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Preface

We would like to present, with great pleasure, the inaugural volume-6, Issue-12, December 2020, of a scholarly journal, *International Multispeciality Journal of Health*. This journal is part of the AD Publications series *in the field of Medical, Health and Pharmaceutical Research Development*, and is devoted to the gamut of Medical, Health and Pharmaceutical issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Medical, Health and Pharmaceutical as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical statistics investigations. Its mission is to become a voice of the Medical, Health and Pharmaceutical community, addressing researchers and practitioners in below areas

Clinical Specialty and Super-specialty Medical Science:

It includes articles related to General Medicine, General Surgery, Gynecology & Obstetrics, Pediatrics, Anesthesia, Ophthalmology, Orthopedics, Otorhinolaryngology (ENT), Physical Medicine & Rehabilitation, Dermatology & Venereology, Psychiatry, Radio Diagnosis, Cardiology Medicine, Cardiothoracic Surgery, Neurology Medicine, Neurosurgery, Pediatric Surgery, Plastic Surgery, Gastroentrology, Gastrointestinal Surgery, Pulmonary Medicine, Immunology & Immunogenetics, Transfusion Medicine (Blood Bank), Hematology, Biomedical Engineering, Biophysics, Biostatistics, Biotechnology, Health Administration, Health Planning and Management, Hospital Management, Nephrology, Urology, Endocrinology, Reproductive Biology, Radiotherapy, Oncology and Geriatric Medicine.

Para-clinical Medical Science:

It includes articles related to Pathology, Microbiology, Forensic Medicine and Toxicology, Community Medicine and Pharmacology.

Basic Medical Science:

It includes articles related to Anatomy, Physiology and Biochemistry.

Spiritual Health Science:

It includes articles related to Yoga, Meditation, Pranayam and Chakra-healing.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within

that community who supported the idea of creating a new Research with *IMJ Health*. We are certain that this issue will be followed by many others, reporting new developments in the Medical, Health and Pharmaceutical Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IMJ Health* readers and will stimulate further research into the vibrant area of Medical, Health and Pharmaceutical Research.

Dr. Kusum Gaur (Chief Editor)

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She has awarded with WHO Fellowship for IEC at Bangkok. She has done management course from NIHFW. She has published and present many research paper in India as well as abroad in the field of community medicine and medical education. She has developed Socio-economic Status Scale (Gaur's SES) and Spiritual Health Assessment Scale (SHAS). She is 1st author of a book entitled "Community Medicine: Practical Guide and Logbook.

Research Area: Community Medicine, Biostatics, Epidemiology, Health and Hospital Management and Spiritual Health.

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Dr. AMER A. TAQA

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Research Area: Dental Science.

Dr. I.D. Gupta

Dr. I. D. Gupta is Professor Psychiatry and working as additional Principal and Dean of student welfare in SMS Medical College, Jaipur.

He is recipient of Prof. Shiv Gautam oration award by Indian Psychiatric Society. He has done training in YMRS at Monte Carlo and BPRS at Singapore. He has been President Indian Psychiatric Society, Rajasthan State Branch. He is author of "Psycho Somatic Disorder" chapter in 1st edition post graduate text book of Psychiatry by Vyas and Ahuja. He has also worked with National Mental Health Programme and has a lot of publication.

Research Area: Community Mental Health, Psycho somatic and liaison Psychiatry.

Dr. Lokendra Sharma

Dr. Lokendra Sharma is Associate Professor Pharmacology and working as Nodal officer of SMS Medical College, Jaipur.

He is recipient of WHO Fellowship award on Poison Patient Management at Vietnam. He is resource faculty for Experimental Toxicology and Basic Course for Medical Education. He is presented and published a lot of research articles at national and international level.

Research Area: PHARMACOLOGY

Dr. Anuradha Yadav

Dr. Anuradha Yadav is working as Professor Physiology, SMS Medical College, Jaipur (Rajsthan) India. She is a popular medical teacher and research scholar who had many publications in indexed journals. **Research Area:** CVS & CNS physiology, Medical Education and Spiritual Health.

Dr. Rajeev Yadav

Dr. Rajeev Yadav is working as Associate Professor Community Medicine, SMS Medical College, Jaipur (Rajsthan) India. He is member of Research Review Board of the Institute.

He has authored a book entitled "Community Medicine: Practcal Guide and Logbook".

Research Area: His area of Interest are Epidemiology, Biostatistics and Spiritual Health.

Prof. Dillip Kumar Parida

Professor and Head in the Department of Oncology, AIIMS, Bhubaneswar.

He has done the Professional Training in Japan (Oska University, NIBI, AHCC Research Association, Hyogo Ion Beam Center), ESTRO Fellowship in Denmark and India(AIIMS Delhi, BARC Mumbai, SCB Medical College-Cuttak, MKCG Medical College-Berhampur).

Research Area: Brachytherapy, Total Skin Electron Irradiation, Palliative Radiotherapy, Stereotactic & Conformal radiotherapy, Radiation Cell Biology, Cancer Genetics.

Dr. Praveen Mathur

Dr. Praveen Mathur is working as Professor- Pediatric Surgery and is recipient of Commonwealth Fellowship in Pediatric Laparoscopy from Uk and fellowship award in minimal access Surgery (FMAS). He has done Clinical observer ship in the Department of Pediatric Surgery, Johns Hopkins University, Baltimore, USA. (2008). He has presented and published a number of research articles at national and international level. He is reviewer of prestigious Journal of Pediatric Surgery (JPS) and World Journal of Gastroenterology, Journal of neonatal Surgery (JNS).

Research Area: Pediatric Surgery & Laparoscopy.

Dr. Lokendra Sharma

Dr. Lokendra Sharma is Associate Professor Pharmacology and working as Nodal officer of SMS Medical College, Jaipur.

He is recipient of WHO Fellowship award on Poison Patient Management at Vietnam. He is resource faculty for Experimental Toxicology and Basic Course for Medical Education. He is presented and published a lot of research articles at national and international level.

Research Area: PHARMACOLOGY.

Dr Rajeev Sharma (MS; FMAS; FIMSA; FCLS)

He is working as Professor, Department of Surgery, Government Medical College, Chandigarh, India. He has done FMAS, FIMSA and FCLS along with MS (Gen Surgery).

He has about 50 international and national publications to his credit. He has held various positions in the Association of Minimal Access Surgeons of India (AMASI) from time to time. He has also acted as instructor of various AMASI skill courses held at different places in India. He has established Surgical Technique learning centre at GMCH Chandigarh for imparting training to the budding surgeons in the field of minimal access surgery. He is also the reviewer in the subject in various journals.

Research Area: Minimal Access Surgery.

Dr Anshu Sharma (MS ANATOMY)

She is Presently working as assistant professor in the department of Anatomy, GMCH, Chandigarh. She has many publications in various national and international journals. She is executive member of Anatomical Society of India (ASI) and North Chapter of ASI. She is also a member of editorial board of Journal of Medical College Chandigarh.

Research Area: Congenital Malformation, Developmental Anatomy.

Dr. Rajeev Yadav

Dr. Rajeev Yadav is working as Associate Professor Community Medicine, SMS Medical College, Jaipur (Rajsthan) India. He is member of Research Review Board of the Institute.

He has authored a book entitled "Community Medicine: Practical Guide and Logbook".

Research Area: His areas of Interest are Epidemiology, Biostatistics and Spiritual Health.

Dr. Dilip Ramlakhyani

Dr. Dilip Ramlakhyani working as Associate professor Pathology and member of IT Committee of Sawai Man Singh Medical College, Jaipur (Raj) India. He has published many articles in indexed journals.

Dr. Virendra Singh

Dr. Virendra Singh worked as Supernatant and head of department of Pulmonary Medicine, SMS Medical

College, Jaipur (Rajsthan) India.

He has gone abroad for many training courses and to present research papers. He had been chairman of

Research Review Board of SMS Medical College, Jaipur. He is a great research scholar and had published

book related to his faculty and had many publications in indexed journals.

Dr. Mahesh Sharma

Dr. Mahesh Sharma is a Principle specialist General Surgery in Rajasthan State Government, India.

He has been PMO of district hospitals for more than 15 years. He has gone abroad as observer of many of

training related to his speciality. He has published and present many research paper in India as well as

abroad.

He has developed Spiritual Health Assessment Scale (SHAS) with Dr. Kusum Gaur.

Research Area: General Surgery, Health and Hospital management and Spiritual Health.

Dr. Ravindra Manohar

Professor Community Medicine, working as head of department of PSM,SMS Medical College, Jaipur

(Rajsthan) India.

Previously he has worked in BP Kiorala Institute of Medical Sciences, Nepal. He has visited CDC Atlántica

for a Statistical workshop. He has been convener of MBBS and PG exams. He is a research scholar and had

many publications in indexed journals.

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Research Area: Pediatric Surgery & Laparoscopy.

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Evaluation of the Inter-Premolar Width Changes in the Mandible using Three Different Commercial Arch Wires: A Prospective Clinical Study

Mohamed Helmi Saleh¹, Mohamed Abdultawab², Hussien Naseef³

¹Assistant Professor of Orthodontics, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

²Master's Degree Student, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

Abstract—

Objective: The aims of this study isto evaluate the inter-premolar width changes in the mandible using three different commercial orthodontic arch wires.

Subjects and methods: Thirty patients including both males and females have been allocated in this study. Three orthodontic wires including NiTi, copper NiTi, and Beta-Titanium wires had been used. A special observational technique including cbct had been used to make an evaluation for the interpremolar width before and after the aligning stage.

Results: The results showed a highly significant increase in post-treatment inter premolar width in CNA and NITI groups; compared to Cu NITI group; with highly significant statistical difference (p < 0.01 respectively).

Conclusion: The present study showed that there is a high significant increase in the inter-premolar width in the mandible, when comparing the wires in the groups (NiTi, CNA, and copper niti). Also, the gender of the patient does not seem to have an impact of the gender on post-treatment efficacy of each wire.

Keywords— Mandible, Commercial Arch Wires, NiTi, copper NiTi, Beta-Titanium wires.

