gingival crevicular fluid are good indicators for a clinical diagnosis of periodontal disease.[3] Matrix metalloproteinase-8 is a poly morpho nuclear leukocyte (PMN)-type collagenase involved in periodontal tissue degradation in periodontal disease. It is stored in specific granules within PMNs and is released when PMNs are triggered. MMP-8 is the major collagenase involved in periodontitis.

Thus, the levels of *Porphyromonas gingivalis* and MMP-8 are good clinical indicators of periodontal disease in these adolescents. Supervised preventive

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programs have been very effective in reducing plaque and gingival inflammation in people with Down Syndrome.[5] The use of antimicrobial agents can be a useful aid in plaque control for these individuals.[6]

Most of the studies on the oral health of DS children and adolescents have reported on their salivary parameters, dental caries and oral hygiene.6-8 Therefore, the objective of this clinical study was to comparatively evaluate MMP-8 levels in gingival crevicular fluid and *Porphyromonas gingivalis* in the dental plaque of subjects with Down syndrome and healthy controls. The aim was also to assess whether tooth brushing, with or without the use of chlorhexidine, influences the levels of MMP-8, *Porphyromonas gingivalis*, dental plaque and gingival health of subjects with DS.

II. Methodology

This randomized controlled trial included twenty children and adolescents, aged 9-16-year-old with Down Syndrome selected from Divya Downs Developmental Trust, Bangalore. Before the study, ethical approval and clearance were obtained from the Institutional Ethics Review Board of our institution. A written permission had been obtained from the concerned authorities of the school. The nature of the study was explained to the authorities, and prior informed written consent had been taken from the parents/caretakers of all the subjects. A proforma had been used to gather demographic data, medical and drug history. The exclusion criteria were: (1) those on long term medication, (2) those who were very uncooperative and were unable to cooperate, (3) severe intellectual disability present along with Down syndrome, (4) association with any other medically compromised conditions and (5) those who had undergone oral prophylaxis in the preceding six months.

Twenty healthy controls who were age and gender-matched had been selected from the Department of Pedodontics and Preventive Dentistry at our institution, to obtain estimates of MMP-8 and *Porphyromonas gingivalis* for comparison. Therefore, there were two groups: Group I: Twenty subjects with Down syndrome and Group II: Twenty healthy subjects.

Oral examination was done by a single trained and calibrated examiner under artificial light using a sterile dental mirror and WHO CPITN probe. In Group I, the plaque index (PI) had been recorded using *Silness and Loe* index9 and gingival health had been assessed using *Loe and Silness* gingival index (GI).10

a) Collection of baselines gingival crevicular fluid (GCF)

Gingival crevicular fluid (GCF) samples (2µl) were collected using sterile microcapillary pipettes (0.01mm). Pooled GCF samples had been collected from the mesial surfaces of first permanent molars by placing the tip of the micropipette at the entrance of the gingival sulcus for 30 seconds. Matrix metalloproteinase-8 (MMP-8) levels were analyzed from the samples that were wrapped in aluminum foil, stored at -80°C.[11]

b) Assessment of matrix metalloproteinase-8

The levels of matrix metalloproteinase-8 (MMP-8) were estimated by subjecting the collected GCF samples to enzyme-linked immunosorbent assay (ELISA).11ELISA was performed based on the protocol in the Fine test Human Matrix Metalloproteinase-8 kit (Wuhan Fine Biological Technology Co., Ltd.).The plate was washed twice before adding standard, sample, and control(zero) wells. 0.1ml of solution was then aliquoted into 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.3125ng/ml, 0.156ng/ml, standard solutions into the standard wells. 0.1ml of sample/ standard dilution buffer was added into the control well. 0.1ml of the test samples were added into test sample wells. The plate was sealed and incubated at 37°C for 90 minutes. The excess fluid was removed using absorbent filter papers. 0.1ml of biotin-detection antibody working solution was added into the above well; the plate was sealed and incubated at 37°C for 60 minutes. The plate was then washed thrice with wash buffer. 0.1ml of HRP-Streptavidin Conjugate (SABC) working solution was added into each well, and the plate was sealed and incubated at 37°C for 30 minutes. The seal was then removed, and the plate content was discarded, the plate was then washed 5 times with wash buffer. 90µl of tetra methyl benzidine (TMB) substrate was added into each well. The plate was sealed and incubated at 37°C in the dark for 15-30 minutes. Stop solution (50µl) was added into each well and mixed thoroughly. The color changed to yellow immediately. The optical density (OD) absorbance was read at 450nm on a microplate reader immediately (within 30 minutes) after adding the stop solution. The calculation was done as: (The relative OD 450) = (the OD 450 of each well) – (the OD 450 of Zero well). The MMP-8 concentration of the samples was interpolated from the standard curve.

c) Collection of baseline subgingival plaque

Subgingival plaque samples were collected from the mesial and buccal sites of first permanent molars using sterile curettes. The plaque samples had been then transferred into sterile Eppendorf tubes containing buffer solution, stored at -80° C for further analysis of *Porphyromonas gingivalis*.[12]

d) Assessment of Porphyromonas gingivalis

The presence of *Porphyromonas gingivalis* had been microbiologically evaluated by suspending the plaque sample in 1ml of saline. Aseptically 0.1ml of the suspension was transferred to Tryptic soy broth (TSB culture medium) and incubated under anaerobic conditions overnight for 24 hours at 37°C. The growth of the bacteria was measured spectrophotometrically by

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reading its optical density (OD) at 600-nanometer wavelength and expressed as colony forming units (CFU) per ml.[13]

Following the collection of baseline plaque and GCF samples, oral prophylaxis was carried out in both groups.

e) Preparation of Chlorhexidine (0.12%) gel

A gel consisting of hydroxy propyl methylcellulose (HPMC), glycerin and water was prepared. Water was added to HPMC, followed by vigorous mixing until the HPMC became miscible with water. Glycerin was then added to this mixture and mixed well to form a gel. Further, a commercially available chlorhexidine (CHX) gel (2%) (Unilab Chemicals and Pharmaceuticals Pvt. Ltd., India) was added to this gel and stirred using a magnetic stirrer to obtain 60 g of 0.12% CHX gel.

f) Periodic application of the Chlorhexidine (0.12%) gel for the first three months

Following oral prophylaxis, this indigenously prepared CHX gel (0.12%) was applied only in Down syndrome subjects, by gently massaging 0.3-0.5g of the gel over the buccal and palatal/lingual surfaces of gingiva using sterile cotton swabs. The time of application was between 10 am to 11 am. They were instructed not to drink, eat, or rinse their mouth for 30 minutes following the gel application. Regular application of CHX gel was carried out by the same examiner once in 15 days over 3 months. A total of 6 applications were done. During this period, parents and the children were given oral health education inside the school premises and were taught the proper methods of tooth brushing.

g) Collection of the second sample of GCF and subgingival plaque

At the end of 3 months, the second sample of GCF and the subgingival plaque was collected only from the Down syndrome subjects (Group I) in the same manner as described earlier.

h) Oral hygiene reinforcement for the next three months

Oral hygiene reinforcement comprising of oral health education on proper tooth brushing techniques followed by a demonstration of the same was carried out for the parents as well as the children at their school premises. It was carried out by the same examiner once in 15 days over the next 3 months.

i) Collection of the third sample of GCF and subgingival plaque

At the end of the sixth month, after successful completion of periodic oral hygiene reinforcement, a third sample of GCF and the subgingival plaque was collected from all the subjects in the same manner.

j) Tests used for statistical analysis

Data obtained was tabulated and subjected to statistical analysis using Independent student t-Test for the comparison of mean MMP-8 and *Porphyromonas gingivalis* levels between control and Down syndrome group, Friedman's Test for the comparison of mean MMP-8 levels between different time intervals in Down syndrome group, Wilcoxon Signed Ranked Post hoc Test had been used for the pairwise comparison of mean difference in MMP-8 levels between different time intervals, repeated measures of ANOVA Test had been used for the comparison of mean *Porphyromonas gingivalis* optical density levels between different time intervals in Down syndrome group and Bonferroni's Post hoc Test had been used for the pairwise comparison of mean difference in *Porphyromonas gingivalis* optical density levels between different time intervals. Pearson's correlation had been used to study the association between MMP-8 and *Porphyromonas gingivalis*.

III. Results

The mean levels of baseline MMP-8 in Group I was 1.253±0.268 ng/ml, which was significantly higher than that of 0.028±0.022 ng/ml in Group II ($p<0.001$). The mean levels of baseline *Porphyromonas gingivalis* in Group I was 0.317±0.035 CFU/ml, which was significantly higher than that of 0.048±0.04 CFU/ml seen in Group II ($p<0.001$) (Table 1). There was a significant difference observed in the mean levels of MMP-8 and the mean levels of *Porphyromonas gingivalis* at different time intervals($p<0.001$) (Tables 2 and 3). Pair-wise comparison of MMP-8 levels in GCF at different time intervals in Group I showed a significant difference between baseline and three months and between baseline and six months ($p<0.001$) (Table 4). Pair-wise comparison of *Porphyromonas gingivalis* in dental plaque at different time intervals in Group I showed a significant difference between baseline and three months (Table 5).

A significant difference was observed in the mean plaque index (PI) and mean gingival index (GI) scores at different time intervals in Group I (Table 6). Pair-wise comparison of mean plaque index (PI) scores and mean gingival index scores (GI) at different time intervals in Group I showed a significant difference between baseline and three months and between baseline and 6 months (Tables 7 and 8). Pearson's correlation showed a significant association between MMP-8 and *Porphyromonas gingivalis* at three months (Table 9).

IV. Discussion

Individuals with Down syndrome are more likely to develop the aggressive periodontal disease at an earlier age than the general population.4,14,15 Subjects with severe intellectual disability and very uncooperative

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children were excluded from the study to facilitate the collection of samples. Sampling was done only after establishing a friendly relationship between the examiner and subjects. Alterations in levels of MMP-8 and *Porphyromonas gingivalis* were avoided by including only those DS individuals who had not undergone oral prophylaxis in the preceding six months.

Dental plaque was recorded using Silness and Loe plaque index because it ignores the coronal extent of plaque and assesses only the thickness of plaque at the gingival area of the tooth. It has good validity and reliability for both mechanical anti-plaque procedures and chemical agents. Loe and Silness gingival index were used because it is simple to use, reliable and can determine the severity of gingivitis. [9,10]

A sensitive and specific marker for periodontal tissue destruction is gingival crevicular fluid. It can be obtained in a non-invasive manner with minimal discomfort to the patient and consists of both locally synthesized and systemically derived molecules.[16] In this study, pooled GCF samples were collected from the mesial surfaces of first permanent molars, as these teeth are the first permanent teeth to erupt and are susceptible to periodontitis.

