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Mycological comparative study between single maxillary denture fabricated from Rapid heat cure acrylic resin material and conventional acrylic resin material modified by titanium dioxide nanoparticles. A Randomized clinical trial

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ABSTRACT

In vivo study that was conducted to assess the degree of candida colonization on maxillary single dentures fabricated from two different denture base materials one of them was fabricated from rapid heat cured acrylic resin and the other fabricated from conventional heat cured acrylic resin modified with titanium dioxide nanoparticles. Seventeen patients with completely edentulous maxilla were randomly selected according to inclusion and exclusion criteria, each patient received two dentures made of rapid heat cured acrylic denture base and modified conventional heat cured acrylic denture base with titanium dioxide nanoparticles then candida samples were taken from the surface of oral mucosa at the crest of the ridge using sterile cotton swabs. Statistical analysis was performed using SPSS 20®1, Graph Pad Prism®2 and Microsoft Excel 20163. Data was represented as mean and standard deviation for quantitative data, Data were explored for normality by using Shapiro Wilk and Kolmogorov Smirnov normality test which revealed that all data is parametric data (P-value > 0.05). Accordingly, comparison between two groups was performed by Independent t test, while comparison between before and after was performed using Paired t -test. In this study we concluded that: addition of titanium dioxide nanoparticles will decrease candida colonization on dentures.

Keywords: In vivo study, Maxillary single dentures, Acrylic resin.

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INTRODUCTION

The single denture opposed by natural dentition is often a greater challenge than complete dentures. Single dentures are more often fabricated in the maxillary arch, as maxillary teeth are usually lost before their mandibular antagonists.

A situation of isolated edentulous maxilla opposed by mandibular dentition is considered to be more difficult due to position of the remaining natural teeth. As a result, single maxillary dentures may be less stable and retentive than full conventional complete dentures. When natural teeth alone make up the opposing dentition, the plane of occlusion of the single denture should be maintained in order to retain maximum stability in function.

Acrylic resins are commonly used for denture fabrication since they exhibit adequate physical, mechanical, and esthetic properties. However, the oral deposits and Microorganisms adhere to different denture base materials with different capacities and lead to several undesirable effects, unaesthetic appearance, unpleasant tactile sensation, taste, and odor. The type of material and its physical properties may influence the attachment of the microorganisms, as well as irregularities in denture base materials. Recently cadmium free rapid cure acrylic resin have been developed and used in dentures construction.

Various nanoparticles (NP) have been added to denture base materials to induce antifungal property. It was found that titanium dioxide nanoparticles has catalytic effect on candida adherence to denture surfaces, low toxicity, high stability and efficiency.(Pant, et al. 2011)[1]

Evaluation of the candidal adhesion facilitates the choice of denture base material, either with or without modification by added titanium nanoparticles.

MATERIALS AND METHOD

PICO

- P: Maxillary single denture patients.
- I: Conventional heat cured acrylic resin modified with Titanium dioxide nanoparticles denture base material.
- C: rapid heat cured acrylic resin denture base material.
- O: candida colonization count.

Patient selection:

Seventeen patients were selected from the out-patient clinic of Prosthodontic department, Faculty of oral and dental medicine, Cairo University. All patients were completely edentulous in the maxillary arch and fully or partially dentate in the mandibular arch restored with fixed or removable prosthesis. (Figure 1)

Figure 1: Fully edentulous maxillary arch opposing fully dentate Mandibular arch.

Inclusion criteria:

1. Patients ages ranged from 45 to 55 years of age.
2. Free from any systemic disease that could affect candidal count.
3. Healthy mucosa without any signs of inflammation or flabby tissue.
4. Angle class I maxilla-mandibular relation.

Exclusion criteria:

1. Severely atrophied ridges.
2. Patients with fibrous tissue in the oral vestibule.
3. Patients with xerostomia.
4. Uncontrolled diabetic patients and Allergic patients to acrylic resin.
5. Patients with bad habits as severe clenching or bruxism, drug or alcohol addiction, moderate or heavy smoking (more than 10 cigarettes per day).
6. Previous history of radiotherapy or chemotherapy.

Patient allocation and randomization:

Informed consent:

The participants received oral and written information about the study and written informed consent was signed by the patients before their participation in the study.

Patient will receive two dentures:

1. One denture made of rapid heat cure acrylic resin.
2. One denture made of modified heat cured acrylic resin with titanium dioxide nanoparticles.

Randomization:

A computer generated two table including the calculated sample size of population was created by the aid of randomization. Dentures were divided into two groups, group (A) represent dentures fabricated from rapid heat cured acrylic resin and group (B) represent

dentures fabricated from titanium dioxide nanoparticles added to conventional acrylic resin material. Participants were randomly received dentures either from group (A) or group(B) by simple randomization with a 1:1 allocation ratio .

Blinding technique:

The study was designed to be double blinded (for the patient and for the assessor including the Statistician).but unfortunately, it couldn't be blinded for the assessor due to the resultant color difference between the two types of dentures. (The modified one is whitish in color due to the addition of titanium dioxide nanoparticles).

Patient history:

A- Medical history:

Medical history was taken to exclude patients with: any systemic disease that could affect candidal count, uncontrolled diabetes and allergy to acrylic resin.

B- Dental history:

The cause of extraction whether it was due to caries or periodontal disease as the cause of extraction may alter the treatment plan.

Extra oral examination:

Facial profile was assessed and temporomandibular joint was examined through digital examination in order to discover any lesions or swilling and any manifestations of candidiasis

Intraoral examination:

Figure 2: Completely edentulous maxilla and dentate mandibular arch

Soft tissue examination: through digital and visual examination of the covering mucosa. Hard tissue examination: through digital and visual examination to determine if there were sharp bony edges, bony projections, bony undercuts and prominent median palatine raphe.

Radiographic examination:

Figure 3: Shows panoramic x-ray

Routine preoperative panoramic x-rays were taken to detect absence of any remaining roots or any lesions that may affect denture retention and stability.