I. INTRODUCTION

One of the targets of orthodontic treatment is to provide improvement in the dental health; this is also obtained through positioning the teeth into the best functional balance using fixed appliances. Consequently, an increasing in the number of adult patients who seeking orthodontic treatment is shown, not only for aesthetic purposes, but also due to recent development in socioeconomic conditions.^{1,2}

Researchers have attempted to define and direct appropriate dental arch forms. With the coming of the pre-adjusted straight wire appliance, trials have been made to create a design and start commercializing arch-wires with ideal arch form.³

Tabulation of arch forms has implicated various geometric and complex mathematical formulas. The changes that it may happen in the form of the dental arch have suggested showing an effect on the stability and the periodontium. To decrease the chances of treatment relapse, multiple methods have been suggested, including preservation of the mandibular incisors in their pre-treatment position and preservation of the original arch form.^{3,4}

³Assistant Professor of Orthodontics, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

At first, the form of the arch is shaped by the configuration of the supporting bone, then the eruption of teeth, it is further adjusted by the surrounding musculature and functional forces. If this arch form is changed during orthodontic treatment, there will be tendency to get back to its pre-treatment shape. Multiple studies have mentioned the return of the canine and molar widths to pre-treatment position during the post-retention phase if the original arch form is altered. Consequently, the preservation of original arch form rather than arch modification is generally recommended to reduce the relapse tendency.⁵

Nickel-titanium (NiTi) arch wires are commonly used in the initial aligning stage of orthodontic treatment, as these wires own greater elasticity and resilience with low elastic modulus and rigidity. As from the presentation of NiTi arch wires into field of orthodontics, multiple elements have been added in order to obtain clinical benefits, copper considered to be one of these elements that have been added to NiTi, which is give a lowering in the loading stress while displaying relatively high unloading stress, which can result in more effective orthodontic tooth movement. In the mid of the 90s, the copper NiTi wires rushed the market, and were distributed with three transition temperature, the first type is the superelastic (27°C), the other two types are the heat activated (35°C and 40°C), also the presence of the copper give the wire a better defined transition temperature, which increase the efficiency of tooth movement.

In 1979, new wire presented to orthodontic field, these wires called Beta-titanium (β -Ti) alloy wires; these wires achieved a great publicity, due its biocompatibility, resistance to corrosion, and low stiffness. Despite the fact that there are multiple and different brands of arch wires in Egypt, only a few of them can be used safely to avert post treatment relapse. From that concept this study focused on evaluation of the inter-premolar width of the mandible using three different orthodontic wires.

II. SUBJECTS AND METHODS

Ethical consideration had been taken from the Ethical committee in the faculty of medicine, Al-Azhar University in Egypt. This study conducted on the patients that visited specialized orthodontic clinic, in the faculty of dentistry, Alazhar University. Thirty orthodontic patients including both genders had been joined into this study. By using a special randomization tool from this website: https://www.graphpad.com, the patients divided into three groups through randomization. The patients allocated equally into three groups, as group A included ten samples treated with (CNA) wire, group B included 10 patients treated with (NiTi) wire, and group C included 10 patients treated with 10 patients treated with (NiTi) wire. Eligibility of the patients had selected according to the following inclusion and exclusion criteria

2.1 Inclusion criteria:

- 1. The age of the patients ranging from 14 to 20 years old.
- 2. Proper oral hygiene with adequate nutritional routine.
- 3. Medically free from any systematic or genetic diseases that may interfere with normal growth.

- 4. Absence of any growth abnormality and bone metabolic disorders.
- 5. The patient has full permanent dentition.
- 6. Patients who have mild to moderate crowding.

2.2 Exclusion criteria:

- 1. Patients who have retention in the deciduous teeth.
- 2. Patients who don't attend more than two successive appointments.
- 3. Patients who don't follow the researcher's instructions.
- 4. Patients who will need extraction one or group of teeth as a part of orthodontic treatment.

2.3 Diagnosis and records:

According to the standard routinely procedures that's done in the clinic of orthodontic department, a record for the patient had been taken. The record contained:

- 1. Intra oral photography.
- 2. Extra oral photography.
- 3. Panorama.
- 4. Lateral cephalometric radiograph.

All of that records done before and after orthodontic treatment, except for the photographs, as it done additionally during orthodontic treatment. A cone beam computed tomography for the mandible had been done before beginning of orthodontic treatment and after the finishing of aligning stage.

2.4 B- Bonding Procedure

The bonding technique was done in a similar method to this study⁹:

- 1. The teeth were cleaned and dried to be prepared for the next step in bonding procedures.
- 2. The etching applied on the enamel for 30 seconds using 37% phosphoric acid (etching gel, 3M, Monrovia, CA, USA).
- 3. The bonding agent (Transbond XT, 3M, Monrovia, CA, USA) was applied on the tooth.
- 4. The composite (Transbond XT, 3M, Monrovia, CA, USA) was applied on the bracket base. The composite was polymerized by using LED lamp (Opticore L3; MarslevByvej, Denmark)

2.5 Wires loading

Then the orthodontics wires prepared and loaded in the brackets:

TABLE 1
THE LOADING OF WIRES AMONG GROUP

Group A	Group B	Group C
CNA wires (Ortho organizers inc, Carlsbad, CA) had been loaded	copper NiTi wires (Henry Schein® Orthodontics)	NiTi wires loaded (Ortho organizers inc, Carlsbad, CA)

The sequences of wires that it's loaded those sizes had used 0.014", 0.016", 0.018" and 0.016*0.022".

2.6 Observational method

According to this study⁹, same observation method had been used. As, the present study used cone beam computed tomography to create an evaluation.

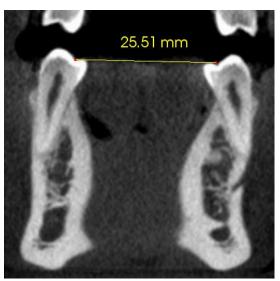


FIGURE 1: Measuring the inter-premolar width

III. RESULTS

TABLE 2
MEAN OF INTER-PREMOLAR WIDTH IN THE 3 GROUPS.

Variable	CNA group (10)	Cu NITI group (10)	NITI group (10)
	Mean ± SD	Mean ± SD	Mean ± SD
IPW0(mm)	34.7 ± 2	31 ± 2.99	33.68 ± 1.98

IPW0: Inter Premolar width before treatment.

 $\begin{tabular}{ll} Table 3\\ Socio-demographic data among 30 patients seeking orthodontic treatment: \\ \end{tabular}$

Variables		Frequency (%)
Age (years)		17.2 ± 1.76*
	Female	19 (63.3%)
Gender	Male	11 (36.7%)

^{*} $Mean \pm SD$.

This table shows that; the mean age of all patients was (17.2 ± 1.76) years. Regarding gender of the patients, the majority (63.3%) of patients were females; while (36.7%) were males.

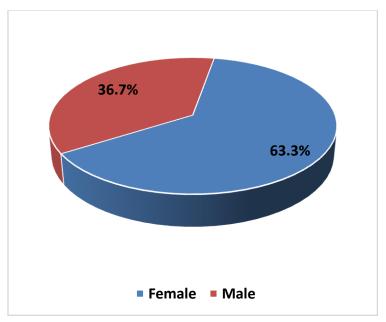


FIGURE 2: Gender among 30 patients seeking orthodontic treatment

TABLE 4
COMPARISON BETWEEN THE 3 GROUPS AS REGARDS SOCIO-DEMOGRAPHIC DATA USING ANOVA AND CHI SQUARE TESTS

Variable		CNA group (10)	Cu NITI group (10)	NITI group (10)	ANOVA test
		Mean ± SD	Mean ± SD	Mean ± SD	P value
Age (years)		17.6 ± 1.6	16.8 ± 2.1	17.4 ± 1.5	= 0.588
Variable		CNA group (10)	Cu NITI group (10)	NITI group (10)	Chi square test
					P value
Gender	Female Male	6 (60%) 4 (40%)	6 (60%) 4 (40%)	7 (70%) 3 (30%)	= 0.8663

ANOVA: analysis of variance. *Percentage of Column Total.

Comparative study between the 3 groups revealed non-significant difference as regards age and sex of the patients (p > 0.05).

 ${\bf TABLE~5}$ Comparison between the 3 groups as regards post-aligning stage data using ANOVA test

Variable	CNA group (10)	Cu NITI group (10)	NITI group (10)	ANOVA test
	Mean ± SD	Mean ± SD	Mean ± SD	P value
IPW0-IPW6(mm)	37.3 ± 1.1	34.3 ± 2	35.7 ± 2.1	= 0.004**

IPW0: Inter-premolar width before treatment, IPW6: Inter-premolar width after aligning stage

Comparative study between the 3 groups revealed; highly significant increase in post-treatment of IPW in CNA and NITI groups; compared to Cu NITI group; with highly significant statistical difference (p < 0.01 respectively).

3.1 Impact of gender on post-treatment efficacy of each wire:

TABLE 5
IMPACT OF GENDER ON POST-TREATMENT EFFICACY OF EACH WIRE USING STUDENT'S T TEST:

	Females (CNA group)	Males (CNA group)	Student's t
Variable	(6)	(4)	test
	Mean ± SD	Mean ± SD	P value
IPW6	37.48 ± 0.97	37.1 ± 1.44	= 0.626
	Females (Cu NITI group)	Males (Cu NITI group)	Student's t
Variable	(6)	(4)	test
	Mean ± SD	Mean ± SD	P value
IPW6	34.4 ± 2.4	34 ± 1.67	= 0.746
	Females (NITI group)	Males (NITI group)	Student's t
Variable	(7)	(3)	test
	Mean ± SD	Mean ± SD	P value
IPW6	35.3 ± 2.44	36.6 ± 0.9	= 0.399

IPW6: Inter-premolar width after aligning stage

Regarding CNA group: Gender had non-significant effect on IPW in CNA group (p > 0.05 respectively).

Regarding Cu NITI group: Gender had non-significant effect on IPW in Cu NITI group (p > 0.05 respectively).

Regarding NITI group: Gender had non-significant effect on, IPW, in NITI group (p > 0.05 respectively).

