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University of Baghdad
College of Dentistry

Effectiveness of a Hydroxyapatite-Containing Toothpaste Versus Mouthwash in Preventing White-Spot Lesions During Fixed-Appliance Orthodontic Treatment: A Randomized Clinical Trial

A Protocol Submitted to
the Council of the College of Dentistry, University of Baghdad in
Partial Fulfillment of Requirements for the Degree of Master of
Science in Orthodontics

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Certification of the Supervisor

This is to certify that the preparation and organization of this protocol entitled **"Effectiveness of a Hydroxyapatite-Containing Toothpaste Versus Mouthwash in Preventing White-Spot Lesions During Fixed-Appliance Orthodontic Treatment : A Randomized Clinical Trial "** had been made by the master student **Heba Tahseen Almasri** under my supervision, at the Department of Orthodontics/College of Dentistry/University of Baghdad in partial fulfillment for the Degree of Master of Science in Orthodontics.

Supervisor's By

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College of Dentistry/

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2025

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Introduction:

Orthodontic treatment is widely recognized as an effective approach to improve dental and facial aesthetics, which play a crucial role in overall attractiveness and psychosocial well-being. A recent systematic review and meta-analysis confirmed that orthodontic therapy enhances facial attractiveness by approximately 9% compared with untreated individuals. However, despite these aesthetic benefits, fixed appliance therapy is associated with several side effects, most notably the development of white spot lesions (WSLs) **(Kouskoura et al., 2022).**

The placement of fixed orthodontic appliances significantly increases dental biofilm accumulation around brackets and wires. Patients undergoing fixed-appliance orthodontic treatment show a higher concentration of plaque, particularly on the labial surfaces of anterior teeth, compared to pre-treatment levels. This accumulation is attributed to the brackets acting as retention sites, making oral hygiene maintenance more challenging and increasing the risk of enamel demineralization, gingival inflammation, and the WSLs **(Kozak et al., 2021).**

WSLs represent the earliest stage of dental caries, characterized by subsurface demineralization of the enamel, which appears as opaque, non-cavitated white areas

on the tooth surface. These lesions indicate the onset of enamel decay and may progress to cavities if left untreated **(Lopes et al., 2024)**.

Fluoride has been widely used in toothpastes and mouthwashes for caries prevention; however, excessive or prolonged exposure has been associated with potential adverse effects. These include dental fluorosis, characterized by white or brown discoloration of teeth **(Choi et al., 2012)**.

Therefore, hydroxyapatite (HAP)-containing oral care products have emerged as a safe and effective alternative. Clinical evidence demonstrates that HAP can remineralize enamel, reduce the formation of WSLs, and provide preventive outcomes comparable to or better than fluoride, without the associated systemic or local side effects **(Amaechi et al., 2019)**. Therefore, incorporating HAP-based toothpaste or mouthwash into daily oral hygiene routines during orthodontic treatment offers a biocompatible and reliable strategy to minimize enamel demineralization and improve clinical outcomes.

Hydroxyapatite (HAP)-containing oral care products have been increasingly recognized for their ability to remineralize enamel and reduce the formation of WSLs. Clinical evidence confirms that HAP-containing toothpaste can re-mineralize enamel lesions effectively while enhancing tooth brightness without adverse effects **(BMC Oral Health, 2022; Paszyńska et al., 2023).** In addition, a mouthwash can significantly reduce interdental gingival bleeding, highlighting its role as an effective adjunct to mechanical oral hygiene **(Saliasi et al., 2018)**. Systematic reviews and meta-analyses have reported that mouth rinses can provide a benefit beyond mechanical oral hygiene alone in preventing plaque accumulation and gingivitis **(Araujo et al., 2015)**. These findings underscore the relevance of incorporating both HAP-based oral care products and mouthwashes as preventive and supportive measures in modern dental care protocols.

