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Evaluation of Adding Chlorhexidine Containing Varnish to the Conventional Preventive Protocol on Caries Risk Assessment for Special Needs Adolescents: A Comparative Study

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KEYWORDS

Caries risk assessment, Chlorhexidine, preventive protocol, special needs, special dental care.

ABSTRACT:

Aim: Adolescents with special needs are very prone to caries progression. (1) providing them with intensified preventive regimen could be an effective intervention to mitigate the risk . This study aimed to compare a chlorhexidine-containing varnish to the conventional preventive protocol (fluoride toothpaste, mouthwash and fluoride varnish without chlorhexidine) on caries risk assessment for special needs adolescents over a 10 -month follow up period.

Materials and methods: A total of 22 participants were divided into two groups according to the tested regimen. Cariogram parameters were collected to generate individual caries profiles. A risk-based preventive program was implemented, and caries profiles were generated again at the end of 10 months. At the end of 10 months, caries profile was generated again. Statistical analysis was performed with a significance level set at $P \leq 0.005$.

Results: Regarding the main outcome, the chance to avoid new caries, a statistically significant difference was found before and after applying any preventive program For the "Plaque amount" Cariogram parameter, there was a significant reduction in the measured score after administration of the protocol containing chlorhexidine ($p<0.05$).

Conclusion: The regular use of preventive regimens is effective in improving caries-related factors in this study. Additionally, the inclusion of chlorhexidine was effective in reducing plaque amounts without diminishing the effect of fluoride.

INTRODUCTION

The global population living with a disability was estimated at 1.3 billion between 2021 and 2023, with 12 million in Egypt alone..[1-3] These patients are more susceptible to dental caries due to factors such as poor oral hygiene, motor limitations and lack of accessible dental services coupled with inadequate skills and knowledge among healthcare professionals. Consequently, preventive oral health regimens are required to mitigate the risk [1, 3-7]

While the use of conventional tooth brushing and fluoride has been shown to improve oral health, more intensive programs may be necessary to achieve better outcomes and avoid the need for surgical interventions [8-11]. Chlorhexidine, often referred to as the "gold standard" among antibacterial agents, has been studied for 30 years. Even in low concentrations, it may reduce the development of new carious lesions. [12-14] This study introduces a novel approach for managing adolescents with special needs by implementing an intensive program that includes chlorhexidine alongside the conventional protocol. This could represent a significant step in enhancing preventive regimens.

Journal of Chemical Health Risks
www.jchr.org
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Chlorhexidine or topical fluoride were applied as varnishes, which deliver small doses to multiple sites, ensuring prolonged action.[4,15] Cariogram, a software program which assesses caries risk by considering both protective and risk factors, was utilized to estimate risk of caries progression and impact of the intervention. Given the limited evidence-based information about adding chlorhexidine to preventive regimens for special needs patients, the aim of this randomised controlled trial was to compare a Chlorhexidine-containing varnish with conventional preventive protocols on bacterial inhibition, plaque adherence, and caries prevention..

METHODS AND MATERIALS

All participants were prescribed fluoridated toothpaste (Signal® cavity fighter, Dubai, United Arab Emirates) containing 1450 ppm fluoride, and mouthwash (B-fresh, Pharopharma, Egypt) containing 0.05 % sodium fluoride. For the control group, topical fluoride varnish (Fluor protector, Ivoclar Vivadent, UK) with 1400 ppm fluoride was applied. For the intervention group, topical varnish containing 0.3 % chlorhexidine and 1400 ppm fluoride was applied. (Cervitec F, Ivoclar Vivadent, UK).

Methods

Study registration

The study protocol was approved by the Research Ethics Committee, Faculty of Dentistry, xxx University, and in accordance with the Declaration of Helsinki and its later modifications. The study was registered on clinical trials in 24/07/2020 (www.clinicaltrials.gov) with I.D**.:** NCT 04485312

Sample size calculation

A power analysis was conducted to ensure adequate power for a 2-sided statistical test of the null hypothesis that a preventive protocol containing chlorhexidine would be no different to conventional preventive treatment for adolescent patients with special needs. . According to the results of **Patil et al. in 2011**[17] in which the probability of patients within the green sector (chances to avoid new lesions) (81-100%) of the comparator regimen was 0.87, probability patients within the green sector (61-80%) was 0.11 and probability of patients within the green sector (41-60%) was 0.02 with effect size w=1.14 (n=8). If the estimated probability of patients within the green sector (81-100%) of the intervention regimen was 0.8, probability patients within the green sector (61-80%) was 0.1 and probability of patients within the green sector (41-60%) was 0.1 with effect size w=0.98 (n=10). By adopting an alpha (α) level of 0.05 (5%), power=80%. The predicted sample size (n) was a total of 9 for each group. To account for possible dropouts during follow-up intervals, the sample size was increased by 20%, resulting in a total of 22 cases, i.e., 11 for each group. Sample size calculation was performed using G*Power 3.1.9.7.

Study design

Based on the sample size calculation, this study employs a parallel-arm design with an equivalence framework and a 1:1 allocation ratio, involving a total of 22 Egyptian adolescents with special needs, aged 13-18 years. These participants were full-time residents at disability schools and had mild to moderate disabilities according to the WHO categorization. Participants with a history of allergy to any drugs or chemicals used in the study, those wearing appliances, those on antibiotics, and those taking medications that interfere with saliva secretion were excluded. Informed consent was obtained from the participants and their caregivers.

Simple randomisation was performed according to a checklist that divided the participants into two groups, labelled A and B. Randomisation was generated using the website www.randomization.com. Allocation of participants to different preventive groups was done using sealed opaque envelopes to ensure complete concealment. Participants were further classified according to their risk profile as detailed in the demographic data analysis. Treatments were administered based on the assigned groups as described in the procedures.