IV. DISCUSSION

The increasing in demands for orthodontic treatment by adult patients is not focusing only on aesthetic purposes, but also due to recent improvements and development in socioeconomic conditions. This new perspective increased the demands to investigate both skeletal and dental changes in soft tissue morphology of adult individuals, considering the increasing search for orthodontic and orthognathic treatment. Understanding these changes may help to know if that notified changes took place primarily due to orthodontic relapse or are part of the natural process of development and maturation. ¹⁰⁻¹²

The retention in orthodontic treatment considered to be the last stage, which this stage aims to preserve the teeth in their corrected positions after the completion of orthodontic tooth movement, since the change in inter-premolar width may influence the rate of stability, hence, the present study focused on evaluating the inter-premolar width in the mandible after the levelling and aligning stage, which is a major stage in orthodontic treatment.¹³

Devinder Preet et al.¹⁴ performed a study in 2014 to create an evaluation in the pre-treatment and post-treatment arch parameters in both upper and lower arches in orthodontic patients that had their treatment using extraction of first premolar teeth. They found that the overall arch width changes within the Classes I and II were also significant except inter-premolar arch width changes in the maxillary arch both in Class I and Class II div 1 subjects and the interincisal arch width changes in Class I subjects in the mandibular arch. Which it shows that there is a significant change in the inter-premolar in the mandible in both class I and class II

cases? Well, this come in agreement with the findings of the present study, despite that there are multiple differences in the methodology and study design.

In assessing the stability of the mandibular arch following orthodontic treatment, Gardner and Chaconas, examined the clinical records of 74 non extraction and 29 extraction patients. They found that the inter-second premolar width increases during treatment. Also, the findings that co-related to non-extraction cases come in agreement with findings if the present study. 14,15

Eunkoo Kim performed a study in 2003 to evaluate both arch widths and smile esthetics. Their findings included that there is decrease in widths in both arches in the extraction sample, whereas this dimension increased in the non-extraction subjects. The results that's come in relation to extraction samples come in disagreement with the results of the present study, as the present study showed that there is an increase in the inter-premolar width, these disagreement may be because that the samples included extraction cases while the samples of the present study focused on non-extraction cases. However, the findings of Eunkoo Kim et al. that's related to non-extraction cases come in agreement with the findings of present study, as they showed that the inter-premolar width had increased. Long-term studies assessed the postoperative changes of orthodontically treated cases. In general, there is a tendency towards continuous reduction in the width and length of dental arches, with increase in crowding, overbite and over jet. The greatest problem has been the inability to determine whether these changes occur primarily as a result of orthodontic treatment, or if they are part of the natural maturation process. ^{16,17}

Unfortunately, there is not much articles that focused on the comparison between those wires, however, multiple studies have focused on evaluation the changes that it may happen in the arch dimension, but it's difficult to create a comparison between the present study and those studies, due the major differences in the study design, methodology, and observational methods. Consequently, it recommended obtaining further investigations in this sector. ¹⁸⁻²⁰

V. CONCLUSION

The present study showed that there is a high significant increase in the inter-premolar width in the mandible, when comparing the wires in the groups (NiTi, CNA, and copper niti). Also, the gender of the patient does not seem to have an Impact of gender on post-treatment efficacy of each wire.

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Performing Maxillofacial Surgeries during Covid19: Current Challenges and Possible Solutions

Natheer Ayed Jassem

BDS, F.I.C.M.S, Lecturer of oral and maxillofacial surgery, Faculty of Dentistry, Albayan University

Abstract— The world is witnessing an invasion from a new corona virus, which resulted in more than one million of deaths. Most of the sectors such industrial, economy, and tourism are facing a crisis, hence the workers in the field of medicine, considered to be the barrier to fight this invasion. This new virus seems to have two main transmission routes: direct and contact, which it will open a high chance of infection among professional health providers, especially, surgeons and dentists. Maxillofacial and dental surgeons, considered to be essential professional health experts that perform, multiple surgeries and dental procedures every day, consequently, these professions will exhibit a high risk of getting infected by Covid19, due to that, this review article aimed to discuss the possible ways that it may help in optimizing the level of infection control.

Keywords—Covid 19, corona virus, Maxillofacial Surgeries, health providers, dental surgeons.

I. INTRODUCTION

Wuhan witnessed unidentified disease which characterized by multiple symptoms such as tiredness, pneumonia, lack of appetite, and vomiting.^{1,2} Then in the following months this new diseases spread to Europe, Middle east, and most of the part of world, which led the World Health Organization to declare a pandemic alert in march 2020.³

Covid-19 considered to be the name for this disease, which is originally caused by a Severe Air Respiratory Syndrome (SARS) Coronavirus 2 (SARS-CoV-2).⁴ According to both genetic and epidemiological research, the diseases begin its the invasion from animal to human, then subsequent to start from human to human.^{4,5}

According to world-meter website, there are more than one million death until 21 November 2020. Hence, multiple health organizations and authorities around the world such as, Center for Disease Control and Prevention have instruct the dentists and their teams to give a regulation for their services to provide them with guidance which will enable them to obtain protection for their patients from this infection.⁶

Performing dental and maxillofacial surgeries may put the dentist and his/her team in a high risk of transmission to Covid19, as they may deal with Asymptomatic (carrier) patients as well as patients with an acute respiratory illness may present for dental treatment at outpatient dental settings. Hence, in this review article, we focused in the challenges, precautions and solutions, that the dental and maxillofacial surgeon should be aware of to decrease the chances of the transmission of this pandemic disease.

II. SYMPTOMS

Patients which have COVID-19 will show multiple clinical symptoms such fever, dry cough, and myalgia.⁸ But there are other clinical symptoms have been recorded:

- 1. Dyspnoea.^{8,9}
- 2. Fatigue.¹⁰
- 3. Headache. 11
- 4. Nausea/vomiting. 12
- 5. Sore throat.¹³
- 6. Rhinorrhoea. 14
- 7. Patchy shadows and ground glass opacity in the lung, which is revealed by chest CT.15
- 8. Hemoptysis.¹
- 9. Shortness of breath. 16-18

Despite that the COVID-19 initially has been divided into four types: mild, moderate, severe, and critical cases, there is increasing evidence that multiple infections of COVID-19 are asymptomatic, but they can transmit the virus to others.¹⁹

III. PATHOGENESIS

In the same way of its previous family member SARS-CoV, this new virus attack the cells by utilize angiotensin-converting enzyme 2 (ACE2) as its receptor, because ACE2-mediated angiotensin II (Ang II) degradation plays a vital role in the pathogenesis of severe lung failure after a viral infection, the seriousness of the virus infection is correlated to the level of maturation and binding capacity of ACE2. 19,20

IV. ROUTES OF TRANSMISSION

The main routes of transmission of Sars-Cov2 are two routes:

- 1.The first route include the transmission through a direct pathway. That's happen when the infected person goes sneezing or coughing near the non-infected person.²¹
- 2. The second route include the transmission through contact with oral, nasal, and eye mucous membranes.^{21,22}

Despite that the common clinical manifestations of this virus do not contain symptoms that it relates to eye, the analysis of conjunctival samples from confirmed and suspected cases of Covid-19 suggests that the transmission of this virus is not restricted to the respiratory tract, and that eye exposure may provide an effective way for the virus to enter the body. ^{21,23}

V. IMPACT OF COVID-19 ON ORAL AND MAXILLOFACIAL SURGERIES

The pandemic of COVID-19 has affected all aspects of life including all economic activity, travel, governance, and education, dentistry and oral surgery are also not spared. 24,25

Balaji SM ²⁴ stated that Oral and maxillofacial surgeons need to revisit their infection control protocols before fulfilling their professional and moral obligation. Before encouraging treatment, globally advocated COVID-19 sterilization and disinfection protocol need to be followed.

It recommended that all avoidable and non-emergency procedures can be postponed, until COVID-19 is contained. Some surgeries can be performed such as surgeries that demands

airway management, stopping bleeding, surgeries of patients who need drainage of infections that is resistant to antibiotics, and on co-surgeries where a delay in performing the surgery will impact the survival period. If the procedure requires immediate treatment, it would be safer to assume them to be COVID-19 positive till proven otherwise and use appropriate guidelines.²⁴

VI. PATIENT EVALUATION

Pre-check triage should be formed to classify the patient status in relation to covid-19¹. The patient evaluation contained some steps:

- 1. The triage starts by calling the patient to make an appointment or to register their elective hospital admission. From the Oral and Maxillofacial Surgery Department, through a simple checking-questionnaire, patients must be identified as having a high risk of infection and, consequently, adopt protective measures.²⁶
- 2. The presence of these symptoms should be investigated temperature of the patient showed an increase above 37.5 °C, or any type of previous mentioned symptoms, Forehead thermometer (for no contact) is highly recommended for the screening procedure. ²⁶
- 3. If a patient stated that he/she has been to an area that showed a history of positive COVID-19 cases within the past 14 days, quarantine for at least 14 days is suggested. In areas where COVID-19 spreads, nonemergency dental practices should be postponed.¹
- 4. Before the procedure of evaluating the patient inside the clinic, operating room or hospitalization area, it is essential to clearly explain to him/her which procedures are prioritized and which are preferably avoidable.²⁶

VII. ORAL AND MAXILLOFACIAL SURGEON PROTECTION

7.1 Health care personal exposure risk:

The nature of work of maxillofacial surgeons make them work in the area of head and neck, same as other surgeons such plastic surgeries, in addition to the their ancillary staff, this will put them in a high risk of exposure to covid-19.²⁷⁻³⁰

Chigurupati R et al.²⁷ stated that there some considerations in "How to Protect Healthcare Personnel". These considerations include:

- 1. Execute source control-facemasks for everyone entering a healthcare centers such as, health care personal, patients, visitors), regardless of symptoms.
- 2. Actively screening everyone for fever and symptoms of COVID-19.
- 3. Create barriers to terminate the contact with patients at triage.
- 4. Control the numbers of staff performing patient care.
- 5. Assure hand hygiene.
- 6. Follow standard and transmission-based precautions.

- 7. Use proper personal protective equipment, including (powered air-purifying respirator or surgical respirator masks, face shield, eye protection, fluid-resistant gowns, booties) for aerosol-generating procedures.
- 8. Understand sequence of donning and doffing of personal protective equipment and mask fitting.