Denture construction:

Dentures were constructed following the conventional manner Maxillary and Mandibular preliminary impressions were made for each patient using irreversible hydrocolloid alginate impression material in suitable prefabricated stock trays. Impressions were poured immediately to obtain study casts on which special trays were constructed. The occlusal plane of the lower arch was adjusted using yurkstas metal "U" shaped occlusal template. A clear acrylic resin template was fabricated over the modified stone cast following Bruce technique. The inner surface of the template is coated with pressure indication paste and was placed over the patient's natural teeth. Interferences were readily noted through the template and were removed by reshaping enameloplasty. The process was repeated until the template was seated properly.

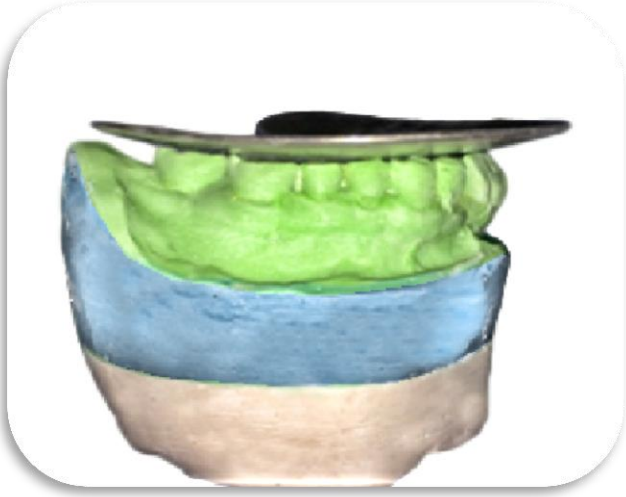

Figure 4: Adjustment of the occlusal plane of the lower teeth by yurkstas "U" shaped occlusal template.

Maxillary secondary impressions were made using high viscosity (putty) silicone impression material. Border molding is done to make the putty take the shape of the vestibule. Final wash of the upper secondary impression was done using medium viscosity silicone impression material, and the border molding is done again to the wash.

Figure 5: Maxillary final impression.

Boxing of the Final impressions was done, then it was poured using extra hard stone. (Figure 6). Then the upper master casts were duplicated using duplicating material. (Figure 7), the mold was poured using extra hard stone. (Figure 8). Indices were engraved on master casts for lab remount procedure.

Figure 6: Maxillary master cast.

Figure 7: Silicone mold of the maxillary master cast and the duplicate cast.

Face-bow record was taken to mount the maxillary cast on a semi-adjustable articulator.(Figure 8) Centric relation at the proper vertical dimension of occlusion was recorded using wax registration method for both casts and semi-anatomic artificial teeth were set in a balanced occlusion. (Figure 9). After setting of the artificial teeth the denture was tried in the patent mouth.

Figure 8: Face bow record to mount the maxillary cast.

Figure 9: Setting of teeth was done on the semi-adjustable articulator

The powder of the rapid heat cure acrylic resin and the monomer were mixed according to the manufacturer instructions, when it reached the dough stage it was packed in the flask using one of the master casts. Another mix of the heat cured acrylic resin was done with addition of 5% by weight Titanium dioxide (TiO2) nanoparticles**.** were mixed thoroughly to the acrylic powder by hand mixing then they were mixed with the monomer till it reached the dough stage and it was packed in the flask using the other master cast.

Processing was done by direct immersion of the packed flasks in boiling water immediately after packing for a short time then the water is brought back to a full boil for 20 minutes.

Processed dentures were removed from the flasks and a laboratory remount was done for both dentures to correct the occlusal errors that have occurred during processing (Figure 10).

Figure 10: Laboratory remount

Finishing and polishing were done to both dentures**.** The standard mechanical polishing technique was used by using Slurry of medium grit pumice mixed in 1:1 ratio of water was used with cloth wheel for 60s on the polishing lathe. (Figure 11 A & B).Then the dentures were checked intra-orally for extension, retention, stability, phonetics and esthetics.

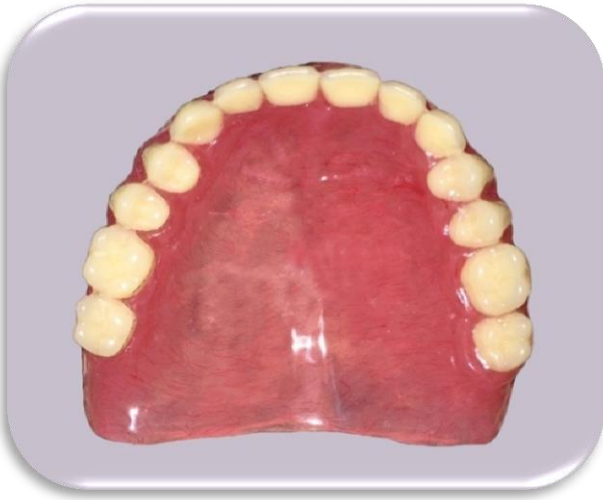

Figure 11: A. Rapid heat cured acrylic denture base resin.

Figure 11: B. Modified heat cure acrylic denture base resin with titanium dioxide nanoparticles.

CANDIDA EVALUATION:

1 Collecting the candida:

Sterilized disposable cotton swabs were used to collect the microorganisms from the patient upper arch after dentures' insertion. Swabs were collected from the tissue surface covering the anterior alveolar bone of the upper arch. (Figure 12). Anther swab was collected from an area

of (1cm x 1cm) dimension in the anterior area of internal surface of the maxillary denture using sterile cotton swab. It was done by rotating the swab firmly and laterally against the denture for 30 seconds to collect the plaque.

A rotating motion with the swab held firmly and laterally against the tissues was used to collect the plaque to ensure proper adherence of the plaque. These swabs were immediately transferred to the commercially available sterile swab holders and placed in the refrigerator for no more than two hours before plating.

Figure 12: Swab.

2 Swabs:

Four swabs were collected from every patient as following:

1. At the time of first denture insertion samples were collected from oral mucosa (crest of the ridge) to evaluate the candida albicans counts at normal condition.
2. One month after first denture insertion, samples were collected from oral mucosa (crest of the ridge) e to evaluate candida albicans colonization count for the first denture.
3. After one month resting period, samples were collected from oral mucosa (crest of the ridge) to ensure that the oral microbial flora return to the normal condition then the other (second) denture was inserted.
4. One month after insertion of the second denture samples were collected from mucosa (crest of the ridge) evaluate candida albicans colonization count for the second denture.

