The microhardness measurement and polarized light microscopic evaluation for enamel surface after debonding of brackets using different orthodontic adhesive materials (A comparative in vitro study)

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Abstract:

This study was carried out to compare the effects of different orthodontic adhesive materials on the micro-hardness of human enamel surface with different incubation period and to evaluate the morphology of enamel surface before and after orthodontic treatment using polarized light microscope.

The sample includes one hundred twenty sound maxillary arid mandibular first premolar teeth which have been extracted for orthodontic treatment purpose from (13-19) years old patients. The samples were divided into one as a control group and three experimental groups (I.II.III) according to the type of the orthodontic adhesive materials Reliance orthodontic products No-Mix، Advantage No-Mix. Orthodontic bonding system No-Mix) respectively.

Each group consists of 30 samples and each group subdivided into three subgroups (10 sample) according to the time of incubation (2 weeks.,4 weeks and 8 weeks) and each subgroup divided to (5 upper, 5 lower).

The study showed that enamel decalcification was associated with fixed orthodontic appliance thereby and the hardness of enamel is highest with group III (orthodontic bonding system No-Mix) followed by group 1 (Reliance orthodontic products) and group II (Advantage No-Mix).

The study indicate that there was non significant difference between group II (Advantage No-Mix and group III (Orthodontic bonding system No-Mix) after 2 and 4 weeks period times bonding in reduction of micro hardness values in both upper and lower teeth-while there is high significant difference between them in micro hardness values after 8 weeks period times bonding in both upper and lower teeth.

The result of polarized light microscope finding show destruction of the perikymata of enamel during 2 weeks period time and increased in destruction during 4 weeks period time but during 8 weeks period time there is remineralization only in group 111 f Orthodontic bonding system No-Mix) .

Keywords:

Microhardness, enamel surface, orthodontic adhesive material, polarized light microscope, morphology of enamel surface.

Introduction:

Enamel demineralization is an undesirable, but common complication of orthodontic fixed appliance therapy. Several studies had reported a significant increase in the prevalence and severity of demineralization after

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orthodontic therapy compared with controls [1,2]. The teeth most commonly affected are molars, maxillary lateral incisors، mandibular canines and premolars [3]. In the environment in which they function, most of orthodontic appliances have the potential to damage enamel either by decalcification or by caries [4]. White spot lesions or decalcification's are early manifestations of the carious process resulting from subsurface demineralization [1,5,6]

Many previous studies have shown that the initial lesion is a surface softening of the enamel rather than a subsurface lesion with a surface layer, such lesion regress rapidly in vivo in contrast to subsurface lesions in which fluoride may cause precipitation in the surface layers, thus arresting further demineralization [7,8].

Since the acid etch technique of enamel was introduced, the direct bonding, of orthodontic brackets has became a routine clinical procedure in orthodontics, resulting in a considerable improvement in. a esthetic and oral hygiene, and a reduction in chair working time [9]. Direct bonding of orthodontic brackets has become more and more important because of ongoing development of the bonding technique, aesthetic and design parameters of the bracket bases [10].

The hardness of the enamel is related to its high degree of inorganic contents [11], on the other hand demineralization or loss of minerals from the enamel (described as an enlargement of the micro space arising from complete or partial dissolution of the crystals to be associated with the decrease in the hardness of the enamel) had been reported to be one of the predisposing factors in the initiation and progress of dental caries [11-14]. Up to our knowledge this study is the first Iraqi experimental study to evaluate the micro hardness of human enamel after exposure of three types of orthodontic adhesive materials (No-Mix type), to give a clear result about the effect of time on the micro hardness of human enamel. We used the polarized light microscope to study the surface morphology after debonding, because of the health and esthetic consideration is recommended before and after orthodontic treatment.

The Aims of the Study are to evaluate and compare the effect of the different orthodontic adhesive [related to three different companies (one paste system)] on the microhardness of human enamel, during orthodontic treatment.

Materials and Methods:

The sample

A total sample was include one hundred twenty sound maxillary and mandibular first premolar teeth، which has been extracted for orthodontic treatment purposes from (13-19) years old. Iraqi patients were used in this study. They obtained from oral surgery department in hospitals، dental College University of Baghdad and from private dental clinics.

All the teeth were used in this study sound, not restored, with no cracks, no white spot or any other visual defect on the tooth surface, the extracted teeth were washed gently with water by hand and stored in non-ionized distilled water in closed container to which 1% thymol was added till the time of bonding [15].