7.2 Personal Protective Equipment:

The epidemic of **human immunodeficiency virus** in the previous year's such as 1980s giving a new concept to understand the "Universal Precautions", that go to a suggestion that all patients were an infection risk, and care should be taken.²⁷ Monje Gil et al.²⁶, described the personal protective equipment for healthcare professionals and workers, which is modulated by World Health Organization European Centre for Disease Prevention and Control:

- 1. **Protection in outpatient clinic area**: If the Patients showed no symptoms and negative to COVID-19, the type of personal protective equipment would include surgical mask / filtering facepiece1, and gloves. However, suspected patients who showed a respiratory symptom or confirmed to be positive COVID-19, will have more preparation, as their type of personal protective equipment will include FFP2 mask, coat, gloves, surgical cap, and eye protection (sealed glasses/face mask).
- 2. Protection inside the office of oral and dental surgery and hospitalization ward: If the activity of the patients showed no symptoms and confirmed negative to COVID-19, the type of personal protective equipment will include surgical mask / filtering facepiece1 and gloves. While if there is a direct activity in suspected patients who is showing symptoms or confirmed positive to COVID-19, the type of personal protective equipment will include filtering facepiece2 mask, coat, gloves, surgical cap, protection (sealed glasses/face mask). However, and the aerosol-forming operations in a suspected patient who shows symptoms or confirmed COVID-19 positive, will have more protection measure, hence the type of personal protective equipment will includes filtering facepiece3 mask, coat, gloves, disposable surgical cap, eye protection (sealed glasses/face mask), and apron.
- 3. **Protection in the surgery room:** If the patients showed no symptoms and are negative to COVID-19, the type of personal protective equipment will include filtering facepiece2 mask, sterile surgical gloves, disposable surgical cap, and eye protection (sealed glasses/face mask). The direct activity in suspected patients who showed symptoms or COVID-19 positive will include special preparation, hence the type of personal protective equipment will include filtering facepiece3 mask, waterproof sterile surgical gown, sterile surgical gloves, double disposable surgical cap, and eye protection (sealed glasses/face mask).

As a general rule, before applying any type of operation or accepting patients, a SARS-CoV-2 test should be done. Patients who have an emergency condition or any type of urgent intervention which doing the test will consume much time should be considered as potentially infectious. Since, there are a huge amount of patients that are asymptomatic but in the same time they are Covid-19 positive, Hence its essential initially to considered all of the patient

are Covid-19 positive. 26,30 To make sure the ideal protection of operating room personnel, if a patient confirmed his/her infection with COVID-19 will be going under treatment or any procedure, personal protective equipment must be used together with the usual operating room clothing. The covid-19 seems to contain two major pathways of transmission: direct and contact, the personal protective equipment must perfectly and completely cover the skin, especially the area of the body which exhibit a greater risk such as mouth and nose, taking into consideration that aerosols could be created during different operational procedures in the oro-maxillofacial area. 26

The glass that is used to provide a protection for the eyes should fit over and around the eyes or personal lenses and be indirectly ventilated to prohibit penetration of splashes or aerosols. The protective screens provide barrier protection for the area of the face and related mucous membranes, as it works as an alternative to protective glasses. In the condition which it may exhibit a superior risk of aerosolization, an approved protective screen should be used. Personal protective equipment will be indispensable in any surgical operation in the field of oral and maxillofacial area and also in other operations room's procedures such as, canalizations, and regional anesthesia. Hence, it's vital to determine which personnel which apply the protection which is suitable for every activity such as the activities in the ambulatory, elective operation, and ward of hospitalization. All of the surgeons, anesthetists, surgeons' assistants, and scrub-nurses must have all the stuff to guarantee a proper condition of ultimate safety. In all cases, the number of the workers, and staff members inside the surgery room should be minimal.^{26,31} The circulating nurse and surgery room assistants will not require superior protection as the surgical-mask could be enough, However filtering face piece 2 mask is always recommended, as they may approach the patient in a risky surgical maneuvers or they may go through operations that may generate aerosols. It's essential that the professional surgical team and assisting staff should have previous supervised professional training in placement and removal personal protective equipment before applying a real operation.²⁶

7.3 Antiseptic Agents:

Vergara-Buenaventura et al.³², suggested to use pre-operational mouthwashes in dental health services centers and clinics to minimize this new corona viral load to decrease the rate of cross-infection risk while treating patients during the pandemic. The antimicrobial effect of povidone iodine (PVP-I) used in the operations, as it applied to provide a skin preparation for the surgery, since the free iodine has the ability to inactivate proteins, oxidize nucleic acids, and destroy microbes..²⁷ The effect of its action has been well demonstrated through many in vitro studies against multiple viruses, including SARS-CoV, MERS-CoV, and influenza virus A (H1N1).³²⁻³⁵ Recent investigations have suggested that 0.23% PVP-I mouthwash for at least 15 seconds before procedures may reduce salivary viral load, indicating its use in COVID-19-positive patients.^{32,36,37}

7.4 Operating room preparation:

Using the operation rooms with negative pressure will be an ideal choice to decrease the level of the risk of the infection.³⁸ But the operation rooms are usually created with positive

pressure air circulation. A superior air exchange cycle rate (≥ 25 cycles/h) help in decrease the load of viruses in the operation rooms.³⁹

The number of operation's gears and tools should be kept minimally, only the mandatory tools should be kept. Once the operation begins, all strategies must be done and prepared to use the current equipment that's already inside the operation room and decrease the staff's movement in and out the operation room, in order to decrease the level of risk of infection. Standard anaesthetic trolleys should be substituted with devoted pre-prepared ones with minimal but suitable stock. All demanded surgical tools and materials such as stitches, and blades must be previously prepared in a sterilizable steel-wire basket. Special infection risk health waste containers should be used for sharp disposable instruments that got infected during surgical procedures. To preserve proper hand hygiene, alcoholic solution should always be there. All surgical team members should enter the surgery room timely to decrease time spent within the operation room itself, hence, they should not go out the surgery room until the operation is completed, and once they left the room, they should not enter again.³⁸

7.5 Linen management:

Linen can be exhibit with microbes due to that, it should be handled carefully, and transportation procedure should be done with a high care, in purpose to prevent diffusion of infection. Disposable laundries considered to be much more preferable if it's possible. The procedure of handling the laundries and linen should be done with the personal protective equipment and should not put on the floor, but directly to the special containers, then the containers sealed and sent to cleaning and sterilization.³⁸

VIII. CONCLUSION

Working and performing the daily routine surgeries seems to be difficult through the period of covid-19. Multiple consideration should be done to create an ideal working environment, hence the surgeons and their team have a lot of new duties, such as re-visiting their infection control protocols, using special personal protective equipment, and prioritizing their surgeries. Consequently, all of that consideration may help in optimizing the level of infection control of the operation room during Covid19 period.

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Ginkgo Biloba Polypeptide Preparation Key Technology Research

Lin Niu¹, Zhengyun Wang², Shuping Li³, Kaiyan Li⁴, Yanlin Liu⁵, Guangyu Liu^{6*}

^{1,2}Jiangsu Agri-animal Husbandry Vocational College, Taizhou, Jiangsu, 225300, China
 ³Jiamusi University, Jiamusi, Heilongjiang, 154007, China
 ⁴Jinling Institute of Technology, Nanjing, Jiangsu, 210046, China
 ^{5,6}CSPC Zhongnuo Pharmaceutical (taizhou) Co., Ltd., Taizhou, Jiangsu, 225312, China
 *Corresponding authors: Liu Guangyu, (1977 -), male, senior engineer, master of engineering Research direction: International Pharmaceutical Engineering Management.

Abstract— In this study, ginkgo powder was used as the research object, and neutral protease and flavor protease were selected to study the degree of hydrolysis of ginkgo protein. Using single factor test and orthogonal test, the results show that the addition amount of neutral protease is 1.6mg/ml, pH is 7.0, enzymolysis temperature is 44°C, and the reaction time is 4h, the best hydrolysis degree is 4.68%; flavor; The added amount of protease is 7.2mg/ml, pH is 6.5, enzymolysis temperature is 55°C, and the reaction time is 6h. The best hydrolysis degree is 24.95%; finally, the ginkgo polypeptide is obtained by separation and purification by 3kDa filter membrane. The yield rate was 8.73%, and the obtained filtrate was concentrated by a rotary evaporator to prepare a polypeptide concentrate.

Keywords—Ginkgo peptide; Enzyme hydrolysis; Membrane separation and purification.

I. Introduction

Ginkgo biloba, also known as ginkgo, is a precious medicinal and food homologous substance with high edible and medicinal value. In addition to rich nutrients, ginkgo also contains many special biologically active functional factors. It can be used as a tonic or as a food. Studies have shown that Ginkgo contains 17 kinds of amino acids. Among them, there are 8 essential amino acids, accounting for about 38% of the total amino acids. The osmotic pressure of peptides is lower than that of free amino acids, the absorption rate is high, and the characteristics of low antigen properties, etc., will not cause adverse reactions such as diarrhea and allergies. Ginkgo protein is enzymatically hydrolyzed into polypeptides, and ginkgo polypeptides have functional properties such as antioxidant, easy absorption, cholesterollowering properties, immune activity, and promotion of metal ion absorption^[1]. The principle of enzymatic hydrolysis of protein is to break the peptide bond in the protein to turn the large long-chain protein in the protein into peptides and amino acids of different lengths. Enzymatic hydrolysis of protein has the advantages of low cost, mild reaction conditions, high reaction efficiency, and short time, no damage to amino acids, high product purity, and easy separation. The hydrolysis of proteins generally requires more than two complex proteases to be selected according to the structural and functional characteristics of the biologically active peptide. At present, a lot of research has been done on the preparation of peptides by protease hydrolysis. The peptides prepared by Wu Mengmeng used alkaline protease to hydrolyze the spirulina protein, and the optimal enzymatic conditions were pH 7.0, enzymatic hydrolysis temperature 55°C, reaction time 2.7h, and the ratio of enzyme addition to substrate concentration was 4300U/g, the degree of hydrolysis can reach 26.8%^[2]. Zhu Yanhua et al. obtained the best alkaline protease enzymatic hydrolysis of corn gluten meal through research and preparation of corn peptides: substrate concentration 4%, pH 9.5, enzyme addition amount 1000 U/g, temperature 55°C hydrolysis 4h^[3]. Two types of polypeptides are separated by protease hydrolysis of casein and soybean

protein: multi-branched amino acid oligopeptides and low aromatic amino acid polypeptides. The principle of ultrafiltration is a membrane separation process with pressure difference as the driving force. The separation and purification of peptides uses ultrafiltration technology. When the enzymatically hydrolyzed liquid passes through the membrane surface under a certain pressure, the microporous structure on the membrane surface selectively separates the hydrolysate, and small molecules permeate the membrane to obtain ultrafiltrate of small molecule polypeptides^[4]. Ginkgo biloba polypeptide can be prepared by separating and purifying the ginkgo protein through a filter membrane by enzymatic hydrolysis.