Culturing Medium*:*

Sabouraud Dextrose Agar:

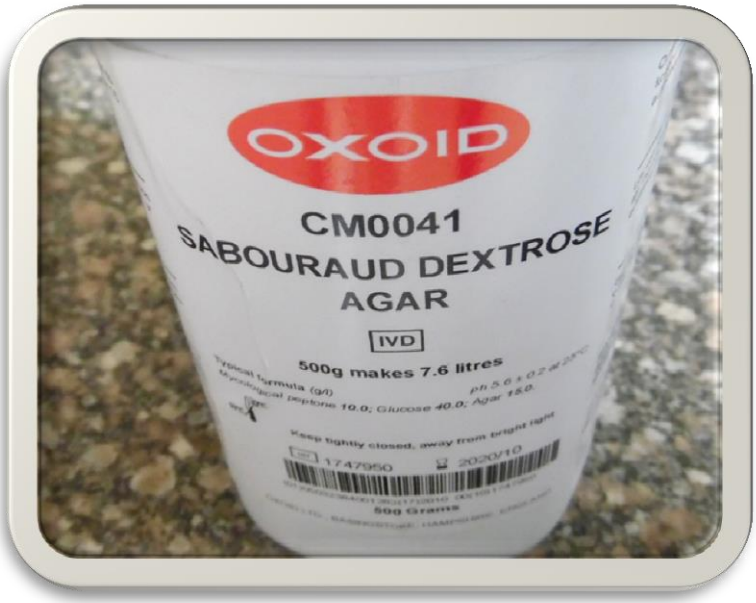

Figure 13: Sabouraud Dextrose agar.

Composition of Sabouraud Dextrose Agar:

Table 1: Sabouraud Dextrose agar ingredients.

Ingredients	gm/L
Dextrose (Glucose)	40 gm
Peptone	10 gm
Agar	15 gm
Distilled Water	1000 ml

Preparation of sabouraud dextrose agar:

The Sabouraud Dextrose Agar (SDA) is presented as powder and mixed with distilled water (65 grams /1000 ml distilled water), (Figure 13). Boiling of the mixture was done till it dissolved completely. The petri plates is sterilized by autoclaving at 15 lbs. pressure (121oc) for fifteen minutes, then cooled down to 40- 50oc. After that Sabouraud Dextrose Agar (SDA) is mixed well and poured into the sterile petri plates.(Figure 14).

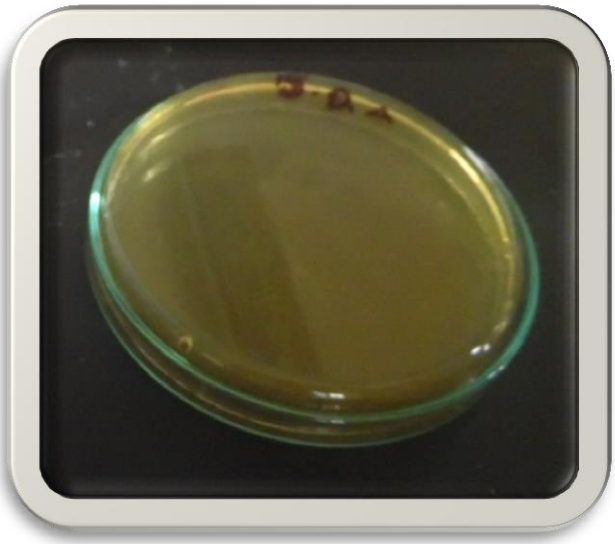

Figure 14: Petri dish before colonization

Culturing and incubation procedure:

Each swab is incubated immediately in sterile tube containing 1 ml of sterile saline for fifteen minutes, (Figure 15). and so undiluted samples were achieved, Using the pre-adjustable micropipette, a 20 µL of the undiluted sample was then plated on the SDA medium. (Figure. 16).

Figure 15: Sterile tube containing the swab.

Using sterile glass rods the sample is spreaded to facilitate the platting and the spreading procedure. After covering the plate and letting it to dry for one minute it is inverted immediately before being placed in incubator for 24 hours at 37 °C. (Figure 17) Negative samples of Culture plates was never discarded unless it was investigated for an extended 48 hours period.

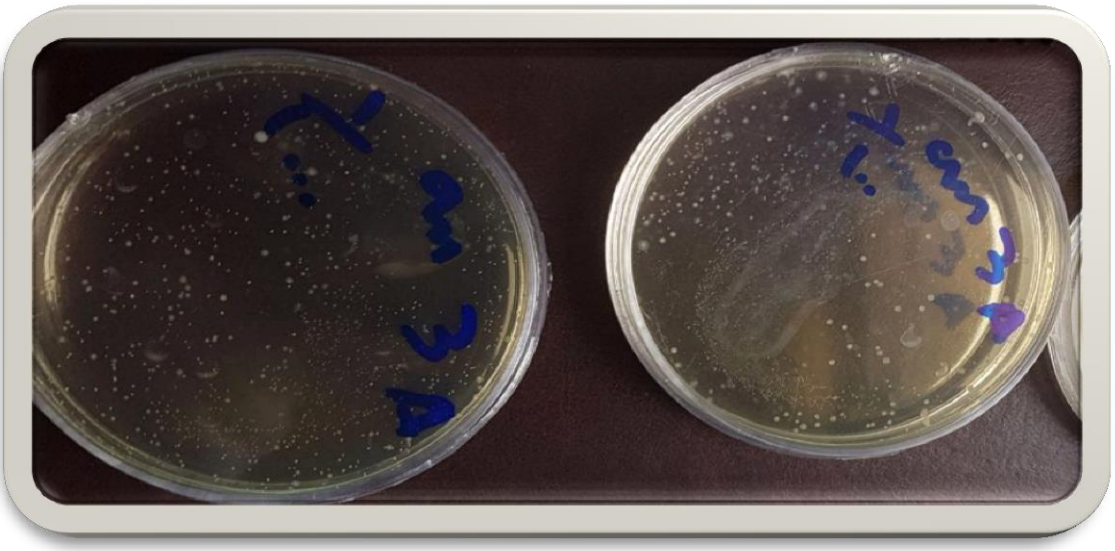

Figure 16: Candida colonies on the plates.