Enzyme-linked immunosorbent assay (ELISA) was used in this study for the quantification of MMP-8. This assay measures total collagenase i.e. pro- and active enzyme as well as collagenase in complex with Tissue inhibitors of metalloproteinase (TIMP). A fundamental problem with bioassays is that they lack specificity, i.e. they are unable to distinguish between two enzymes that degrade the same substrate, thus leading to inaccurate measurements with elevated enzyme levels.[17] Therefore, a commercially available ELISA kit specific for MMP-8 and with good sensitivity was used.[18]

Spectrophotometric analysis was employed to assess the growth of *Porphyromonas gingivalis.* It is estimated by optical density, which is based on the fact that an increase in the number of bacteria, results in less light transmitted. It is a simple, rapid, low cost and non-destructive method.[19]

In individuals with DS, there is a need for an oral hygiene regime that is simple, easy, economical and acceptable to both patient and caregiver.[20] Cumbersome tooth brushing techniques, and flossing, may be difficult to practice, due to reduced manual dexterity.21 Anti-plaque chemical agents such as chlorhexidine gluconate along with tooth brushing has proved to be useful in reducing plaque and gingivitis.[21] However, how it is delivered may be critical to a successful outcome.[22] Mouth rinses may not be suitable for use in DS, due to their inability to rinse the mouth and low gag reflex. The Application of CHX gel in trays has not been well accepted in children with intellectual disabilities.[21] Higher concentrations of CHX (0.2% or 1%) have been reported to cause mucositis, superficial mucosal erosions and burning sensation.18 In this study, a structured plaque control regime was implemented in subjects with DS. Tooth brushing which is a simple yet effective method for reducing plaque and gingivitis was followed. Good compliance was achieved by using a convenient and simple technique of massaging 0.12% CHX gel over the gingiva. CHX, a cation, interacts and forms salts of low solubility with anions, such as sodium lauryl sulphate (SLS) and sodium monofluorophosphate (MFP) present in dentifrices. To optimize the anti-plaque effect of CHX, an interval of 2 hours was given between tooth brushing and application of CHX gel.23 Long term use of CHX has side effects like extrinsic staining,[18] offensive taste, and altered taste sensation. Hence, the gel application was carried out fortnightly, and only for an initial period of three months.

Several studies have shown higher plaque accumulation and greater severity of gingivitis in DS children compared to healthy children.[6,7,24] In the present study, there was a significant decrease of 35.4% in plaque scores from 1.47 to 0.95, at the end of three months. On discontinuation of CHX gel application, plaque score increased by 28.4% to 1.22. Similarly, moderate gingivitis (GI score=1.48) that was seen at the beginning showed a significant reduction in inflammation resulting in mild gingivitis (0.99) after three months. Subsequently, due to the increase in plaque, there was a reversal to signs of moderate gingivitis (1.24) at six months.

The baseline MMP-8 level in GCF of DS (1.253 ng/ml) was significantly higher than that of healthy controls (0.028 ng/ml), which is in accordance with earlier reports.[11,12] This is probably due to the increased inflammatory response seen in DS and due to the presence of gingivitis. There was a significant eight-fold reduction in the MMP-8 level in subjects with DS after three months of following both mechanical and chemical plaque control. CHX inhibits the activities of MMP-8 thereby, indicating its anti-proteolytic properties.[25] On discontinuation of CHX gel application, MMP-8 level was seen to increase by 16.65% to 0.413 ng/ml.

Our study found the levels of *Porphyromonas gingivalis* to be significantly higher in DS. This microorganism thrives in individuals with poor oral hygiene and high plaque accumulation. *Porphyromonas gingivalis* stimulates periodontal cells to produce inflammatory mediators such as prostaglandin E_2 (PGE_2), matrix metalloproteinases (MMPs), and proinflammatory cytokines including interleukin (IL)-1, IL-6 and IL-8.[26] An association between *Porphyromonas gingivalis* and MMP-8 was also seen in our study.

In the present study, tooth brushing along with CHX gel application in subjects with DS decreased *Porphyromonas gingivalis* by 38.9% (from 0.316 CFU/ml to 0.193 CFU/ml) at the end of three months. This significant reduction was not long-lasting, because the

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absence of CHX application during the next three months, resulted in a 47.7% increase in *Porphyromonas gingivalis*.

Gingival massaging of CHX gel can mechanically disrupt the biofilm on teeth, dispersing the agents throughout the gingiva, stimulating blood circulation to the gingival tissues and thereby strengthening its immune response. The most important unique property of CHX is its substantivity or oral retentiveness.[27] CHX also has the ability to neutralize *Porphyromonas gingivalis*. The di-cationic positively charged CHX is attracted to the negatively charged phosphate containing compounds in the bacterial cell wall. This alters the integrity of the bacterial cell membrane and makes CHX get attracted to the inner cell membrane and binds to phospholipids causing leakage of low molecular weight compounds like potassium ions. The cytoplasm of the cells get coagulated and chemically precipitated due to the formation of phosphate complexes which include adenosine triphosphate and nucleic acids leading to bacterial death.[28]

Individuals with DS need more assistance from caretakers with their daily oral health care. In our study, reinforcement of tooth brushing through instructions, monitoring, and continuous motivation was carried out in the presence of parents/ caregivers and schoolteachers. The visits were interactive, and parents discussed their child's oral health.

The results of this study indicated that professional treatment along with regular tooth brushing and CHX gel (0.12%) application for a short duration brought about an improvement in gingival health. However, in order to obtain long-lasting effects, periodic application of CHX gel in low concentration may be necessary in addition to mechanical plaque control.

V. Conclusion

The levels of MMP-8 in GCF and levels of *Porphyromonas gingivalis* in subgingival plaque of Down syndrome children were significantly higher than that of healthy children. The gingival health of Down syndrome children showed improvement following tooth brushing, use of 0.12% chlorhexidine gel and oral hygiene reinforcement. It indicates the need for continuous mechanical and chemical plaque control measures along with regular monitoring in Down syndrome children.

Legends of Tables

Table 1: Comparison of mean levels of MMP-8 and *Porphyromonas gingivalis* between the groups

Group	MMP-8 levels Mean ± SD (ng/ml)	*Porphyromonas gingivalis* levels Mean ± SD (CFU/ml)
Down syndrome (Group I)	1.253 ± 0.268	0.317 ± 0.035
Control (Group II)	0.028 ± 0.022	0.048 ± 0.04-
t	20.375	22.474
p value	<0.001*	<0.001*

*p<0.001 is significant

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Table 2: Comparison of mean MMP-8 levels at different time intervals in Group I

Time	MMP-8 levels Mean ± SD (ng/ml)	H	p value
Baseline	1.253 ± 0.268	34.300	p<0.001*
At 3 months	0.155 ± 0.147		
At 6 months	0.413 ± 0.319		

*p<0.001 is significant

Table 3: Comparison of mean *Porphyromonas gingivalis* levels at different time intervals in Group I

Time	*Porphyromonas gingivalis* levels Mean ± SD (CFU/ml)	H	p-value
Baseline	0.3167±0.035	61.819	p<0.001*
At 3 months	0.1936±0.051		
At 6 months	0.2858±0.044		

*p<0.001 is significant

Table 4: Pair-wise comparison of MMP-8 levels in gingival crevicular fluid at different time intervals in Group I

Time	Time	MMP-8 levels Mean difference (CFU/ ml)	95% confidence interval		p-value
			Lower	Upper	
Baseline	At 3 months	1.0970	0.914	1.280	<0.001*
	At 6 months	0.8390	0.560	1.118	<0.001*
At 3 months	At 6 months	-0.2580	-0.435	-0.081	0.001

*p<0.001 is significant

Table 5: Pair-wise comparison of *Porphyromonas gingivalis* in dental plaque levels at different time intervals in Group

Time	Time	*Porphyromonas gingivalis* levels Mean difference (CFU/ ml)	95% confidence interval		p-value
			Lower	Upper	
Baseline	At 3 months	0.1230	0.0900	0.1560	<0.001*
	At 6 months	0.0310	0.0060	0.0560	0.01
At 3 months	At 6 months	-0.0920	-0.1240	-0.0610	<0.001*

*p<0.001 is significant

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Table 6: Comparison of mean plaque index (PI) and gingival index (GI) scores at different time intervals in Group I

Index	Time			H	p-value
	Baseline	At 3 months	At 6 months		
Plaque Index Score (Mean± SD)	1.47 ± 0.36	0.95±0.28	1.22±0.29	38.100	<0.001*
Gingival Index Score (Mean± SD)	1.48 ± 0.35	0.99 ± 0.38	1.24 ± 0.39	39.077	<0.001*

*p<0.001 is significant

Table 7: Pair-wise comparison of mean plaque index (PI) scores at different time intervals in Group I

Time	Time	Plaque index score Mean difference	95% CI for Diff.		p-value
			Lower	Upper	
Baseline	At 3 months	0.52	0.42	0.62	<0.001*
	At 6 months	0.25	0.16	0.34	<0.001*
At 3 months	At 6 months	-0.28	-0.34	-0.21	0.001

*p<0.001 is significant

Table 8: Pair-wise comparison of mean gingival index (GI) scores at different time intervals in Group I

Time	Time	Gingival index score Mean difference	95% CI for Diff.		p-value
			Lower	Upper	
Baseline	At 3 months	0.50	0.42	0.57	<0.001*
	At 6 months	0.25	0.18	0.31	<0.001*
At 3 months	At 6 months	-0.25	-0.29	-0.21	0.001

*p<0.001 is significant

Table 9: Association between MMP-8 levels and *Porphyromonas gingivalis*

Time	r score	p value
At Baseline (DS)	0.115	0.315
At 3 months	1.000	0.000*
At 6 months	-0.047	0.422

*p<0.001 is significant

Why this paper is important to Pediatric dentists?

- This study indicates the need for continuous mechanical and chemical plaque control measures in Down syndrome children.
- Pediatric dentists can opt for a 0.12% Chlorhexidine gel to be implemented in their practice for these children.
- Pediatric dentists can regularly educate the parents and children and conduct oral hygiene reinforcements in these children.

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Improvement of Surgical Treatment with Combined Sculoorbital Injuries

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Summary- *Purpose of the research:* Improving surgical treatment of bottom orbital fractures in children, with the use of auto-cartilaginous block, without destroying the integrity of the rib and collagen membrane.

Material and methods: We performed 12 operations according the proposed methods. During analyzing the results of the restoration of the orbital bottom published by most authors, the obvious problem is a lack of common criteria for evaluating the effectiveness of surgical treatment. Perhaps, this circumstance explains it and significantly differences presented in the data when different authors use the same methods of plastic surgery of the lower orbital wall.

Keywords: *bottom fractures of the orbit, surgical treatment, auto-cartilaginous block.*

GJMR-J Classification: *NLMC Code: WU 300*



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Improvement of Surgical Treatment with Combined Sculoorbital Injuries

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Summary- ***Purpose of the research:*** Improving surgical treatment of bottom orbital fractures in children, with the use of auto-cartilaginous block, without destroying the integrity of the rib and collagen membrane.

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Keywords: bottom fractures of the orbit, surgical treatment, auto-cartilaginous block.

I. Actuality

Despite the significant successes of maxillofacial surgery, the rehabilitation of patients with bottom orbital fractures is one of the actual problems of modern maxillofacial surgery and ophthalmology in particular. In the structure of traumatic brain injuries, fractures of the lower wall of the orbit account for 7.9% [1].

One of the main problems of bottom orbital fractures is enophthalmos of the eyeball, accompanied with prolapse and sharp limitation of eye movements. The typical reason for limiting movement of the eyeball is the interposition of the orbital tissue and perforation in the maxillary sinus, followed by a partial or complete decrease function of the visual analyzer, leading to disability. According to B.L. Polyak(in 1972), with the trauma of the above localization, damage of the visual analyzer occurs in 57% of cases [2].