Material and equipments

1- Three types of orthodontic adhesive material (No-mix adhesive types)
a- Orthodontic bonding system No-mix (Dentaurum. Germany)

b- Advantage No-mix (ortho-organizer. USA)
c- Reliance orthodontic products (Rely-a-bond, USA)
2- Orthodontic brackets (Dentaurum. Germany(
3- Storage material:
a- De-ionized distilled water (al-Mansur factory, Iraqi product(
b- Thymol/°\ Q added to distal water
4-Testing equipment
a- Universal research microscope (Adolph 1. Buhler INC. USA)
b- Light microscope
c- Polarized light microscope
d- Zwick universal testing machine

Methods

Preparation of the teeth

The teeth were gently clean with water to remove the debris or any stain and examined for any visible cracks using visible light cure device with aids of magnifying eye lens (x10) and there root surfaces were notched by using contra-angle high speed hand piece with carbide fissure bur for retention and the teeth were stored in distilled water at room temperature to which 1% thymol was added.

Mounting of teeth

A glass slab was placed on the surveyor, then the teeth was fixed on the glass slide in vertical position using sticky wax at the root's apex so to be parallel to analyzing rod of the surveyor [15]. The tooth is fixed to the glass slide at the middle third of buccal surface which market by surveyor using sticky wax after that we use polyvinyl chloride plastic ring put it around fixed tooth to glass slab in such a way that the lingual surface of the crown facing plastic ring.

The powder and liquid of cold-cure acrylic were mixed and poured around the tooth to the level of plastic ring, after complete setting of cold cure acrylic we separate the plastic ring from the glass slab. The buccal surface of tooth with the level of acrylic surface .The specimens were stored in wet media (artificial saliva) to prevent dehydration of teeth at 37°c [16].

Acid etching procedure

Etching the buccal surface of enamel of teeth for 60 second using 37% phosphoric acid solution with one disposable brush for each tooth according to the manufacture instruction after that we rinsing with a copious amount of water for 30 second and then the teeth are dried with an oil free steam of air 20 second [17]. The buccal enamel surface of the etched tooth appears chalky white in color. The process of bonding is done according to the manufacture instruction.

Sample grouping

The sample was divided randomly into one control group and three experimental groups according to the type of the orthodontic adhesive. Each group consist of 30 samples and each group subdivided into three subgroups (10 samples and-each subgroup divided to upper and lower (5-5) with three times intervals (2,4 , 8) weeks.

Debonding of brackets

This procedure carried out by using zwick universal testing machine in the central organization for standardization and quality in Baghdad city mounting apparatus with hole and

chisel and rod have been specially made for this study.

Testing procedure (Micro hardness measurement)

Testing the hardness measurement of all control group-samples were recorded at first to be a control, and after 2 weeks from the starting of the treatment the microhardness values of all experimental group samples were recorded after debonding of brackets. The general procedure for testing the microhardness included the application of a standardized force or weight to the surface, and such force will produce symmetrical shaped in dentition which can be measured under the microscope. The universal research microscope Südph 1. Buhler 1 NC) was used with Vickers indentor [17].

The specimen were mounted on the glass microscope and fixed, for accurate well defined indentation a flat surface must be used. Consequently all tests in this study were performed on enamel surface [18].

Also after 4 weeks from bonding of brackets. The microhardness values of all samples of this group were recorded using the same method described and this sequence was repeated at the end of the 8 weeks.

The procedure to measure Vickers Hardness Number (V.H.N) according to the pilot study the load applied was 100 gram for 30-seconds. For every sample the recording of each measurement were converted into (V.H.N) by the equation.

$$(V.H.N) = 1845 \times \frac{\text{Load (100gm)}}{(D \times 0.168)^2}$$

1845=constant number

D= diameter of the indentation

Samples preparation for polarized light microscope

Following the microhardness test procedure completed twenty samples selected two from control group and six from two weeks period times, six from four weeks period times, six from eight weeks period times. One from lower subgroup and one from upper subgroup. The roots of the teeth were cut from the crowns with diamond disk under abundant water-cooling. The crowns of teeth will cut by diamond bur at the middle area of middle third of buccal surface (cross section) and smoothing cutting area by use sandpaper disk with cooling water after that we dry the cutting area and fixed it to glass slide by using special adhesive (epofix hardener) and leave it on the bench about one day to be dry after that we cut the remnant of tooth by use cutting disk with cooling water about half mm away from the glass slide to make thin section finally the glass slide with thin section (0.3 mm) wash with water and dry then examine under polarized light microscope.