Figure 17: Incubator.

Identification and counting of candida colonies:

Colony forming units per sample on SDA plate (CFU/sample) is the measuring method used to count the Number of Candida colonies. Its calculated as following (CFU/ml = Total number of colonies counted in the plate X 50). Primary identification of candida was carried out after growth of characteristic creamy convex yeast colonies on SDA.

If the number of colonies above 200 colony, The plates were split into sectors of equal size (lines were drawn on the bottom of the plate), all colonies were counted in one sector and then multiplied by the number of sectors.

RESULTS

Statistical analysis:

Statistical analysis was performed using SPSS $20_{®1}$, Graph Pad $Prism_{®2}$ and Microsoft Excel 2016_3. Data was represented as mean and standard deviation for quantitative data, as presented in (9) tables and (6) graphs. Data were explored for normality by using Shapiro Wilk and Kolmogorov Smirnov normality test which revealed that all data is parametric data (P-value >

0.05). Accordingly, comparison between two groups was performed by Independent t test, while comparison between before and after was performed using Paired t -test. Moreover, percentage of change was calculated using the following formula

$$= \frac{(V_2 - V_1)}{|V_1|} \times 100$$

Where

V1: CFU value before denture insertion.

V2; CFU after 1 month from denture insertion.

The results of the present study will be presented under the following headings:

I. Normality test.

II. Demographic data:

a) Age.

b) Gender.

c) Distribution of age among gender.

III. Analytical Data:

a. Group I (single maxillary dentures fabricated from rapid heat cured acrylic resin):

- Before denture insertion.
- After 1month of denture insertion.
- Difference between before and after denture insertion.

b. Group II (single maxillary dentures fabricated from the conventional heat cured acrylic resin denture base material modified by titanium dioxide nanoparticles:

- Before denture insertion.
- After 1month of denture insertion.
- Difference between before and after denture insertion.

c. Comparison between group I and group II:

- Before denture insertion.
- After 1month of denture insertion.
- Difference between before and after denture insertion.

Normality test:

Exploration of the given quantitative data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality. As Listed in table (2) and showed in figure (18), it was revealed that the significant level (P-value) was insignificant as P-value > 0.05 which indicated that alternative hypothesis was rejected, and the concluded data originated from normal distribution (parametric data) resembling normal Bell curve.

Table 2: Normality Exploration of both groups:

		Normality Exploration	
		P value	Indication
Group I	Before	>0.05	Normal (Non-parametric)
	After 1 month	>0.05	Normal (Non-parametric)
Group II	Before	>0.05	Normal (Non-parametric)
	After 1 month	>0.05	Normal (Non-parametric)

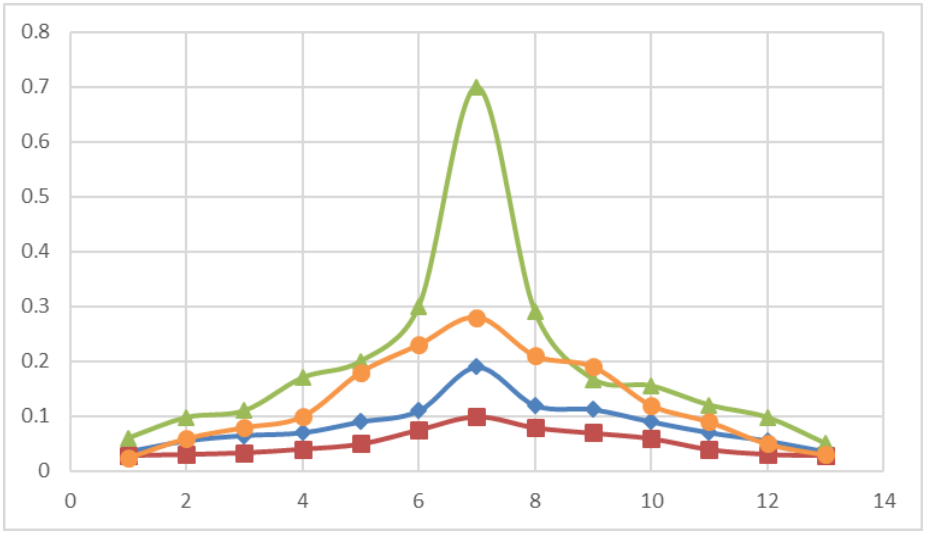

Figure 18: Normality bell curve.

Demographic data:

Age:

The mean age in this study was 49.5 with 3.5 standard deviation, the minimum age was 45 year and the maximum age was 55 year as presented in a table (3) and figure (19). The age in this study divided into two ranges; the first range from 45 to 50 which represented 58.3 %, and the second range more than 50 years with 41.7 %, comparison between two ranges was performed using Chi square test and revealed insignificant difference between them as P<0.05, as presented in table 4 and figure

(20).

Table 3: Mean ± Standard deviation, minimum and maximum age in this study:

Age	N	Min.	Max.	M.	SD.
	12	45	55	49.50	3.55

N; count Min; minimum Max; maximum M; mean SD; standard deviation.

Table 4: Comparison between different age ranges:

Age Range	N	%	P value
45-50	7	58.3	0.41 ns
>50	5	41.7	
Total	12	100.0	

N; count % percentage. ns; non-significant.