The participants, assessors, and statistician were blinded to the interventions and comparator assessment methods. However, the principal investigator could not be blinded due to the difference in colour and consistency of the materials. Additionally, no information was exchanged among the research team members throughout the entire study period.

Participant preparation

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Scaling and polishing procedures were performed on all participants. Both protocols were applied every 3 or 6 months according to the participants' caries risk profile—high, moderate, or low risk respectively—as illustrated in the CONSORT flow diagram **(Figure 1).** [18] All participants were advised to brush their teeth twice daily using a circular motion for a minimum of 2 minutes (30 seconds per quadrant to reach the optimal effect on plaque reduction) using fluoride toothpaste. Supervised brushing was done with the help of their caregivers'. Additionally, mouthwashes were used after brushing with fluoride toothpaste for at least 30 seconds, twice daily [19-21]. All participants were residents of educational facilities who also prepared and provided their meals, minimising dietary variations between groups.. Health education and dietary counselling were provided to caregivers once per month to ensure patient adherence to the guidelines.

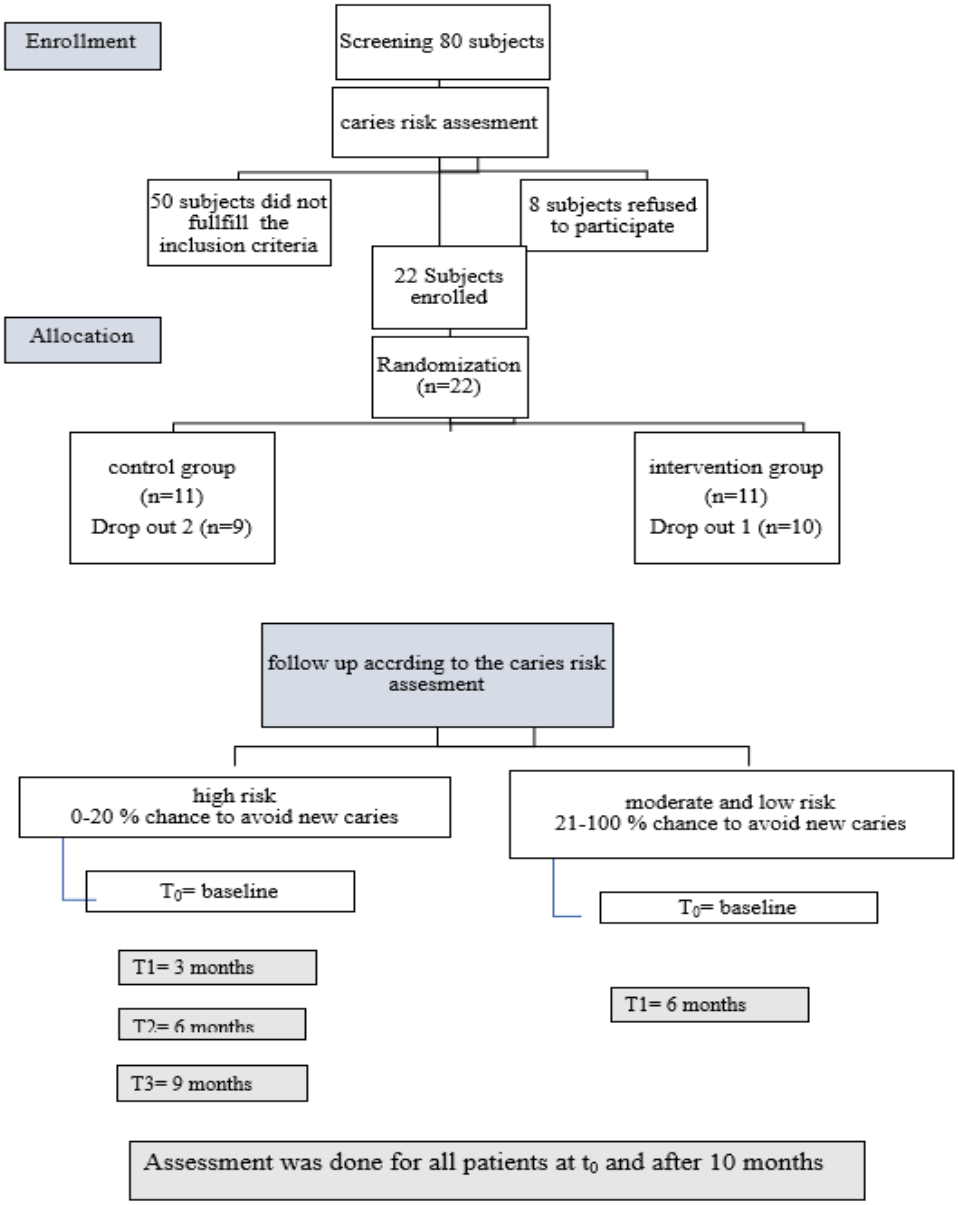

Figure 1: consort flow diagram

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JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Cariogram's caries related factors

A visual-tactile clinical examination was performed by the researcher in natural daylight, and caries diagnosis was conducted according to WHO diagnostic criteria. The DMFT (Decayed, Missing, and Filled Teeth) index was calculated. For related diseases, a score of one was assigned as a standard for all participants, given their special needs. Participants were asked about their regular diet regimen. Plaque was evaluated using the Silness-Loe index.[22] Stimulated saliva samples were collected by asking the patient to spit accumulated saliva into calibrated flasks (**Fig.2a).** If a participant was unable to spit, saliva was aspirated using a plastic syringe over a period of 5 minutes and collected in the flasks (**Fig.2b).** The Bacterial count (CFU/ml) for mutans streptococci was determined using MSB culturing media. [23] . Unstimulated saliva was collected to measure buffer capacity. Regarding the fluoride program, participants were asked about their oral hygiene routines involving any fluoride use. To avoid subjective bias, clinical judgement was uniformly set to 1 for all patients.