The problem of surgical treatment of injuries lower wall of the orbit in maxillofacial surgery started to study in detail a little more than 20 years ago, although some works have been encountered before. Also, very interesting information on the diagnosis and treatment of fractures of this localization is presented in the writings of V.A. Belchenko (1988), V.A. Stuchilova (1988), Yu.A. Medvedev (1984, 1992), F.T. Temerkhanova (2000) [3, 4, 5, 6, 7].

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The main purpose of surgical treatment for fractures of the lower orbital wall is to restore its anatomical integrity and functional perfection, which is achieved either by repositioning and holding the fragments in the correct position, or by replacing a bone defect using of transplants.

A so-called “blow-out” or isolated fracture is distinguished into a separate group (J.M.Converse, B. Smith 1956) [8] when sharply increases intraorbital pressure as a result of a blow to the eyeball, which leads to damage of thin lower walls. Moreover, the eyeball may remain intact (S.N.Bessonov, 2001) [9].

Experimental researchhas shown that fractures caused by wave-like deformation are limited to the front half of the inner part of the bottom of the orbit, do not extend to the medial wall, and are not accompanied by infringement of soft tissues.

Using a traditional x-ray examination does not provide information about the state of the deep sections of the orbit and a lower group of extraocular muscles,also it is impossible to determine the dislocation of the eyeball. In this regard, computed tomography has become an integral part of diagnostic research. The necessity of compulsory computed tomography for all patients with the trauma of this localization was indicated by E.K. Kolesnikova [10], 1995; S.H. Miller (1972) [11], N.A. Rabukhina (2006) [12].

a) Purpose of the research

To improve surgical treatment of bottom orbital fractures with the use of auto-cartilaginous block, without destroying the integrity of the rib and collagen membrane in children.

II. Material and Methods

In the department of pediatric maxillofacial surgery at the clinic of the Tashkent State Dental Institute, 12 patients with this pathology were admitted for the period 2012-2017. The age of patients ranged from 8 to 17 years. We performed 12 operations using the proposed method. This method is different for its ease of execution and its technical characteristics justify itself in the anatomical and functional restoration of the damaged area of the face providing good aesthetic results.

We carried out antibacterial and general health-improving therapy in the postoperative period. 2-3

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weeks after the operation and then for three months, physiotherapy was recommended, including magneto and laser therapy.

a) Clinical example

Patient A., was hospitalized to the pediatric maxillofacial surgery at the clinic of the Tashkent State Dental Institute (TSDI) with a mild diagnosis case of closed craniocerebral injury. Brain contusion. Soft tissue trauma of the periorbital region on the right. The concussion of the visual organ is moderate fracture of the orbital bottom.

b) Complaints on admission

Swelling in the upper and lower eyelids of the right eye, limitation, and pain during movement of the eyeball.

Anamnesis Morbi: A patient was injured during a football match.

Status local: Soft tissue edema of the upper and lower eyelids of the right eye determined on visual examination. The skin of the upper and lower eyelids is cyanotic, palpation is painless. The movement of the right eye is limited to the upward and outward side. On palpation, nasal bones are without pathology.

On (multi-slice spiral computed tomography) MSCT diagnostics determined lower wall's fracture of the right eyeball and punching of the right eyeball to a depth of 1.5-2 cm. in the maxillary sinus.



a b c

Fig. 1: a, b, c - MSCT sections of the orbital lower walls' fracture with perforation of fiber in the right maxillary sinus;

c) Clinical example

Based on (multi-slice spiral computed tomography) MSCT diagnostics and general condition of the child, it was planned operation "Removing of the soft tissues from maxillary sinus to right orbit by eliminating defect of the lower wall using with an auto-cartilage and collagen membrane, under general intubation anesthesia.

d) Stages of our operation method

The operative site is carefully cleanedunder intubation anesthesia.

A cut line is drawn with a medical felt-tip pen under the ciliary edge and sequentially IX-X of the rib area, soft tissues of the palate are infiltrated with anesthetics.



d e f

Fig. 2: d - view of the patient before surgery, restriction of the eyeball movement; e– marks of cut lines withfelt-tip pen above and below ciliary edge; f - raising eye globe

The skin incision is made underciliary edge of the lower eyelid and exfoliates 1.5-2.0 cm, then infraorbital muscle and periosteum dissected and raised with a special Farabeuf surgical instrument.

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The lower wall of the orbit is revised carefully, the interposition and perforated ocular fiber is pulled out. If it is not possible to completely fiber is removed from the maxillary sinus, in this case, the "window" is opened from the oral cavity side and this auxiliary to remove soft tissues from both sides.

In the region IX-X of the costal arch on the skin, an incision until the cartilaginous part of these ribs is made. A block of split cartilage with a thickness of 0.2-0.4 mm is taken without destroying the integrity of the ribs. A wound is seed layer-by-layer.

Cartilaginous block is prepared to close the defect in orbital lower wall; the above part is closed with bone-cartilaginous from the collagen membrane, which prevents further relapses, perforations, and interposition of the optic tissue in the maxillary sinus. Soft tissues are sewed in layers, and intradermal sutures are put.

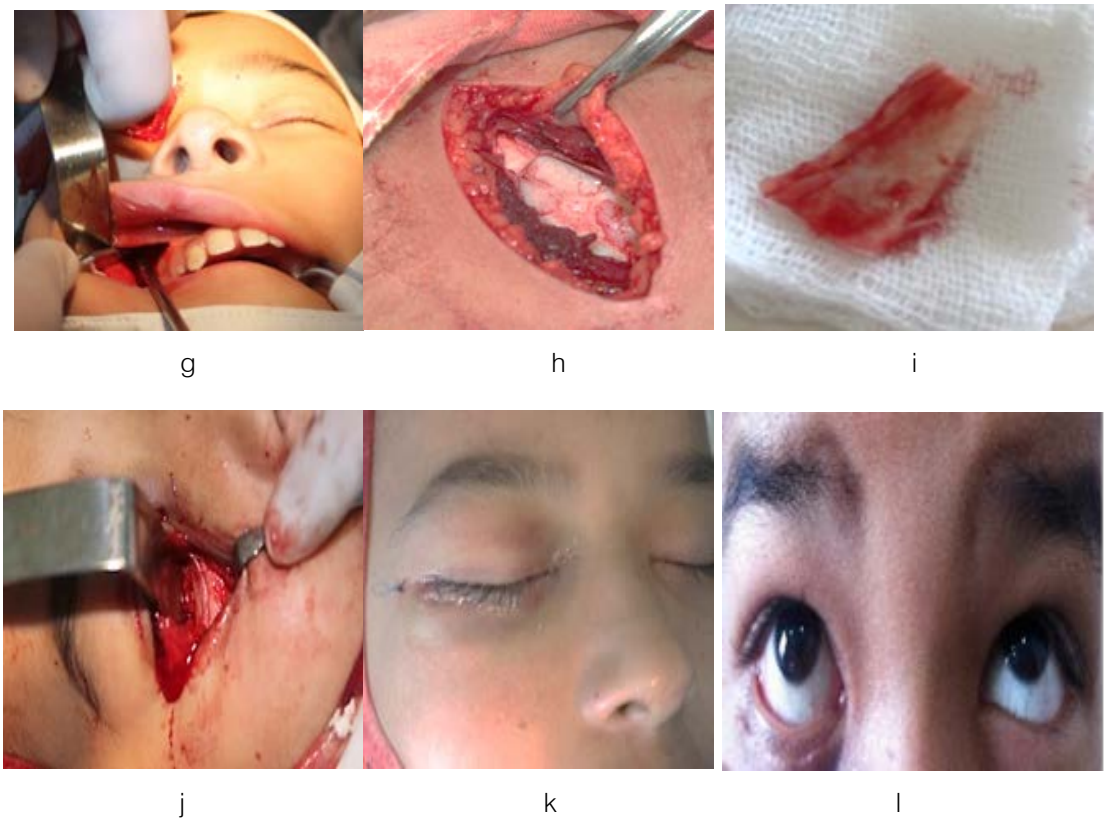


Fig. 3: g - opening the "window" in the anterior wall of the maxillary sinus; h, i - taking the auto-cartilaginous block; j - defect correction with an auto-cartilage graft and closing of the defect with a collagen membrane; k - stitching of the skin; l–view of patient after surgery on 14th day.

III. Analysis of Results

This type of operation showed that after surgical treatment of the patients with fractures of the orbital lower wall, the general state of health improved, the intensity of sickliness (OR pain) decreased significantly, asymmetry of the eyeballs during eye movement disappeared. The formation of an aesthetic scar was noted under the ciliary edge of the eyelid. Using an auto-cartilaginous block without destroying the integrity of the rib and it makes it possible to heal the postoperative field quickly. Also, there is a complete closure of the collagen membrane with bone-cartilaginous combination, which prevents further relapses, perforation and interposition of the orbital tissue in the maxillary sinus at the same time.

a) Findings

Thus, our experience should be evaluated as positive to achieve good anatomical, functional, and aesthetic results in the surgical treatment of children with fractures of the orbital bottom.

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Association between Periodontitis and Severe Asthma: The Role of IgG Anti-*Porphyromonas gingivalis* levels

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Abstract- Asthma and periodontitis are both very prevalent worldwide. Although the association between these diseases has been investigated, the biological mechanism underlying this association, especially the role of biological mediators, remains unclear. Thus, the aim of this study was to evaluate serum levels of anti-*Porphyromonas gingivalis* IgG in subjects with and without severe asthma. A case-control study involving 169 individuals consisted of subjects with severe asthma in addition to others without asthma (control group). An indirect enzyme linked immunosorbent assay (ELISA) was performed to measure serum levels of IgG specific to *Porphyromonas gingivalis*. Bacterial DNA was extracted from subgingival biofilm samples and real-time polymerase chain reaction (RT-PCR) analysis was performed to quantify *Porphyromonas gingivalis* levels.

Keywords: *asthma, periodontitis, immunoglobulin G.*

GJMR-J Classification: *NLMC Code: WU 242*



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Association between Periodontitis and Severe Asthma: The Role of IgG Anti-*Porphyromonas gingivalis* levels

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Abstract- Asthma and periodontitis are both very prevalent worldwide. Although the association between these diseases has been investigated, the biological mechanism underlying this association, especially the role of biological mediators, remains unclear. Thus, the aim of this study was to evaluate serum levels of anti-*Porphyromonas gingivalis* IgG in subjects with and without severe asthma. A case-control study involving 169 individuals consisted of subjects with severe asthma in addition to others without asthma (control group). An indirect enzyme linked immunosorbent assay (ELISA) was performed to measure serum levels of IgG specific to *Porphyromonas gingivalis*. Bacterial DNA was extracted from subgingival biofilm samples and real-time polymerase chain reaction (RT-PCR) analysis was performed to quantify *Porphyromonas gingivalis* levels. No statistically significant differences were observed between case and control groups with respect to levels of IgG specific to *Porphyromonas gingivalis* whole extract (p=0.484) or rHmuY protein (p=0.903). The relative quantification of *Porphyromonas gingivalis* was similar between the groups with and without asthma (p=0.553). When participants were regrouped according to a diagnosis of periodontitis, no significant differences were detected between subjects with and without periodontitis, either in serum levels of IgG specific to *Porphyromonas gingivalis* whole extract (p=0.789) or specific to rHmuY (p=0.630). However, participants with chronic periodontitis had lower levels of *Porphyromonas gingivalis* in their biofilm than individuals without periodontitis (p=0.001). We hypothesize that the occurrence of asthma exerts an influence over *Porphyromonas gingivalis* colonization in the biofilm, as well as modulates the production of IgG specific to this pathogen in periodontitis.