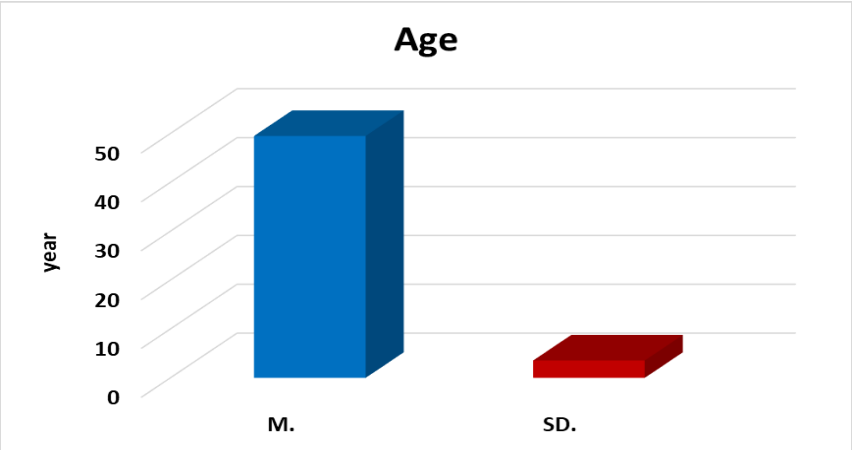

Figure 19: Mean ± Standard deviation of age.

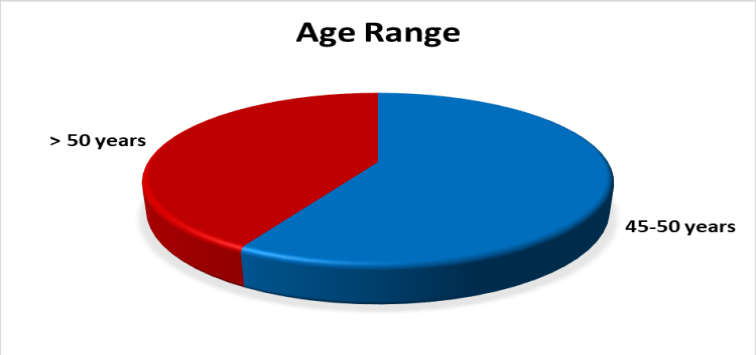

Figure 20: Different age ranges.

Gender:

In this study, males represented 58.3 % while females represented 41.7%, comparison between them was performed using Chi square test which revealed insignificant difference between them as P>0.05 as presented in table (5) and figure (21).

Table 5: Gender distribution:

Gender	N	%	P value
Male	7	58.3	0.41 ns
Female	5	41.7	
Total	*12*	*100.0*	

N; count *% percentage.* *ns; non-significant.*

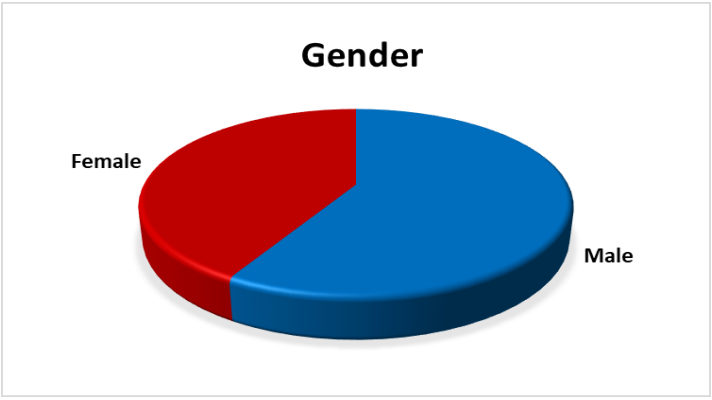

Figure 21: Gender distribution.

Distribution of age among gender:

Age distribution among gender revealed that, 45- 50 years age range represented 57.1% & 42.9% in male and female respectively, while >50 years age range revealed 60% & 40% in male and female respectively as presented in table (6) and figure (22). Comparison between male and female gender was performed using Chi square test which revealed in significant difference in both ranges. Also, comparison between different ranges was performed using Chi square test which revealed insignificant difference in both male and female gender as presented in table (6).

Table 6: Age distribution among gender:

Age Range	Gender				P value
	Male (n=7)		Female (n=5)		
	N	%	N	%	
45-50	4	57.1%	3	42.9%	0.61 ns
>50	3	60.0%	2	40.0%	0.51 ns
P value	0.91ns		0.92 ns		

N; count % percentage. ns; non-significant.

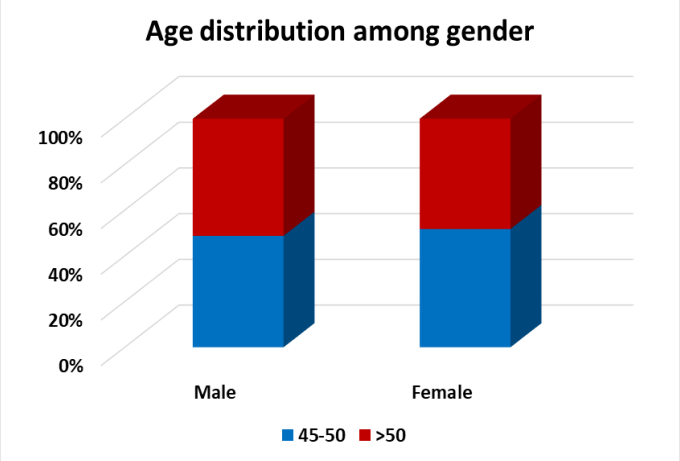

Figure 22: Age distribution among gender.

Analytical Data:

a) Group I (single maxillary dentures fabricated from rapid heat cured acrylic resin):

In group I, before denture insertion the mean ± standard deviation CFU was 6.08 ± 3.8, minimum was 1 and maximum was 15, while after 1 month of denture insertion the mean ± standard deviation CFU was 11.08 ± 4.6, minimum was 4 and maximum was 20 as presented in table 7.

The mean difference in CFU between before denture insertion and after 1 months was 5.00 ± 2.04 as it was increased from 6.08 ± 3.85before denture insertion to 11.08 ± 4.62 after 1 month. Also, the percent of change was calculated and revealed 83.3% increase in CFU as presented in table 8 and figure 23

Table 7: CFU in group I before and after 1 month of denture insertion:

Group I	N	Min.	Max.	M.	SD.	P value
Before Denture Insertion	12	1	15	6.08	3.85	0.008*
After 1 month	12	4	20	11.08	4.62	

*N; count Min; minimum Max; maximum M; mean SD; standard deviation. *significant difference.*

Table 8: Mean difference and percentage of change in CFU regarding after 1month group I:

Group I	M.	SD.
Before	6.08	3.85
After	11.08	4.62
Difference	5.00	2.04
Percent of change	83.3%	

M; mean SD; standard deviation.