Research Question:

In patients undergoing fixed-appliance orthodontic treatment, which intervention hydroxyapatite-containing toothpaste, hydroxyapatite mouthwash, or conventional oral hygiene is most effective in preventing the development of white-spot lesions?

Aim and Objectives:

Aim:

To evaluate and compare the effectiveness of a hydroxyapatite-containing toothpaste, a hydroxyapatite-containing mouthwash, and conventional oral hygiene products in preventing or reducing the incidence of white spot lesions (WSLs) during fixed-appliance orthodontic treatment.

Primary Objectives:

To evaluate the preventive effectiveness of two enamel remineralizing methods compared to the conventional method in reducing the risk of WSLs on the labial surfaces of upper and lower incisors, and canine teeth during six months of orthodontic treatment by monitoring:

1. Severity of WSLs using the Gorelick index.

2. Enamel integrity and early demineralization using Diagnodent Pen scores.

3. Incidence of WSLs assessed using photographic documentation.

Secondary Objectives:

1. To assess oral health status using Gingival Bleeding Index (GBI), and Plaque

Index (PI).

2. To measure salivary pH changes over the study period.

Hypothesis:

Null Hypothesis (H0):

There is no significant difference in the incidence or severity of white-spot lesions among patients using hydroxyapatite toothpaste, hydroxyapatite mouthwash, or conventional oral hygiene during fixed-appliance orthodontic treatment.

Alternative Null Hypothesis (H1):

There is a significant difference in the incidence and severity of white-spot lesions among patients using hydroxyapatite toothpaste or hydroxyapatite mouthwash, with either intervention potentially showing superior preventive effectiveness.

Methodology

Study Design:

This study is designed as a multicenter, parallel, randomized clinical trial. Each participant will be randomly assigned to one of the three groups:

• **Group A**: Participants will be instructed to brush their teeth three times daily with the hydroxyapatite-containing toothpaste and to rinse twice daily with a placebo mouthwash.

• **Group B**: Participants will be instructed to brush their teeth three times daily with a conventional fluoride toothpaste and to rinse twice daily with a hydroxyapatite-containing mouthwash.

• **Group C:** Participants will be instructed to brush their teeth three times daily with a conventional fluoride toothpaste and to rinse twice daily with a placebo mouthwash.

All interventions and assessments will be conducted throughout the study period. The same participant (orthodontic patient) will be evaluated at baseline (before bonding) and at 1, 3, and 6 months after the placement of orthodontic appliances.

Participants will receive standardized oral hygiene instructions, and all assessments of white-spot lesions will be performed using Diagnodent Pen, Gorlick index (GI), digital photographs, Gingival Bleeding Index (GBI), Plaque Index (PI), and salivary pH measurements to minimize variability and ensure consistent data collection.

Randomization and Allocation Concealment:

Participants will be randomized into three parallel groups with an equal allocation ratio of 1:1:1 using a computer-generated randomization list created with GraphPad QuickCalcs. Allocation concealment will be achieved using sequentially numbered, opaque, sealed envelopes (SNOSE) prepared by an independent researcher not involved in recruitment. Each envelope will contain:

1. A group allocation card (A, B and C).
2. The assigned oral hygiene product in an opaque sealed bag.
3. Written instructions for product use.

Envelopes will remain sealed until the day of bonding, at which point the clinician will open the envelope to reveal the participant's group and provide the assigned intervention. The allocation list will be securely stored by the independent researcher and will not be accessible to the study team until after data collection and primary

statistical analysis are completed.

Blinding:

This study will be conducted under a double-blind design. Both the principal investigator and the participants will be blinded to group allocation. The toothpaste and mouthwash bottles will be coded and identical in appearance, color, and packaging, ensuring that neither the clinician nor the participant can identify which formulation (hydroxyapatite-containing or conventional) is being used. The code will be kept by an independent researcher who is not involved in the study procedures or data analysis and will only be revealed after the completion of data collection.