Statistical Analysis

In present study the following statistical methods were used to analyze the results:

1- Descriptive statistics: includes means and standard deviations of all the groups.

2- One way analysis of variance (ANOVA) to test any statistically significant difference between the three different adhesive types and the three testing time periods.

Results:

Measurements of Microhardness

Descriptive statistics include means and standard deviation of all variables measured including the three adhesives (Reliance Orthodontic product. Advantage no-mix and Orthodontic bonding system) at different time intervals (2 weeks, 4 weeks, 8 weeks) for microhardness.

Table (1) represent the individual microhardness measurement (VHN) of reliance orthodontic product group includes (control, two weeks, four weeks, and eight weeks period times).

Table (1): Microhardness measurements (V.H.N) of reliance orthodontic product group (I) samples

Group (I) Reliance orthodontic product					
No.		Control	2 weeks	4 weeks	8 weeks
2	U	430	326	280	230
1	L	429	325	273	225
4	U	439	329	278	240
3	L	435	340	263	237
6	U	431	335	275	227
5	L	425	328	268	231
8	U	430	335	272	229
7	L	438	337	279	239
10	U	440	330	278	228
9	L	439	327	275	334
AV	U	434	331	276.6	230.8
	L	433.2	331.4	271.6	233.2

Table (2) represent the individual microhardness measurements (VHN) of advantage no-mix group (control, two weeks, four weeks and eight weeks period times).

Table (2): Microhardness measurements (V.H.N) of advantage No-mix group (H) samples.

Group (II) Advantage No-mix					
No.		Control	2 weeks	4 weeks	8 weeks
1	U	439	383	240	220
2	L	433	370	233	218
3	U	430	355	225	210
4	L	434	385	238	223
5	U	435	360	239	218
6	L	440	365	236	213
7	U	440	357	228	221
8	L	436	362	237	215
9	U	431	361	227	212
10	L	435	359	234	216
AV	U	435	363.2	231.8	216.2
	L	435.6	368.2	235.6	217

Table (3) represents the individual microhardness. measurement (VHN) of orthodontic bonding system group include (control, two weeks, four weeks and eight weeks period times

Table (3): Microhardness measurements (V.H.N) of orthodontic bonding system group (III) samples

Group (III) Orthodontic bonding system					
No.		Control	2 weeks	4 weeks	8 weeks
1	U	439	368	234	280
2	L	435	355	239	275
3	U	440	365	225	260
4	L	429	369	240	277
5	U	431	352	235	262
6	L	430	362	230	265
7	U	428	359	227	273
8	L	425	354	232	268
9	U	427	367	238	270
10	L	432	363	236	272
AV	U	433	362.2	231.8	269
	L	430.2	360.6	235.4	271.4

Table (4) represent descriptive statistic of the different groups and period of times for upper samples.

Table (4): The means of microhardness values of the three treatment groups 2, 4 and 8 weeks with their control values (Upper)

Group / Time	I	II	III	Grand Mean
Control	434	435	433	434
2weeks	331	363.2	362.2	352.13
4weeks	276.6	.231.8	231.8	246.73
8weeks	230.8	216.2	269	238.67
Grand Mean	318.1	311.55	324	317.88

Table (4) the result demonstration that in group I and group II the lowest value of (V.H.N) in eight weeks period times after debonding comparative with control samples, while in group III the lowest value of (V.H.N) in 4 weeks period times after debonding comparative with control samples.

Table (5) represents descriptive statistics of the different groups and period of times for lower samples.

Table (5): The means of microhardness values of the three treatment groups 2, 4 and 8 weeks with their control values (Lower).

	I	II	III	Grand Mean
Control	433.2	435.6	430.2	433
2 weeks	331.4	368.	360.6	353.4
4weeks	271.6	235.6	235.4	247.53
8weeks	233.2	217	271.4	240.53
Grand Mean	317.35	314.1	324.4	318.62

The result indicated that the lowest value of (V.H.N) in eight weeks period times appear in group I and group II only in comparative with control group. While the lowest value of (V.H.N) in 4 weeks period times appear in group III in comparative with control samples.

Table (6) descriptive statistics including mean, standard deviation, coefficient of variances, maximum and minimum values of (V.H.N) for upper and lower samples in group I.