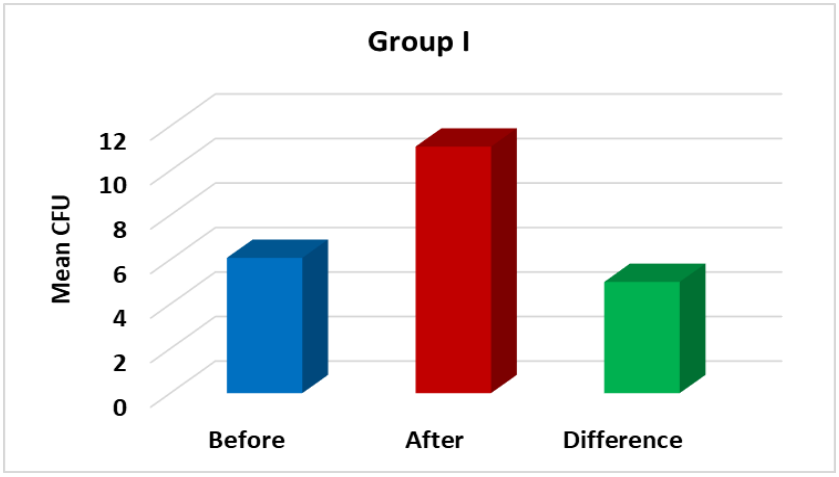

Figure 23: Mean CFU in group I.

b) Group II (single maxillary dentures fabricated from the conventional heat cured acrylic resin denture base material modified by titanium dioxide nanoparticles:

In group II, before denture insertion the mean ± standard deviation CFU was 6.08 ± 3.8, minimum was 1 and maximum was 15, while after 1 month of denture insertion the mean ± standard deviation CFU was 5.67 ± 3.5, minimum was 4 and maximum was 20 as presented in table (9).

The mean difference in CFU between before denture insertion and after 1 months was - 0.42 ±1.16 as it was decreased from 6.08 ± 3.85before denture insertion to 5.67 ± 3.57after 1 month. Also, the percent of change was calculated and revealed 6.6% decrease in CFU as presented in table 10 and figure 24.

Table 9: CFU in group II before and after 1 month of denture insertion:

Group I I	N	Min.	Max.	M.	SD.	P value
Before Denture Insertion	12	1	15	6.08	3.84	0.79 ns
After 1 month	12	1	13	5.67	3.57	

N; count Min; minimum Max; maximum M; mean SD; standard deviation. Ns; non-significant

Table 10: Mean difference and percentage of change in CFU regarding after 1month group II:

Group II	M.	SD.
Before	6.08	3.84
After	5.67	3.57
Difference	- 0.42	1.16
Percent of change	6.66%	

M; mean SD; standard deviation.

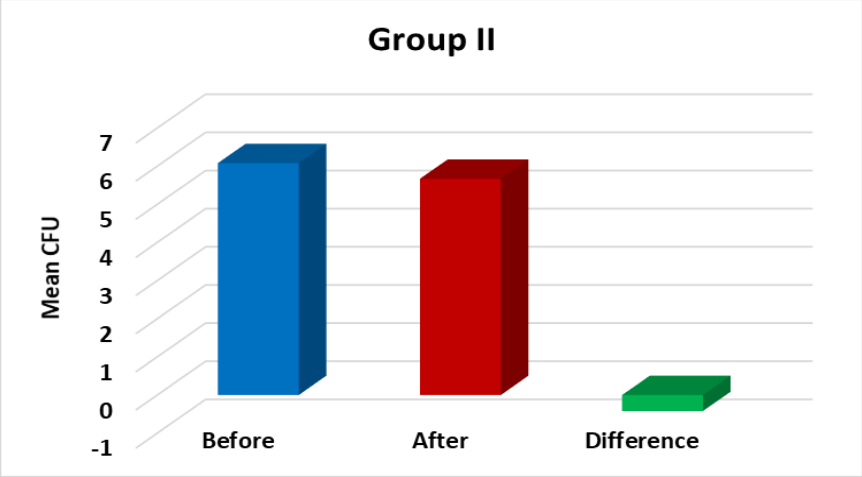

Figure 24: Mean CFU in group II.

c) Comparison between group I and group II:

Before denture insertion:

Comparison between mean CFU before denture insertion was performed using Independent t-test which revealed insignificant difference as P>0.05 as presented in table (11) and figure (25).

Table 11: Comparison between group I and group II regarding mean CFU before denture insertion:

	Before denture insertion		P value
	M	SD	
Group I	6.08	3.848	1.00 ns
Group II	6.08	3.848	

M; mean SD; standard deviation. Ns; non-significant.

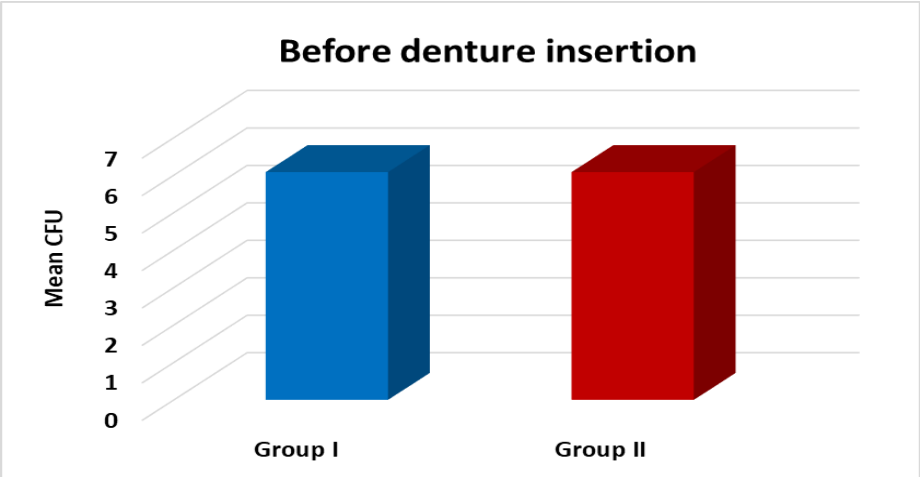

Figure 25: Mean CFU before denture insertion.