All clinical assessments, including Gorelick Index (GI), Diagnodent Pen measurements , digital photography, Gingival Bleeding Index (GBI), Plaque Index (PI), and salivary pH, will be conducted without knowledge of participants' assigned group.

Settings:

The study will be conducted at the Department of Orthodontics, University of Baghdad, as well as in selected private and public dental clinics that provide fixed-appliance orthodontic treatment. All participants will be treated using conventional metal brackets to ensure standardization of the orthodontic appliance type. All procedures, including oral examinations and sample collections, will follow standardized protocols to ensure consistency and uniformity across all study sites.

Subjects / Materials:

Participant Recruitment:

Participants will consist of patients requiring fixed-appliance orthodontic treatment due to malocclusion, crowding, or spacing issues.

Informed Consent:

Written informed consent will be obtained from all participants prior to enrollment. Participants will receive a clear explanation of the study objectives, procedures, potential benefits, and possible risks. They will be informed that participation is voluntary and that they may withdraw at any time without affecting their ongoing treatment. Confidentiality of participant information will be maintained throughout the study and in all subsequent publications.

Inclusion Criteria:

All included participants will be drawn from those who will receive comprehensive orthodontic treatment and fulfill the following inclusion criteria:

1. Complete permanent dentition up to the first premolar, with no intended extractions in orthodontic treatment plan.
2. Good overall health.
3. Good oral hygiene: full-mouth plaque score < 20%.
4. No bleeding upon probing after 30 seconds.
5. Discontinuous band of plaque at the gingival margin.
6. Normal stimulated salivary flow rate.
7. Normal buffer capacity (final pH between 6.0 and 7.0).

Exclusion Criteria:

1. History of previous orthodontic treatment.

2. Bleaching or topical fluoridation within the last six months.
3. Severely rotated any of the study teeth (limiting the appearance of facial surfaces).
4. Visible signs of caries, fluorosis, hypocalcification, or other developmental defects.
5. Restoration on the labial surface of the study teeth.
6. Systemic or endocrine conditions (e.g., cardiac pacemakers, diabetes mellitus).
7. Craniofacial anomalies and clefts.

Sample Size:

The sample size for the present study was determined to provide 80% statistical power at a 5% significance level ($\alpha = 0.05$) to detect a 1-unit difference in the decalcification index (Gorlick index) **(Hoffman et al, 2014)**. Accordingly, the current study will include a total of 75 participants, equally distributed into three groups of 25 subjects each, to ensure adequate statistical power and to account for potential dropouts.

Procedure / Intervention:

• **Group A**: Participants will be instructed to brush their teeth three times daily with the hydroxyapatite-containing toothpaste and to rinse twice daily with a placebo mouthwash.

• **Group B**: Participants will be instructed to brush their teeth three times daily with a conventional fluoride toothpaste and to rinse twice daily with a hydroxyapatite-containing mouthwash.

• **Group C**: Participants will be instructed to brush their teeth three times daily with a conventional fluoride toothpaste and to rinse twice daily with a placebo

mouthwash.

Toothbrushing Instructions:

All participants will receive oral and written instructions, as well as a demonstration video, on proper toothbrushing technique using their assigned toothbrush. The technique applied will be the Modified Bass method, in which the toothbrush is positioned at the gum margin at a 45° angle, performing small vibratory movements while simultaneously moving along the dental arches to ensure that all accessible tooth surfaces are thoroughly cleaned **(Farook, 2023).**

Each toothbrushing session will be performed for 2minutes, allocating approximately 30 seconds for each quadrant. Participants will be instructed to maintain this routine three times daily throughout the study period.

Mouthwash Protocol:

All participants will be instructed to use the mouthwash according to the following standardized protocol to ensure consistency across the study:

• Group A: Placebo mouthwash (purified water, flavoring agents).

• Group B: Hydroxyapatite-containing mouthwash.

• Group C (control group): Placebo mouthwash (purified water, flavoring agents).