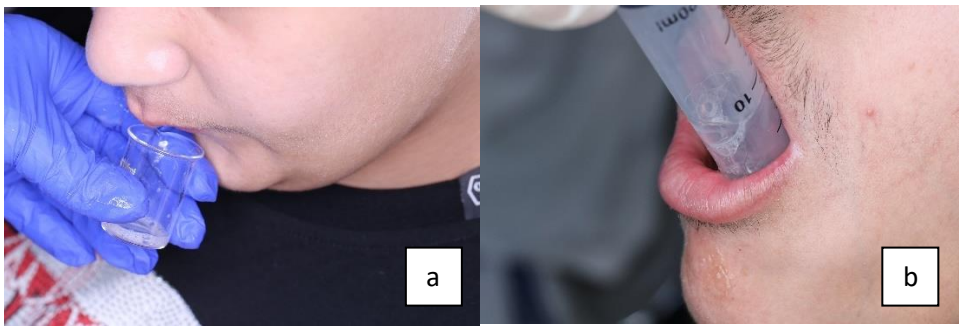

Figure 2a: Collecting stimulated saliva in the calibrated small flask & b: Aspirating stimulated saliva using plastic syringe.

Risk based preventive program

After obtaining Cariogram results for each patient, a risk-based preventive programme was implemented for both the control and intervention groups. [17,24,25] Before the application of varnishes, plaque was removed using a toothbrush without toothpaste and volunteers were asked not to eat or drink except water for one hour before the visit The teeth were then rinsed with water and dried using an air syringe and gauze. Cotton rolls were used for teeth isolation, and the varnish was applied using a micro-brush according to the manufacturer's guidelines. For each participant, 0.3 ml of varnish was aspirated by a syringe and dropped in a dish then applied to all teeth and allowed to dry for 30 seconds **(Fig. 3).** Patients were instructed to avoid spitting for 2 hours.[26]. After 10 months (one academic year) the caries risk profile was reassessed .

Figure 3: Varnish application using brush

Data confidentiality and quality control

All data related to the study were stored in locked cabinets with restricted access. Data entry was performed by the principal investigator and reviewed by a member of the research team. All data were stored on a computer and encrypted with a password to ensure accurate data

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

entry and protect against misuse. Data were also backed up on an additional storage device to prevent loss. An audit was conducted by one of the authors to ensure the quality of the research methods, sampling techniques, and interventions. The names and personal data of the participants did not appear on the protocol forms and will be securely maintained for 10 years after the trial to protect participants' privacy and civil rights.

Data collection

Baseline data collection included obtaining medical and dental histories for each patient and completing examination charts. Outcome data were collected using Cariogram scores, which were evaluated and checked at follow-up periods and after 10 months. To maximise patient retention and compliance with follow-up visits, contact information (telephone number and address) was collected from the patient or caregiver, and they were called before each visit. The importance of the study and adherence to preventive measures were explained to both the patient and caregiver.

Statistical analysis:

Age data were analysed for normality using the Shapiro-Wilk test, presented as mean and standard deviation values, and compared using the independent t-test. Categorical and ordinal data were presented as frequency and percentage values. Independent categorical data were compared using Fisher's exact test. Repeated measurements were analysed using the marginal homogeneity test. Intergroup comparisons for ordinal data were analysed using the Mann-Whitney U test. Intragroup comparisons were analysed using the Wilcoxon signed rank test. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed using R statistical analysis software version 4.1.3 for Windows.

RESULTS

The clinical trial was conducted on 22 cases, which were randomly and equally allocated to one of the two study groups (i.e. 11 cases each). There were 2 dropouts in the control group and 1 in the intervention group. In the control group, 6 (66.6%) of the participants were male and 3 (33.3%) were female. In the intervention group, 6 (60%) of the participants were male and 4 (40%) were female; the difference between the groups was not statistically significant (p=0.670). The mean age of the participants was 15.9±1.8 years in the control group and 14.8±2.1 years in the intervention group, with no statistically significant difference (p=0.206). There was also no significant difference between the groups regarding the risk profile categorisation into low, moderate, and high-risk groups (p=0.356). In the control group, 6 (66.7%) cases had high risk, while 3 (33.3%) had moderate risk. In the intervention group, 4 (40%) cases had high risk, and 6 (60%) had moderate risk.

Regarding the chance to avoid new caries, a statistically significant difference was found between before and after applying any preventive program while no statistically significant difference was found between trial arms. A correlation between different Cariogram sectors revealed that there was a strong positive correlation between green sector and red and light blue sectors scores (r_s=0.764 and 0.926). There was a moderate positive correlation between green sector and yellow and blue sectors scores (r_s=0.499 and 0.639). All correlation were statistically significant ($p<0.001$) (**table1**).

The results revealed that the parameters affected by both regimens were the red sector and the light blue sector. For "Streptococcus mutans" parameter, there was a significant reduction in scores after administration of both preventive regimens. For "Amount of plaque" parameter, there was a significant score reduction in the intervention group (p=0.034) (**table2**). For the light blue sector "Fluoride program" parameter, there was a significant score reduction in both groups ($p<0.05$), while for other parameters, the difference was not statistically significant ($p>0.05$) **(table3).** As for the yellow sector (circumstances) and the blue sector (Diet), there was no statistically significant difference between tested groups ($p>0.05$).