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I. Introduction

Severe asthma and periodontitis are chronic diseases highly prevalent worldwide, affect individuals ≥ 18 years [(1,2)].

Asthma, an inflammatory disease affecting the airways, is characterized by increased bronchial responsiveness and reversible airflow limitations, i.e. frequent episodes of shortness of breath and gasping, thoracic oppression and coughing[(2)]. Of the individuals affected by asthma, 10% suffer from its severe form, leading to negative economic and social impacts. The recurrent suffocation experienced results in human suffering and decreases in quality of life[(3)].

Among the chronic diseases that are predominant nowadays, the relationship between asthma and oral health has been a relevant topic of discussion [(4)]. The presence of chronic infections seems to influence the pathogenesis of asthma, and a wide range of phenotypes and endophenotypes have been observed[(5)].

Many studies have demonstrated a positive association between asthma and periodontitis[(6–10)]. However, other studies found a negative association and attempted to explain this using the hygiene hypothesis. Accordingly, a high prevalence of oral infectious diseases may contribute to decreases in the incidence of asthma and other allergic diseases. Thus, exposure to oral bacteria, including periodontal disease pathogens, could play a protective role in the development of asthma and allergies by polarizing the immune response[(13,14)].

Recently, a new model describing the pathogenesis of periodontitis was proposed, in which the onset of periodontal disease is promoted by a synergistic and dysbiotic microbial community, instead of a specific group of bacteria referred to as periodontopathogens. Some bacteria present at low levels in the microbiota can affect the entire community. Due to their important role in the development of dysbiosis, these are denominated "keystone pathogens"[(15)]. Of these, one of the most studied is

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Porphyromonas gingivalis, a Gram-, strictly anaerobic and asaccharolytic bacterium belonging to the Bacteroidaceae family, has been consistently associated with human periodontitis[15,16].

Confirming the role of *Porphyromonas gingivalisin* the etiology and pathogenesis of periodontitis, high levels of specific IgG against this bacteria were found in the sera of affected individuals[17,18]. Moreover, individuals with chronic periodontitis presented higher levels of IgG against *Porphyromonas gingivalis* antigens, such as HmuY and the gingipains proteinases, than healthy individuals, or those with gingivitis[17–21].

Due to the public health relevance of these two highly prevalent chronic diseases, asthma and periodontitis, and the gaps in knowledge regarding associations between them, specifically concerning the role of biological molecules, the present study aimed to investigate associations between these two chronic diseases, as well as to evaluate levels of IgG specific to the crude extract of *Porphyromonas gingivalis,* and HmuY, a lipoprotein of this bacteria.

II. Material and Methods

a) Study Design

The present non-matched case-control study was performed to evaluate the serum humoral immune response against *Porphyromonas gingivalis* in severe asthma. The case group consisted of individuals with severe asthma, while individuals in the control group did not have asthma. The case: control ratio was 1:2.

b) Participants and study area

Asthmatic participants were enrolled at the ProAR clinic (Program for Asthma Control in Bahia), located in Salvador, Bahia, Brazil. This study was approved by the IRB of the Feira de Santana State University (protocol no. 43131615.3.0000.0053).

Study volunteers were informed of the research protocol and required to sign a term of free and informed consent. Interviews were conducted to obtain background information regarding socioeconomic status, medical history, lifestyle and health habits, as well as oral hygiene habits and frequency of dental visits.

c) Selection criteria

Individuals were seen at the ProAR clinic, part of the multidisciplinary municipal health center (Centro de Saúde Carlos Gomes), located in Salvador, Bahia, Brazil, were selected based on a diagnosis of severe asthma. For each participant in the case group, two other non-asthmatic individuals were selected from the patient pool of the municipal health center for inclusion in the case group, provided they resided in the same neighborhood as the case participant. To not introduce bias, all control individuals were recruited from the dental services division of the municipal health center on days in which a periodontal specialist was not present.

d) Sample size calculation

Sample size was calculated based on a previously reported proportion of individuals with periodontitis (61.9%) within a group of severe asthmatic patients versus 27.1% in a group without asthma[6]. Thus, considering an odds ratio of 4.37,a significance level of 95%, a power of 80% for two-tailed testing, and a case: control ratio of 1:2, the minimum sample size was 54 individuals for the CASE group and 108 individuals for the CONTROL group.

e) Periodontal Diagnosis

Individuals were considered to have periodontitis when at least four teeth presented one or more sites meeting all of the following conditions: probing depth ≥ 4mm, clinical attachment level ≥ 3mm, and bleeding on probing after stimulation[22]

f) Diagnosis of Severe Asthma

The diagnosis of asthma was made by GINA, 2012[7]. Before the inclusion, a revision in the medical records of each participant was realized, and only those who received a diagnosis by two specialists and confirmed by spirometry were included.

g) Peripheral blood collection

Peripheral blood (5 mL) was collected by venipuncture in the antecubital fossa from all participants and stored in Vacutainer tubes (BD, SP, Brazil) with clot activator. The tubes were centrifuged for 10 min to obtain sera, which was stored at -20^{o} until analysis.

h) Antigen obtainment

P. gingivalis ATCC33277 was grown in Brucella broth supplemented with menadione and L-cysteine, and then somatic immunogenic proteins were obtained by centrifugation (18). The recombinant protein HmuY (rHmuY) of *Porphyromonas gingivalis* was cloned from *Escherichia coli* using a plasmid as a cloning vector, then purified and characterized.

i) ELISA to measure IgG against antigens of Porphyromonas gingivalis

An enzyme linked immunosorbent assay (ELISA) was used to evaluate the humoral immune response against a crude extract of *Porphyromonas gingivalis* and the rHmuY protein using an indirect measure of IgG levels in the sera of the participants.

A 96-well plate was coated with 5μg/mL of antigen (*P. gingivalis* crude extract or rHmuY) and incubated overnight at 4^{o}C. After washing with phosphate-buffered saline (PBS), blocking was performed using 5% skim milk (Molico, Araçatuba, Brazil). After reincubation for 2h at 37^{o}C, the plates were washed twice, and diluted sera (1:1000) was placed in each well, followed by an incubation period of 1h at 37^{o}C. All wells were then washed five times with PBS and reincubated with anti-human IgG conjugated with

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peroxidase (Sigma Aldrich, USA) diluted at 1:25000 for 1h at 37°C. After five additional washes, a chromogenic substrate (H_2O_2-TMB) was added, and the reaction was stopped using 2N H_2SO_4. IgG levels were measured by optical density (OD) using a microplate reader (PR2100 Bio-Rad, USA) at wavelengths ranging from 450- 620 nm.

j) Biofilm Collection

Following periodontal examinations, subgingival biofilm was collected using a periodontal curette from the site with the greatest probing depth in each sextant (Hu-Friedy, USA). Samples were pooled in a microtube containing sterile PBS (one tube for each patient), and, after centrifugation, pellets were stored at -20°C until DNA extraction.

k) Bacterial DNA Extraction and Genotyping

Bacterial DNA was extracted from subgingival biofilm samples using a PureLink™ Genomic DNA Mini Kit (Invitrogen) by the manufacturer's protocol.

The relative quantification of *P. gingivalis* was performed using the TaqMan® probe quantitative real-time polymerase chain reaction (qPCR) method. The probe sequence used in the reaction was: 5'- 6-FAM-CRA ACA GGA TTA GAT ACC CTG GTA GTC CRC-BHQ1 -3'. The primer sequence was: *forward* - 5'- GAC TGA CAC TGA AGC ACG AAG -3' and *reverse* - 5'- GCT TGA CGG TAT ATC GCA AAC TC -3'. The PCR mix (final volume of 12.5 µl) consisted of: 10xbuffer (1.25µl) containing 50mM$MgCl_2$(0.38µl),4x2.5mM(1µl), 10 µM forward primer (0.38 µl), 10 µM reverse primer (0.38 µl), 5U/µl Taq (0.05 µl), RNase-free water (6.33 µl), 10 µM probe (0.25 µl) and DNA (2.5 µl). Reactions were performed using an initial denaturation cycle at 94°C for 1 min, 45 cycles at 94°C for 20 sec, and 58°C for 35 sec.

l) Statistical Analysis

Descriptive statistical analysis was carried out using the Student T-test for continuous variates and the chi-square test, or Fisher´s exact test, for dichotomous variates. Comparisons of IgG levels were made using the Mann- Whitney test. The association between periodontitis and severe asthma was evaluated by logistic regression, using the backward strategy to select confounders. All statistical analyses were performed using the SPSS v21 statistical package, and the results were considered statistically significant when $p \leq 0.05$.

III. Results

A total of 169 individuals were included in the study. The group with severe asthma (case) consisted of 53 Participants (31.4%), while 116 participants (68.6%) were included in the group without asthma (control). The mean age in the case group was 49.5±12.1 years, versus 43.95±11.4 years in the control group. In the case group, 45 (84.9%) participants were female, and eight (15.1%) were male, while the control group consisted of 100(86.2%) females and 16 (13.8%) males. No statistically significant differences were detected between the groups in terms of age (P=0.17) or sex (P=1.0), nor concerning the socioeconomic and demographic characteristics evaluated (Table 1), demonstrating homogeneity regarding these covariates.

Table 2 delineates the distribution of aspects related to lifestyle habits and oral health in the groups with and without severe asthma. No statistically significant differences were observed between cases and controls concerning these aspects, except for mouth-breathing habit (P <0.01).

Table 3 lists characteristics related to the general health status of the participants. While homogeneity was observed between the case and control groups, statistically significant differences were seen about diagnoses of periodontitis (P<0.01) and hypertension (P<0.01).

A positive association between periodontitis and severe asthma was observed (OR = 6.73 [2.57-17.64]), even after adjusting for mouth breathing, hypertension, body mass index (BMI) and practicing physical activity (OR=7.96 [2.65-23.9]). The frequency of periodontitis in the case group was 30.8% versus 6%, in the control group, i.e, the chance of having severe asthma was almost eight times higher among individuals with periodontitis.

The humoral evaluation found ,no statistically significant differences in levels of IgG specific to the crude extract (P=0.48) or rHmuY (P=0.90) between the case and control groups, as illustrated in Figures 1 and 2, respectively. Moreover, IgG levels specific to the crude extract (P=0.79) and to rHmuY (P=0.63) were similar among individuals with or without periodontitis, as shown in Figures 3 and 4, respectively.

Also, we found no statistically significant differences in the relative amount of *Porphyromonas gingivalis* (P=0,05) among participants with severe asthma and those without asthma (Figure 5). Unexpectedly, significantly lower relative amounts of *Porphyromonas gingivalis* (P<0,001) were observed in the biofilm of individuals with periodontitis in comparison to those without periodontitis (Figure 6).

