Oral Response to a Newly Formulated Autopolymerized Complete Denture Base Material (Microbiologial and Cytological Study)

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Abstract: _Statement of problem_: *Several alternative methods to conventional compression-molding processing for denture base material have been developed to increase the adaptation of the denture base, such as fluid resin techniques. These offered improved adaptation, dimensional stability, reduced cost and simple procedure but had low strength, higher solubility and high residual monomer levels which is related to mucosal irritation and sensitization of tissues.* _Subjects and Methods:_ *Fourteen Completely Edentulous patients were selected. Patients were divided into two groups equally.one group were treated using auto-polymerized pour-type acrylic resin denture base material and the second group were treated using the conventional heat cured acrylic resin (PMMA) complete denture base material. Microbiological and cytological samples were collected. Data were statistically analyzed.*_Results:_*When comparing the two studied groups regarding mean difference of the level of both pro inflammatory salivary cytokines (TNF-α & IL-6) after delivery of the new denture, group I levels were significantly higher than group II levels at each period separately (½ hour, 1 hour 2 hours, 1 day, 3 days,1 week and 2 weeks)and when comparing between the two studied groups regarding the mean values of the number of keratinized epithelial cells before and after two months of denture delivery, it was found that there was no significant difference between them.* _Conclusion:_ *Immunologically, Auto-polymerized pour-type acrylic resin denture base material elicited higher immunological response at the beginning due to its chemical composition and leaching of higher amount of residual monomer. Cytologically, both denture base materials showed an increase in the number of keratinized epithelial cells with no significant difference between them.*

Keywords: pour-type acrylic resin, Complete denture, SalivaryCytokines, TNF-α, IL-6, keratinized epithelial cells

1. Introduction

Loss of teeth, which may be due to trauma, dental diseases, pathology, or otherwise not only alters the psychological thoughts of the patients but also disturbs the esthetics, phonetics, and functional occlusion[1].

Replacement of missing teeth is highly essential in order to restore the defect and regain function as best as possible [2]. Polymers are used in the manufacture of dentures.[3][3][3] Among the polymer materials introduced in prosthetic dentistry, Polymethyl methcrylate (PMMA) is the only proven material for a successful denture base on account of its optimal physical properties and excellent esthetics with relatively low toxicity compared to other plastic denture base materials [3–6].

Several techniques are utilized in resin polymerization method, such as heat polymerization method, pouring method, injection pressing method, and microwave activated polymerization method. Improvements have been made in each method to strive towards a system with high accuracy and reliability [7,8] .

Compression molding with heat activation in a water bath for resin polymerization is the conventional method to process dentures. However, polymerization shrinkage of the resin and distortion of the denture base due to thermal stress is virtually unavoidable during the processing of dentures. These adverse effects cause movement of the artificial teeth position and increase the gap between the denture base and underlying mucosa, resulting in an ill-fitting denture [5].

Several alternative methods to conventional compression-molding processing for denture base have been developed to increase the adaptation of the denture base, such as fluid resin techniques[9].

The pouring method of denture base resin was developed in the 1960s using agar hydrocolloids as investment material and has been one of the most popular polymerization techniques because of three merits. It is simple to use, less time consuming, and it offers better adaptation accuracy than the heat polymerization method[9–12].

The principal difference is in the size of the polymer powder or beads. Small particle size results in a fluid mix. The mix is quickly poured into the mould and allowed to polymerize under pressure [13].

Denture base is in continuous contact with the great part of the oral mucosa. It is important to evaluate the effect of the residual monomer, which has been shown to leach into water saliva or artificial saliva, on the oral mucosa that is adjacent to the denture base. Leaching residual monomer have been

suggested to cause irritation of oral mucosa, irritation or even an allergic reaction[14].

Salivary cytokine levels are very likely to provide useful information of the presence of disease, epithelial behaviour, the local inflammatory response, and carcinogenesis [15][16].

Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factor. Cytokines are produced by a broad range of cells, including immune cells like macrophages, T-lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. Cytokines are important in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction[17,18].

Both InterLeukin-6 and Tumor Necrosis Factor- alpha are pro-inflammatory cytokines characterized by multi directional action which regulates cell functions and reactions, phagocytosis and the killing of microorganisms. [19,20].

In the edentulous patients, the mucosa covering the crest of the residual ridge is classified as masticatory mucosa which is formed by keratinized stratified squamous epithelium and the adjacent narrow layer of connective tissue known as lamina propria .The oral mucosa under dentures usually present some changes in the continuous renewal process of maturation and differentiation of keratinocytes, and this process is observed varying as histological changes to clinical abnormalities as a process of adaptation of the epithelium in order to protect the adjacent structures [21,22].