DISCUSSION

All disease factors were assessed using Cariogram [27] which incorporates parameters with different weights to clarify the primary cause of caries. Cariogram graphically illustrates an individualized caries risk profile, aiding communication with the patient. [28,29] and

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

predicting caries development more accurately than any single-factor model.[30]

Two types of varnish were used. Fluor Protector varnish was applied in the control group. It promotes remineralisation by forming calcium fluoride deposits in saliva, relying on the bioavailability of fluoride from the varnish and calcium from saliva. Additionally, it adheres to tooth surfaces, mucosa, and dental plaque, reducing demineralisation and inhibiting acid-producing bacteria due to its antibacterial properties. [31-34] In the intervention group, Cervitec F varnish was used. This varnish contains antibacterial agents added to the fluoride varnish (0.3% chlorhexidine plus 1450 ppm fluoride), which effectively target mutans streptococci and reduce plaque. Chlorhexidine retention in the oral cavity is crucial for its effectiveness but can cause adverse effects like tooth discolouration, changes in oral microbiota, and altered taste, especially with continuous use for more than 7 days in vehicles like mouthwashes. These adverse effects were avoided in this study by using chlorhexidine varnish every 3 or 6 months, according to each patient's caries risk profile. [15] Both varnishes allow for prolonged contact and slow release, reaching non-accessible areas more effectively than other methods. [35-39]

Demographic analysis of the participants revealed homogeneity in age and caries status in the selected population. [40] The results showed that using any preventive regimen was successful in reducing the chance of developing new caries, which aligns with the findings of other researchers. [17,25,41,42] The strong positive correlation between green, red and light blue sectors scores and moderate positive correlation between green, yellow and blue sectors , indicate that reducing bacteria and increasing protective factors decrease the likelihood of developing new cavities.. This highlights the importance of intensifying protective factors for special needs patients as there was a significant reduction in cariogenic bacteria after administrating both preventive regimens. For amount of plaque parameter, there was a significant score reduction only in the intervention group (p=0.034), demonstrating the efficacy of chlorohexidine-containing preventive regimen in caries prevention for special needs patients. This result is in total agreement with Huang et al. ,[43] who found that bacterial count is related to plaque amount, strongly affecting caries risk since the plaque film, full of bacteria in close contact with the tooth surface, initiates the caries process.[31,41,44-46] This finding also partially agrees with Doitchinova et al. [47] and completely agrees with Senneby et al. [30] who found a strong relationship between plaque index, diet frequency and increased the caries risk .

The significant reduction in plaque amount in the intervention group supports the idea that chlorhexidine is a leading antiplaque agent.[48-52] .This finding contrasts with Clavero et al. [53] who found that cervitec F has no effect on plaque index scores. Other studies suggest that the effect of Cervitec F may not be observable 12 weeks after treatment due to its short-term action. [54,55]

Regarding the light blue sector, participants' commitment to the fluoride program resulted in no significant difference between the two regimens. Comparable results were found for saliva secretion rate and saliva buffer capacity, which may be explained by the fact that the ingredients of fluoridated agents used do not directly influence salivary secretion rate, pH, or buffer capacity. This finding aligns with other studies [17,25,56,57] suggesting the addition of saliva promoting agents to the preventive protocol.

The yellow sector showed no statistically significant results as the DMF and the health status of adolescent special needs patients changed only mildly during the one-year follow-up. [17,25] The blue sector also showed no statistically significant results, disagreeing with some studies [58,59] , likely due to differences in ethnicity and eating habits or the difficulty in controlling food intake among most disabled patients. However, the moderate correlation between the blue and green sectors suggests that strict oral hygiene measures can counteract the effects of fermentable carbohydrates.

Finally, the overall results indicated that the only significant difference was in the amount of plaque, with the chlorhexidine-containing preventive regimen being more effective than the conventional regimen. Thus, the results partially support the null hypothesis and confirm the importance of an intensified caries preventive regimen for special needs patients

CONCLUSIONS

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JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Given the limitations of this primary study, including the limited sample size of the special needs population, the challenging circumstances, the work environment with special needs, and the difficulties in managing these patients, the following conclusions can be drawn. Firstly, regular use of preventive regimens using different vehicles is effective in improving caries-related factors. Secondly, the addition of chlorhexidine was effective in reducing plaque without diminishing the effect of fluoride.

Therefore, it is recommended to apply the novel intensified preventive regimen that includes chlorhexidine in addition to the conventional protocol for caries prevention in adolescents with special needs. This new approach could enhance the effectiveness of existing preventive measures.

Further studies are needed to evaluate the long-term effects of different caries preventive products and related factors on larger sample sizes. This will help achieve a better understanding of the ideal clinical protocols recommended for caries prevention in this population.

List of abbreviations

WHO: world health organization

DMFT: decayed, missed, filled teeth index

CFU/ML: the colony forming unit

MSB: Mitis Salivarius Bacitracin agar

Statements and Declarations

Competing interests:

The authors declare that they have no conflicts of interest

Ethics approval and consent to participate

This study was proposed to the Conservative Department, Faculty of Dentistry, Cairo University. Sample size calculation was revised by the Medical Biostatistical Unit (MBU). The protocol of the study was approved by the Research Ethics Committee (CREC), Faculty of Dentistry, Cairo University in June 2020 with approval number xxxx, and in accordance with the Declaration of Helsinki and its later modifications. The study was registered on clinical trials in July 2020 (www.clinicaltrials.gov) with I.D**.:** NCT 04485312**.**

Informed consent with an easy Arabic language as mother language of participants was signed by the adult participants and for minors the informed consent was signed by the parent and/or legal guardian. An informed consent was obtained from all subjects and/or their legal guardian(s) for publication of identifying information/images in an online open-access publication.