II. MATERIALS AND METHODS

2.1 Materials and equipment

Ginkgo biloba is analytical pure sulfuric acid, boric acid, hydrochloric acid, ethanol, sodium hydroxide, petroleum ether produced by Sinopharm Chemical Reagent Co., Ltd. Shanghai Enzyme Link Biotechnology Co., Ltd. produces chemical reagent flavor protease, neutral protease, papain, and alkaline protease. pH meter (EL20), CJ78-1 type magnetic stirrer, PRIMOR type refrigerated centrifuge, HH-S2 type digital display constant temperature water bath, DHG9101.2s type electric heating constant temperature blast drying oven, DFT-100 type 100g portable high-speed Chinese medicine Crusher, LABCONCO type freeze drying system, RE-5203 type rotary evaporator.

2.2 Method

2.2.1 Preparation of Ginkgo Powder

To dry the water in the ginkgo, use a portable high-speed Chinese medicine grinder to crush the ginkgo into powder. Because ginkgo acid in ginkgo is a harmful component, it can cause serious allergic reactions, nerve damage, gene mutations and other harms. Ginkgo acid is easily soluble in alcohol. According to the mass-volume ratio of 1:3, take 500g of ginkgo powder and 1500ml of 85% alcohol and mix well, discard the yellow alcohol solution and soak it with alcohol until it is colorless, then use a freeze-drying system After drying, ginkgo powder with ginkgolic acid removed is obtained^[5].

2.2.2 The technological process of preparing Ginkgo polypeptide concentrate

Ginkgo powder \rightarrow hydrolysis \rightarrow enzyme inactivation (water bath 100°C, 10min) \rightarrow centrifugation (8000r/min, 15min) \rightarrow take supernatant \rightarrow ultrafiltration(3kDa) \rightarrow take filtrate \rightarrow concentrate (rotary evaporator 55°C) \rightarrow finished product (Refrigerate for later)

2.2.3 Operation points

- 1) Hydrolysis Weigh ginkgo powder and 250mL Erlenmeyer flask, prepare a suspension with water according to the ratio of 1:10, and stir evenly. Put it in a constant temperature water bath at 100°C for pre-denaturation for 10 minutes, adjust the required pH with 1mol/L NaOH and 1mol/L HCL^[6], add enzyme to the flask, put it in a constant temperature water bath, and hydrolyze for a certain period of time, The optimal conditions for regulating enzymes.
- 2) Enzyme inactivation after the hydrolysis, the flask containing the sample is placed in a jacketed water bath, and the enzyme is inactivated at 100°C for 10 minutes.

- 3) **Centrifugation**: The hydrolysate after enzyme inactivation is rapidly cooled, the centrifuge speed is set to 8000r/min, and the working time is 15min. After the centrifugation, the supernatant is taken for later use.
- 4) **Ultrafiltration**: Put the supernatant into an ultrafiltration cup, adjust the air pressure to 0.1-0.2kPa, ultrafiltration for about 8 hours, and remove the filtrate.
- 5) **Concentration**: The lower filtrate after ultrafiltration is concentrated under reduced pressure with a rotary evaporator at 55°C. When the concentration of the solution reaches 30-40%, it is taken out and collected.

2.2.4 Neutral protease hydrolysis single factor test

- 1) The effect of neutral protease addition on the degree of hydrolysis of Ginkgo biloba peptides. The addition of neutral protease is 0.8mg/ml, 1.0mg/ml, 1.2mg/ml, 1.4mg/ml, 1.6mg/ml. ML, 1.8mg/ml, 2.0mg/ml were used to investigate the degree of hydrolysis of Ginkgo polypeptide by neutral protease.
- 2) The effect of enzymolysis temperature on the degree of hydrolysis of Ginkgo biloba peptides. The reaction temperature of enzymatic hydrolysis was 40°C, 44°C, 48°C, 52°C, 56°C, 60°C, 64°C to investigate the effect of neutral protease on Ginkgo biloba polypeptide. Degree of hydrolysis.
- 3) The effect of pH of enzymatic hydrolysis on the degree of hydrolysis of Ginkgo biloba polypeptide. The pH of enzymatic hydrolysis was 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 to investigate the degree of hydrolysis of Ginkgo biloba polypeptide by neutral protease.
- 4) The effect of enzymolysis time on the degree of hydrolysis of Ginkgo biloba polypeptide. The enzymatic hydrolysis time was 1h, 2h, 3h, 4h, 5h, 6h, and 7h to investigate the degree of hydrolysis of Ginkgo biloba polypeptide by neutral protease.

2.2.5 Orthogonal test design of neutral protease hydrolyzing ginkgo polypeptide

Ginkgo powder was initially hydrolyzed with neutral protease, and an orthogonal experiment was designed according to the addition amount of neutral protease, enzymolysis time, pH, and enzymolysis temperature (Table 1).

TABLE 1
ORTHOGONAL TEST TABLE OF NEUTRAL PROTEASE HYDROLYSIS

NO.		Factor		
NO.	A amount (mg/ml)	B time (h)	СрН	D temp (°C)
1	1.4	3	6.5	48
2	1.6	4	7.0	52
3	1.8	5	7.5	56

2.2.6 Flavour protease hydrolysis single factor test

1) The influence of the added amount of flavor protease on the hydrolysis degree of Ginkgo biloba peptides. The added amount of flavor protease is 1.0mg/ml, 2.0mg/ml, 3.2mg/ml, 4.5mg/ml, 6.0mg/ml, 7.2mg/ml, 9.0mg/ml to investigate the degree of hydrolysis of ginkgo polypeptide by flavor protease.

- 2) The effect of enzymatic hydrolysis temperature on the hydrolysis degree of Ginkgo biloba polypeptide. The enzymatic hydrolysis reaction temperature was 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C to investigate the hydrolysis degree of Ginkgo biloba polypeptide by flavor protease.
- 3) The effect of pH of enzymatic hydrolysis on the degree of hydrolysis of Ginkgo biloba polypeptide. The pH of enzymatic hydrolysis was 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 to investigate the degree of hydrolysis of Ginkgo biloba polypeptide by flavor protease.
- 4) The effect of enzymolysis time on the degree of hydrolysis of Ginkgo biloba polypeptide. The enzymolysis time was 1h, 2h, 3h, 4h, 5h, 6h, and 7h to investigate the degree of hydrolysis of Ginkgo biloba polypeptide by flavour protease.

2.2.7 Orthogonal test design for hydrolyzing ginkgo polypeptide by flavor protease

The flavour protease was used to initially hydrolyze the ginkgo powder, and the orthogonal experiment was designed according to the addition amount of flavour protease, enzymolysis time, pH, and enzymolysis temperature (Table 2).

TABLE 2
FLAVOUR PROTEASE HYDROLYSIS FACTOR LEVEL TABLE OF GINKGO BILOBA POLYPEPTIDE

NO	Factor				
NO.	A amount (mg/ml)	B time (h)	СрН	D temp (°C)	
1	5.4	4	6.0	45	
2	7.2	5	6.5	50	
3	9.0	6	7.0	55	

- 1) The determination of amino nitrogen content adopts potentiometric titration (GB 5009.235-2016)
- 2) Protein determination method, national standard method, Kjeldahl method (GB 5009.5-2016).
- 3) Degree of protein hydrolysis:

 $\label{eq:decomposition} \text{Degree of hydrolysis (\%)} = \frac{\text{Amino acid nitrogen content after enzymolysis} - \text{Amino acid nitrogen content before enzymolysis}}{\text{Total nitrogen content in raw materials}} \times 100\%$

III. RESULTS AND ANALYSIS

3.1 Neutral protease hydrolysis test

3.1.1 The effect of neutral protease addition on the degree of hydrolysis of ginkgo powder

The hydrolysis degree of ginkgo protein is 1.6% when the amount of enzyme is 0.8mg/ml; the degree of hydrolysis of ginkgo protein is 2.2% when the amount of enzyme is 1.0mg/ml; the hydrolysis of ginkgo protein when the amount of enzyme is 1.2mg/ml The degree of hydrolysis of Ginkgo protein is 3.1% when the amount of enzyme is 1.4mg/ml; when the amount of enzyme is 1.6mg/ml, the degree of hydrolysis of Ginkgo protein is 3.3%; when the amount of enzyme is 1.8mg/ml The degree of hydrolysis of ginkgo protein is 1.4%; when the addition of enzyme is 2.0mg/ml, the degree of hydrolysis of ginkgo protein is 3.4% (Figure 1); the reaction curve gradually rises and tends to level, so the addition amount of neutral protease is 1.4mg/ m L, 1.6 mg/ml and 1.8 mg/ml are the horizontal factors in the orthogonal experiment.

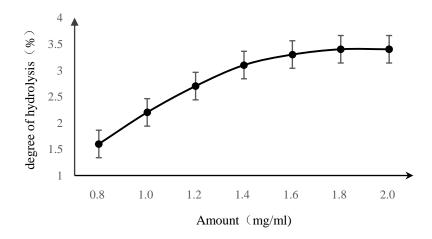


FIGURE 1: The effect of neutral protease addition on the degree of hydrolysis of ginkgo powder

3.1.2 The effect of enzymatic hydrolysis temperature on the degree of hydrolysis of ginkgo powder

When the enzymolysis time is 36°C, the hydrolysis degree of Ginkgo biloba protein is 2.8%; when the enzymolysis time is 40°C, the hydrolysis degree of Ginkgo biloba protein is 3.2%; when the enzymolysis time is 44°C, the hydrolysis degree of Ginkgo protein is 3.5%; the enzymolysis time is 48. The degree of hydrolysis of Ginkgo protein at 50°C is 3.7%; the degree of hydrolysis of Ginkgo biloba protein at 52°C is 3.6%; the degree of hydrolysis of Ginkgo protein at 56°C is 3.4%; the hydrolysis time of Ginkgo biloba is at 60°C. The temperature is 3.2%; it can be seen from the figure that the temperature gradually increases from 36°C to 48°C, and the reaction is a rising curve. When the enzymolysis temperature is 48°C, it is the optimum enzymolysis temperature and the hydrolysis degree of Ginkgo protein is the largest. When the temperature rose from 48°C to 60°C, the reaction showed a decreasing curve (Figure 2). Therefore, 44°C, 48°C and 52°C are selected as the horizontal factors in the orthogonal experiment for the enzymolysis temperature of neutral protease.