Histologic studies of the effect of wearing dentures on the keratinization of the mucosa of the crest of the residual ridges have produced conflicting results. However, most studies indicate that wearing complete dentures doesn't seem to be harmful to the epithelium, but reduced thickness of the keratinized surface is noted [23].

Exfoliative cytology, as method of investigation, is based on monitoring the exfoliated cells of the mucosa, which reflect many of the features of the underlying tissues and also have a valuable role in the diagnosis of certain local and systemic disease.[24] . It is a practical,simple, quick, painless, non-invasive, bloodless, inexpensive, and repeatable technique that enables the investigator to obtain an overview of the whole working field and provide the opportunity for repeated sampling without injuring the tissues[25] .

This study was aimed to evaluate, both microbiologically and cytologically, oral response to auto-polymerized pour-type acrylic resin denture base material and the conventional heat cured acrylic resin complete denture base material.

2. Subjects and Methods

Fourteen Completely Edentulous male patients were selected from Prosthodontic Department, Faculty of Dentistry, Alexandria University. The fourteen selected patients were divided equally into two groups:
- **Group I (study group):** received complete dentures, which were constructed of auto-polymerized acrylic resin(Vertex™ Casatveria, Vertex-DentalB.V. Headquarters The Netherlands)using the pour technique.
- **Group II (control group):** received complete dentures, which were constructed of conventional poly methyl methacrylate acrylic resin using the compression molding technique.

All selected patients were completely edentulous aged from 40 to 60 years with no previous denture experience. They were non-smokers, with good oral hygiene and free from any systemic diseases. They had no signs of inflammation (such as: redness & swelling). They had well-formed maxillary and mandibular residual ridges covered with healthy mucosa and acceptable maxillo-mandibular relationship.

All clinical & laboratory steps for denture construction were done for every patient according to standardized conventional technique.

For group I, Complete Dentures were constructed using auto-polymerized acrylic resin which was supplied in the form of powder and liquid, it was supplied in different shades. The auto-polymerized acrylic resin is based on the pour type technique.

The waxed denture was placed on the plate of the flask and the flask was closed.Then the gel of 48ºC was poured in the flask very gently.

Figure 1: The waxed denture was placed on the plate of theflask

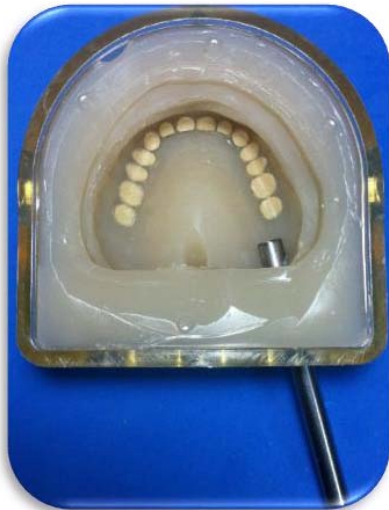The flask was cooled down and the gel washardened. Three channels were inserted by making use of largest canal cutter.

Figure 2: Three channels were inserted by making use of largest canal cutter

17g powder and 10 ml liquid was mixed for 20 seconds and then poured with a thin straight line in the center opening of the flask. The flask was left for 8 minutes until the surface of the pouring channels became matt.

The flask was placed in the pressure vessel in the position so that the pouring channels were above the water surface and polymerized at 55 °C for 30 minutes at 2.5 bar pressure

After polymerization, the flask was allowed to stand outside the pressure vessel for 30 minutes. Finishing and polishing of the denture was done.

Figure 3: The flask was placed in the pressure vessel

Figure 4: The finishedauto-polymerized pour resin denture

Complete dentures constructed of Heat cure acrylic resin (PMMA) were processed using the conventional compression molding technique and cured by long curing cycle. The dentures were finished and polished in the conventional manner.

3. Salivary Cytokines Measurement

Collection of whole unstimulated saliva (WUS) from every patient in the two groupswas done using standard techniques according to Navazesh[26] cytokine profiles.

Salivary samples were obtained in the morning by requesting participants to swallow first, then tilt their head forward, and expectorate all saliva into a 5ml sterile centrifuge tubes for 5 minutes without swallowing.

The first sample was collected directly before delivery of the new denture. The other samples were collected after delivery of the new denture by 1/2, 1 hour, 2 hours, 1 day, 3days, 1 week & 2 weeks

Participants were instructed not to eat, drink, or use mouth rinse at least 2 hours prior to salivary sample collection.

The saliva samples were frozen at - 70°C till used later on for cytokine detection by the ELISA method. All samples were centrifuged at 4500 g for 20 min and supernatant of each sample was separated to be used in the assay.

Cytokines in saliva samples were assayed using Enzyme-linked immunosorbent assay (ELISA):
o TNF-α was measured using TNF-α -ELISA. (TNF-α Elisa kit-96 T, BE55001 - I B L International – Hamburg, Germany)
o IL-6 was measured using IL-6- ELISA. (IL-6 Elisa kit-96 T, BE53061 - I B L International – Hamburg, Germany).