Consent for publication

The authors certify that they have obtained all appropriate informed consent forms from patients. An informed consent with an easy Arabic language as mother language of participants was signed by the adult participants and for minors the informed consent was signed by the parent and/or legal guardian. An informed consent was obtained from all subjects and/or their legal guardian(s) for publication of identifying information/images in an online open-access publication.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, on reasonable request.

REFERENCES

1. World health organization. disability and health. 2018 https://www.who.int/news-room/fact-sheets/detail/disability-and-health
2. World health organization. Disability and health. 2023. https://www.who.int/en/news-room/fact-sheets/detail/disability-and-health.
3. Waldman, H. B., Al-Khatib, S., and Perlman, S. P. Dentistry for Egyptians with Special Needs. Journal of Dental and Oral Health. 2019; 3:3, 1–3. DOI: 10.21608/adjalexu.2021.45370.1110
4. XIONG J, CHEN X, WU H. Research progress on dental materials for preventing root caries. Journal of Prevention and Treatment for Stomatological Diseases. 2024:64-9. DOI: 10.12016/j.issn.2096-1456.2024.01.010.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

5. Devinsky O, Boyce D, Robbins M, Pressler M. Dental health in persons with disability. Epilepsy & Behavior. 2020 Sep 1;110:107174. doi.org/10.1016/j.yebeh.2020.107174.
6. da Rosa SV, Moysés SJ, Theis LC, Soares RC, Moysés ST, Werneck RI, Rocha JS. Barriers in access to dental services hindering the treatment of people with disabilities: a systematic review. International journal of dentistry. 2020;2020(1):9074618. doi.org/10.1155/2020/9074618.
7. Lam PP, Du R, Peng S, McGrath CP, Yiu CK. Oral health status of children and adolescents with autism spectrum disorder: A systematic review of case-control studies and meta-analysis. Autism. 2020 Jul;24(5):1047-66. doi.org/10.1177/1362361319877337.
8. Lebrun-Harris LA, Canto MT, Vodicka P, Mann MY, Kinsman SB. Oral health among children and youth with special health care needs. Pediatrics. 2021 Aug 1;148(2). doi.org/10.1542/peds.2020-025700
9. Duangthip D, Crystal YO, Chu CH. Oral health and policy for patients with special health care needs. Frontiers in Oral Health. 2021 Dec 9;2:802255. doi.org/10.3389/froh.2021.802255
10. Featherstone JD, Crystal YO, Alston P, Chaffee BW, Doméjean S, Rechmann P, Zhan L, Ramos-Gomez F. Evidence-based caries management for all ages-practical guidelines. Frontiers in Oral Health. 2021 Apr 27;2:657518. doi.org/10.3389/froh.2021.657518
11. Bab NB, Rahman RN, Mohamed S, Radzi NA, Yusof N. Effectiveness of oral health interventions among children and adolescents with mental disorders: a systematic review. Special Care in Dentistry. 2024 Mar 13. doi.org/10.1111/scd.12990.
12. Kotsanos N, Sulyanto R, Ng MW. Dental Caries Prevention in Children and Adolescents. InPediatric Dentistry 2022 Jan 28 (pp. 247-280). Cham: Springer International Publishing. doi.org/10.1007/978-3-030-78003-6_12
13. Molina G, Zar M, Dougall A, McGrath C. Management of dental caries lesions in patients with disabilities: Update of a systematic review. Frontiers in Oral Health. 2022 Oct 28;3:980048. doi.org/10.3389/froh.2022.980048
14. Kamal D, Hassanein H, Akah M, Abdelkawy MA, Hamza H. Caries preventive and antibacterial effects of two natural mouthwashes vs chlorhexidine in high caries-risk patients: A randomized clinical trial. J Contemp Dent Pract. 2020 Dec 1;21(12):1316-24. doi.org/10.5005/jp-journals-10024-2986
15. Deus FP, Ouanounou A. Chlorhexidine in dentistry: pharmacology, uses, and adverse effects. International dental journal. 2022 Jun 1;72(3):269-77. doi.org/10.1016/j.identj.2022.01.005
16. Baik A, Alamoudi N, El-Housseiny A, Altuwirqi A. Fluoride varnishes for preventing occlusal dental caries: A review. Dentistry journal. 2021 Jun 3;9(6):64. doi.org/10.3390/dj9060064
17. Patil, Y.B., Hegde-Shetiya, S., Kakodkar, P.V., and Shirahatti, R. : Evaluation of a preventive program based on caries risk among mentally challenged using the Cariogram model. Community dental health, 2011 20: 1–6. doi:10.1922/CDH_2605. doi:10.1922/CDH_2605Patil0606
18. Wang Z, Rong W, Xu T. Effect of fluoride varnish in preventing dental caries of first permanent molars: a 24-month cluster randomized controlled trial. International journal of environmental research and public health. 2022 Dec 11;19(24):16656. doi.org/10.3390/ijerph192416656
19. Zendron MP, Rocha AD, Simões MS, Santana CM, Bolan M, Cardoso M. Fluoride varnish in dentistry: A bibliometric analysis of the 100 most-cited

