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"Anti-Microbial Efficacy of Chlorhexidine Hexametaphosphate Coated Elastomeric Modules.''

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Abstract:
Background*: Orthodontic ligatures are potential vector that may be used for local delivery of antimicrobial agent to prevent WSL in orthodontic patients. Coating elastomeric ligatures with antimicrobial CHX-HMP nanoparticles could provide a sustained dose of anti-microbial delivery eliminating dependence on patient compliance. there is no literature evidence on anti-microbial effect of CHX-HMP NPs coated ligatures in orthodontic patients.*
Aims & Objectives*: To evaluate and compare antimicrobial efficacy of elastomeric modules functionalized with CHXdg and CHX-HMP and elution of CHX over a function of time in aqueous medium*
Materials & Methodology*: An effective total sample of 30 was obtained. F or each group (n=2) 15 participants were allocated. Oral prophylaxis was done in all patients before placing elastomeric modules in 1st visit to bring base line plaque index of patients to 0. Arch wires were tied with differently functionalized elastomeric modules. A total of 620 silver-colored polyurethane elastomeric ligatures (3M Unitek) were rinsed in DIW and allowed to air dry for 1 hour beforeuse. 310 ligatures were immersed in ethanol for 60 minutes under agitation. Immediately after conditioning, ligatures were immersed 5mM CHX-HMP for 10 minutes under agitation. Another set of 310 ligatures were immersed 5mM CHXdg for 10 minutes under agitation. Followed by a final immersion in DIW for 10 seconds to remove any unbound material and air drying for at least 1 hour before further use. In this way 2 sets of (n=300 per group over 8 weeks period) elastomeric ligatures were functionalized with either CHXdg or CHX- HMP aqueous suspensions. After functionalization, two groups of ligatures (n=10) were placed into individual UV-transparent cuvettes suitable for ultraviolet spectrophotometry. Amount of released CHX from coated ligatures was studied for a period of 56 days over 12 intervals. A single ligature was placed in an individually labeled cuvette and 2.5 ml of deionized water was added to submerge the ligature. Cuvettes were kept sealed at ambient room temperature (24°C) and medium was collected for evaluation of CHX release on 12 intervals. Entire volume was collected on each time point and then cuvette was refilled with 2.5 ml deionized water. Collected media was kept in sealed cuvettes and stored in freezer at 0° F until sample collection was completed. Absorption at 260 nm was measured by spectrophotometry from 200 µL of collected samples to determine amount of released CHX. Standard solutions of 0–50 µm CHX was prepared as a reference and to calibrate CHX concentrations[19]. Cumulative CHX release at conclusion of 8thweek period was determined.*
Microbial count was assessed at end of 1st week (T0), at end of 4th week (T1) and at end of 8th week (T2). Swab was inoculated into tube containing 2ml of BHI broth for bacterial isolation and identification; it was incubated at 37°C for 2 hrs. After 2 hours, 10µl of broth was inoculated onto Blood agar for Streptococcus mutans isolation. Cultured plates were incubated at 37°C for 24 hours for Streptococcus mutans. After bacterial growth, colony morphologies evaluated, counted and measured in CFU per mL.
Results:
Highest CHX release in Chlorhexidine-dg group was observed on day 1 (63.13±0.81 µmol/L) with a consistent reduction in release at subsequent observations before the release decayed down to zero on 35th day for CHX-dg group. In CHX-HMP group highest release was observed on day 1 (111.74±1.76 µmol/L) with a consistent reduction in release at subsequent observations with least mean values recorded on 56th day (16.21±0.52 µmol/L).
Conclusion:
CHX-HMP nanoparticles coated elastomeric ligatures incubated inwater released aqueous CHX beyond a period of 8 weeks. CHXdg coated elastomeric ligatures stopped elution of CHX by35th day of incubation period. CHX-HMP nanoparticle coated elastomeric ligatures were capableof inhibition of Streptococcus

mutans growth. Significant reduction of Plaque scores was observed with CHX-HMP coated elastomeric ligatures.

Keywords: *Chlorhexidine, elastomeric modules, anti-microbial*

I. INTRODUCTION

Fixed orthodontic appliance is composed of brackets or bands which are bonded to tooth surface with composite or Glass Ionomer Cement (GIC). Arch wires are tied to brackets with help of elastomeric ligature ties or stainless-steel ligature ties. Out of several iatrogenic effects of orthodontic treatment, One most common effects is WSLs as the fixed appliance compromises oral hygiene and promotes plaque accumulation.[1] Dental plaque consists of a variety of microbes and among them, *Streptococcus mutans* is most virulent and associated with white spot lesions, caries and gingival inflammation.[2] WSLs are defined as 'subsurface enamel porosity from carious demineralization.[3] With WSLs developing within 4 weeks of appliance placement.[4] Although there is evidence of remineralisation after removal of fixed appliance, but baseline pretreatment levels are not regained. Moreover, fixed orthodontic appliances lead to increase in number of pathogenic microbes, increasing the incidence of caries development.[5] If WSLs are left untreated, may cause progression into dental caries. It was stated that incidence of new dental caries lesions in patients who are undergoing fixed orthodontic treatment is 45.8%.[3] Moreover, periodontal diseases and gingival diseases can develop if deposited plaque is not removed properly, and these diseases lead to loss of tooth in severe cases.[6]

Oral hygiene practice include mechanical and chemical methods are most important preventive method in controlling WSLs. Mechanical methods include proper brushing, flossing etc. chemical methods include mouthwashes and fluoride toothpastes, fluoride gels etc. Chemical agents could be used in addition to mechanical agents such as brushing and flossing during active phase of orthodontic treatment to reduce bacterial plaque accumulation, gingivitis and periodontitis.[7] The most common method of managing WSLs by dental professionals is by fluoride mouth wash, to counsel patient about brushing habits and to maintain proper oral hygiene. This fluoride mouth wash causes additional problems as it causes formation of fluorapatite crystals which prevents remineralisation of WSLs. As an alternative to these other mouthwashes are available[1] in market, chlorhexidine was highly effective in reduction of pathogenic microorganisms like Streptococcus mutans thus reducing dental plaque.[8]