4. Cytological Smear Examination

Cytological smear was taken from each patient of both groups before delivery of the new denture and after two months of denture delivery from the mucosa covering the crest of the mandibular alveolar ridge.The smear was then spread on a clean glass slide and immediately fixed in 96% ethyl alcohol.

The slides were labeled with patient's name, date, and site of the smear. The smears were stained by papanicolaou technique [27]. The stained slides were examined by the digital camera attached to the light microscope at a magnification 400 mounted with a calibrated ocular grid. The count of fully keratinized stained cells was calculated. The clumped cells were not included in the statisticalanalysis because the piling of cells prevented their identification and quantification.

Results were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described in Newton as range (minimum and maximum) mean and standard deviation[28]. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test and revealed a normally distributed data. Accordingly, Comparison between two independent data was done using independent t-test; Comparison between multiple data was done using ANOVA with repeated measures and Post Hoc test was assessed using Dunnett's test.Results were quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level. A p-value of less than 0.05 was considered statistically significant.

Table 1: Comparison between the two studied groups regarding mean difference of salivary TNF - α in pg/ml at different period of follow up

		Before insertion (Baseline)	½ hour	1 hour	2 hours	1 day	3 days	1 weeks	2 weeks		
Group I	Min. – Max.	5.86-6.78	0.0-1.37	0.81-1.99	1.79-3.18	11.95-14.03	8.41-11.77	0.3 - 1.15	0.02-0.81	F	21.7
	Mean ± SD	6.54±0.31	0.71±0.55	1.35±0.46	2.40±0.50	13.24±0.79	9.88±1.19	0.58±0.54	0.34±0.33	P	0.001 *
Group II	Min. – Max.	5.91-7.65	-0.21-0.78	-1.07-1.23	1.19-2.32	10.94-12.23	4.26-5.20	-0.31-0.84	-0.91-0.31	F	22.65
	Mean ± SD	6.83±0.63	0.22±0.32	0.24±0.92	1.92±0.35	11.61±0.51	4.74±0.37	0.29±0.43	0.20±0.42	P	0.0001 *
P			0.132	0.001*	0.0001*	0.001*	0.0001*	0.061	0.109		

5. Ethical Approval

This study protocol was approved by the research ethics committee of the Faculty of Dentistry, Alexandria University, Egypt.

6. Ethics, consent and permissions

All the patients signed an informed consent form before participation in this study.

7. Consent to publish

All the patients who participated in the study provided consent to publish the data obtained from them duringthe study.
F: value for F test (ANOVA)
Statistically significant at p ≤ 0.05

8. Results

As shown in table (1) and figure (5), the mean difference of the level of salivary tumor necrosis factor alpha (TNF- α) of group I was at its highest value after one day of the new denture delivery (13.24±0.79) pg/ml and lowest after two weeks of denture delivery (0.34±0.33) pg/ml.

It showed significant increase from 1hr, 2hrs, till one day of denture delivery, P= 0.035*,0.001*,0.0001* respectively. Then it decreased significantly after 3 days and 1 week, P= 0.0001*, 0.041* respectively and finally it returned back to normal values by the end of 2 weeks

The mean difference of the level of salivary tumor necrosis factor alpha (TNF- α) of group II was in its highest value after one day of the new denture delivery (11.61±0.51) pg/ml and lowest after two weeks of denture delivery (0.20±0.42) pg/ml.

It was increased insignificantly from the beginning at the same day (1hour and 2 hours) P = (0.104 ,0.098) respectively and it increased significantly after 1day of delivery P = (0.0001*). Then it declined significantly after 3 days and 1 week of delivery P= (0.001*, 0.046*) respectively. After two weeks it decreased close to a normal value showing statistically non-significant difference from the baseline P= (0.098).

When comparing the mean difference of the level of salivary cytokines (TNF- α) for patients of both groups after delivery (half hour, one hour, two hours, 1 day, 3 days, 1 week and 2 weeks). It was found that group I (study group) was significantly higher than group II (control group) at 1hr, 2hrs, 1day, 3 days P=(0.001*,0.0001*,0.001*,0.0001*) respectively. And it was insignificantly higher at 1 week, & 2 weeks P=(0.0611,0.109) respectively.
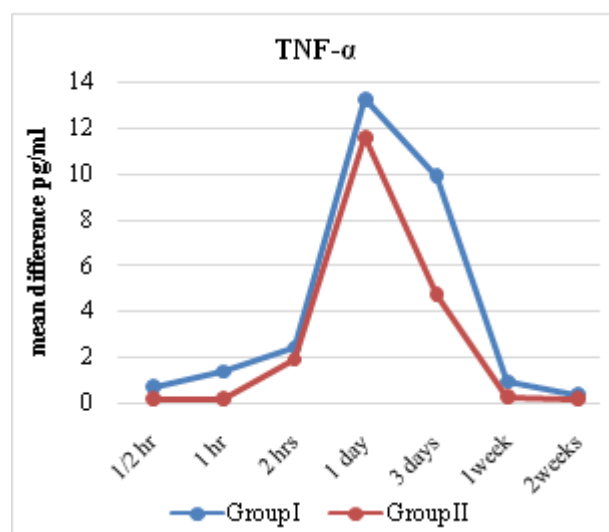