papers. Caries Research. 2023 Nov 10;57(3):189-96.
20. Soldo M, Matijević J, Malčić Ivanišević A, Čuković-Bagić I, Marks L, Nikolov Borić D, Jukić Krmek S. Impact of oral hygiene instructions on plaque index in adolescents. Cent Eur J Public Health. 2020 Jun 1;28(2):103-7. doi.org/10.21101/cejph.a5066
21. Abbass, M.M.S., Mahmoud, S.A., El Moshy, S., Rady, D., Abubakr, N., Radwan, I.A., Ahmed, A., Abdou, A., and Al-Jawaldeh, A. The prevalence of dental caries among Egyptian children and adolescences and its association with age, socioeconomic status, dietary habits and other risk factors. A cross-sectional study. 2020; https:// doi.org/10.12688/f1000research.17047.1
22. Silness, J. and Loe, H. Periodontal Disease in Pregnancy II. Correlation between Oral Hygiene and Periodontal Condition. Acta Odontologica Scandinavica.1964; https// DOI.org/10.3109/00016356408993968
23. Sharma C, Khajuria RR, Singh R. Salt water rinse and chlorhexidine against oral microbes. Journal of Advanced Medical and Dental Sciences Research. 2022 Apr 1;10(4):65-8. doi: 10.21276/jamdsr
24. Brown N, Foley C, Flanagan C, Fujita T, Harford S. Fluoride varnish applications provided in general dental practice for children of primary school age in three areas of the UK. British Dental Journal. 2024 Mar 22;236(6):469-74. doi.org/10.1038/s41415-024-7171-8
25. AbdAllah E. A., Metwalli N. E., & Badran A. S. Effectiveness of a one-year oral health educational and preventive program in improving oral health knowledge and oral hygiene practices of a group of Autistic Egyptian children and their caregivers. Future Dental Journal. 2018; https://doi.org/10.1016/j.fdj.2018.02.001
26. Dhyppolito IM, Nadanovsky P, Cruz LR, de Oliveira BH, Dos Santos AP. Economic evaluation of fluoride varnish application in preschoolers: A systematic review. International Journal of Paediatric Dentistry. 2023 Sep;33(5):431-49. doi.org/10.1111/ipd.13049
27. Güler Y, Derelioğlu SŞ, Yılmaz S. Caries risk assessment in children with different rates of vitamin d deficiency, using cariogram model. Int J Child Health Nutr. 2023 Apr 18;12:23-31. DOI: 10.4103/jiaphd.jiaphd_28_23
28. Ramya K, Suresh S, Pachava S, Pavani NP, Pydi SK, Viswanath V. Caries Risk Profiles in 13–15-year-old Schoolchildren in Guntur City Using Cariogram: A Cross-sectional Study. Journal of Indian Association of Public Health Dentistry. 2024 Jan 1;22(1):66-70. DOI: 10.4103/jiaphd.jiaphd_28_23
29. Taqi M, Zaidi SJ. Predictive validity of the reduced Cariogram model for caries increment in non-cavitated and cavitated lesions: cohort study. BMC Oral Health. 2023 Oct 24;23(1):790. doi.org/10.1186/s12903-022-02383-z
30. Senneby A, Fransson H, Foresight Research Consortium, Vareman N. What is risk? The challenge of defining 'risk'in caries risk assessment. Acta Odontologica Scandinavica. 2023 Nov 17:1-5. doi.org/10.1080/00016357.2023.2275032
31. Yadav, Rakesh Kumar, et al. "Comparative evaluation of remineralizing effect of fluoride and non fluoride agents on artificially induced caries using different advanced imaging techniques." Journal of Conservative Dentistry 25.1 2022: 26-31. doi.org/10.4103/jcd.jcd_426_21
32. Erkmen Almaz, M., & Akbay Oba, A. Antibacterial activity of fluoride varnishes containing different agents in children with severe early childhood caries: a randomised controlled trial. Clinical Oral Investigations. 2020; https:// doi.org/10.1007/s00784-020-03300-w
33. Gupta, A., Sharda, S., Nishant, Shafiq, N., Kumar, A., & Goyal, A. Topical fluoride-antibacterial agent combined therapy

versus topical fluoride monotherapy in preventing dental caries: a systematic review and meta-analysis. European Archives of Paediatric Dentistry: Official Journal of the European Academy of Pediatric Dentistry.2020; https://doi.org/10.1007/s40368-020-00561-7