Chlorhexidine (CHX) is a biguanide class of drugs that acts as a antimicrobial agent against gram negative and gram-positive bacteria's and yeasts.[9] And is used in medicine and dentistry, as a mouth rinses as CHX-digluconate (CHXdg).[9,10] CHX being cationic in nature, is attracted to negatively charged bacterial cell wall and binds to inner membrane. This increases permeability of cell wall, thereby causing loss of cell components, precipitation of bacterial cytoplasm, and cell death.[11] This is a rapid process, occurring within 20 seconds of exposure, causing most damage.[12] CHX acts by damaging cell membrane involving phospholipid-bilayer at bothlow and high concentrations causing congealing of cytoplasm. CHX does not encourage development of bacterial resistance as CHX is a broad-spectrum antimicrobial and antifungal agent.[10,12] Commonly used form of CHX is Chlorhexidine digluconate (CHXdg), which readily dissolves in water, and therefore, easy to formulate into mouth rinses and other aqueous topical agents.[11] Main advantage of CHX is sustained substantivity over longer periods results in prolonged antimicrobial effects due to combination of CHX with hydroxyapatite of tooth enamel, oral mucosa, and oral bacteria, which results in prolonged release over 12– 24 hrs.[13,14] However, because saliva is continuously released into mouth, effects of such products do not last long. If antimicrobial materials were able to remain inside oral cavity for prolonged periods, they would avoid dental disease throughout orthodontic treatment.[6] Studies conducted by Wood et al[15] and Barbour et al[16] to develop CHX releasing materials utilizing hexametaphosphate (HMP) nanoparticles (NPs). Sodium HMP is a cyclic inorganic phosphate used in food industry and dental field due to its ability to inhibit formation of dental calculus and prevent formation of extrinsic stains.[17,18] Research indicated that Chlorhexidine hexametaphosphate (CHX-HMP) nanoparticles (NPs) can be affixed to substrate materials to get prolonged release of antimicrobially active CHX.[16]

Orthodontic patients often visit dental office to change elastic ligatures of fixed orthodontic treatment. Orthodontic ligatures are potential vector that may be used for local delivery of antimicrobial agent to prevent WSL in orthodontic patients. Ligatures are close to enamel and are regularly changed during orthodontic treatment. Coating elastomeric ligatures with antimicrobial CHX-HMP nanoparticles could provide a sustained dose of anti-microbial delivery eliminating dependence on patient compliance.

Wood NJ et al[15] reported that titanium, glass, elastomeric wound dressing and ethylene-vinyl acetate (EVA) polymer specimens were coated with CHX-HMP nanoparticles that provided continuous release of soluble-CHX over 50 days without reaching a plateau.[15] Subramani et al reported that coating of Orthodontic elastomeric chains (OEC) with antimicrobial CHX-HMP nanoparticles, serve as means to reduce WSLs by inhibiting microbes causing formation of WSL.[19] Yasmin et al[11] reported a sustained CHX release of 200µM

over 8 week period from CHX-HMP treated elastomers without change in their mechanical properties and ethanol conditioning enhanced CHX-HMP uptake by elastomers.

However, there is no literature evidence on anti-microbial effect of CHX-HMP NPs coated ligatures in orthodontic patients. Hence this study was done to evaluate and compare antimicrobial efficacy of elastomeric modules conditioned with CHXdg and CHX-HMP in addition to insitu evaluation of CHX elution from functionalized elastomeric ligatures over 8-week duration.

II. AIM: -

To evaluate and compare antimicrobial efficacy of elastomeric modules functionalized with CHXdg and CHX-HMP and elution of CHX over a function of time in aqueous medium.

OBJECTIVES: -

1. To evaluate antimicrobial effect of CHXdg functionalized elastomeric modules at 1 week after appliance placement (T0), 4th week (T1) and 8th week (T2) interval.
2. To evaluate antimicrobial effect of CHX-HMP functionalized elastomeric modules at 1 week after appliance placement (T0), 4th week (T1) and 8th week (T2) interval.
3. To compare antimicrobial efficacy between the CHXdg and CHX- HMP conditioned elastomeric modules.
4. To evaluate elution of CHX from CHXdg functionalized elastomeric modules over 56 days at 12 intervals(1,2,3,5,7,14,21,28,35,42,49,56).
5. To evaluate elution of CHX from CHX-HMP functionalized elastomeric modules over 56 days at 12 intervals(1,2,3,5,7,14,21,28,35,42,49,56).
6. To compare elution of CHX over a function of time between the CHXdg and CHX-HMP conditioned elastomeric modules.

This present clinical trial was conducted in Department of Orthodontics and Dentofacial Orthopedics, SIBAR Institute of Dental Sciences, Guntur, & was approved by Institutional Ethics Committee (Ref No: 1/IEC-SIBAR/CIR/21).

***Inclusion criteria*:**

1. Patient within age group of 18 – 25 years.
2. Patients with permanent dentition.
3. Orthodontic cases treated by non-extraction method.

***Exclusion criteria*:**

1. Subjects who have used antibiotics 3 months prior to study.
2. Patient with history of smoking and any periodontal dis
3. Patient with any systemic disorders.
4. Prior use of any mouthwash for 10 consecutive days in last 3months.
5. Patient with carious lesion, restoration, visible cracks andenamel hypoplasia.
6. Pregnant women.

Sample size:

- Determined by G*Power version 3.1.9.2 software with following parameters:
- Effective size - 0.56
- Confident Interval - 95%
- Power - 80%

An effective total sample of 30 was obtained. Based on 1:1 allocation, for each group (n=2) 15 participants were allocated. 30 subjects of both genders receiving orthodontic treatment with pre-adjusted edgewise appliances and havingequal base line plaque index score were randomly included in study. Informed consent was procured from all patients who participated in study.

Patients were assigned into 2 groups: -

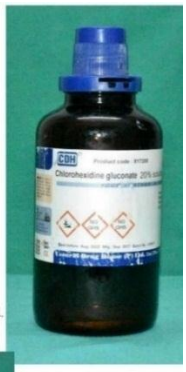
1. Group I: Brackets will be ligated with elastomeric modules functionalized with CHXdg.
2. Group II: Brackets will be ligated with elastomeric modules functionalized with CHX-HMP.

Materials:

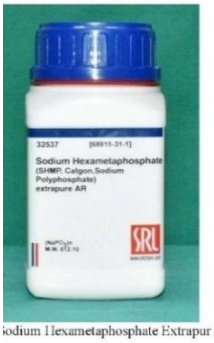
1. 3M™ AlastiK™ QuiK StiK™- Elastomeric modules
2. Chlorhexidine gluconate - Central Drug House (P) Ltd.

3. Sodium Hexametaphosphate Extrapur AR - Sicso ResearchLimited Pvt. Ltd.
4. Ethanol - Changsu Hongsheng Fine Chemicals Co. Ltd.
5. Deionized water (DIW)
6. Brain Heart Infusion (BHI) broth medium

M™ AlastiK™ QuiK StiK™- Elastomeric modules.