Figure 5: Comparison between the two studied groups regarding mean difference of salivary **TNF - α** at different period of follow up

As shown on table (2)and figure (6), the mean difference of the level of salivary Interleukin 6 (IL - 6) of group I was in its lowest value after two weeks of the new denture delivery (0.11±0.37) pg/ml and highest after one day of delivery (6.31±0.35) pg/ml.

It showed significant increase from the beginning till first day (1hr, 2hrs, 1day) P=0.037*,0.002*,0.0001*. Then it decreased significantly after 3 days, 1 week P= 0.001*,0.034* respectively. And finally it returned back to normal values by the end of 2 weeks P= 0.071.

Table 2: Comparison between the two studied groups regarding mean difference of salivary IL-6in pg/ml at different period of follow up

		Before insertion (Baseline)	½ hour	1 hour	2 hours	1 day	3 days	1 weeks	2 weeks		
Group I	Min. – Max.	1.96-2.47	- 0.20-0.37	- 0.03-0.81	0.74-2.13	5.57-6.63	3.11-4.34	0.03-0.83	-0.47-0.72	F	20.6
	Mean ± SD	2.28±0.17	0.15±0.20	0.50±0.26	1.23±0.51	6.31±0.35	3.98±0.46	0.33±0.23	0.11±0.37	P	0.001 *
Group II	Min. – Max.	2.17-2.58	-0.21-0.78	0.09-0.54	0.37-1.08	4.70-5.55	2.08-3.89	0.05-0.39	-0.15-0.28	F	18.11
	Mean ± SD	2.37±0.15	0.22±0.32	0.32±0.17	0.78±0.22	5.06±0.27	2.95±0.72	0.24±0.12	0.06±0.19	P	0.002 *
P			0.351	0.041*	0.001*	0.001*	0.004*	0.079	0.092		

F: value for F test (ANOVA)
Statistically significant at p ≤ 0.05

The mean difference of the level of (IL-6) of group II was in its lowest value after two weeks of delivery of the new denture (0.06±0.19) pg/ml and highest after one day of denture delivery (5.06±0.27) pg/ml.

It increased insignificantly from the beginning at the same day (1hour and 2 hours) P = (0.289,0.061) respectively and it increased significantly after 1day of delivery P = (0.001*). Then it declined significantly after 3 days and 1 week of delivery P = (0.001*,0.047*) respectively. After two weeks it decreased close to a normal value showing statistically non-significant difference from the baseline P = (0.210).
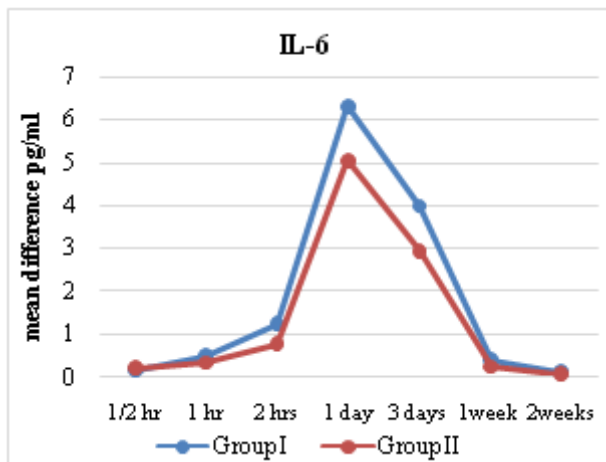

Figure 6: Comparison between the two studied groups regarding mean difference of salivary **IL-6** at different period of follow up.

When comparing the mean difference of the level of salivary cytokine (IL- 6) for patients of both groups after delivery (half hour, one hour, two hours, 1 day, 3 days, 1 week and 2 weeks). It was found that group I (study group) was significantly higher than group II (control group) at 1hr, 2hrs, 1day, 3 days P= (0.041*,0.001*,0.001*,0.004*) respectively. And it was insignificantly higher at 1 week & 2 weeks P= (0.079,0.092) respectively.