- Frequency of use: twice daily after brushing.
- Dosage: 15 ml of mouthwash per use.
- Rinsing duration: Participants will rinse the mouthwash for 30 seconds and then expectorate without swallowing.
- rinsing instructions: Participants will be instructed not to eat, drink, or rinse with water for at least 30 minutes after using the mouthwash to maximize its

effect.

- Additional Instructions: Participants should shake the bottle well before each use.

Patient Reminder System:

To enhance adherence to the assigned oral hygiene regimen, all participants will be reminded weekly through one of the social media platforms. These messages will remind the participants in all groups to perform their assigned oral hygiene protocols. The messages will include brief instructions and practical tips for proper use the assigned toothpaste and mouthwash, and participants will be encouraged to confirm completion of their daily oral hygiene routine. This system is designed to improve compliance and ensure standardized adherence throughout the study period.

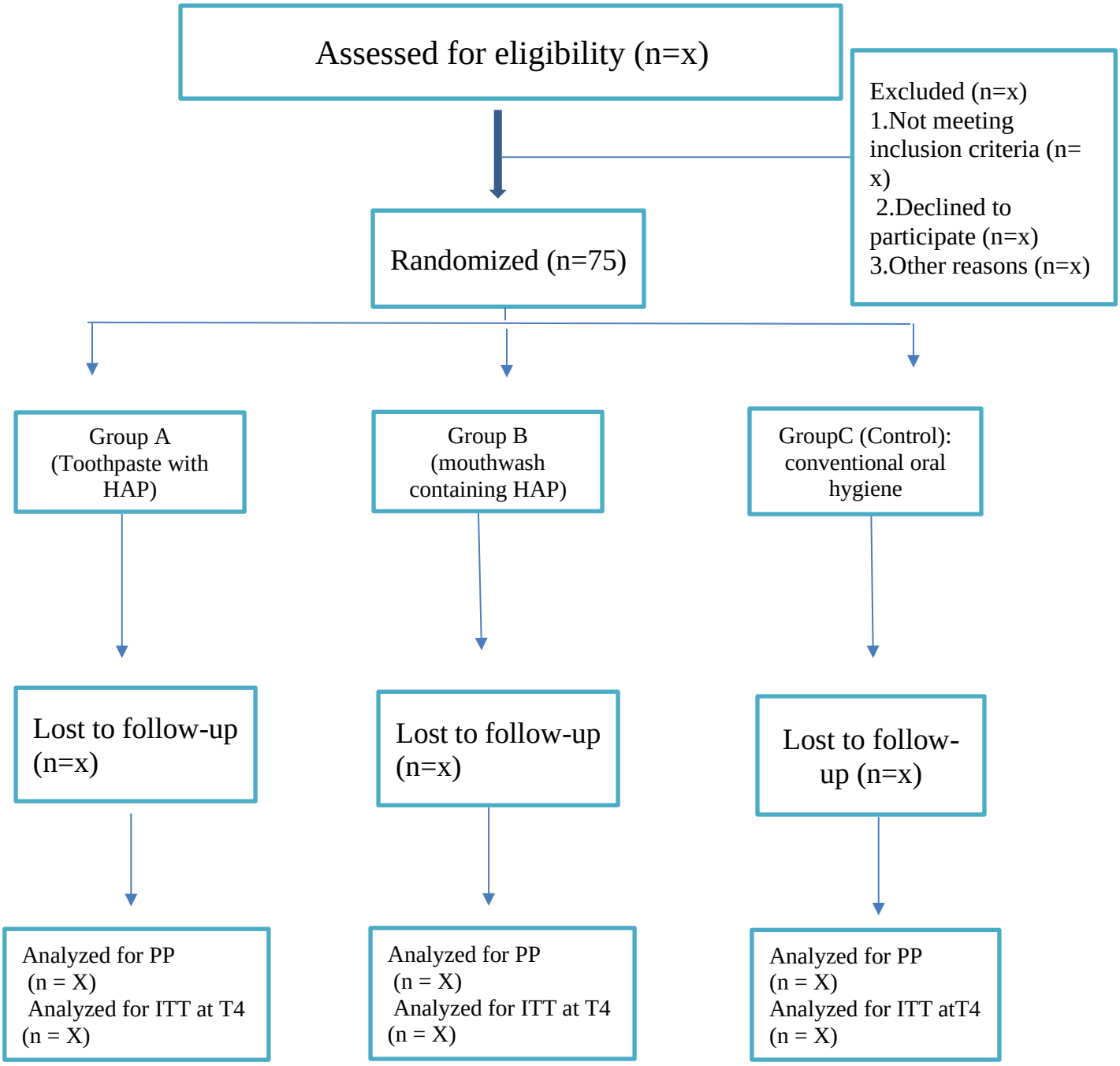

Figure 1: The intended CONSORT Flow Diagram of the Current Study.

CONSORT = Consolidated Standards of Reporting Trials, PP = Per-Protocol Analysis (includes participants who fully adhered to the study protocol), ITT = Intention-to-Treat Analysis (includes all randomized participants, regardless of adherence), T4 = Time point4 (fourth follow-up visit).

Assessment of WSLs and Oral Health:

White Spot Lesions (WSLs) and oral health parameters will be evaluated at baseline, 1 month, 3 months, and 6 months using DIAGNOdent Pen readings, standardized digital photographs, Gorelick Index, Plaque Index (PI), and Gingival Bleeding Index (GBI). All assessments will follow standardized protocols to ensure consistency across participants.

Photography and Gorelick Index Assessment

All patients' teeth were photographed using a digital camera. After drying with compressed air, three digital photographs (frontal, right, and left lateral) of each tooth were taken. A single investigator captured all images to ensure consistency in image quality. Photographs were taken at approximately 20° perpendicular to the buccal tooth surfaces **(Papadimitriou et al., 2024).**