IV. Discussion

The main finding of the present study was the establishment of a strong association between periodontitis and asthma, even after adjusting for confounders, indicating that individuals with periodontitis are more likely to suffer from asthma. Another relevant result was similar production levels of IgG specific to *P. gingivalis* among the participants, regardless of whether they had severe asthma, periodontitis, or neither of these conditions. While the

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literature indicates that individuals with periodontitis are expected to harbor significantly higher levels of IgG specific to *Porphyromonas gingivalis*[17], the present results were divergent. This would seem to suggest that the presence of severe asthma may influence the humoral response to *Porphyromonas gingivalis*, the relative quantities of *Porphyromonas gingivalis* were found to be significantly lower in the subgingival biofilm of individuals with chronicperiodontitis.

To date, the literature is controversial regarding the relationship between periodontitis and asthma. Several studies have shown positive associations [7,23–26] in individuals with periodontitis, who are five times as likely to present bronchial inflammation[6], or have a three times greater chance of developing severe asthma[27]. By contrast, other studies have reported either a negative association[13] or demonstrated the absence of any relationship between these diseases[11,12].

Possibly due to this lack of concordance, there are no reports in the literature that attempt to confirm this association on a molecular level. To an effort to investigate this association, we sought to evaluate serum levels of IgG against antigens of *Porphyromonas gingivalis,* a keystone microorganism in periodontal dysbiosis[15,28], which is prevalent in deep periodontal pockets[16].

In contrast to the findings reported herein, previous studies have shown that individuals with periodontitis present high levels of IgG specific to *Porphyromonas gingivalis* extract, as well as toitslipoprote in HmuY. The literature indicates a remarkable discrepancy regarding IgG levels in individuals with a clinical diagnosis of periodontitis, those with gingivitis, and others with sound periodontal health[18,19,35]. Nevertheless, is important to emphasize that these studies did not include individuals diagnosed with any other diseases, nor those taking medications, such as corticosteroids, which like this eliminated agents capable of modulating host response.

Given this fact, it is possible to speculate that, in individuals with chronic periodontitis, the presence of severe asthma may modulate the production of IgG specific to *Porphyromonas gingivalis*, which reinforces the bidirectional association between these two diseases. Regardless, further investigation is necessary to clarify whether this modulation occurs by way of immune system regulation.

Furthermore, it is possible that the similarities seen in levels of IgG specific to the *Porphyromonas gingivalis* extract and HmuY, in individuals with and without severe asthma, may be related to possible interactions between periodontal tissues and the drugs used to control the clinical symptoms of asthma[23,25]. The immunosuppressant effect of corticosteroids can influence the host immune response seen in periodontal tissues, including the production of IgG[36].

Moreover, it is possible that the presence of asthma provokes changes in the periodontal microenvironment, which may alter the colonization, in the subgingival biofilm, of members of the microbial community. Herein, surprisingly, the relative quantities of *Porphyromonas gingivalis* were found to be higher in individuals without periodontitis.

As previously mentioned, this microorganism is capable of modulating host defense by altering the growth and development of the entire microbial community, i.e. eliciting destructive changes in the relationships among its members, which is normally homeostatic. *Porphyromonas gingivalis* is considered to be a keystone pathogen that, when present even in low amounts in the biofilm, can provoke, an imbalance between host response and the biofilm, which may favor the onset and progression of periodontitis[15].

It is important to consider that some studies argue that the presence of periodontitis may offer protection against asthma. This is justified by the hygiene hypothesis, which holds that fewer opportunities for infection are responsible, at least in part, for increases in the prevalence of allergic diseases[11–13].

In this context, a study reported that *Porphyromonas gingivalis* can reduces inflammation in the airways, although this effect did not affect the hyperreactivity of these airways[14]. Also, high concentrations of serum IgG against *Porphyromonas gingivalis* were also found to be significantly associated with a lower prevalence of asthma and sibilance [13].

The present study represents an initial attempt to investigate the molecular aspects of the association between periodontitis and asthma. A link between levels of *Porphyromonas gingivalis* in the subgingival biofilm and, the humoral immune response against this bacterium was found. Moreover, the relative quantification of the bacterium was performed via a widely used sensitive technique. Also, the immunoassays employed to evaluate IgG levels were previously standardized, and the capacity to distinguish among a variety of periodontal conditions was also demonstrated[35].

About limitations, due to the present case-control design, there was no way to establish which disease preceded the other, as both are chronic diseases. The possibility of residual confoundment may also exist, since some covariates, such as genetic factors, may not have been considered.

Thus, in light of its limitations, the present study seems to suggest that the occurrence of severe asthma is capable of modulating the colonization of *Porphyromonas gingivalis* in the subgingival biofilm, in addition to the production of IgG specific to antigens of this bacterium (extract and HmuY) in the sera of individuals with periodontitis.

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Tables and Figures

Table 1: Distribution of socioeconomic demographic characteristics among individuals diagnosed with severe asthma and individuals without asthma. ProAR, Salvador, Bahia-Brazil, 2015

	Without asthma (N=116)	Severe asthma (N=52)	
Age (in years)	N (%)	N (%)	p **
18-39	41(35,3%)	13(25%)	0,21
>39	75 (64,7%)	39 (75%)	
Sex	N (%)	N (%)	p**
Female	100 (86,2%)	45 (84,9%)	0,82
Male	16(13,8%)	8(15,1%)	
Race / skin color	N (%)	N (%)	p**
White	13 (11,3%)	6 (11,3%)	1,00
Black / brown	102(88,7%)	47 (88,7%)	
Household density (number of people)	N (%)	N (%)	p**
≤ 3 people	56(48,3%)	27(50,9%)	0,87
> 3 people	60(51,7%)	26 (49,1%)	
Marital status	N (%)	N (%)	p**
With partner	59 (50,9%)	28(52,8%)	0,87

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Without partner	57 (49,1%)	25(47,2%)	
Family income (in minimum wage)	N (%)	N (%)	p**
< 2 minimum wage	49(42,2%)	30 (56,6%)	0,08
≥ 2 minimum wage	67(57,8%)	30(56,6%)	
City of residence	N (%)	N (%)	p**
Salvador	113 (97,4%)	51(96,2%)	0,19
Interior	3 (2,6%)	2 (3,8%)	

** Diagnosis of periodontitis according to Gomes Filho et al 2007*
*** p: significance level (≤ 0.05) Pearson's chi-square or Fisher's exact test*

Table 2: Distribution of characteristics related to life habits and oral health condition among individuals with severe asthma diagnosis and individuals without asthma; ProAR, Salvador, Bahia-Brazil, 2015

	Withoutasthma (N=116)	**Severe asthma (N=52)**	
Current smoking habit	**N (%)**	**N (%)**	**p****
Not	107 (92,2%)	52(98,1%)	0,17
Yes	9 (7,8%)	1 (1,9%)	
Smoking habit in the past	**N (%)**	**N (%)**	**p****
Not	93 (86,9%)	45 (86,5%)	1,00
Yes	14 (13,1%)	7(13,5%)	
Frequent alcohol use	**N (%)**	**N (%)**	**p****
Not	77 (66,4%)	34 (64,2%)	0,86
Yes	39 (33,6%)	19(35,8%)	
Physical activity practice	**N (%)**	**N (%)**	**p****
Not	84 (72,4%)	30(56,6%)	0,05
Yes	32 (27,6%)	23(43,4%)	
Consultation with the dental surgeon	**N (%)**	**N (%)**	**p****
Never or 1 ≥ year	72 (62,1%)	34 (64,2%)	0,86
<1 year	44(37,9%)	19 (35,8%)	
Oral health guidelines	**N (%)**	**N (%)**	**p****
Not	32 (27,6%)	14(26,4%)	1,00
Yes	84 (72,4%)	39 (73,6%)	
Breathing habit	**N (%)**	**N (%)**	**p****
Not	73 (62,9%)	11(20,8%)	<0,01
Yes	43(37,1%)	42(79,2%)	
Frequency of brushing	**N (%)**	**N (%)**	**p****
<3x per day	50 (43,1%)	21(39,6%)	0,400
3x a day or more	66 (56,9%)	32 (60,4%)	
Flossing	**N (%)**	**N (%)**	**p****
Not	60(51,7%)	26 (49,1%)	0,868
Yes	56 (48,3%)	27(50,9%)	
Oral antiseptic	**N (%)**	**N (%)**	**p****
Not	71(61,2%)	33 (62,3%)	1,00
Yes	45 (38,8%)	20 (37,7%)	

** Diagnosis of periodontitis according to Gomes Filho et al 2007*

*** p: significance level (≤ 0.05) Pearson's chi-square or Fisher's exact test*

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Table 3: Distribution of characteristics related to general health conditions among individuals diagnosed with severe asthma and individuals without asthma. ProAR, Salvador, Bahia-Brazil, 2015

	Without asthma (N=116)	Severe asthma (N=52)	
Hypertension	N (%)	N (%)	p**
Not	95 (81,9%)	29 (54,7%)	<0,01
Yes	21(18,1%)	24(45,3%)	
Diabetes	N (%)	N (%)	p**
Not	109 (94,0%)	46(86,8%)	0,14
Yes	7(6,0%)	7(13,2%)	
Osteoporosis	N (%)	N (%)	p**
Not	115(99,1%)	51(96,2%)	0,23
Yes	1 (0,9%)	2 (3,8%)	
Kidney disease	N (%)	N (%)	p**
Not	115(99,1%)	53 (100%)	1,00
Yes	1 (0,9%)	0(0%)	
Hypercholesterolemia	N (%)	N (%)	p**
Not	110 (94,8%)	46(86,8%)	0,01
Yes	1(0,9%)	7(13,2%)	
Cardiovascular disease	N (%)	N (%)	p**
Not	115(99,1%)	84(93,3%)	0,04
Yes	1 (0,9%)	6 (6,7%)	
Body mass index (weight / height²)	N (%)	N (%)	p**
< 25	42(36,2%)	11 (20,8%)	0,05
≥25	74 (63,8%)	42(79,2%)	
Diagnosis of periodontitis *	N (%)	N (%)	p***
Without periodontitis	109 (94%)	36(69,2%)	<0,01
With periodontitis	7 (6%)	16 (30,8%)	

** Diagnosis of periodontitis according to Gomes Filho et al 2007*
*** p: significance level (≤ 0.05) Pearson's chi-square or Fisher's exact test*

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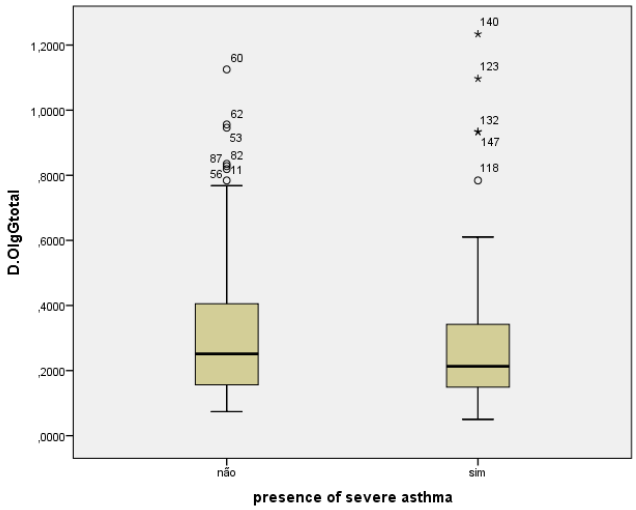


Figure 1: Serum levels of IgG specific to the extract of *Porphyromonas gingivalis* in individuals with severe asthma and without asthma (p=0,482).