As shown in figures (8,10), The mean count of keratinized epithelial cells for group I (study group) were(25.86± 3.53, 38.14± 4.06)before, and after 2 month of denture delivery

respectively. There was statistically significant increase in number of keratinized epithelial cells (P= 0.0015 *)

As shown in figures (9,11), the mean counts of keratinized epithelial cells for group II (control group) were (21.71± 2.87, 34.57± 4.43)before, and after 2 month of denture delivery respectively. There was a statistically significant increase in number of keratinized epithelial cells (P=0.001 *)

Table (3) and figure (7) showed the comparison between the mean values of the number of keratinized epithelial cells of the two studied groups of denture base materials, it was found that there was no significant difference between the two studied groups regarding number of keratinized cells before and after two month of the new denture delivery.

Table 3: Comparison between the mean values of the number of keratinized epithelial cells of the two studied groups

	Cytology			
	Before	*2 months*		
Group I			T	5.5
Min. – Max.	21– 30	32 – 44		
Mean ± SD.	25.86± 3.53	38.14± 4.06	P	0.0015 *
Group II			T	5.9
Min. – Max.	18– 26	28– 41		
Mean ± SD.	21.71± 2.87	34.57± 4.43	P	0.001 *
T	1.9	1.57		
P	0.082	0.141		

t: Student t-test for comparing between the two studied groups
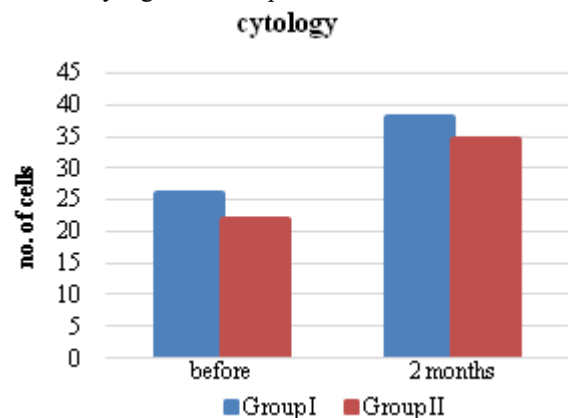*: Statistically significant at p ≤ 0.05

Figure 7: Comparison between the mean values of the

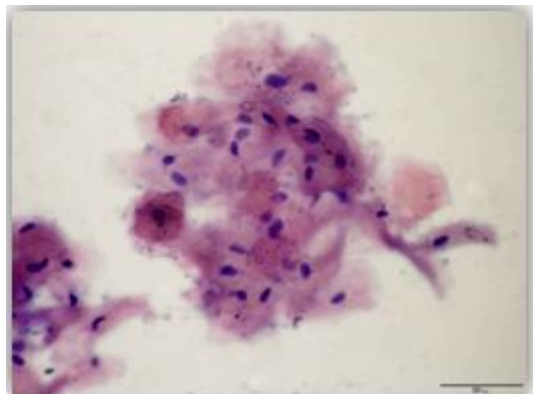number of keratinized epithelial cells of the two studied groups

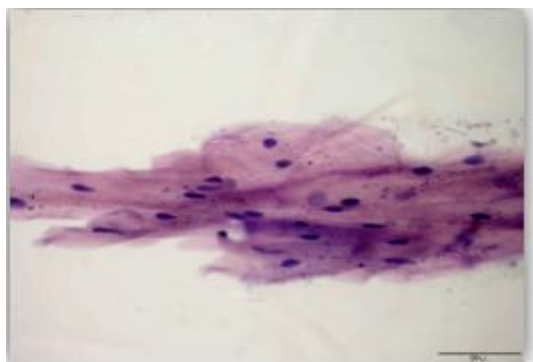**Figure 8:** Few keratinized cells in Group I before Denture insertion

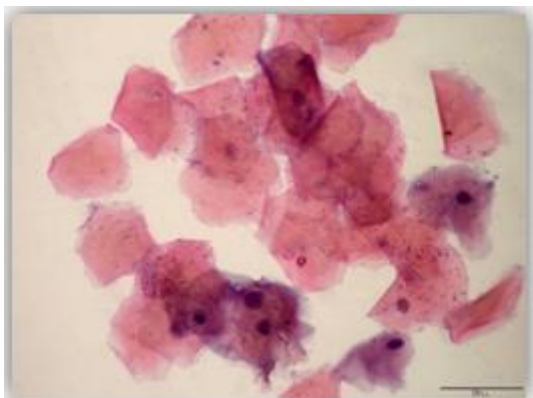**Figure 9:** Few keratinized cells in Group II before Denture insertion

Figure 10: Increased keratinization in Group I After Denture insertion
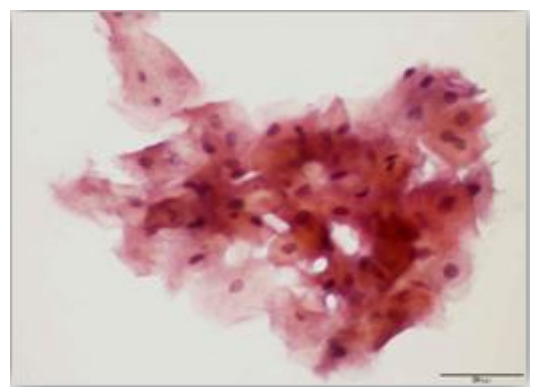