34. Xavier, Giftlin Denny, et al. "Comparative evaluation of remineralization potential of four different remineralization agents on human enamel: An in vitro study." Journal of Conservative Dentistry and Endodontics 27.1 2024: 29-35. doi.org/10.4103/jcde.jcde_113_23
35. Jaganath, Bharath Makonahalli, et al. "Assessment of color changes in teeth and composite resins under the influence of chlorhexidine with and without anti-discoloration system: An: in vitro: study." Journal of Conservative Dentistry 26.1 2023: 52-55. DOI: 10.4103/jcd.jcd_393_22
36. WANG X, PENG X, LI Y, ZHOU X. Research progress on the relationship between free sugars and caries. Journal of Prevention and Treatment for Stomatological Diseases. 2023:813-8. DOI: 10.12016/j.issn.2096-1456.2023.11.008
37. LIU lei, HUANG Lixia, DI Lisha, SU Kui. Clinical Study Effect of fluoride varnish, glass ionomer and resin sealant on the prevention of pit and fissure caries in young children. Journal of Prevention and Treatment for Stomatological Diseases. 2022, 30(2): 117-122. doi.org/10.12016/j.issn.2096-1456.2022.02.007
38. Brookes, Z.L.S., Bescos, R., Belfield, L.A., Ali, K., and Roberts, A. Current uses of chlorhexidine for management of oral disease: a narrative review. Journal of Dentistry.2020; https://doi.org/10.1016/j.jdent.2020.103497
39. Phadnaik, G.M., Ghike, M., Tripathi, R., and Phadnaik, M.B. Chlorhexidine: An Adjunct Anti-microbial in Dental Therapy. Journal of Dental and Medical Sciences. 2020; 19: 37–42. DOI: 10.9790/0853-1907093742
40. CHEN Bei-si , LIU Qiu-lin , CHEN Bo-lin , WU Wei , MA Xue-ling , ZENG Xiao-juan . Effect analysis of topical fluoride varnish on caries prevention of first permanent molars in school-age children[J]. Journal of Prevention and Treatment for Stomatological Diseases ,2017, 25(4): 254-257 doi.org/10.12016/j.issn.2096-1456.2017.04.011
41. LI Yujiao , CHENG Xiaogang , QIAN Fei , PAN Yating , CHEN Liyuan , TIAN Yu . Metagenomic study on the composition and function of oral microorganisms in healthy adults[J]. Journal of Prevention and Treatment for Stomatological Diseases , 2022, 30(8): 533-541 https://doi.org/10.12016/j.issn.2096-1456.2022.08.001
42. Pisarnturakit, P., & Detsomboonrat, P. Comparison of two caries prevention programs among Thai kindergarten: a randomized controlled trial. BMC Oral Health. 2020; https://doi.org/10.1186/s12903-020-01107-5
43. HUANG Xinliang , YAN Jiarong , HUA Fang , HE Hong. Research progress on the relationship between orthodontic treatment and the oral microbiome[J]. Journal of Prevention and Treatment for Stomatological Diseases, 2022, 30(12): 896-901 https://doi.org/10.12016/j.issn.2096-1456.2022.12.010 WANG X, PENG X, LI Y, ZHOU X.
44. Research progress on the relationship between free sugars and caries. Journal of Prevention and Treatment for Stomatological Diseases. 2023:813-8. DOI: 10.12016/j.issn.2096-1456.2023.11.008
45. NING J, HU X, CHENG X. Research progress on oxidative stress regulatory

mechanisms in Streptococcus mutans. Journal of Prevention and Treatment for Stomatological Diseases. 2023:295-300. DOI: 10.12016/j.issn.2096-1456.2023.04.012

46. Lorenz, K., Hoffmann, T., Heumann, C., and Noack, B. Effect of toothpaste containing amine fluoride and stannous chloride on the reduction of dental plaque and gingival inflammation. A randomized controlled 12-week home-use study. Int J Dent Hygiene. 2019; https:// doi.org/10.1111/idh.12392
47. Doitchinova, L., Kirov, D., Nikolova, J., & Topalova-Pirinska, S. Caries Risk Assessment in Adults Using the Cariogram. Journal of Folia Medica. 2020; https:// doi.org/10.3897/folmed.62.e51874
48. ZHOU Qingnan , SHANG Jiajian. Research progress on the relationship between the changes in microbial community composition of plaque and dental caries in children[J]. Journal of Prevention and Treatment for Stomatological Diseases, 2021, 29(4): 267-272 https://doi.org/10.12016/j.issn.2096-1456.2021.04.008
49. WANG R, YU F, YE L. Research progress on multifunctional natural antioxidants in oral therapies. Journal of Prevention and Treatment for Stomatological Diseases. 2023:831-6. DOI: 10.12016/j.issn.2096-1456.2023.11.011
50. Kamath DG, Nadimpalli H, Nayak SU, Rajendran V, Natarajan S. Comparison of antiplaque and anti-gingivitis effects of aloe vera mouthwash with chlorhexidine in fixed orthodontic patients—A randomized controlled trial. International Journal of Dental Hygiene. 2023 Feb;21(1):211-8. **doi.org/10.1111/idh.12615**
51. Al-Maweri SA, Alhajj MN, Deshisha EA, Alshafei AK, Ahmed AI, Almudayfi NO, Alshammari SA, Alsharif A, Kassim S. Curcumin mouthwashes versus chlorhexidine in controlling plaque and gingivitis: a systematic review and meta-analysis. International Journal of Dental Hygiene. 2022 Feb;20(1):53-61..
52. Khobragade, V.R., Vishwakarma, P.Y., Dodamani, A.S., Jain, V.M., Mali, G.V. and Kshirsagar, M.M. Comparative Evaluation of Indigenous Herbal Mouthwash with 0 . 2 % Chlorhexidine Gluconate Mouthwash in Prevention of Plaque and Gingivitis: A Clinico - Microbiological Study. J Indian Assoc Public Health Dent. 2020; https:// doi.org/10.4103/jiaphd.jiaphd_132_19. **doi.org/10.1111/idh.12518**
53. Clavero, J., Baca, P., Paloma González, M., & Valderrama, M. J. Efficacy of chlorhexidine-thymol varnish (Cervitec) against plaque accumulation and gingival inflammation in a geriatric population. Journal of Gerodontology. 2006; https:// doi.org/10.1111/j.1741-2358.2006.00085.x
54. Park KJ, Meißner T, Günther E, Schmalz G, Kottmann T, Krause F, Haak R, Ziebolz D. Arrest of root caries with an adjuvant chlorhexidine–fluoride varnish over a 12-months observation period: a QLF-analyzed, placebo-controlled, randomized, clinical trial (RCT). Odontology. 2022 Jan;110:193-202. doi.org/10.1007/s10266-021-00637-w
55. Mohamed H, Hafez A, ElBaz M. Assessment of inhibition of biofilm formation and plaque bacterial count of fluoride varnish containing chlorhexidine and cetylpyridinium chloride (Cpc) versus conventional fluoride varnish among high caries risk patients: randomized clinical trial. Egyptian Dental Journal. 2022 Apr 1;68(2):1809-18. DOI: 10.21608/edj.2022.111225.1914
56. Hassan, H., Ghali, L., Wildeboer, D., Sarwar, S., Lingström, P., and Carlén, A. Interproximal in situ Plaque pH after a Sugar Challenge in Relation to Caries in Adultsbefore and after Short-Term Use of 1.5% Arginine Toothpaste. Int J Dent Oral