The photographs will be evaluated using the Gorelick Index (GI) as follows:

(0) no white spot lesion present.

(1) visible white spots without surface interruption (mild decalcification).

(2) visible white spot lesion having a roughened surface but not requiring a restoration (moderate decalcification).

(3) visible white spot lesion with surface interruption (severe decalcification).

(4) cavitation **(Gorelick, Geiger & Gwinnett, 1982).**

Assessment of White Spot Lesions Using DIAGNOdent Pen:

In this study, the cutoff values of the DIAGNOdent device will be adopted according to the manufacturer's operating guide to standardize data collection in the clinical

setting. Readings from 0–10 represent sound tooth structure, 11–20 indicate outer half enamel caries, 21–30 correspond to inner half enamel caries, and readings of ≥30 are considered dentin caries **(KaVo Dental, Biberach an der Riss, Germany)**. These values will serve as a reference for monitoring changes in white spot lesions during the study **(Du et al., 2011; Gohar, Ibrahim and Safwat, 2023)**.

Prior to each measurement, the device will be calibrated for every patient. Readings will be obtained from four locations on the labial/buccal surface of each tooth: gingival, occlusal, mesial, and distal, in accordance with the recommendation of (Banks & Richmond, 1994). After drying the tooth surface, the number 2 pen tip will be placed 1 mm from the bracket on the labial surface of the tooth being examined.

The baseline measurement (T0) will be recorded in the patient's dental chart. Subsequent assessments will be performed at 1, 3, and 6 months (T1, T3, and T6) after the placement of the orthodontic appliance. All measurements will be conducted by a single trained examiner to ensure consistency and reliability.

Assessment of Plaque index:

To determine the Plaque Index (PI) of the patients, the thickness of dental plaque was evaluated by probing the mesial, distal, buccal, and palatal surfaces of all teeth using a Williams periodontal probe. The plaque index for each individual was calculated by summing the scores obtained for each tooth and dividing by the number of teeth examined. The classification of the plaque index was based on **Silness & Löe (1964)**:

• PI 0: No plaque in the area adjacent to the gingiva.