Figure 11: Increased keratinization in Group II After Denture insertion

9. Discussion

The patient selection for this study was restricted to male patients to avoid any influence of sex hormone variations on the oral epithelium, with average age ranging from 40 to 60 years. The patients beyond 60 years of age were not included in this study because of probable changes which may occur in oral mucosa due to senility. Patients whose histories revealed no systemic disorders were selected to eliminate the possible influence of systemic diseases and drug intake on oral mucosal tissues [29,30].

The fourteen selected Patients were divided into two groups equally. One group (study group) was treated using the auto-polymerized pour-type acrylic resin complete denture base material and the second group (control group) was treated using the conventional heat cured acrylic resin (PMMA) complete denture base material.

All steps of fabricating complete denture were done as conventional technique. Clinical remount was done to adjust the occlusion to avoid the irritation resulting from cuspal interference [31].

The long curing cycle of compression molding technique was selected to process acrylic dentures as it was concluded to give the lowest value of residual monomer [32,33].

Before delivery of the dentures to patients of both groups, the acrylic dentures were 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 [34,35].

Patients were shown how to clean their dentures, in addition to oral and written instructions. Denture hygiene protocol comprised of: (i) brushing internal and external surfaces of the dentures for 2 min with a nonabrasive denture cleanser then rinsing with water. (ii) Cleaning the mouth after removing the dentures (v) Soaking dentures overnight in a mild antiseptic denture soaking solution. (vi) Rinsing dentures before putting them back in the mouth[36].

Saliva samples were collected from patients of both groups I and II at 8 different periods; before denture delivery and after denture delivery (1/2, 1 and 2 hours, 1 day, 3 days, 1 week, 2 weeks) for both microbiological &immunological study. Salivary cytokines were detected and quantified by the enzyme linked immunosorbent assay (ELISA) for its excellent analytical performance and the ability to be automated[37].

Detection of salivary cytokines levels before delivery of the new denture was important since there were no estimated values for normal levels of cytokines in the saliva of healthy edentulous patients in the literature. and we need these values to compare it with the level of cytokines after delivery of the new denture .and thus to conclude the tissue reaction of mucosa to the denture base materials.

In this study the levels of pro-inflammatory salivary cytokines (TNF- α, IL-6) increased significantly from 1hr, 2hrs, till one day of denture delivery. Then it decreased

significantly after 3 days and 1 week and finally it returned back to normal values by the end of 2 weeks

These results were in agreement with Baker et al.[38] who detected higher amounts of methyl methacrylate (MMA) in the saliva of subjects wearing dentures made from auto-polymerized resins compared with heat-cured resins

These results were also in agreement with Goncalves et al. [39] who evaluated the in situ levels of residual MMA monomer of an autopolymerized acrylic resin in forty volunteers. High concentrations of residual monomer during the first 24h of use were observed.

On the other hand, the levels of these cytokines in the saliva of group II patients increased insignificantly after delivery at the same day. And then increased significantly 1 day after delivery. After two weeks it decreased close to normal value showing statistically non-significant difference from the baseline.

These Results were in agreement with Sofou et al[40] who monitored the monomer release from three different types of heat cured acrylic resin in different storage conditions (artificial saliva, water, and ethanol/water) every 24 h for 5 days at room temperature. They found that methyl methacrylate (MMA) leached out from all the acrylic resins examined; the level of which seems to be time-dependent, decreasing over a period of 5 days.

The high levels of pro-inflammatory salivary cytokines (TNF-α and IL-6) that were found in saliva of both studied groups may be linked to their protective effect against tissue inflammation caused by release of residual monomer[41] .

The results were also in agreement with Lung and Darvell[42] who found that most residual monomer was lost in the first few days of insertion but became almost constant after 2 weeks.

Diagnostic cytology, as a method of investigation depends on the fact that cells exfoliated reflect many of the features of the underlying tissue. Cytology offers several advantages in studying the response of epithelia to prosthesis .The cytological study was done because it is a simple, quick, painless and bloodless technique .Therefore it was decided to evaluate tissue changes in response to dentures through microscopic examination of cytological smears[24].

The parameter of evaluation in the cytological observation was the number of keratinized cells ,since the function of keratin is protective in nature and it has been observed that areas under mechanical stress or those are subjected to irritation show changes in the degree of keratinization[29].