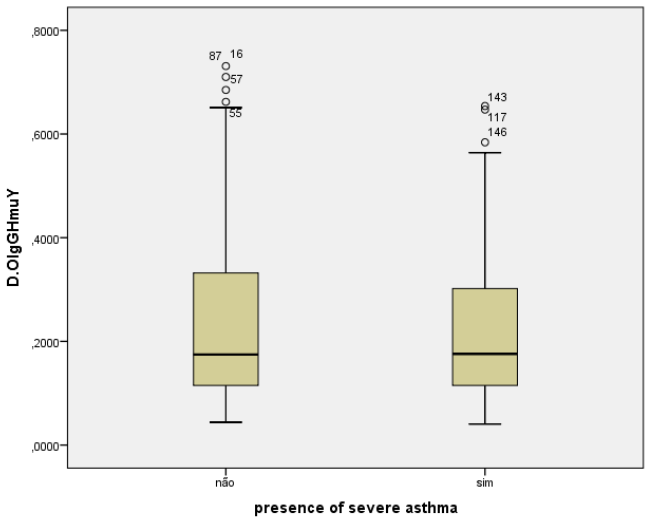


Figure 2: Serum levels of IgG specific to the recombinant protein HmuY of *Porphyromonas gingivalis* in individuals with severe asthma and without asthma (p=0,903).

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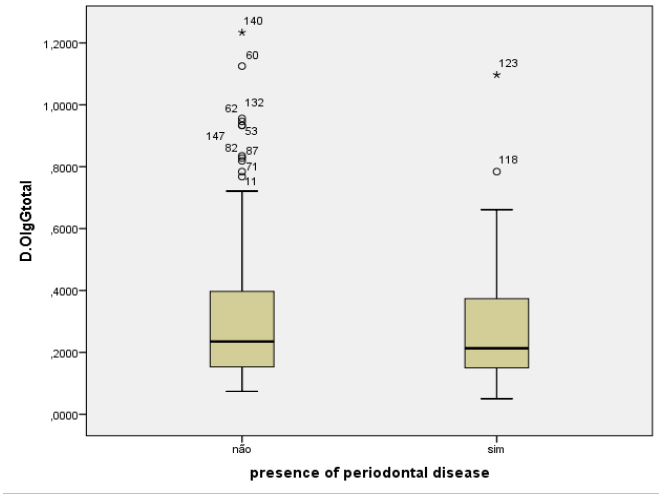


Figure 3: Serum levels of IgG specific to the extract of *Porphyromonas gingivalis* in individuals with and without periodontitis (p=0,789).

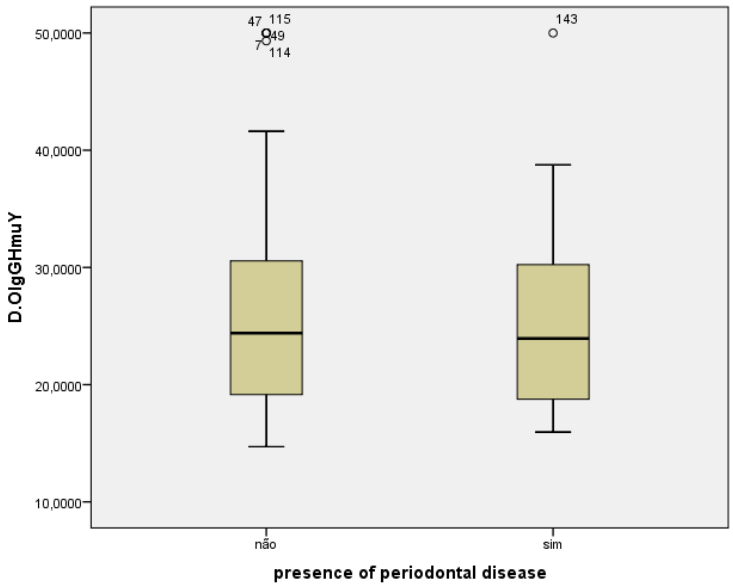


Figure 4: Serum levels of IgG specific to the recombinant protein HmuY of *Porphyromonas gingivalis* in individuals with and without periodontitis (p=0,630).

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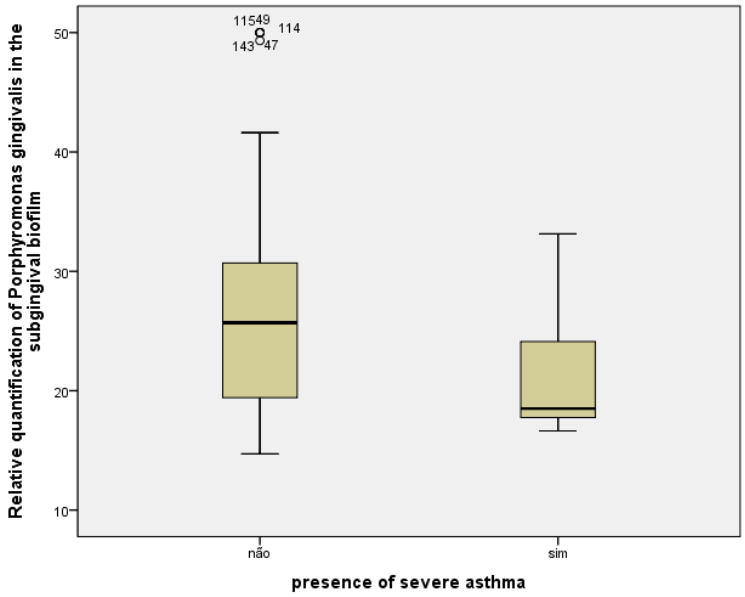


Figure 5: Relative quantification of *Porphyromonas gingivalis* in the subgengival biofilm of individuals with severe asthma and without asthma.

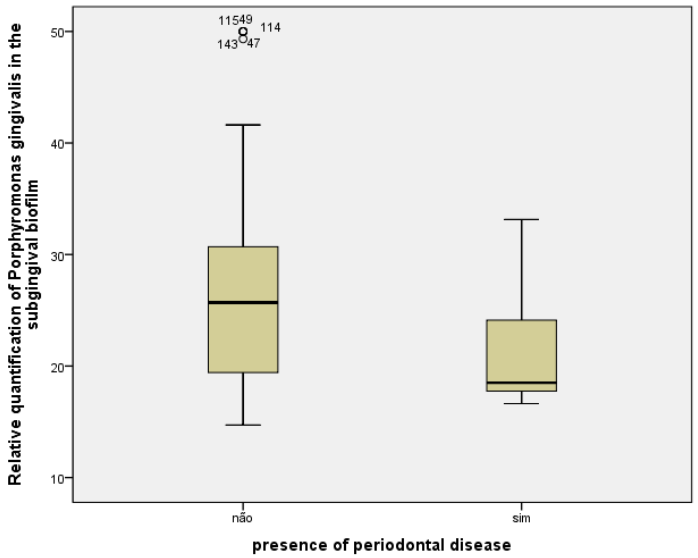


Figure 6: Relative quantification of *Porphyromonas gingivalis* in the subgengival biofilm of individuals with and without periodontitis.

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Evaluation of the Pharyngeal Airway Space Before and After Bi-Lateral Sagittal Split Osteotomy Surgery using Three-Dimensional Cone Beam Computed Tomography

By Sameer Khan, Devaki Vijayalakshmi, K.S. Nagachandran, S. Karthik, Janani Jayapal & Abinaya Somaskandhan

Meenakshi Ammal Dental College

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Material and Methods: The sample consisted of patients (n=7), aged between 21-30 years, having a skeletal Class II with retrognathic mandible and orthognathic maxilla who underwent orthodontic treatment and were advised for BSSO advancement surgery. Pre-surgical CBCT scans were taken a week before the surgery (T0) and the post-treatment records, three months after the surgery (T1). The 3D PAS was reconstructed from the CBCT scans, and the volumetric changes were evaluated.

Keywords: *airway volume, BSSO advancement, CBCT, 3-dimensional, pharyngeal airway space.*

GJMR-J Classification: *NLMC Code: WU 300*



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Sameer Khan [α], Devaki Vijayalakshmi [σ], K.S. Nagachandran [ρ], S. Karthik [ω], Janani Jayapal [¥] & Abinaya Somaskandhan [§]

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Result: The PAS volume was derived, and the pre-surgical values (T0 mean=14083.19mm3) were compared with the post-surgical values (T1 mean=18067.26mm3), and a significant increase by 28.29% in the volume was evident ($t=4.51$, $p=0.04 < 0.05$) for a mean mandibular advancement of 4.7mm.

Conclusion: Our findings suggest that bilateral mandibular advancement surgery in patients with retrognathic mandible increases the PAS volume anatomically and enhances the breathing functionally.

Keywords: airway volume, BSSO advancement, CBCT, 3-dimensional, pharyngeal airway space.

I. Introduction

The upper airway has long been an area of interest in orthodontics, with topics such as the relationships between facial type and airway,[1] airway shape and volume with growth and development[2] and the clinician's potential to modify the airway through treatments like growth modification[3] and surgery for correction of jaw deformities.[4]However, most studies evaluating the airway have been conducted with two-dimensional (2D) cephalograms, providing limited data such as linear and angular measurements, for a complex three-dimensional (3D) structure.[5,6]

Contemporarily, the lateral cephalograms still seem to be the dominating evaluation tool in the field of upper airway research inspite of its disadvantages. The main drawback, without any doubt, is considered as the degradation of a three-dimensional (3D) entity into two dimensions. With the introduction of CBCT, shortcomings of lateral cephalograms have been overcome.[7,8]

Despite the widespread use of CT examinations in clinical practice, this new technology brought along concerns about the exposure to ionizing radiation and its potential hazards. Therefore, radiation dose and strategies for dose reduction, especially for younger patients, have become an important focus of interest.

With the advent of Cone Beam Computed Tomography, lower radiation doses and faster image acquisition times have become possible when compared with conventional computed tomography scans.[9,10] Also the three-dimensional diagnosis of the airway is more reliable with the Cone Beam Computed Tomography with the reduced radiation dose.[11]

The isolation of the hard and soft tissue structures based on the variations in their densities is apparent in a CBCT image, which allows the segmentation and visualization of hollow structures such as the airway, and the analysis of the airway volume are easy with the use of appropriate software.

Previous studies have reported that patients with skeletal class II malocclusion with mandibular retrognathism had a reduced pharyngeal airway space.[12] Mandibular advancement surgery has been advocated as a treatment option for skeletal retrognathism, which has a strong influence on the airway post-surgically. Several studies have reported regarding the Pharyngeal Airway Space (PAS) changes two-dimensionally using lateral cephalograms after

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surgical procedures.[5,6] The purpose of this study is to evaluate the airway volumetric after mandibular advancement surgery using 3D CBCT techniques.

II. Materials and Methods

The study protocol was reviewed and approved by 'The Institutional Ethics Committee of Meenakshi Academy of Higher Education and Research, Meenakshi University' with the Ref MAHER/COE – 250/2014. Before the CBCT scan, the patients were fully informed about the purpose of this study and the radiation risks associated with the scan. All the patients included in this study were from the department of orthodontics, meenakshi ammal dental college and hospital who have reported for orthodontic treatment.