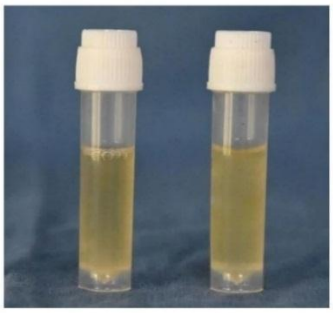


Chlorhexidine gluconate - Central Drug House (P) Ltd.



Sodium Hexametaphosphate Extrapur AR – Sicso ResearchLimited Pvt. Ltd.

Ethanol - Changsu Hongsheng Fine Chemicals Co. Ltd.

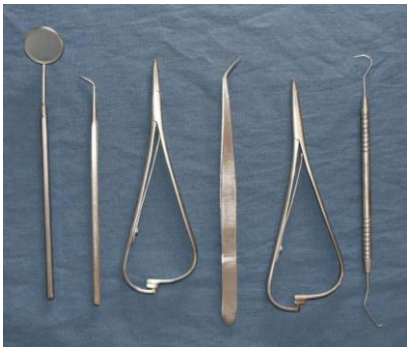


Brain Heart Infusion (BHI) broth medium

Armamentarium:

- Mathew plier
- Tweezers
- Mouth mirror
- Straight probe

Armamentarium for elastomeric module placement and removalof elastomeric module.



***Equipment used*:**

- Spectrophotometric machine - Shimadzu corp.

III. METHODOLOGY:

Oral prophylaxis was done in all patients before placing elastomeric modules in 1st visit to bring base line plaque index of allpatients to 0. Standard oral hygiene instructions were given toall participants. Arch wires were tied with differently functionalized elastomeric modules

***Preparation of CHX-HMP NPs coated elastomeric ligatures*: Flowchart 1**

100mL of 10mM aqueous NaHMP was added to 100mL of 10mM aqueous CHXdg under constant stirring at room temperature and pressure. This resulted in aqueous suspension of CHX–HMP, with CHX concentration of 5mM. For comparison, a 5mM aqueous solution of CHXdg was also prepared.[16]

A total of 620 silver-colored polyurethane elastomeric ligatures (3M Unitek) were rinsed in DIW and allowed to air dry for 1 hour beforeuse. 310 ligatures were immersed in ethanol for 60 minutes under agitation. Immediately after conditioning, ligatures were immersed 5mM CHX-HMP for 10 minutes under agitation. Another set of 310 ligatures were immersed 5mM CHXdg for 10 minutes under agitation. Followed by a final immersion in DIW for 10 seconds to remove any unbound material and air drying for at least 1 hour before further use.[11]

In this way 2 sets of (n=300 per group over 8 weeks period) elastomeric ligatures were functionalized with either CHXdg or CHX- HMP aqueous suspensions. Accordingly Group I sample of patients received ligatures treated with CHXdg and Group II sample received ligatures treated with CHX-HMP. Two sets of elastomeric ligatures (n=10per group) were utilized to check CHX elution from ligatures.

***CHX elution from functionalized ligatures*:**

After functionalization, two groups of ligatures (n=10) were placed into individual UV-transparent cuvettes suitable for ultraviolet spectrophotometry. Amount of released CHX from coated ligatures was studied for a period of 56 days over 12 intervals (1, 2, 3, 5, 7, 14,21, 28, 35, 42, 49 & 56 days). A single ligature was placed in an individually labeled cuvette and 2.5 ml of deionized water was added to submerge the ligature. Cuvettes were kept sealed at ambient room temperature (24°C) and medium was collected for evaluation of CHX release on 12 intervals. Entire volume was collected on each time point and then cuvette was refilled with 2.5 ml deionized water. Collected media was kept in sealed cuvettes and stored in freezer at 0° F until sample collection was completed. Absorption at 260 nm was measured by spectrophotometry from 200 µL of collected samples to determine amount of released CHX. Standard solutions of 0–50 µm CHX was prepared as a reference and to calibrate CHX concentrations[19]. Cumulative CHX release at conclusion of 8thweek period was determined.

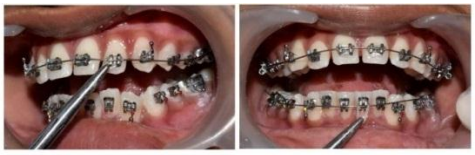
Estimation of Microbial count: -

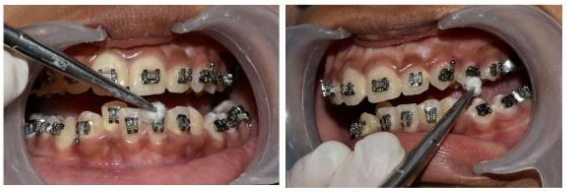
Microbial count was assessed at end of 1st week (T0), at end of 4th week (T1) and at end of 8th week (T2). For process of sample collection for microbial analysis, quadrants were isolated with cotton rolls to avoid saliva contamination before collecting sample. Plaque samples were collected aseptically with sterile cotton

swab moistened with sterile saline from around orthodontic attachments of maxillary pre-molars and lower incisors. Only one cotton swab was used for both regions and only one sample was collected from each patient. Collected swab was transferred aseptically and immediately into sterile tube containing 2 ml of BHI broth medium and it was delivered to microbiology lab.
Swab was then inoculated into tube containing 2ml of BHI broth for bacterial isolation and identification; it was incubated at 37°C for 2 hrs. After 2 hours, 10µl of broth was inoculated onto fresh Blood agar for Streptococcus mutans isolation. Cultured plates were then incubated at 37°C for 24 hours for Streptococcus mutans. After bacterial growth, colony morphologies evaluated, counted and measured in colony forming units per mL (cfu/ml).[20]

Recording of Plaque index: -
Plaque index scores will be recorded in each individual at end of 1st week (T0), at end of 4th week (T1) and at end of 8th week (T2).

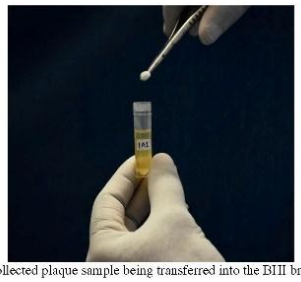
Criteria for plaque index system:[21]
0 = No plaque in gingival area.
1 = A film of plaque adhering to free gingival margin and adjacent area of tooth. Plaque may only be recognized by runninga probe across tooth surface.
2 = Moderate accumulation of soft deposits within gingival pocket, on gingival margin and/or adjacent tooth surface, which canbe seen by naked eye.
3 = Abundance of soft matter within gingival pocket and/or on gingival margin and adjacent tooth surface


Functionalized module being placed in patient.