The cytological smears of both studied groups showed significant increase in number of keratinized epithelial cells after two months of denture insertion, this could be attributed to the protective effect of using the dentures and that finding was in agreement with Zarb GA et al.[43] who indicated by cytological studies that increased amounts of keratinizedmaterial are present in edentulous ridges when the clinical quality of the denture is good, an indication that

well-fitting dentures may be important in maintaining the normal histologic conditions of the mouth.

When comparing the two studied groups before and after 2 months of denture delivery as regards to the count of number of keratinized cells, there was no significant difference; this finding may be attributed to similarity in composition of both denture base materials and leaching out of nearly all residual monomer from the pour-type auto-polymerized complete dentures.

10. Conclusion

Within the limitations of this study, it could be concluded that:

- Auto-polymerized pour-type acrylic resin denture base material was associated with an increase in pro-inflammatory salivary cytokines (TNF- α & IL-6) from the beginning of denture delivery and decreased by the end of follow-up period which was two weeks.
- On the other hand, Heat cured acrylic denture base material was associated with a little increase in these levels.
- Immunologically, Auto-polymerized pour-type acrylic resin denture base material elicit higher immunological response at the beginning due to its chemical composition and leaching of higher amount of residual monomer.
- Cytologically, both denture base materials show an increase in the number of keratinized epithelial cells with no significant difference between them.

References

[1] Zarb GA, Bolender CL, Carlsson GE. Boucher's Prosthodontic Treatment for Edentulous Patients. 11th ed. Mosby; 1997. 337-42 p.
[2] Jagger DC, Jagger RG, Allen SM, Harrison A. An investigation into the transverse and impact strength of "high strength" denture base acrylic resins. J Oral Rehabil. 2002 Mar;29(3):263–7.
[3] Aydogan Ayaz E, Durkan R. Influence of acrylamide monomer addition to the acrylic denture-base resins on mechanical and physical properties. Int J Oral Sci. 2013 Dec;5(4):229–35.
[4] Woelfel JB. Newer materials and techniques in prosthetic resin materials. Dent Clin North Am. 1971 Jan;15(1):67–79.
[5] Takamata T, Setcos JC. Resin denture bases: review of accuracy and methods of polymerization. Int J Prosthodont. 1989;2(6):555–62.
[6] Anusavice KJ, Shen C, Rawls HR. Phillips' Science of Dental Materials. 12th ed. Elsevier Health Sciences; 2013.
[7] Teraoka F, Takahashi J. Controlled polymerization system for fabricating precise dentures. J Prosthet Dent. Elsevier; 2000;83(5):514–20.
[8] Laughlin GA, Eick JD, Glaros AG, Young L, Moore DJ. A comparison of palatal adaptation in acrylic resin denture bases using conventional and anchored polymerization techniques. J Prosthodont. 2001 Dec;10(4):204–11.
[9] LEE C-J, BOK S-B, BAE J-Y, LEE H-H. Comparative

adaptation accuracy of acrylic denture bases evaluated by two different methods. Dent Mater J. 2010;29(4):411–7.

[10] Shepard WL. Denture bases processed from a fluid resin. J Prosthet Dent. Mosby; 1968 Jun;19(6):561–72.

[11] Goodkind RJ, Schulte RC. Dimensional accuracy of pour acrylic resin and conventional processing of cold-curing acrylic resin bases. J Prosthet Dent. 1970 Dec;24(6):662–8.

[12] Takamata T, Setcos JC, Phillips RW, Boone ME. Adaptation of acrylic resin dentures as influenced by the activation mode of polymerization. J Am Dent Assoc. Elsevier; 1989 Aug;119(2):271–6.

[13] Tandon R, Gupta S, Agarwal S. Denture base materials: From past to future. Ijds.in. 2010;(2).

[14] Bural C, Aktaş E, Deniz G, Ünlüçerçi Y, Bayraktar G. Effect of leaching residual methyl methacrylate concentrations on in vitro cytotoxicity of heat polymerized denture base acrylic resin processed with different polymerization cycles. J Appl Oral Sci. 2011;19(4):306–12.

[15] Streckfus C, Bigler L. Saliva as a diagnostic fluid. Oral Dis. Blackwell Publishers; 2002 Mar;8(2):69–76.

[16] Prasad G, McCullough M. Chemokines and cytokines as salivary biomarkers for the early diagnosis of oral cancer. Int J Dent. Hindawi Publishing Corporation; 2013;2013.

[17] Foster JR. The functions of cytokines and their uses in toxicology. Int J Exp Pathol. 2001;82(3):171–92.

[18] Brooks G, Carroll KC, Butel JS, Morse SA, Mietzner TA. Jawetz, Melnick, & Adelberg's Medical Microbiology. 25th ed. McGraw-Hill Medical, 2010.

[19] Brailo V, Boras V, Arambasin AC, Alajbeg IJ, Milenovic A LJT significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. OO 2006; 42: 370–3. No Title.