Inclusion Criteria

i. The patients included in the study were adults within the age group of 21 years to 30 years.
ii. The patients should have a skeletal Class II jaw base with retrognathic mandible and an orthognathic maxilla.
iii. Cephalometric criteria (mean pre-surgical values). SNB 76.40. ANB 4.70. Wit's Appraisal 2.4mm.
iv. The patients should not have undergone any previous surgeries of the oral and maxillofacial region before the study.

Seven patients, who were advised for mandibular advancement surgery using Bilateral Sagittal Split Osteotomy, were selected for this study. The 3D-CBCT was acquired using Planmeca ProFacecone beam 3D imaging system (Planmeca, Helsinki, Finland). The pre-surgical 3D-CBCT was taken within a week before the surgery. The post-surgical 3D-CBCT was taken three months following the Bi-Lateral Sagittal Split Osteotomy. The surgery performed on the patients involved advancement of the mandible, with a mean of 4mm, in the anterior-posterior direction. Rigid fixation was done using mini-plates and screws.

All subjects were examined and oriented to have their heads positioned with the Frankfort horizontal plane parallel to the floor with maximum intercuspation. The whole maxillo-facial complex, (extending from the vault of the skull superiorly, till the level of the thyroid cartilage) was scanned with the Planmeca ProFace Cone-Beam 3D imaging system. With the exposure time of about 14 seconds, the 3D images acquired were reconstructed with 400 microns isometric voxel size, with the tube voltage of about 90 kV and 9mA tube current. Images were examined with the scanner´s proprietary software ROMEXIS (Version 3.0.2.R).

In the explorer view of the software, the image in the coronal section was oriented so that the sagittal slicing plane (Red line) passes through the anterior nasal spine (ANS). This will help to obtain the particular sagittal section which passes exactly through the mid-sagittal plane. In the sagittal section of the image, the following landmarks were marked (Figure: 1)

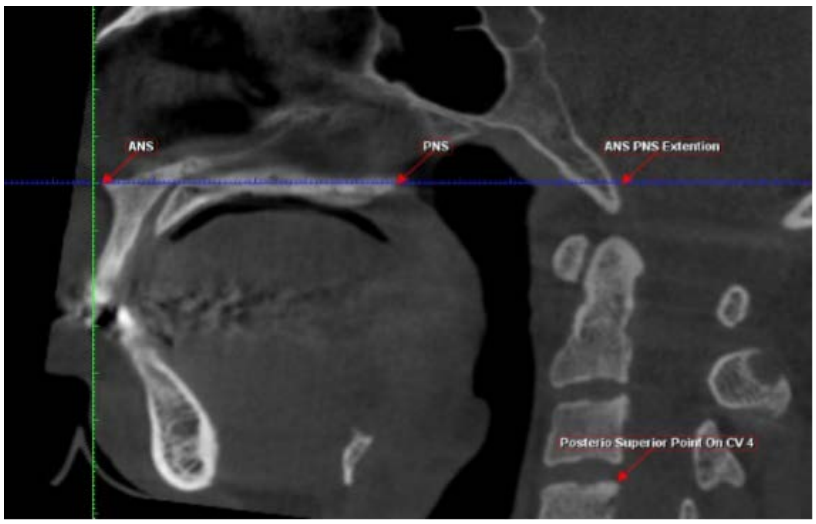


Figure 1: Sagittal section showing orientation to palatal plane with its landmarks (Pre-surgical)

- ANS-Anterior nasal Spine
- PNS-Posterior nasal Spine
- C4up-The most superior point on the posterior border of the body of the C4

Then the sagittal image is rotated so that the palatal plane (ANS-PNS) is parallel to that of the axial slicing plane (Blue Line). Since the airway is not bounded fully with hard tissue structures, the anterior and superior boundary is located at the PNS point and the posterior and inferior boundary is located at the C4up point as it corresponds to the deepest point of the vallecula. Taking these landmarks as reference, the external volume of the airway is drawn using a tool provided by the software.

The airway could be isolated after demarking the total volume of interest required for the study. The ROMEXIS software automatically created the third dimension based on the height and the width of the

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region marked in the two-dimensional view (Figure: 2). Using the 3D region growing tool, the 'air cavity' was selected from the pre-sets. A local threshold level of 70 was used and the particular area of interest in the airway region was selected.

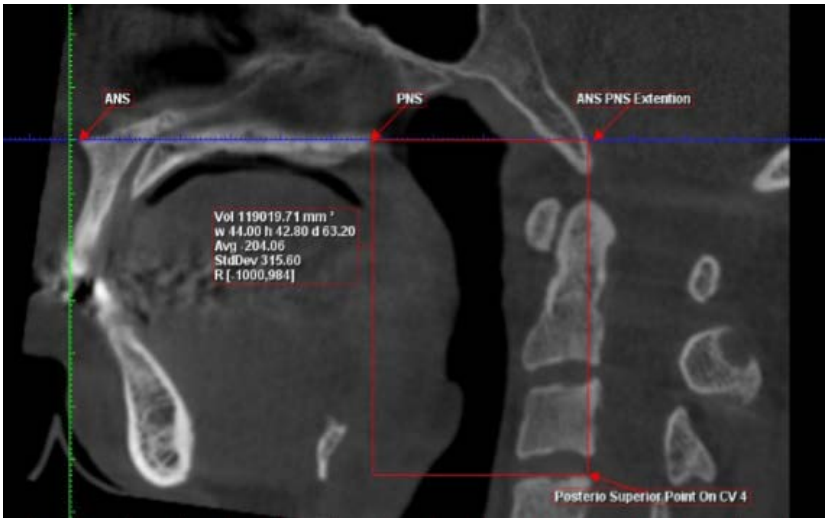


Figure 2: Demarcation of the total volume of interest required for the study (Pre-surgical)

Depending on the density variations, the volume of the selected region is derived and displayed by the software in all the three orientation planes (Figure: 3). Thus, the air cavity was isolated and a 3D rendered image of the airway along with its volume (measured in mm^3) was displayed in the explorer window (Figure: 4a, 4b).

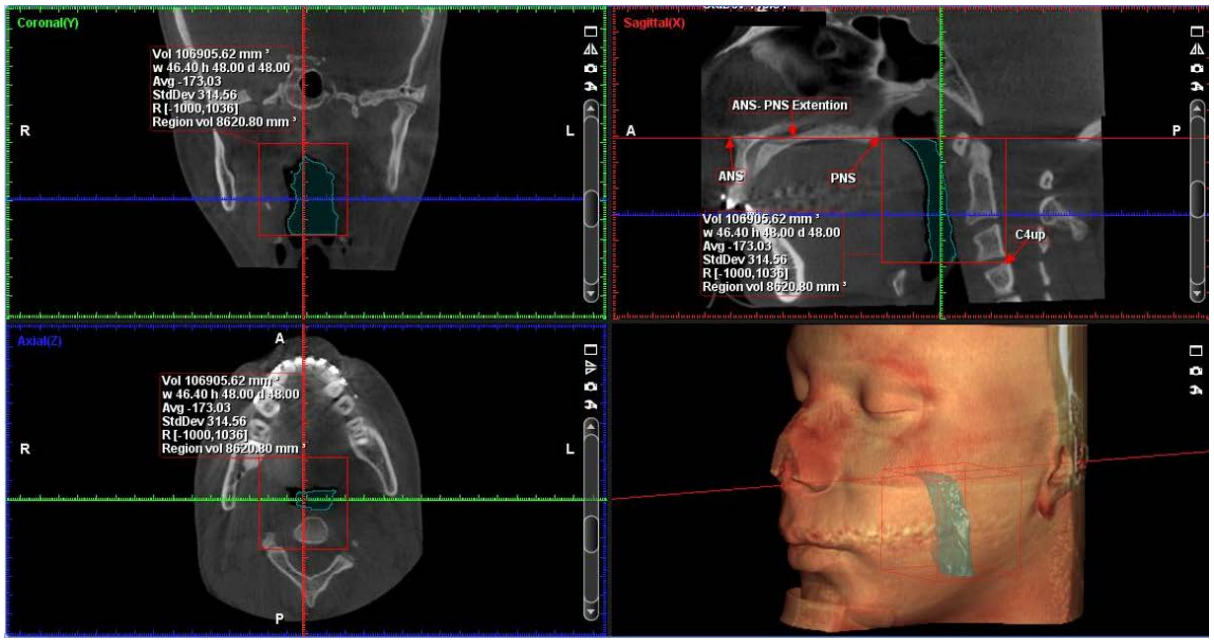


Figure 3: Airway isolated in coronal sagittal, axial and 3D rendered view

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Figure 4(a): Measured Pharyngeal Airway Space volume (mm^3)

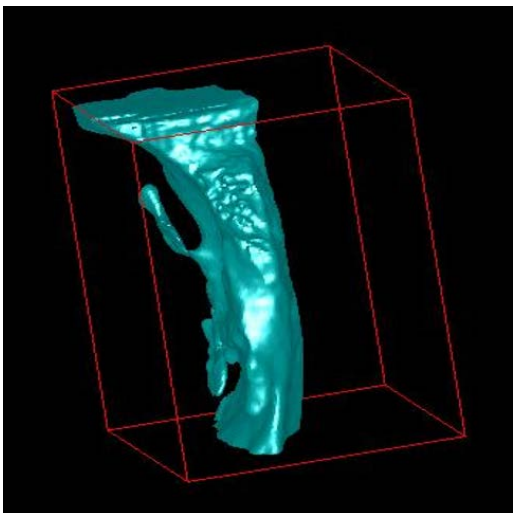


Figure 4(b): 3D rendered view of the isolated Pharyngeal Airway Space (Pre- surgical)

This procedure was carried out by a single operator (S.K) to create and measure the rendered volumes. For reliability purpose, it was repeated by the same operator after a period of two weeks and finally, the average is taken for the calculation.

Descriptive statistics including the mean, and standard deviation for each group were calculated by using SPSS (version 20.0). The Normality test, Kolmogorov-Smirnov and Shapiro Wilk tests results showed that all the variables followed the normal distribution and therefore the parametric test was applied to analyze the data. To compare the mean values between the pre-surgical and post-surgical Pharyngeal Airway Space (PAS) volume paired t-test was applied. A p-Value < 0.05 level of significance was used for the test.

III. Results

The descriptive statistics are summarised in Table-I, II shows the changes in the cephalometric measurements of the craniofacial morphology taken before and after the mandibular advancement surgery. The volumetric increase in the Pharyngeal Airway Space (PAS) for the patients (n=7) showed a significant

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increase from baseline (T0) to 3 months post-surgical (T1) ($t=4.51$, $p=0.04 < 0.05$), which is represented in Table-III. From baseline (T0) to the post-surgical measurements (T1), the total PAS volume for a mean mandibular advancement of 4.7mm showed an increase of 28.29%.Changes in the PAS volume before and after mandibular advancement surgery (volume in mm3) are given in Figure: 5-7.