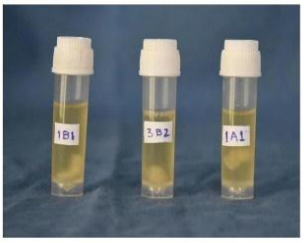

Plaque samples being collected from the incisor andpremolar area for microbiological colony count.

Prepared CHX-HMP solution concentration of 5mM

Prepared CHXdg solution with a CHX concentration of 5mM


Collected plaque sample being transferred into the BHI broth.


Sample being placed in the spectrophotometric machine tocheck the release of CHX from the solution.

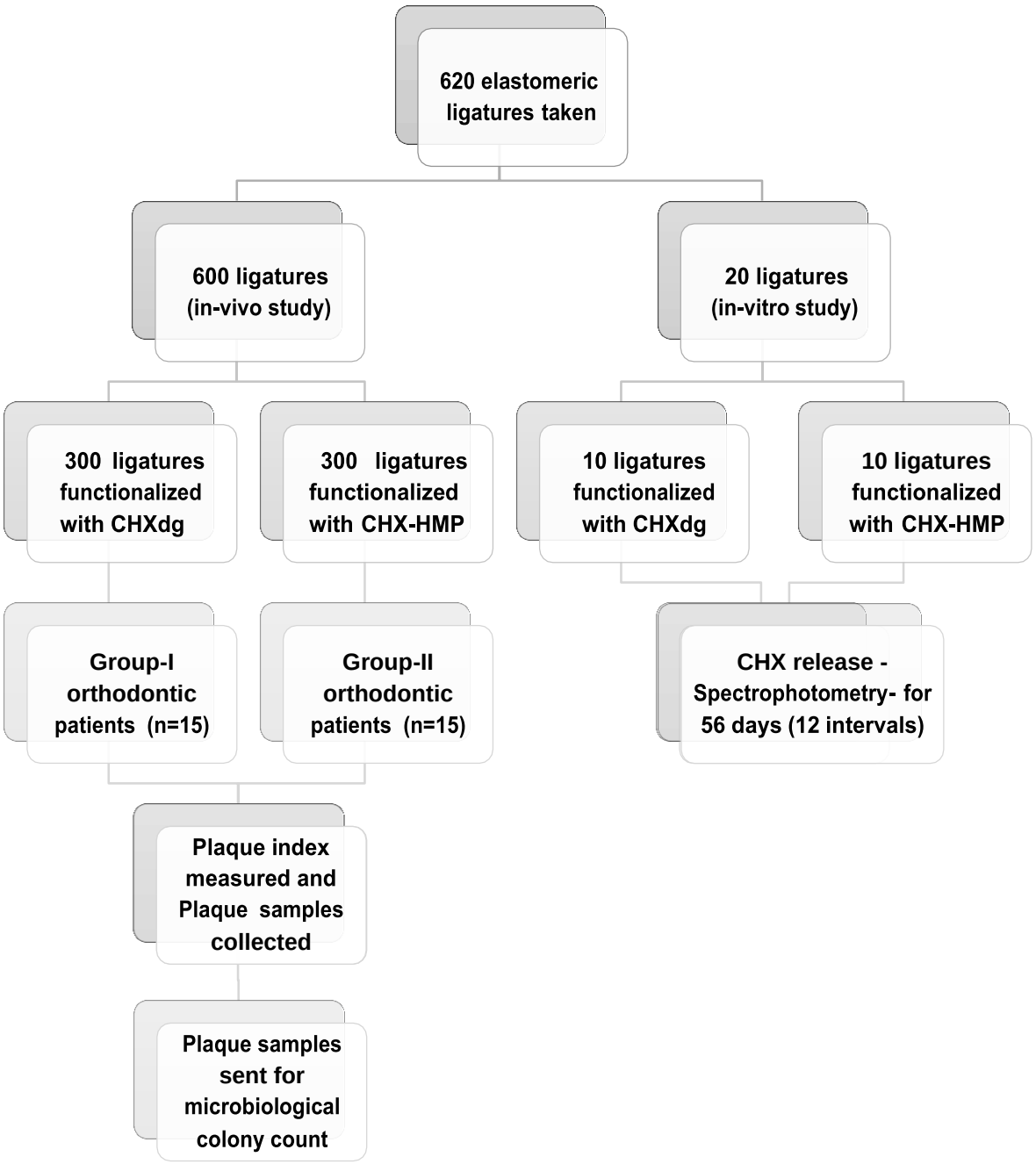


BHI broth with plaque samples sent to microbiologicallaboratory for microbiological colony count.

***Interpretation of Plaque score*: -**

Rating	**Score**
Excellent	0
Good	0.1-0.9
Fair	1.0-1.9
Poor	2.0-3.0

Flow Chart 1: - Methodology



***Statistical analysis*:**
Data were analyzed using IBM SPSS version-20 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics, repeated measures analysis of variance (ANOVA), and independent samples t- tests were done to evaluate study data.

Participant flow
Thirty patients were invited to participate in this clinical trial (15 participants in CHXdg group, 15 participants in CHX- HMP group).

Flow Chart 2: CONSORT flowchart of participants throughеach stage of trial.