[20] Bienvenu J. Exploration of cytokines in inflammation in biological fluids. C R Seances Soc Biol Fil. 1995 Jan 1;189(4):545–55.

[21] Ueno D, Sato J, Igarashi C, Ikeda S, Morita M, Shimoda S, et al. Accuracy of oral mucosal thickness measurements using spiral computed tomography. J Periodontol. 2011 Jun;82(6):829–36.

[22] Wara-aswapati N, Pitiphat W, Chandrapho N, Rattanayatikul C, Karimbux N. Thickness of palatal masticatory mucosa associated with age. J Periodontol. 2001 Oct;72(10):1407–12.

[23] Zarb GA, Hobkirk J, Eckert S, Jacob R. Prosthodontic Treatment for Edentulous Patients Complete Dentures and Implant-Supported Prostheses. 13th ed. Elsevier Health Sciences; 2013. 162 of 464 p.

[24] Kumaresan GD, Jagannathan N. Exfoliative cytology - A predictive diagnostic tool. Int J Pharm Pharm Sci. 2014;6(5):1–3.

[25] Mehrotra R. Oral cytology : a concise guide. 2nd ed. Springer; 2013. 96 p.

[26] Navazesh M. Methods for Collecting Saliva. Annals of the New York Academy of Sciences, 1993; 694: 72–7.

[27] Drury RA, Wallington EA. Carleton's Histological technique. 5th ed. Ch 18. London: Oxford University Press,1980. p 335.

[28] Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, California: Wadsworth: Cengage Learning; 2013.

[29] Jani RM, Bhargava K. A histologic comparison of palatal mucosa before and after wearing complete dentures. J Prosthet Dent. 1976 Sep;36(3):254–60.

[30] Zimmermann ER, Zimmermann AL. Effects of race, age, smoking habits, oral and systemic disease on oral exfoliative cytology. J Dent Res. 1965 Jan;44:627–31.

[31] Hochstedler JL, Shannon JL. A time-saving method for performing a clinical remount procedure with complete dentures. J Prosthet Dent. 1995 Jul;74(1):39–41.

[32] Golbidi F, Asghari G. The Level of Residual Monomer in Acrylic Denture Base Materials. Res J Biol Sci. 2009;4(2):244–9.

[33] Bayraktar G, Guvener B, Bural C, Uresin Y. Influence of polymerization method, curing process, and length of time of storage in water on the residual methyl methacrylate content in dental acrylic resins. J Biomed Mater Res Part B Appl Biomater. Wiley Subscription Services, Inc., A Wiley Company; 2006 Feb;76B(2):340–5.

[34] Urban VM, Machado AL, Oliveira R V, Vergani CE, Pavarina AC, Cass QB. Residual monomer of reline acrylic resins. Effect of water-bath and microwave post-polymerization treatments. Dent Mater. 2007 Mar;23(3):363–8.

[35] Urban VM, Machado AL, Vergani CE, Giampaolo ET, Pavarina AC, de Almeida FG, et al. Effect of water-bath post-polymerization on the mechanical properties, degree of conversion, and leaching of residual compounds of hard chairside reline resins. Dent Mater. Elsevier; 2009 May;25(5):662–71.

[36] Paranhos HF, da Silva CH, Venezian GC, Macedo LD, de Souza RF. Distribution of biofilm on internal and external surfaces of upper complete dentures: the effect of hygiene instruction. Gerodontology. 2007;24(3):162–8.

[37] Bienvenu J. Exploration of cytokines in inflammation in biological fluids. C R Seances Soc Biol Fil 1995; 189: 545-55.

[38] Baker S, Brooks SC, Walker DM. The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: an assay for monomer in saliva. J Dent Res. 1988 Oct;67(10):1295–9.

[39] Gonçalves TS, Morganti MA, Campos LC, Rizzatto SMD, Menezes LM, Nealey ET, et al. Allergy to auto-polymerized acrylic resin in an orthodontic patient. Am J Orthod Dentofac Orthop. Elsevier; 2006 Mar;129(3):431–5.

[40] Sofou A, Tsoupi I, Emmanouil J, Karayannis M. HPLC determination of residual monomers released from heat-cured acrylic resins. Anal Bioanal Chem. 2005 Apr;381(7):1336–46.

[41] Vallittu PK, Miettinen V, Alakuijala P. Residual monomer content and its release into water from denture base materials. Dent Mater. 1995;11(6):338–42.

[42] Lung CYK, Darvell BW. Minimization of the inevitable residual monomer in denture base acrylic. Dent Mater. 2005 Dec;21(12):1119–28.

[43] Zarb GA, Hobkirk J, Eckert S, Jacob R. Prosthodontic Treatment for Edentulous Patients: Complete Dentures and Implant-Supported Prostheses. Elsevier Health Sciences; 2013. 190-207 p.

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