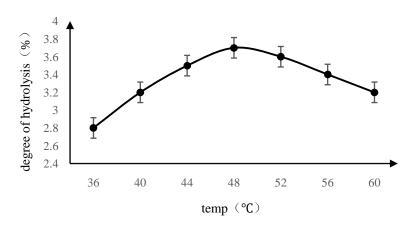


FIGURE 2: The effect of neutral protease hydrolysis temperature on the degree of hydrolysis of ginkgo powder

3.1.3 The effect of pH on the hydrolysis degree of ginkgo powder

When the pH of enzymolysis is 5.5, the degree of hydrolysis of ginkgo protein is 1.8%; when the pH of enzymatic hydrolysis is 6.0, the degree of hydrolysis of ginkgo protein is 2.5%; when the pH of

enzymatic hydrolysis is 6.5, the degree of hydrolysis of ginkgo protein is 3.1%; when the pH of enzymatic hydrolysis is 7.0. The degree of hydrolysis is 3.4%; when the pH of enzymatic hydrolysis is 7.5, the degree of hydrolysis of ginkgo protein is 3.1%; when the pH of enzymatic hydrolysis is 8.0, the degree of hydrolysis of ginkgo protein is 2.5%; when the pH of enzymatic hydrolysis is 8.5, the degree of hydrolysis of ginkgo protein is 1.7%. It can be seen that the pH of the enzymatic hydrolysis gradually increased from 5.5 to 7.0, and the reaction was a rising curve. When the enzymolysis pH is 7.0, it is the optimum pH and the hydrolysis degree of Ginkgo protein is the largest. When the pH of enzymatic hydrolysis rises from 7.0 to 8.5, the reaction becomes a decreasing curve (Figure 3). Therefore, the selection of pH 6.5, 7.0 and 7.5 for the enzymatic hydrolysis of neutral protease is the horizontal factor in the orthogonal experiment.

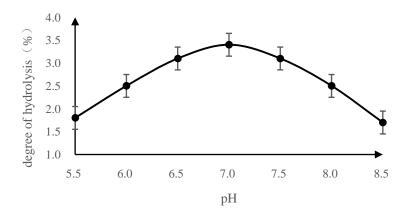


FIGURE 3: The effect of pH of neutral protease hydrolysis on the degree of hydrolysis of ginkgo powder

3.1.4 The effect of enzymolysis time on the degree of hydrolysis of ginkgo powder

When the hydrolysis time is 1h, the hydrolysis degree of ginkgo protein is 1.5%; when the hydrolysis time is 2h, the hydrolysis degree of ginkgo protein is 2.1%; when the hydrolysis time is 3h, the hydrolysis degree of ginkgo protein is 2.6%; when the hydrolysis time is 4h, the hydrolysis degree of ginkgo protein. The degree of hydrolysis is 2.9%; when the hydrolysis time is 5h, the degree of hydrolysis of ginkgo protein is 3.1%; when the hydrolysis time is 6h, the degree of hydrolysis of ginkgo protein is 3.2%; when the hydrolysis time is 6h, the degree of hydrolysis of ginkgo protein is 3.2%; reaction curve It gradually rises and tends to level (Figure 4), so 4h, 5h, and 6h are selected as the horizontal factors in the orthogonal experiment for the enzymatic hydrolysis time of neutral protease.

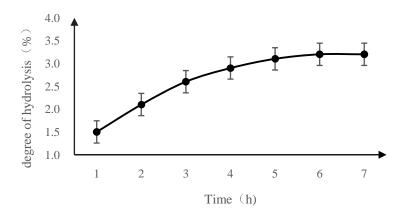


FIGURE 4: The effect of neutral protease hydrolysis time on the degree of hydrolysis of ginkgo powder

3.1.5 Orthogonal test results of neutral protease hydrolyzing Ginkgo powder

It can be seen from Table 3 that B>C>D>A; through the K value, the optimal condition for judging each factor is A2B2C2D1: the addition amount of neutral protease enzyme is 1.6 mg/ml, enzymatic hydrolysis pH7.0, enzyme. The solution temperature is 44°C and the enzymolysis time is 4h^[7]. The best degree of hydrolysis is 4.68% at this time.

TABLE 3
ORTHOGONAL TEST TABLE OF NEUTRAL PROTEASE HYDROLYSIS

S. No.	Factor				DH
S. 140.	A amount (mg/ml)	B time (h)	СрН	D temp (°C)	(%)
1	1.4	3	6.5	44	4.47
2	1.4	4	7.0	48	4.99
3	1.4	5	7.5	52	3.88
4	1.6	3	7.0	52	4.84
5	1.6	4	7.5	44	5.12
6	1.6	5	6.5	48	3.65
7	1.8	3	7.5	48	4.83
8	1.8	4	6.5	52	4.12
9	1.8	5	7.0	42	4.38
K1	4.45	4.71	4.08	4.66	
K2	4.54	4.74	4.74	4.49	
K3	4.44	3.97	4.61	4.28	
R	0.10	0.77	0.66	0.38	

3.2 Flavor protease hydrolysis test

3.2.1 The effect of flavor protease addition on the degree of hydrolysis of ginkgo powder

In the experiment, the hydrolysis degree of ginkgo protein was 19.3% when the addition amount of flavor protease was 1.0mg/ml; when the addition amount of enzyme was 2.0mg/ml, the hydrolysis degree of ginkgo protein was 22.9%; when the addition amount of enzyme was 3.2mg/ml. The degree of hydrolysis of ginkgo protein is 25.1%; the degree of hydrolysis of ginkgo protein is 26.1% when the amount of enzyme is 4.5mg/ml; the degree of hydrolysis of ginkgo protein is 26.9% when the amount of enzyme is 6.0mg/ml; the amount of enzyme is 7.2. The hydrolysis degree of ginkgo protein is 27.5% at mg/ml; the hydrolysis degree of ginkgo protein is 27.5% when the enzyme is added at 9.0 mg/ml; Figure 5 shows that the reaction curve gradually rises and tends to level, so the amount of flavor protease added Choose 4.5mg/ml, 6.0mg/ml and 7.2mg/ml as the horizontal factors in the orthogonal test.

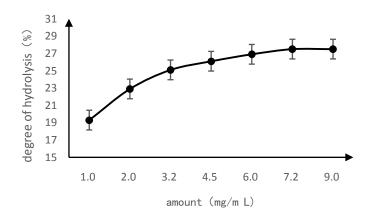


FIGURE 5: The effect of flavor protease addition on the degree of hydrolysis of ginkgo powder

3.2.2 The effect of enzymatic hydrolysis temperature on the degree of hydrolysis of ginkgo powder

In the experiment, the hydrolysis degree of ginkgo protein was 21.2% when the enzymatic hydrolysis time was 35°C; the hydrolysis degree of ginkgo protein was 23.0% when the enzymolysis time was 40°C; the hydrolysis degree of ginkgo protein was 24.4% when the enzymolysis time was 45°C; When the time is 50°C, the hydrolysis degree of Ginkgo biloba protein is 25.2%; when the enzymolysis time is 55°C, the hydrolysis degree of Ginkgo biloba protein is 24.6%; when the hydrolysis time is 60°C, the hydrolysis of ginkgo biloba protein is 23.6%; when the enzymolysis time is 65°C The degree of hydrolysis of ginkgo protein is 22.4%; it can be seen from the figure that the temperature gradually increases from 35°C to 50°C, and the reaction is a rising curve. When the enzymolysis temperature is 50°C, it is the optimum enzymolysis temperature and the hydrolysis degree of Ginkgo protein is the largest. When the temperature rises from 50°C to 65°C, the reaction is a decreasing curve (Figure 6). Therefore, the enzymatic hydrolysis temperature of neutral protease is 45°C, 50°C and 55°C as the horizontal factors in the orthogonal experiment [8].

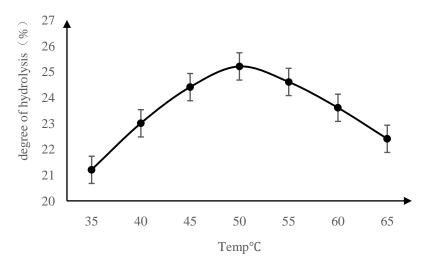


FIGURE 6: The effect of the temperature of flavour protease hydrolysis on the degree of hydrolysis of ginkgo powder

3.2.3 The effect of pH on hydrolysis of ginkgo powder

In the test, the hydrolysis degree of ginkgo protein was 20.4% when the enzymatic pH was 5.0; the hydrolysis degree of ginkgo protein was 21.9% when the hydrolysis pH was 5.5; the hydrolysis degree of ginkgo protein was 23.0% when the hydrolysis pH was 6.0; the hydrolysis pH was 6.5; when the hydrolysis of ginkgo protein is 23.6%; when the hydrolysis pH is 7.0, the hydrolysis degree of ginkgo protein is 23.1%; when the hydrolysis pH is 7.5, the hydrolysis degree of ginkgo protein is 22.1%; when the hydrolysis pH is 8.0, the hydrolysis degree of ginkgo protein is 21.1%. It can be seen from the figure 7 that the pH of enzymatic hydrolysis gradually increases from 5.0 to 6.5, and the reaction is a rising curve. The optimum pH for enzymatic hydrolysis is at pH 6.5, and the degree of hydrolysis for ginkgo protein is the greatest. When the pH of enzymatic hydrolysis rises from 6.5 to 8.0, the reaction is a decreasing curve. Therefore, the selection of pH 6.0, 6.5 and 7.0 for the enzymatic hydrolysis of neutral protease is the horizontal factor in the orthogonal experiment.

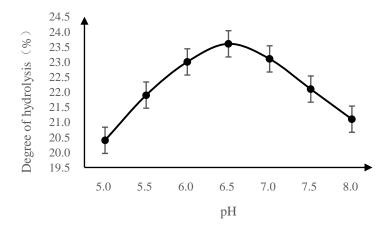


FIGURE 7: The effect of pH of flavour protease hydrolysis on the degree of hydrolysis of ginkgo powder

3.2.4 The effect of enzymolysis time on the degree of hydrolysis of ginkgo powder

In the experiment, the hydrolysis degree of ginkgo protein was 17.9% when the enzymolysis time was 1h; the hydrolysis degree of ginkgo protein was 19.8% when the enzymolysis time was 2h; the hydrolysis degree of ginkgo protein was 21.4% when the enzymolysis time was 3h; the hydrolysis time was 4h when the hydrolysis time is 5h, the hydrolysis degree of ginkgo protein is 23.0%; when the hydrolysis time is 6h, the hydrolysis degree of ginkgo protein is 23.1%; when the hydrolysis time is 6h, the hydrolysis degree of ginkgo protein is 23.1%. Figure 8 shows that the reaction curve gradually rises and tends to level, so 4h, 5h and 6h are selected as the horizontal factors in the orthogonal experiment for the enzymolysis time of neutral protease.