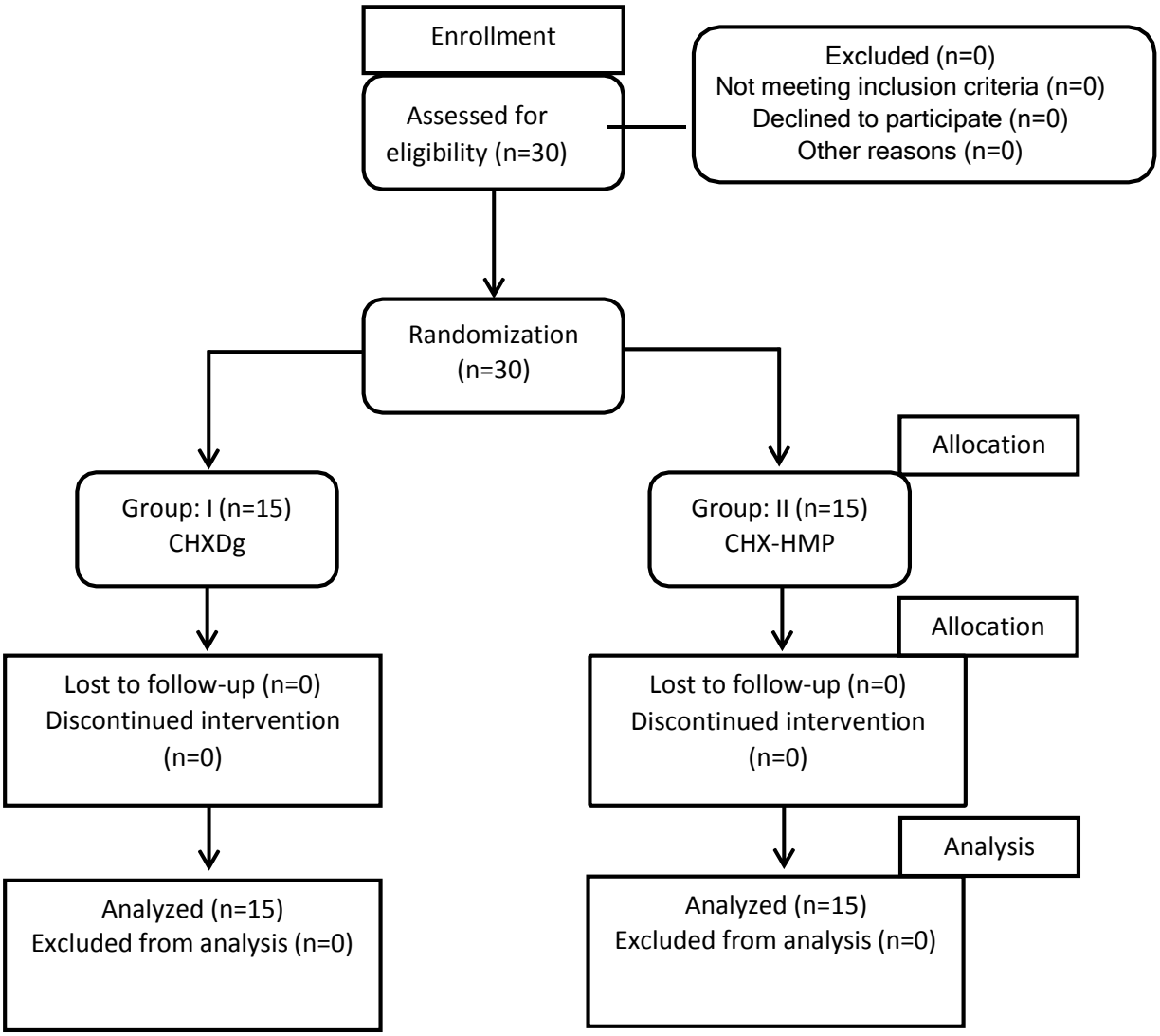
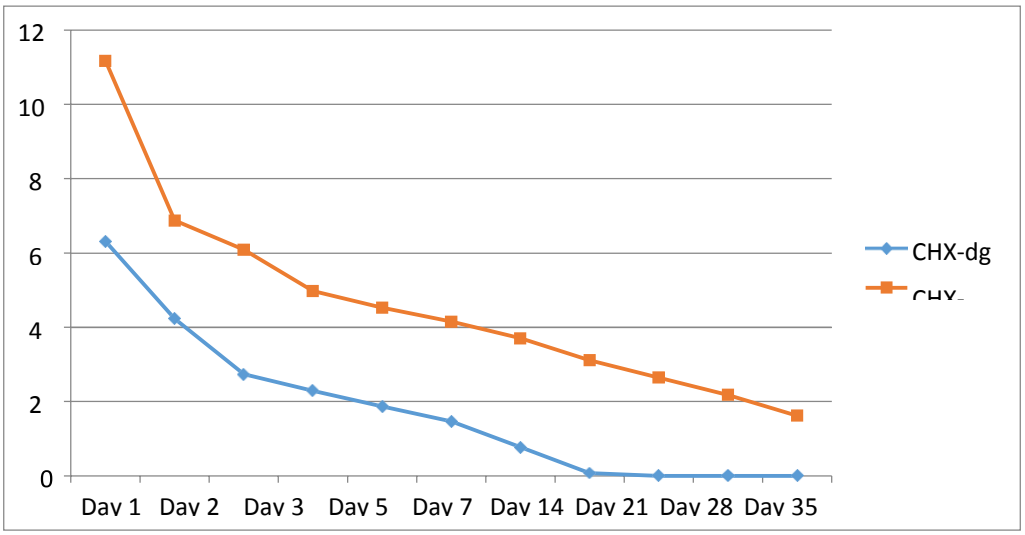


Table 1: Inter-group comparison of chlorhexidine release (µmoles/L) atdifferent time points.

Day	Group		t value	P value
	CHX-dg	CHX-HMP		
Day 1	63.13±0.81	111.74±1.76	78.92	<0.001*
Day 2	42.34±0.54	68.76±0.45	116.9	<0.001*
Day 3	27.36±1.01	60.87±0.575	90.77	<0.001*
Day 5	22.92±0.611	49.75±0.64	95.251	<0.001*
Day 7	18.67±0.603	45.29±0.45	111.206	<0.001*
Day 14	14.67±0.816	41.51±0.53	86.973	<0.001*
Day 21	7.73±0.46	37.07±0.51	133.878	<0.001*
Day 28	0.757±0.286	31.15±0.26	244.48	<0.001*
Day 35	0±0	26.49±0.46	179.88	<0.001*
Day 42	0±0	21.78±0.44	154.298	<0.001*
Day 56	0±0	16.21±0.52	97.118	<0.001*

Independent samples t test; p≤0.05 - statistically significant; *□denotesstatistical significance



Graph 1: Intra Group Comparison of chlorhexidine release (µmoles/L) between thestudy groups.

Table 2: Inter-group comparison of cumulative chlorhexidine release(µmoles/L).

Group	Mean	SD	SE	t value	P value
CHX-dg	197.61	1.88	0.595	-279.85	<0.001*
CHX-HMP	510.66	2.99	0.947		

p≤0.05 considered statistically significant; * denotes statisticalsignificance

Graph 2: Comparison of colony forming units (*10^5cfu/ml) between thestudy groups.