After 1 month of denture insertion:

Comparison between mean CFU after 1 month of denture insertion was performed using Independent t-test which revealed insignificant difference as P>0.05 as presented in table (12) and figure 26.

Table 12: Comparison between group I and group II regarding mean CFU after 1 month of denture insertion:

	After denture insertion		P value
	M	SD	
Group I	11.08	4.621	0.003 *
Group II	5.67	3.576	

*M; mean SD; standard deviation. *significant.*

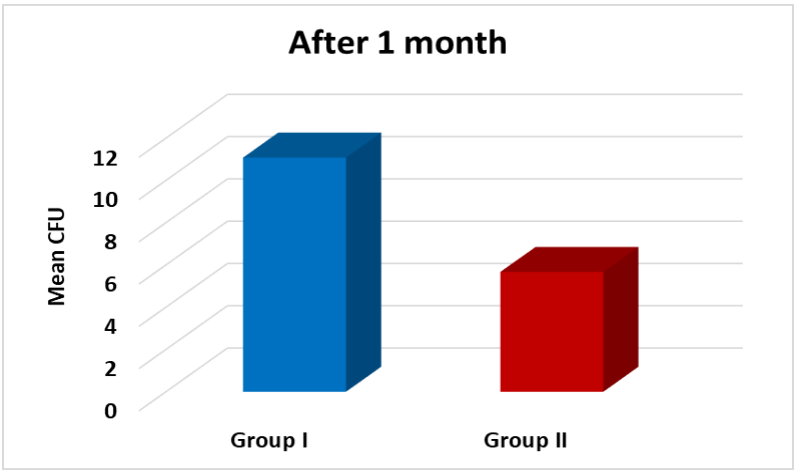

Figure 26: Mean CFU after 1 month of denture insertion.

Difference between before and after denture insertion:

Comparison between mean difference of CFU (difference between before and after 1 month of denture insertion) was performed using Independent t-test which revealed significant difference as P<0.05 as presented in table (13) and figure (27).

Table 13: Comparison between group I and group II regarding mean difference of CFU (difference between before and after 1 month of denture insertion)

	Difference between Before & After denture insertion		P value
	MD	SD	
Group I	5.00	2.045	0.000
Group II	- 0.42	1.165	1*

M; mean , SD; standard deviation,*significant.

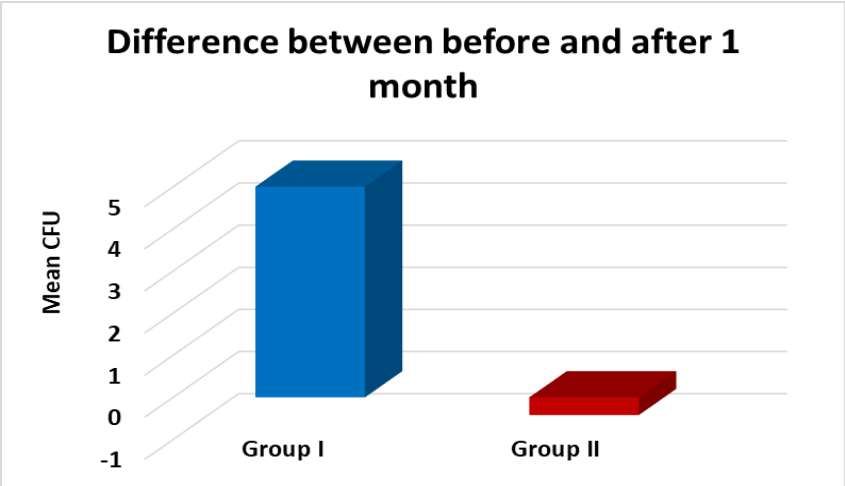

Figure 27: Mean difference of CFU (difference between before and after 1 month of denture insertion).

DISCUSSION

Discussion of methodology:

This randomized clinical trial (RCT), of parallel groups design, was approved by Evidence Based Dentistry Committee, Ethical Committee and Prosthodontics Department Board of Faculty of Dentistry, Cairo University.

In terms of internal validity, randomized clinical trials represent the most scientifically rough study design, when properly performed, as they are best able to control bias and serve as a gold standard of study designs for evaluating treatment efficacy and widely considered as highest level of confirmatory scientific evidence. *(*Friedman, et al. 2010)[19]

Patients ages range from 45-55 years old. Patients over 55 were excluded, as they may suffer from more trauma of oral mucosa and Age may affect the neuromuscular coordination, that leads to less oral hygiene measures and more accumulation of Candida flora. (Boskey and Coleman 2010)[2]

Patients were free from any systemic disease that could affect candid count as it may affect the oral flora for example patients with lowered immunity may cause increase of oral Candida.

Heavy smokers and alcohol addicts were excluded because alcohol abuse and heavy smoking change bacterial community composition, and increase incidence of human oral diseases. (Thomas, et al., 2014)[3]

Antibiotics and steroids intake especially for prolonged consumption affects the immune system and affects oral Candida cont. so, uncontrolled diabetics and patients receiving antibiotics or corticosteroids were excluded from the study. (Martins, et al. 2014)[4]

It was found that chemotherapy and radiotherapy treatment caused major changes in oral flora. So, those patients were excluded as their radio thereby may affect the oral Candida count and alter the final results. (Stringer, et al. 20-15)[5]

Panoramic and periapical radiographs were performed as a complete mouth survey to detect any pathosis that may alter the results or the treatment. (Carr, et al. 2005)[6].

An informed consent was signed by each patient as it is one of the most important steps to make sure that a patient understands the risks and benefits of any medical procedure. Requiring informed consent protects many patients from being forced to participate in medical studies without understanding the risks involved. (Carr, et al. 2005) [6]

Denture construction:

Dentures were constructed following conventional manner Maxillary and Mandibular preliminary impressions were made for each patient using irreversible hydrocolloid alginate impression material in suitable prefabricated stock trays Impressions were poured immediately to obtain study casts on which special trays were constructed. (Yarapatineni, et al. 2013)[7]

The mandibular teeth of the primary casts were adjusted to conform to the occlusal plane using yurkstas metal “U” shaped occlusal template.