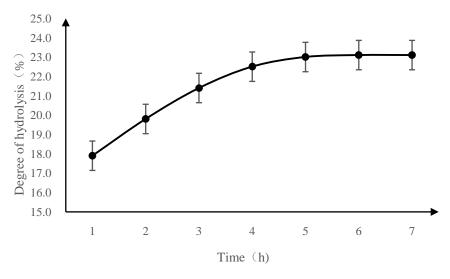


FIGURE 8: The effect of the time of flavour protease hydrolysis on the degree of hydrolysis of ginkgo powder

3.2.5 Orthogonal test design for hydrolyzing ginkgo powder by flavor protease

It can be seen from Table 4 that A>C>B>D, it can be seen from the k value that A3B3C2D3 is better, therefore, the flavor enzyme addition amount 7.2mg/ml, enzymatic hydrolysis pH6.5, reaction temperature 55°C and Enzymolysis time is 6h. As the optimal flavour protease enzymolysis conditions. The best degree of hydrolysis at this time is 24.95%.

22.76

0.77

23.87

3.18

K3

R

Factor DH (%) S. No. D temp $({}^{0}C)$ A amount (mg/ml) B time (h) C pH 4.5 6.0 45 18.94 5 50 2 4.5 22.23 6.5 3 4.5 6 7.0 55 20.89 4 23.51 6.0 4 6.5 55 5 5 6.0 7.0 45 22.04 6 6.0 6 6.0 50 23.12 7 7.2 22.75 4 7.0 50 7.2 8 5 6.0 55 23.87 9 7.2 6 6.5 45 24.98 **K**1 21.73 21.99 20.69 21.98 K2 22.89 22.71 23.57 22.70

21.89

1.68

TABLE 4
ORTHOGONAL TEST RESULTS OF FLAVOUR PROTEASE HYDROLYSIS

IV. CONCLUSIONS AND PROSPECTS

23.00

1.27

In this study, neutral protease and flavor protease were selected to study the degree of hydrolysis of Ginkgo protein. Firstly, neutral protease is selected for single factor test and orthogonal test. The results show that the degree of hydrolysis is the best when the addition amount of neutral protease is 1.6mg/ml, pH is 7.0, enzymolysis temperature is 44°C, and reaction time is 4h. It was 4.68%; the single factor test and orthogonal test were performed with flavor protease. The results showed that the addition amount of flavor protease was 7.2mg/ml, pH was 6.5, enzymatic hydrolysis temperature was 55°C, and the reaction time was 6h. The best is 24.95%; finally, the Ginkgo biloba polypeptide is separated and purified by a 3kDa filter membrane, and the yield of Ginkgo biloba polypeptide is 8.73%.

Taizhou City, Jiangsu Province, China is known as the hometown of ginkgo. Its ginkgo production is abundant but the deep processing is insufficient, and the resource advantages of ginkgo cannot be fully utilized^[9]. This study uses local resource advantages to clarify the mechanism of extraction and separation of Ginkgo biloba polypeptides, and screen the best extraction process for preparing polypeptides by enzymatic hydrolysis of proteins, provide theoretical and scientific basis for the comprehensive utilization of ginkgo, speed up the deep processing of ginkgo, and promote the structure of the ginkgo industry Adjustment and sustainable development^[10]. This research aims to lay the foundation for the research of ginkgo polypeptide health products, and at the same time open up a new way for the comprehensive utilization of ginkgo.

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Assessment of the Inter-Molar Width Changes in the Mandible by using different Orthodontic Arch Wires: A Prospective Clinical Study

Mohamed Helmi Saleh¹, Mohamed Abdultawab², Hussien Naseef³

¹Assistant Professor of Orthodontics, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

²Master's Degree Student, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

³Assistant Professor of Orthodontics, Department of Orthodontics, Faculty of Dentistry, Alazhar University (Boys, Cairo).

Abstract— The demands for orthodontic treatment are in continuous increasing, hence producing an ideal treatment plan need to cover all stages of the treatment carefully, especially the retention and stabilization stage. As, any changing in the arch width through changing the inter-molar width will lead to impair the stabilization of the arch and will lead to relapse. Due to that, the present study performed to evaluate the inter-molar width of the mandible using three different commercial orthodontic wires. Thirty patients had been allocated in this study, using a cone beam computed tomography to create an assessment for the inter-molar width. It concluded that there is an increase in the inter-molar width between pre-treatment and after finishing of aligning stage, also there is a highly significant increase in post-aligning stage between the three groups.

Keywords— Inter-molar width changes, Orthodontic Arch Wires, retention stage, stabilization stage, NiTi wires.

I. INTRODUCTION

Creating a successful treatment depend on multiple factors, as these factors include obtaining a proper diagnosis, in addition to a good treatment plant which it includes both active and retention phases. Also, keeping the tooth in its position after treatment seems challenging in orthodontic field; hence multiple points of views and schools had displayed their solutions for that.¹⁻⁴

During all of orthodontic treatment, the widths of both mandible and maxilla increase during the aligning phase with or without extractions. The higher changes took place in the area of premolars, then in the area of canines and, finally, in the area of molars, due to that, proper determining the arch form of the patient considered to be an essential parameter in creating a stable, functional and esthetic orthodontic treatment result, since failure to keep the arch form might raise the probability of relapse.⁵⁻⁷

In the market, there are multiple orthodontic wires, such Nickel-titanium (NiTi), stainless steel (SS), and beta-titanium wires. NiTi alloys are not rigid like SS or even beta-titanium. NiTi wires are so elastic that it is hard to create loops over them, and even after high deflections, they go back to their genuine shape when the force is removed, and the wires are unhanded.⁸

In the mid of 1990s another type of NiTi wires called Copper-nickel-titanium (CuNiTi) entered the market. By adding copper to the previous nickel-titanium alloy, thermal-activation became so easily controlled. They are marketed based on a different transition temperature: 27 degrees, 35 degrees, and 40 degrees. Therefore, the current study focused on the evaluation of the inter-molar width of the mandible by using three different commercial wires, to understand the effect of each wire on the arch width.^{8,9}

II. SUBJECTS AND METHODS:

Ethical consideration of this study had been obtained from the "Ethical committee in the faculty of medicine, Al-Azhar University in Egypt". This study performed on the patients that visited specialized orthodontic clinic, department of orthodontics, in the faculty of dentistry, Alazhar university. Thirty orthodontic patients including both genders had been joined into this study. By using a special randomization tool from this graphpad website. The patients allocated equally into three groups, as group A included ten samples treated with (betatitanium) wire, group B included ten patients treated with (Copper Nickel titanium) wire, and group C included ten patients treated with (Nickel titanium) wire. Eligibility of the patients had selected according to the following inclusion criteria:

2.1 Inclusion criteria:

- 1. The age group of the patients was 14 to 20 years old.
- 2. Proper oral hygiene.
- 3. Patients with proper nutritional routine.
- 4. Medically free from any systematic or genetic diseases that may interfere with normal growth.
- 5. Absence of any growth abnormality and bone metabolic disorders.
- 6. The patient has full permanent dentition.
- 7. Patients who have mild to moderate crowding.

2.2 Exclusion criteria:

- 1. Patients who miss two successive appointments.
- 2. Patients who don't follow and apply researcher instructions.

2.3 Diagnosis and records:

According to the standard routinely procedures that's done in the clinic of orthodontic department, a record for the patient had been taken. The record contained:

- 1. Intra oral photography: Taken before, during, and after orthodontic treatment.
- 2. Extra oral photography: Taken before, during, and after orthodontic treatment.
- 3. Panorama: taken before and after orthodontic treatment.

- 4. Lateral cephalometric radiograph taken before and after orthodontic treatment.
- 5. A cone beam computed tomography (CBCT): Before treatment and after finishing the aligning stage.

2.4 Bonding and Loading wires:

The bonding procedures include the following steps¹⁰:

- 1. All the teeth cleaned, polished and prepared by using low speed handpiece and pumice free of fluoride.
- 2. Applying of acid etching on the labial and buccal surface of the teeth, using 37 % phosphoric acid (3M, Monrovia, California, United states of America). The application time was thirty seconds.
- 3. The etching gel then got washed, and then adhesive bond applied (Transbond XT, 3M, Monrovia, California, United states of America).
- 4. Curing technique used by applying a light cure (LED lamp (Opticore L3; MarslevByvej, Denmark). The application time is eighty seconds, as there is twenty seconds for every surface (mesial, distal, occlusal, gingival).
- 5. The excess composite then removed carefully using special instrument.

2.5 Wires loading:

Then the orthodontics wires prepared and loaded in the brackets according to table 1:

TABLE 1
THE LOADING OF WIRES AMONG GROUP.

Group A	Group B	Group C
Beta titanium wires (Ortho organizers inc, Carlsbad, CA) had been loaded	copper Nickel titanium wires (Henry Schein® Orthodontics)	Nickel titanium wires loaded (Ortho organizers inc, Carlsbad, CA)

III. OBSERVATIONAL METHOD:

The observational methods used and depend on the superimposition that also applied via using CBCT, in a similar procedure and technique of this study.¹⁰

Each cone beam computed tomography scan was evaluated separately by inputting their DICOM files (Digital Imaging and Communication in Medicine) into a special software called Invivo anatomage version 5.2 (Anatomage Inc. San Jose, California) to obtain required measurements. To create standardization for the parameters, each scan was superimposed on its corresponding scan through using point registration so that evaluation can be done on the same cut for all the scans of the same patient, inter-canine width measurements done by using cups tips respectively. Then the same measurement was taken again at the same exact point (by transitioning to the superimposed post-operative scan (Figure-1), (Figure-2).