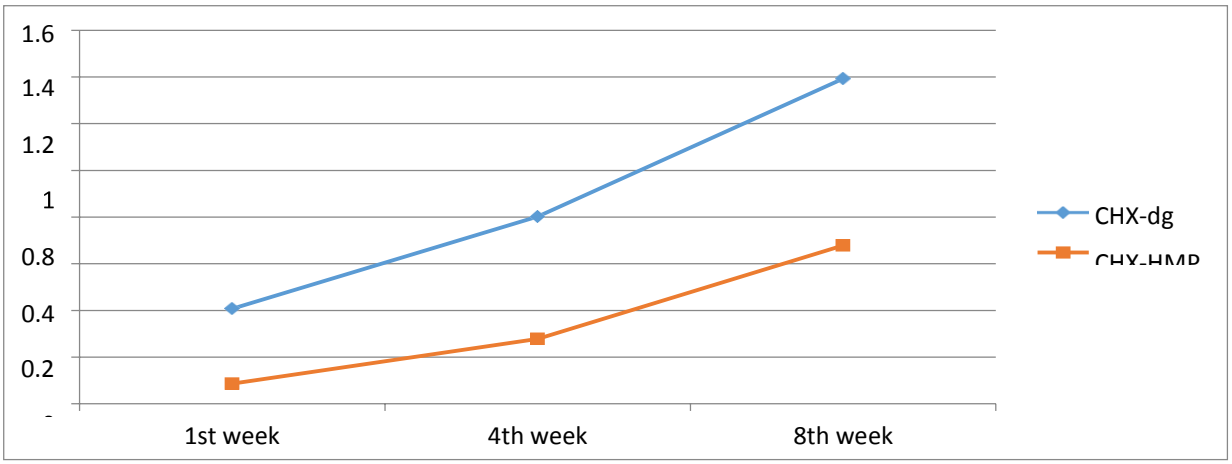


Table 3: Inter-group comparison of % decrease in CHX release with reference to Day 1 at different time points.

Parameter	**Group**	**N**	**Mean**	**Std. Deviation**	**Std. Error mean**	**T value**	**P value**
% decrease Day 2	CHX-dg	10	32.923111	1.0886500	.3442614	-11.68	<0.001*
	CHX-HMP	10	38.445165	1.0247380	.3240506		
% decrease Day 3	CHX-dg	10	56.645668	1.7844717	.5642995	18.84	<0.001*
	CHX-HMP	10	45.516647	.5524280	.1746931		
% decrease Day 5	CHX-dg	10	63.683537	1.0200849	.3225792	17.85	<0.001*
	CHX-HMP	10	55.457754	1.0400273	.3288855		
% decrease Day 7	CHX-dg	10	70.408359	1.1165360	.3530797	25.58	<0.001*

	CHX-HMP	10	59.454148	.7657460	.2421501		
% decrease Day 14	CHX-dg	10	76.748270	1.4876539	.4704375	27.49	<0.001*
	CHX-HMP	10	62.843284	.5875396	.1857963		
% decrease Day 21	CHX-dg	10	87.752941	.7933392	.2508759	65.2	<0.001*
	CHX-HMP	10	66.818746	.6335895	.2003586		
% decrease Day 28	CHX-dg	10	98.798463	.4546282	.1437661	147.18	<0.001*
	CHX-HMP	10	72.111693	.3493732	.1104815		
% decrease Day 35	CHX-dg	10	100.000000	0	0	164.45	<0.001*
	CHX-HMP	10	76.289215	.4559445	.1441823		
% decrease Day 42	CHX-dg	10	100.000000	0	0	104.72	<0.001*
	CHX-HMP	10	80.500173	.5888111	.1861984		
% decrease Day 56	CHX-dg	10	100.000000	0	0	87.77	<0.001*
	CHX-HMP	10	85.485512	.5229380	.1653675		

Independent samples t test; p≤0.05 considered statistically significant; *□denotes statistical significance

Table 4: Inter-group comparison of colony forming units (*10^5cfu/ml)between the study groups.

Time	Group	n	Mean	SD	t value	P value
1st week	CHX-dg	15	0.4073	0.08	13.806	<0.001*
	CHX-HMP	15	0.086	0.008		
4th week	CHX-dg	15	0.802	0.112	14.357	<0.001*
	CHX-HMP	15	0.278	0.084		
8th week	CHX-dg	15	1.392	0.324	8.106	<0.001*
	CHX-HMP	15	0.678	0.104		

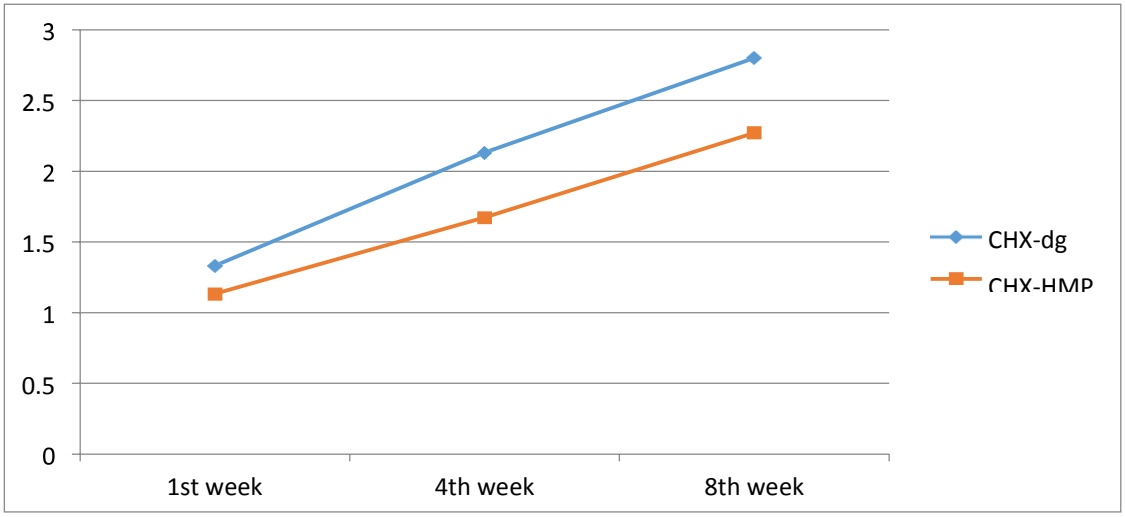
Independent samples t test; p≤0.05 considered statistically significant;
*□denotes statistical significance

Table 5: Inter-group comparison of plaque index scores between thestudy groups.

Time	Group	n	Mean	SD	t value	P value
1st week	CHX-dg	15	1.33	0.488	1.288	0.208
	CHX-HMP	15	1.13	0.352		
4th week	CHX-dg	15	2.13	0.516	2.54	0.017*
	CHX-HMP	15	1.67	0.488		
8th week	CHX-dg	15	2.8	0.414	3.347	0.002*
	CHX-HMP	15	2.27	0.458		

Independent samples t test; p≤0.05 considered statistically significant;
*□ denotes statistical significance

Graph 3: Comparisons for plaque index scores between the studygroups.