A clear acrylic resin template was fabricated over the modified stone cast following Bruce technique. The inner surface of the template is coated with pressure indication paste and placed over the patient’s natural teeth. Interferences are readily noted through the template and are removed by reshaping by enameloplasty. The process is repeated until the template seats properly. (Yarapatineni, et al. 2013)[7]

Maxillary secondary impressions were made using putty rupper base material Final wash of the upper secondary impression was done using medium rubber base material .

The impressions were boxed to preserve the thickness, the shape and the extension of the borders. Then master casts were duplicated using silicone duplicating material to give rise an accurate replica of the master cast. Trial record bases and wax rims were s fabricated over the master casts and duplicate master casts. (Kelly, et, al. 2003)[8]

Face bow record was taken from the patients to mount the maxillary casts on semi adjustable articulator. Jaw relation was taken using wax registration method. (Kelly, et, al. 2003)[8]

Semi anatomic cross linked acrylic teeth were arranged in centric occluding relation, waxed up then, tried in patient's mouth. (Kaur, et al. 2016)[9]

Teeth were arranged, waxed up on the duplicate master cast, and then tried inpatient's mouth to obtain two trial waxed dentures for each patient. The trial waxed dentures were flasked, packed and processed at the same time to avoid variation in dimensional changes occurring during fabrication. (Kaur, et al. 2016)[9]

The dentures were finished and polished then soaked in water for one day to decrease leaching out of monomer inside the patient's mouth with its irritating and adverse effects on oral mucosa. In addition soaking in water resulted in decrease in dimensional changes and compensated the polymerization shrinkage. (Urban, et al. 2009 ; Gharechahi, et al. 2014)[10,11]

At time of denture insertion, pressure indicating paste was used to detect the presence of any pressure areas on the fitting surface of the dentures. Elimination of the detected pressure areas improved fit and adaptation of the dentures. Patients were instructed to clean their dentures after each meal under tap water. Mechanical or chemical means were avoided, which might affect plaque accumulation and its microbial flora. (Pavarina, et al. 2003)[17]

Also patients were instructed to remove their dentures during sleep for 6-8 hrs. This will also give chance for tissue recovery and physiologic stimulation, which is important for the integrity of the oral tissues. (Pavarina, et al. 2003) [17]

Rapid heat cured acrylic resin like the conventional heat cured acrylic have the same problems of porosity and surface roughness, that makes it theoretically have the affinity to retain microorganisms and candid. Unfortunately the ability of rapid heat cured acrylic resin to retain candid and increase its colonization count lack in terms of researches and studies. So in this study we used rapid heat cured acrylic resin to try to know to what degree it will retain candida and helps in its fungal colonization. (Craig, et al. 2002)[12]

Many studies has shown the effect of titanium dioxide nanoparticles on *candida albicans*. As these nanoparticles considered to have an antifungal effect on candida by inhibiting candida colonization. It has been suggested that TiO2 interacts with the candida cells causing its death.

Adding TiO2 nanoparticles to the denture material is supposed to inhibit the colonization and aggregation of *candida albicans*, lowering or preventing the possibility of oral candidiasis and other associated oral conditions. (Ahmed, et al. 2019)[13]

Swabs are a very effective method of collecting candida from patients as it is a non-invasive method with the advantage of being easy and quick and somehow reproducible. Swabs also are non-expensive and not technique sensitive. (Caldwell 2017)[14]

Swabs were taken from three points on the oral mucosa of the patients from the crest of the ridge which is considered a a primary stress bearing area of thee removable denture. In order

to minimize the error of false counting the candida we do calculate the sum of the three points swabs.

Sabouraud Dextrose Agar (SDA) was selected as culture medium as it is used for cultivating pathogenic & commensal fungi and yeasts. The high dextrose concentration and acidic pH of the formula permits selectivity of fungi. (Caldwell 2017)[14]

The results of this study showed difference in candida colonies count that formed on the agar plates from the swabs that were taken from the crest of the ridge. The swaps were taken before the insertion of each denture and after one month interval after insertion. This study showed difference between the two groups in the candida colonies count after the month interval after insertion.

After one month of using the first denture that was constructed from rabid heat cured acrylic risen the results showed increased candida colonization count. That is because rabid heat cured acrylic resin has the disadvantage of having more affinity to retain microorganisms. Because of surface texture of heat-cured resin material the surface of the denture favors to accumulate dental plaque and acts as reservoirs for microorganisms and candida. (Ferracane 2001)[15] roughness of the surface, surface free energy, topography, and micro porosities, are factors that affect candida adhesion to denture base resin materials and thus its colonization affecting the end result count as well as surface charge of the candida and presence of saliva coating. (Al-Bakri, et .al. 2014)[16]

On the other hand after one month of using the second denture that is constructed from acrylic resin modified with titanium dioxide nanoparticles the candid colonization count had slightly decreased.

That is due to the antifungal effect of titanium dioxide nanoparticles that do alter the growth rate of candida albicans and also affect the growth phase of candida albicans by affecting the onset of length of that growth phase (Ahmed, et al. 2019)[13] mechanical and chemical cleansing of dentures was proven not to be sufficiently adequate to eliminate denture contamination of candida, although sufficient cleansing is effective in removal of the dental plaque. (Pavarina, et al. 2003; John F, et al. 2008)[17,18]

CONCLUSION

From this study, we can conclude that Candida *Albicans* can grow and colonize on both denture base materials (rapid heat cured acrylic and acrylic resin modified by titanium dioxide nanoparticles).rapid heat cured acrylic resin has the affinity to increase candida growth and colonization Titanium dioxide nanoparticles can stabilize candida growth and colonization on acrylic denture base .using acrylic resin modified by titanium dioxide

nanoparticles can decrease the incidence of oral candidiasis Titanium dioxide nanoparticles have an antifungal effect .

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