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Health. 2020; https:// doi.org/10.16966/2378- 7090.323
57. Pichugina YA, Maksimova IV, Berezovskaya MA, Afanaseva NA, Pichugin AB, Dmitrenko DV, Timechko EE, Salmina AB, Lopatina OL. Salivary oxytocin in autistic patients and in patients with intellectual disability. Frontiers in Psychiatry. 2022 Nov 24;13:969674. doi.org/10.3389/fpsyt.2022.969674
58. Cascaes AM, da Silva NR, dos Santos Fernandez M, Bomfim RA, dos Santos Vaz J. Ultra-processed food consumption and dental caries in children and adolescents: a systematic review and meta-analysis. British Journal of Nutrition. 2023 Apr;129(8):1370-9. doi.org/10.1007/s40368-019-00468-y
59. WANG Xinyan , PENG Xian , LI Yuqing , ZHOU Xuedong. Research progress on the relationship between free sugars and caries[J]. Journal of Prevention and Treatment for Stomatological Diseases, 2023, 31(11): 813-818 https://doi.org/10.12016/j.issn.2096-1456.2023.11.008

Table (1): Inter and intragroup comparisons of chances to avoid new carious lesions (green sector) and Correlations between green sector and other sector

Interval	**Chances to avoid new carious lesions**	**Green sector**				**u-value u**	**p-value p**
		Control		**Intervention**			
		n	**%**	**n**	**%**		
Before	**Low**	0	0.0%	0	0.0%	**33.00**	**0.356**
	Moderate	3	33.3%	6	60.0%		
	High	6	66.7%	4	40.0%		
After	**Low**	7	77.8%	7	70.0%	**48.50**	**0.780**
	Moderate	2	22.2%	3	30.0%		
	High	0	0.0%	0	0.0%		
	w-value	**0.00**		**0.00**			
	p-value p	**0.006***		**0.002***			

correlation of Sectors		**r_s**
Green	**Yellow**	0.499
	Blue	0.639
	Red	0.764
	Light blue	0.926

*significant ($p<0.05$) r_s: spearman's rank order correlation coefficient

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Table (2): Inter and intragroup comparisons of red sector scores

Parameter	Interval	Score	Red sector				u-value	p-value
			Control		Intervention		u	p
			n	%	n	%		
Amount of plaque	**Before**	**0**	0	0.0%	1	11.1%	**44.00**	**0.968**
		1	2	20.0%	1	11.1%		
		2	2	20.0%	1	11.1%		
		3	6	60.0%	6	66.7%		
	After	**0**	1	10.0%	1	11.1%	**67.50**	**0.065**
		1	0	0.0%	4	44.4%		
		2	7	70.0%	4	44.4%		
		3	2	20.0%	0	0.0%		
	w-value u		**6.00**		**3.50**			
	p-value p		**0.317**		**0.034***			
Streptcoccus mutans	**Before**	**0**	0	0.0%	0	0.0%	**45.00**	**1**
		1	0	0.0%	0	0.0%		
		2	0	0.0%	0	0.0%		
		3	9	100.0%	10	100.0%		
	After	**0**	0	0.0%	0	0.0%	**27.00**	**0.156**
		1	3	33.3%	7	70.0%		
		2	3	33.3%	2	20.0%		
		3	3	33.3%	1	10.0%		
	w-value u		**0.00**		**0.00**			
	p-value p		**0.024***		**0.005***			

*significant ($p<0.05$)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

Table (3): Inter and intragroup comparisons of light blue sector scores

Parameter	Interval	Score	Light blue sector				u-value u	p-value p
			Control		Intervention			
			n	%	n	%		
Fluoride program	**Before**	**0**	0	0.0%	0	0.0%	**44.00**	**0.968**
		1	1	11.1%	0	0.0%		
		2	6	66.7%	9	90.0%		
		3	2	22.2%	1	10.0%		
	After	**0**	9	100.0%	10	100.0%	**45.00**	**1**
		1	0	0.0%	0	0.0%		
		2	0	0.0%	0	0.0%		
		3	0	0.0%	0	0.0%		
	w-value u		**0.00**		**0.00**			
	p-value p		**0.006***		**0.002***			
Salivary secretion rate	**Before**	**0**	9	100.0%	9	90.0%	**49.50**	**0.720**
		1	0	0.0%	1	10.0%		
		2	0	0.0%	0	0.0%		
		3	0	0.0%	0	0.0%		
	After	**0**	8	88.9%	9	90.0%	**44.50**	**0.968**
		1	0	0.0%	0	0.0%		
		2	1	11.1%	1	10.0%		
		3	0	0.0%	0	0.0%		
	w-value u		**1.00**		**2.00**			
	p-value p		**0.317**		**0.655**			
	Before	**0**	8	88.9%	10	100.0%	**50.00**	**0.343**

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 963-977 | ISSN:2251-6727

		1	1	11.1%	0	0.0%		
Salivary buffering capacity		**2**	0	0.0%	0	0.0%		
	After	**0**	9	100.0%	10	100.0%	**45.00**	**1**
		1	0	0.0%	0	0.0%		
		2	0	0.0%	0	0.0%		
	w-value u			**0.00**		**0.00**		
	p-value p			**0.317**		**1**		

*significant ($p<0.05$)