• PI 1: Thin film of plaque along the gingival margin, not visible to the naked eye.

• PI 2: Moderate accumulation of plaque visible on the gingival margin and in the gingival pocket.

• PI 3: Dense accumulation of plaque covering the gingival margin and filling the gingival pocket.

Assessment of Gingival Bleeding Index (GBI):

To evaluate the Gingival Bleeding Index (GBI), gingival bleeding was assessed on the mesial, distal, buccal, and palatal/lingual surfaces of all teeth using a Williams periodontal probe. The probe was gently inserted along the gingival margin and inside the gingival pocket, and bleeding was recorded approximately 30 seconds after probing. The index for each individual was obtained by summing the scores for all teeth and calculating the mean.

The classification of the Gingival Bleeding Index was based on **Löe & Silness (1963)**:

• GBI 0: Healthy gingiva with no bleeding.

• GBI 1: Slight bleeding on probing on one or more surfaces.

• GBI 2: Moderate bleeding observed on multiple surfaces.

• GBI 3: Profuse or spontaneous bleeding involving most or all surfaces.

Saliva Collection and pH Measurement:

Salivary samples will be collected from the participants. At the first stage,

participants will be given a paraffin wax to chew, which stimulates saliva production, and they will be asked to swallow the pooled saliva. Then, they will be instructed to spit their saliva into a saliva-collecting tube for five minutes. At each designated time point, the pH of saliva will be measured using pH test strips. The strips will be soaked in the saliva for one minute, and the resulting color change, indicating pH values, will be recorded by comparing it with the accompanying reference chart **(Alkarad, Alkhouli and Dashash, 2023; Campus et al., 2024).**

• **Patient compliance**: monitored with a diary and product usage checks.

Timetable:

Phase	Duration / Time Points	Description
Recruitment & Screening	1 month	Patient selection, informed consent, baseline assessment.
Baseline Assessment	Week 0	Initial evaluation of WSLs using Diagnodent Pen, digital photos, Gorelick Index, GBI, PI, and salivary pH.
Intervention & Follow-up	6 months	Participants receive assigned oral care regimen (toothpaste and mouthwash). Measurements at 1 month, 3 months, and 6 months for WSLs and oral health parameters.
Data Analysis	1 month	Statistical analysis of primary and secondary outcomes.

Stopping Roles:

• Any patient developing severe adverse reactions to the products.

- Patient withdrawal of consent.

Budget and Funding:

The study is self-funded

Ethical Approval:

The protocol will be submitted to ethics committee

Data Management and Analysis

Further Considerations

Pilot study:

A pilot study will NOT be conducted

Dissemination:

Postgraduate Thesis

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University of Baghdad
College of Dentistry

Research Ethics
Committee

Ref. number:1139

Date: 1-12-2025

Decision of the Research Ethics Committee

To Heba Tahseen Almasri and Asst. Prof. Dr. Noor Muhammed Hasan Garma

The Research Ethics Committee of the College of Dentistry, University of Baghdad has reviewed the submitted research project outlined below for ethical approval.

Project No.	1139425
Project title	Effectiveness of a Hydroxyapatite-Containing Toothpaste Versus Mouthwash in Preventing White-Spot Lesions During Fixed-Appliance Orthodontic Treatment: Randomized Clinical Trial
Decision	Approved

This decision was made based on the following submitted items that have been received and reviewed by the committee:

- Study protocol.
- Application form and checklist for research that involves human subjects (if applicable).
- Patient information sheet and consent form (if applicable).
- Application form and checklist for research that involves animal experiments (if applicable).

Salwan Bede

Prof. Dr. Salwan Y. Bede BDS, FIBMS

Chairman of the Research Ethics Committee

Email: ethical.approval@codental.uobaghdad.edu.iq