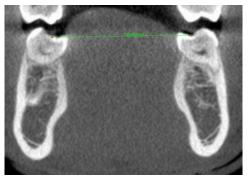


FIGURE 1: Pre operatively measured inter-molar width

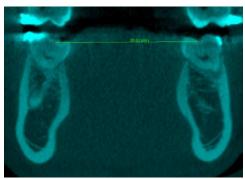


FIGURE 2: Post operatively measured inter-molar width

IV. RESULTS:

4.1 Statistical Methodology

Data input, processing and analyzing of statistics was done via MedCalc ver. 18.2.1 (MedCalc, Ostend, Belgium). Data were displayed and proper analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. P-values less than 0.05 (5%) was considered to be statistically significant.

P- value: level of significance

P > 0.05: Non-significant (NS).

P < 0.05: Significant (S).

P < 0.01: Highly significant (HS).

TABLE 2
SOCIO-DEMOGRAPHIC DATA AMONG 30 PATIENTS SEEKING ORTHODONTIC TREATMENT:

Variables		Frequency (%)	
Age (years)		17.2 ± 1.76*	
Gender	Females	19 (63.3%)	
	Males	11 (36.7%)	

^{*} $Mean \pm SD$.

TABLE 3

COMPARISON BETWEEN THE 3 GROUPS AS REGARDS SOCIO-DEMOGRAPHIC DATA USING ANOVA AND

CHI SQUARE TESTS:

Variable		CNA group (10)	Cu NITI group (10)	NITI group (10)	ANOVA test	
		Mean ± SD	Mean ± SD	Mean ± SD	P value	
Age (year	rs)	17.6 ± 1.6	16.8 ± 2.1	17.4 ± 1.5	= 0.588	
Variable		CNA group	Cu NITI group	NITI group	Chi square test	
		(10)	(10)	(10)	P value	
Gender	Female	6 (60%)	6 (60%)	7 (70%)	= 0.8663	
	Male	4 (40%)	4 (40%)	3 (30%)	- 0.8003	

ANOVA: analysis of variance. *Percentage of Column Total.

Comparative study between the 3 groups revealed non-significant difference as regards age and sex of the patients (p > 0.05).

4.2 Arch measurements & bone density data:

TABLE 4
MEAN OF INTER-MOLAR WIDTH OF THE 3 GROUPS AS REGARDS PRE-TREATMENT

Variable	Beta-Titanium (10)	Copper Nickel titanium (10)	Nickel titanium (10)
	Mean ± SD	Mean ± SD	Mean ± SD
IMW (mm)	40 ± 1.7	37.7 ± 1.37	39.98 ± 1.76

IMW: Inter Molar Width.

Variable	Beta-Titanium (10)	Copper Nickel titanium (10)	Nickel titanium (10)	ANOVA test
	Mean ± SD	Mean ± SD	Mean ± SD	P value
IMW (mm)	40.34 ± 1.5	38.1 ± 1	40.5 ± 1.48	= 0.001**

IMW: Inter Molar Width.

Comparative study between the 3 groups revealed; highly significant increase in post-aligning IMW in CNA and NITI groups; compared to Cu NITI group; with highly significant statistical difference (p < 0.01 respectively).

4.3 Factorial ANOVA table and multi-variate graphs revealed that:

We found marked increase in IMW in Cu CNA and NITI groups; compared to CNA group; during the serial pre and post-aligning measurements.

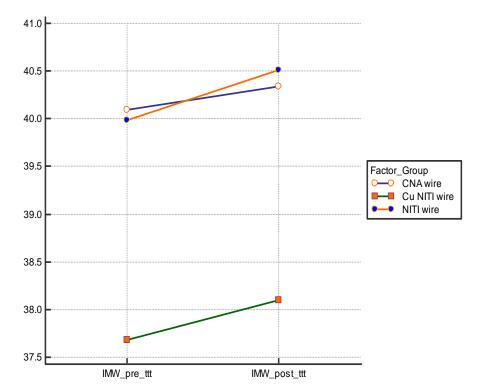


FIGURE 3: Comparison between the 3 groups of patients regarding serial IMW assessments.

INITIAL OF GENERAL ON TOST TREATMENT EFFICIAL OF EACH WIRE COING STODENT STREET.				
	Females (Beta-Titanium)	Males (Beta-Titanium)	Student's t	
Variable	(6)	(4)	test	
	Mean ± SD	Mean ± SD	P value	
IMW	40.5 ± 1.47	40 ± 1.72	= 0.647	
	Females (Copper Nickel titanium)	Males (Copper Nickel titanium)	Student's t	
Variable	(6)	(4)	test	
	Mean ± SD	Mean ± SD	P value	
IMW	38.46 ± 1.08	37.5 ± 0.96	= 0.211	
	Females (Nickel titanium)	Males (Nickel titanium)	Student's t	
Variable	(7)	(3)	test	
	Mean ± SD	Mean ± SD	P value	
IMW	40.2 ± 1.67	41.13 ± 0.83	= 0.417	

TABLE 6
IMPACT OF GENDER ON POST-TREATMENT EFFICACY OF EACH WIRE USING STUDENT'S t TEST:

Regarding CNA group: Gender had non-significant effect on IMW, in CNA group (p > 0.05 respectively).

Regarding Cu NITI group: Gender had non-significant effect on IMW in Cu NITI group (p > 0.05 respectively).

Regarding NITI group: Gender had non-significant effect on IMW, in NITI group (p > 0.05 respectively).

V. DISCUSSION

The causes of the high increase in demanding for orthodontics treatments is not coming back for the aesthetic purpose only, but it includes other multiple reasons, such as improvement and development in the social and economic status of the patients. Well, this opened a new challenge to understand the stability of the dental arches, since any changes in the arch dimension, will lead to a relapse post-orthodontic treatment.¹¹

Consequently, in this study it focused to understand the effect that it may happen on the inter-molar width of the mandible by using different orthodontic arch wires. There are multiple orthodontic arch wires in the markets, hence it focused to evaluate the influence of these wires during the aligning stage and compare that effect between them. The present study included thirty patients, which is divided into three groups, as every group contained ten patients using certain wires. In this study we used a CBCT as an observational method, since this is showed that measurements of distance between anatomical land marks using CBCT software packages revealed superior accuracy when compared with distances measured with a digital caliper.¹²

The results of the present study showed that a clear increase in the inter-molar width between the three groups, also the gender seems to have no influence in the inter-molar width after finishing of the aligning stage. However, the previous tables from results showed that there is increase in the inter-molar width between pre-treatment and after the finishing of aligning stage.

The retention in orthodontic treatment considered to be the last stage, which this stage aims to preserve the teeth in their corrected positions after the completion of orthodontic tooth

movement, since the change in inter-premolar width may influence the rate of stability, hence, the present study focused on evaluating the inter-premolar width in the mandible after the levelling and aligning stage, which is a major stage in orthodontic treatment.¹³

Germec-Cakan et al. performed a study on arch-perimeter changes in patients with Class I malocclusion that go under orthodontic treatment using both extractions or non-extractions approaches, they found that the maxillary and mandibular inter-molar widths decreased in the extraction group, however in the non-extraction group, the inter-molar widths minimized but arch perimeters did not change significantly. Well these results come in disagreement with our results, as in the present the study it found that the inter-molar width has increased despite that the patients went in non-extraction approach as a line of treatment. These differences may come back to difference in the study design and methodology between the two studies.¹⁴

Kim and Gianelly, creates a comparison of the changes of arch-width and smile esthetics in thirty non-extraction and thirty patients with extraction approach. They found that inter-molar width reduced in both mandibular and maxillary arch in the extraction group and increased in the non-extraction group. According to non-extraction group, their findings come in agreement with the results in the present study.¹⁵

Unfortunately, there is not much articles that focused on the comparison between those wires, however, multiple studies have focused on evaluation the changes that it may happen in the arch dimension, but it's difficult to create a comparison between the present study and those studies, due the major differences in the study design, methodology, and observational methods. Consequently, it recommended obtaining further investigations in this sector. ¹⁶⁻¹⁸

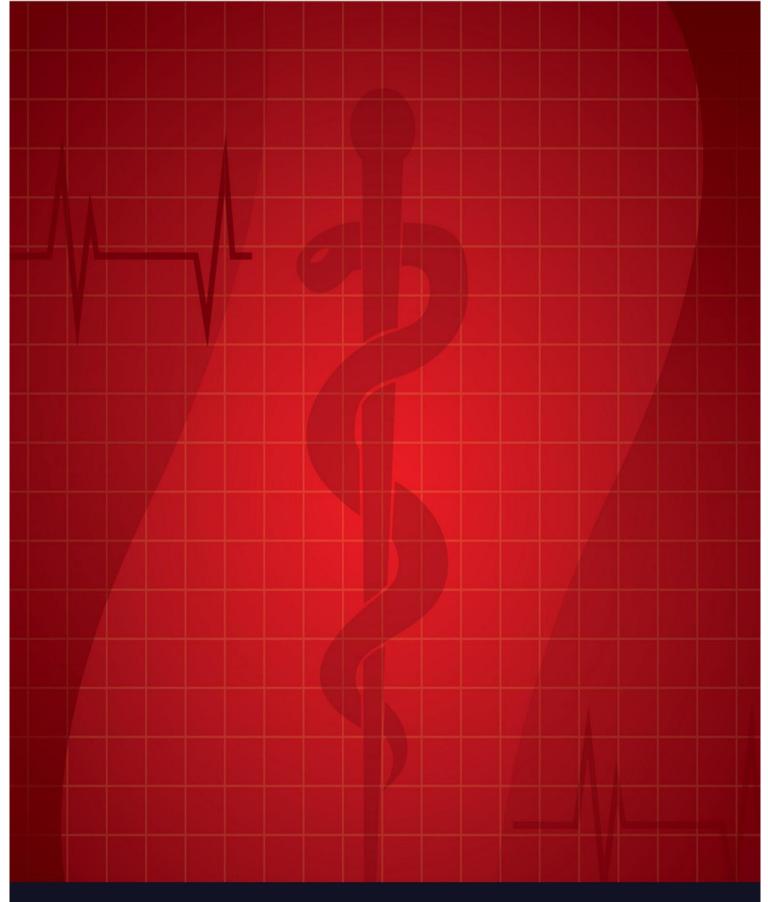
VI. CONCLUSION:

The present study concluded that there is an increase in the inter-molar width between pretreatment and after finishing of aligning stage, also there is a highly significant increase in post-aligning stage between the three groups.

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