IV. RESULTS:

Highest CHX release in Chlorhexidine-dg group was observed on day 1 (63.13±0.81 µmol/L) with a consistent reduction in release at subsequent observations before the release decayed down to zero on 35th day for CHX-dg group. In CHX-HMP group highest release was observed on day 1 (111.74±1.76 µmol/L) with a consistent reduction in release at subsequent observations with least mean values recorded on 56th day (16.21±0.52 µmol/L). Intra-group differences in both groups were statistically significant as analyzed with ANOVA (Tables 1). Table 2 shows inter-group comparison of chlorhexidine release (µmoles/L) at different time points. At all-time points in this study, CHX-HMP demonstrated significantly higher cumulative release of chlorhexidine compared to CHX-dg group. Except at Day 2 Percentile decrease relative to day 1 at 11 different time intervalsbetween CHX-dg and CHX- HMP groups, from Day 2 to Day 56, percentage decrease was significantly higher in CHX-dg group compared to CHX-HMP group. (Table 3)

For CFU In both groups (Table 4, Graph 2), least mean values were documented at 1st week recording with a consistent increase in two subsequent recordings at 4th and 8th weeks, with significantly lesser colonies in the CHX-HMP group at each given time point. for PI scores in both groups, least mean values were documented at 1st week with a statistically significant consistent increase in two subsequent recordings at 4th and 8th weeks & significantly lesser mean PI scores in CHX-HMP group at 4th and 8th week time points. There was no significant difference in mean plaque index scores between groups at 1 week time point. (Table 5, Graph 3)

V. DISCUSSION:

Current practices in WSL management by dental professionals were investigated in earlier studies.[22,23] Commonly recommended method was administration of fluoride mouth rinse after brushing.[22] Patients were encouraged use fluoride mouth rinse by 85% of orthodontists, 69% general dentists and 76% orthodontists suggested in-office fluoride treatment for severe WSLs immediately after fixed orthodontic treatment.[23] This treatment cause additional problems since use of fluoride treatment after formation of WSL result in formation of fluorapatite crystals which prevents remineralization of WSLs. As an alternative, MI paste has been recommended for treatment of WSL. Recaldent, the active ingredient of MI Paste, is a complex of casein phosphopeptides and amorphous calcium phosphate (CPP-ACP) that increases level of calcium phosphate in dental plaque to promote remineralization of enamel.[24]

But prevention of microbial buildup is preferred alternative to treatment. Chlorhexidine was widely preferred by dentists and Orthodontists for prevention of WSLs in patients undergoing orthodontics treatment. CHX diacetate demonstrated CHX release over 8 days when incorporated into some kind of material.[25] However, side effects such as unpleasant taste, undesirable tooth discoloration, burning sensation and dryness in mouth demotivate patients to use this mouthwash.[26,27]

Treat posed by microbiological evolution of antibiotic resistance is of grave concern to international community. Development of antimicrobial methods that do not support emergence of such resistance is strongly urged. It has been found that while specific populations of microbes can become less sensitive to CHX when exposed to rising environmental concentrations, these changes can be undone when CHX stimulus is removed, indicating that changes are reversible and do not actually represent true resistance.[40] Because of this,

CHX employed as CHX digluconate salt, easily soluble in aqueous solutions, has been regarded as promising candidate for creation of antimicrobial substances and devices that do not increase need for antibiotics.[10] By soaking in CHX digluconate solutions, biological materials have been transformed into antibacterial substances..[41,42] It is also used in form of CHX-diacetate, and has been introduced as dry crystalline powder to variety of materials with aim of conferring them antimicrobial characteristics.[26,27]

NaHMP is cyclic inorganic phosphate used in food industry and dental field due to its ability to inhibit formation of dental calculus and prevent formation of extrinsic stains.[17,18] A novel salt of CHX, CHX-hexametaphosphate (CHX-HMP), has been reported as material that provides sustained release of constituent CHX when exposed to aqueous environment.[16] and does not reach equilibrium in sealed system after 8 weeks. An initial period of rapid CHX release was followed by slower and gradual CHX release.[11] Attributing to physical and chemical properties of this salt, it can be employed as a component of composite materials. Provided the composite has a degree of water permeability, substrate composite can provide sustained release of CHX under aqueous conditions. Dose and duration of release are influenced by variety of factors such as doping, local physicochemical conditions (such as flow, temperature, ionic strength, and other ions), and host substrate.[11] Studies showed specimens of glass, titanium, elastomeric wound dressing and ethylene vinyl acetate (EVA) polymer were successfully coated with CHX-HMP NPs that provided continuous elution of soluble CHX over a period of 50 days without reaching a plateau.[15,16] Antimicrobial property of released soluble CHX was shown by growth inhibition of methicillin- resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Streptococcus gordonii.[16,28] These studies established that CHX- HMP nanoparticle can be affixed to materials with release of antimicrobially active CHX. As CHX and Sodium HMP has been used widely in dentistry as antibacterial mouthwash and anticalculus agent and has been effective against oral microbes causing WSLs, coating of orthodontic materials such as orthodontic elastomeric chains (OEC) with antimicrobial CHX-HMP nanoparticles provide means to reduceWSLs by inhibiting microbes[19]

Polyurethane is a polymer used for medical device production due to its exceptional physical and mechanical properties and good biocompatibility.[29] Polyurethane elastomeric chains are preferred option to close spaces and correct rotations in orthodontic mechanotherapy.[30,31] Orthodontic treatment increases risk of caries due to accumulation of plaque as well as bacterial flora modification.[32] CHX-diacetate has been associated for application on dental implant to minimize risk of infection in earlier days following intervention.[33,34] Antibacterial polyurethane nanocomposites using CHXdg have also beendeveloped.[35] Huynh TTN et al in their in-vitro research reported preparation, mechanical, and physicochemical characterization of CHX-diacetate loaded polyurethane biomaterial for local delivery ofchlorhexidine.[36] Catalbas B et al[37] and Dalli M. et al[38] showed that treating orthodontic elastomeric chains with CHX gel did not affect mechanical properties of orthodontic elastomeric chains either in-vitro nor in-vivo. Although gel formulation provided momentary antibacterial action. Padois K et al, in their in-vitro study used advanced drug delivery system based on chlorhexidine loading into polyurethane elastomeric orthodontic chains for sustained release of antimicrobial drug during orthodontic treatment.[39] CHX-salt could diffuse through polyurethane wall and elastomeric orthodontic chains showed a sustained CHX release over longer period suggesting it as good treatment modality. Subramani K. et al[19] concluded CHX-HMP nanoparticle coated orthodontic elastomeric chains released chlorhexidine over time period of at least 28 days and this elution is capable of antibacterial effect thus promising clinical applications in orthodontic mechanotherapy. Kamarudin Y et al[11] concluded CHX-HMP conditioned elastomeric ligatures showed sustained release of chlorhexidine up to 8 weeks proving sustained localized antimicrobial delivery around orthodontic attachments thus reducing patient compliance in controlling WSLs and providing an effective anticariogenic effect.