Table I: Volumetric measurements of the Pharyngeal Airway Space before and after mandibular advancement surgery and their mean values of the patients (n=7)

Patients	Pre-Surgical Airway Volume (mm^3) (T_0)	Post-Surgical Airway Volume (mm^3) (T_1)	Increase in volume (mm^3) (T_1)- (T_0)	Percentage of variation (%)
Patient 1	14832.45	16645.08	1812.63	12.22
Patient 2	8246.72	10917.12	2670.4	32.38
Patient 3	13206.72	20093.57	6886.85	52.15
Patient 4	17946.11	23677.06	5730.95	31.93
Patient 5	13351.42	20075.36	6723.94	50.36
Patient 6	14124.56	16031.62	1907.06	13.50
Patient 7	16874.34	19031.01	2156.67	12.78
Mean	**14083.19**	**18067.26**	**3984.07**	**28.29%**

Table II: Variables describing the craniofacial morphology of the samples both pre-surgical and postsurgical.

Mean Variables	Pre Surgical (T_0)	Post-Surgical (T_1)	Difference (T_1)-(T_0)
SNB °	76.4	81.3	4.9
ANB °	4.7	2.4	2.3
Wit's (mm)	2.4	-0.9	-3.3

Table III: Paired differences & the test results of the study

Post – Pre surgical airway volume	Mean Paired Differences		t – Value	p-Value
	Volume (mm^3)	Percentage of variation (%)		
	3984.07	28.29%	4.51	0.04

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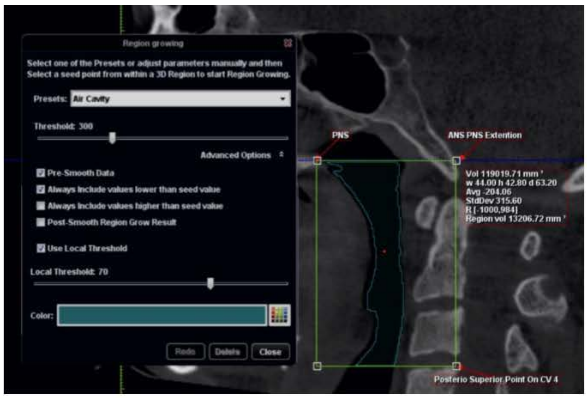
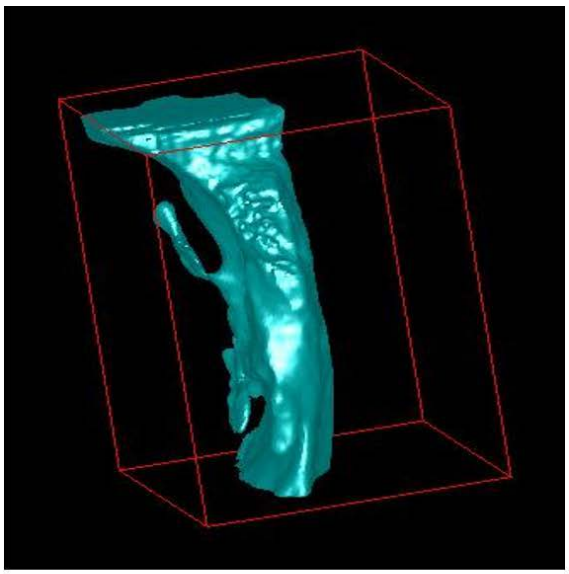




Figure 5(a) *Figure 5(b)*

Figure: 5(a): Pre-surgical & 5(b): Post-surgical Pharyngeal Airway Space



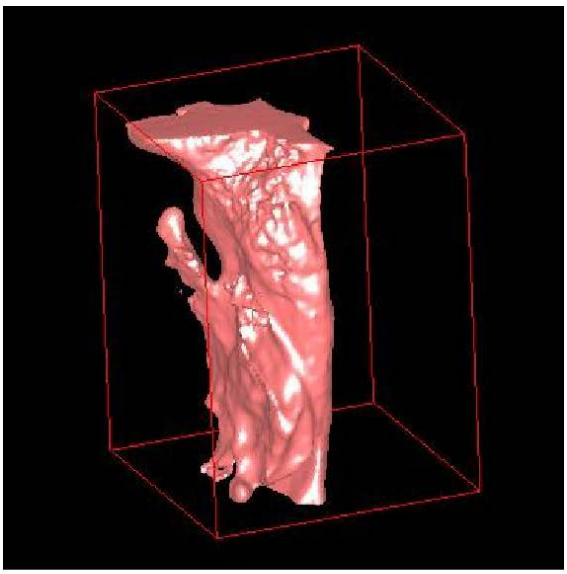


Figure 6(a) *Figure 6(b)*

Figure: 6(a): Pre-surgical & 6(b): Post-surgical 3D rendered view of the Pharyngeal Airway Space

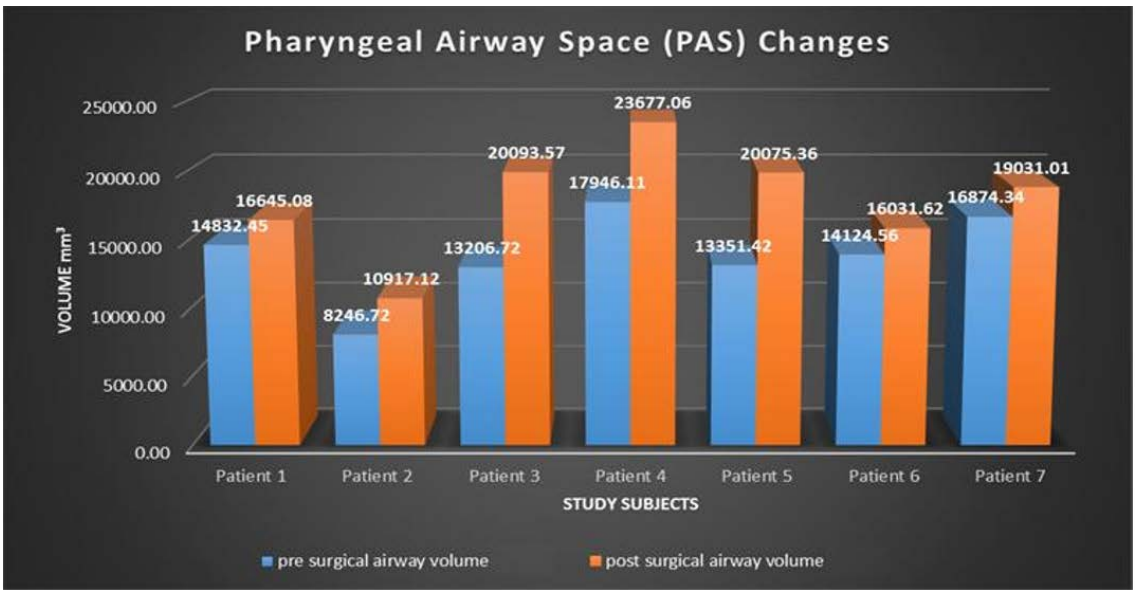


Figure 7: Pharyngeal Airway Space volume changes before and after mandibular advancement surgery (volume in mm^3)

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IV. Discussion

Several two-dimensional studies have evaluated the relationship between the airway, head posture, craniofacial morphology, and the positioning of the jaw bases.[1,2,13,14] However many drawbacks have been reported regarding the assessment of the airway using the lateral cephalograms. Some of them include, assessment of a three-dimensional structure using two-dimensional imaging technique, measurements in "area" rather than "volume" and superimposition of structures.

To overcome these drawbacks, many studies have utilized the Computed Tomography (CT) Scan for the assessment of the Pharyngeal airway.[15-17] However the ionizing radiation and potential hazards of increased radiation dose with conventional CT Scan have limited its use in assessing the Pharyngeal airway. On the contrary CBCT imaging provides an adequate imaging quality with low radiation doses and shorter exposure time. Recently many studies have also been reported regarding the accuracy of CBCT in assessing the Pharyngeal Airway volume over the conventional lateral cephalograms, and CT Scans.[8,11]

Aboudara et al[8] evaluated the PAS volumes determined from cephalograms and compared them with 3D-CBCT records. The 3D PAS analysis yields reproducible and clinically relevant data while being superior to cephalometric analysis. In the present study, Pharyngeal Airway Space (PAS) volume was analyzed by using the software PLANMECA ROMEXIS. The threshold value in the analysis software is an important tool in determining the boundaries of the airway to be measured. By varying the threshold value, the filling degree of the airway can get altered, and hence the measurements might be overestimated or underestimated when compared with the actual volume. Alves et al[18] evaluated the influence of threshold value in measuring the actual airway volume and reported that the accurate prototype airway volume could be obtained with thresholds between 70 and 75 when using the Dolphin 3D software. In the present study, Romexis Software was used; for the assessment of the airway volume, the software is provided with a pre-set local threshold value of 70, which was similar to that as suggested by Alves et al. and the software automatically calculated the airway volume in cubic millimeters.

Studies have reported that the variation in the anteroposterior jaw relationships show different pharyngeal airway volumes and patients with a retrognathic mandible have a relatively lesser airway volume when compared to the airway volume to that of a Class I skeletal base.[2,18]

Treatment modalities for the correction of mandibular retrognathism in Class II patients in growing individuals include functional appliance therapy to advance the mandible where as in adults, surgical mandibular advancement is the only option available, both of which will have a strong influence in the airway volume.[19,20] Li et al. compared growing class II div1 patients and patients treated with twin block appliance and found an improvement in the oropharyngeal and the hypopharyngeal airway along with the anterior positioning of the hyoid bone after functional appliance treatment.[21]

Several studies have reported the improvement of the PAS in Class II patients with mandibular retrognathia after mandibular advancement surgery.[22,23] Hernández et al. have stated that the mandibular advancement surgery results in greater improvement in the Pharyngeal airway volume due to the advancement of the skeletal attachment of the suprahyoid muscles and tendons that play a major role in the widening of the PAS.[24]

Turnbull et al. described a similar effect in their cephalometric study demonstrating significant enlargement in the PAS after mandibular advancement surgery. It was noted that the volume increase is most pronounced in the lower and middle thirds of the PAS, which is related to post-operative advancement of the tongue, hyoid, and associated structures.[25]Kochel et al. in their 3D CBCT study assessed the Pharyngeal airway changes after mandibular advancement surgery in adult Class II patients and reported an increase in the PAS by 45.6%.[26]

The improvement in the PAS in our study showed an increase of 28.29% for a mean mandibular advancement of 4.7mm. This was less when compared to that of the studies reported by Hernández et al.[24] and Kochel et al.[26] Probably, the reason for the reduced degree of improvement in our study is due to lesser sample size. Also, the degree of mandibular advancement might influence on the magnitude of the airway volume improvement, and Hernández et al.[24] have already reported that in their future study, they might try to evaluate a correlation between the magnitude of the skeletal forward movement and the increase in the PAS volume.

The present study evaluated the airway volume, three dimensionally using cone beam computed tomography in patients with skeletal Class II malocclusion with mandibular retrognathism. However, the limitations of the current study include limited sample size, and short-term follow-up. Moreover, the reliability of the software was not carried out.

V. Conclusion

The following conclusions can be drawn from the present study:

1) Mandibular advancement surgery increased the Pharyngeal Airway Space (PAS) in patients with the retrognathic mandible.

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2) With a mean mandibular advancement of 4.7mm, the PAS showed about 28.29% of improvement, which was statistically significant.

Our findings suggest that bilateral mandibular advancement surgery in patients with retrognathic mandible is a viable treatment option that not only improves the facial profile and esthetics but also enhances the functional characteristics of the airway by increasing its volume.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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