There is no evidence in literature regarding efficacy of CHX-HMP functionalized ligatures; in-vivo conditions as CHX concentration in oral environment influenced by dilute effect of saliva and food being consumed by orthodontic patients. Hence our study aimed at evaluating antimicrobial efficacy of CHX-HMP functionalized elastomeric ligatures while simultaneously comparing it with concentrations of CHX-elution in; in-vitro conditions over a duration of 8 weeks.

In current study greatest amount of CHX elution occurredon day 1 in both test groups (CHXdg - 63.13 µmoles/L); (CHX- HMP -111.74 µmoles/L). Both groups showed a consistent reduction on subsequent observations with CHX-dg group exhibiting no elution from 35th day of observation, while CHX-HMP functionalized elastomeric ligatures showed elution beyond 8-week period. Values of CHX elution in this study are in concurrence with values reported by Subramani K et al[19] with respect to 5mM CHX-dg and 5mM CHX-HMP functionalized elastomeric modules on day 1 and day 28 of observation period. Outcomes of current research are in agreement with outcomes of Subramani et al[19] andKamarudin Y et al[11] who concluded there wasn't any CHX elution after 28th day from 5mM CHXdg functionalized elastomeric ligatures and elution continued beyond 28th day and 8-week duration in 5mM CHX- HMP functionalized elastomeric ligatures. 5mM CHX-HMP demonstrated higher release of CHX compared to 5mM CHXdg functionalized

orthodontic ligatures although there was decreasing intensity of elution as a function of time in both groups. Percentage decrease as a function of time was higher in CHX-dg group compared to CHX-HMP group reflecting sustained release of CHX over time in CHX-HMP functionalized elastomeric ligatures. When coated with CHX-HMP-5, more CHX was bound to elastomeric ligatures using HMP-nanoparticles and HMP nanoparticles promoted slow and steady release of CHX which extends treatment period with antimicrobial. This shows that HMP-nanoparticle is effective as carrier for CHX coatings and for slow, long term continual release.[19] In current study functionalization of orthodontic ligatures with 5mM CHX-HMP was enhanced by solvent conditioning using ethanol. Literature shown ethanol as best organic solvent in impregnating biomedical polymers with antimicrobial nanoparticles compared to acetone conditioning. Ethanol softens surface layer of ligature without effecting bulk thus enhancing uptake of CHX-HMP at surface and in near subsurface region. Ethanol conditioning showed a minimum degree of chemical degradation in terms of discoloration without effecting physical and mechanical properties of orthodontic ligatures, whereas conditioning with acetone caused decrease in lumen size and swelling of elastomeric ligature and significant effect on force and extension of ligature at rupture.[11]

Microbial colony counts in current study were estimated following 1st week of start of orthodontic treatment after an initial oral prophylaxis in an attempt to bring baseline plaque scores to zero in both treatment groups. Outcomes of present clinical trialshowed S. mutans colonies increased over a function of time in both groups. Minimum values were documented at 1st week and there was consistent increase at 4th and 8th weeks. Increase in number of CCU was significantly higher in CHX-dg group when compared to CHX-HMP group at all time points. Results reflect effect of HMP nanoparticles as effective carrier for chlorhexidine from elastomeric ligatures to local orthodontic environment. Microbial evaluation from confirms sufficient and extended CHX release to be effective against S. mutans, primary microbes that cause WSLs and dental decay. Results of this study are in agreement with Subramani K et al[19] who concluded from their in-vitro study, CHX eluate is capable of inhibiting S. mutans and L. rhamnosus. While present study has utilized CFU, Subramani K et al[19] in their study used zone of inhibition calculation for evaluation of antibacterial activity of released CHX. Sustained antimicrobial efficacy can be expected to be effective as long as their remains CHX-HMP nanoparticles to deliver soluble CHX. Since release mechanism is on dissolution this will be inherently affected by maximum nanoparticle coverage that can be applied to substrate used in oral environment be it either implants or orthodontic auxiliaries. It has been proven in previous studies primary risk period for colonization of implant surface with microbes is soon after placement of implant and in such situations CHX offers effective treatment of peri-implant mucositis since CHX release for weeks of months after implant surgery is of high utility.

This property of polymeric biomaterial to exhibit sustained release of CHX over period of prolonged duration has created widespread use in diverse applications such as intra-vitreal devices for treatment of eye inflammation and disease as antibiotic coating for urethral catheters and in implants of various kinds for prevention and control of local infection.[43,44] Above modality of CHX nanoparticle impregnation on polymeric orthodontic auxiliaries can be employed to prevent iatrogenic effects of orthodontic treatment such as WSLs and dental caries.

***LIMITATIONS OF THE STUDY*:**

Shortcomings of study include failure to include SEM evaluation of elastomeric ligatures to study surface changes in coated ligatures. SEM analysis shows coating characteristics of nanoparticle deposit either inhomogeneous or homogeneous. Pattern of coating depends on conditioning of polymer wherein acetone conditioning led to inhomogeneity, while ethanol conditioning led to homogeneity. Moreover, this study did not include adverse effects of conditioning polymers with organic solvents and functionalization with CHX-HMP nanoparticles on chemical degradation, physical properties and mechanical properties of orthodontic elastomers. Primary strength of elastomeric ligatures is tensile strength which retains full engagement of arch wire within bracket slot.

Following are salient conclusions from present study.

VI. CONCLUSION:

- CHX-HMP nanoparticles coated elastomeric ligatures incubated inwater released aqueous CHX beyond a period of 8 weeks.
- CHXdg coated elastomeric ligatures stopped elution of CHX by35th day of incubation period.
- CHX-HMP nanoparticle coated elastomeric ligatures were capableof inhibition of Streptococcus mutans growth.
- Significant reduction of Plaque scores was observed with CHX-HMP coated elastomeric ligatures.

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