Table (6): Descriptive statistics including mean, standard deviation, coefficient of variances, minimum and maximum values of (V.H.N) (Group I / Reliance orthodontic product)

Upper						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	434	5.05	1.16	430	440
2 weeks	5	331	3.94	1.19	326	335
4 weeks	5	276.6	3.13	3.13	272	280
8 weeks	5	230.8	5.26	2.28	227	240
Lower						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	433.2	6.02	1.39	425	439
2 weeks	5	331.4	6.66	2.01	325	340
4 weeks	5	271.6	6.23	2.29	263	279
8 weeks	5	233.2	5.50	2.36	225	234

Table (6) show that the highest value of coefficient of variance in 8 weeks period times is (2.28) for the upper and (2.36) for the lower where is the mean and standard deviation for upper (434, 526) and (433.2. 6.66) for lower samples While the lowest value of coefficient of variance in control group (1.39)for lower .(1.13) in 4 weeks for upper where is the lowest value of mean and standard deviation for upper (230.8, 3.13) and (233.2, 5.50) for lower samples.

Table (7) descriptive statistics including mean, standard deviation, coefficient of variances, maximum and minimum values of (V.H.N) for upper and lower samples in group II.

Table (7): Descriptive statistics including mean, standard deviation, coefficient of variances, minimum and maximum values of (V.H.N). (Group II /Advantage No-mix).

Upper						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	435	4.35	1.04	430	440
2 weeks	5	363.2	11.32	3.12	355	383
4 weeks	5	231.8	7.12	3.07	225	240
8 weeks	5	216.2	4.92	2.28	210	221
Lower						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	435.6	2.7	0.62	433	440
2 weeks	5	368.2	10.23	2.78	359	385
4 weeks	5	235.6	2.07	0.88	233	238
8 weeks	5	217	3.81	1.76	213	223

The result shows that the highest value of coefficient of variance in 2 weeks period times is (3.12) for upper samples and (2.78) for lower samples, where is the mean and standard deviation for upper (435, 11.32) and (435.6, 10.23) for lower samples.

While the lowest values of coefficient of variance in control group (1.04) for upper and (0.62) for lower where is the mean and standard deviation (216.2,4.53) for upper (217. 2.07) for lower samples.

Table (8) descriptive statistics including mean, standard deviation, coefficient of variances, maximum and minimum values of (V.H.N) for upper and lower samples in group III.

Table (8): Descriptive statistics including mean, standard deviation, coefficient of variances, minimum and maximum values of (V.H.N). (Group III /Orthodontic bonding system).

Upper						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	433	6.12	1.41	427	440
2 weeks	5	362.2	6.69	1.85	352	368
4 weeks	5	231.8	5.54	2.39	225	238
8 weeks	5	269	8.19	3.04	260	280
Lower						
	No.	Mean	S.D	C.V%	Min.	Max.
Control	5	430.2	3.7	0.86	425	435
2 weeks	5	360.6	6.19	1.72	354	369
4 weeks	5	235.4	4.34	1.84	230	240
8 weeks	5	271.4	4.93	1.82	265	277

Table (8) shows that the highest value of coefficient of variance in 8 weeks period times is (3.04) for upper and (1.84) in 4 weeks period times for lower samples. Where is mean and standard deviation (433, 8.19) for upper and (430.2, 6.19) for lower samples.

While the lowest values of coefficient of variance in control group is (1-41) for upper and (0.86) in control group for lower samples. Whereas mean and standard deviation are (231.8, 5.54) for upper and (235.4, 3.7) for lower samples.

Comparison between groups and different period

Table (9): Comparison between the different groups and period times for upper jaw.

ANOVA	Sum of square	d.f	Mean square	F-value	P-value
Between period	389906.2	3	129968.7	392.65 ***	0.000
Between groups	1551.4	2	775.7	2.34	N.S
Within	17863	54	330.80		
Total	409320.2	59			

N.S: Non Significant at level P>0.05
*** : Highly Significant difference at level P<0.001

Two ways ANOVA was performed to test the comparison between different group and period times. Result showed significant difference at level P<0.001 between periods, and non significant difference at level P>0.05 between group.

Polarized light microscope finding

The type of the section of sample used in this study is ground section with 0.3 mm in thickness and magnifications of micrographs of samples are (x2.5, x6.3).

Figures (1, 2.3) exhibit the typical feature of normal enamel, this micrograph is taken to the part of enamel not exposed to the orthodontic adhesive when the brackets are removed after two weeks period times, four weeks period times and eight period times for all samples of treated group in most teeth when visually inspected there is alteration of the enamel surface under the brackets range from slight loss of translucency to district white spots.

The destruction of enamel surface in samples during (two weeks, four weeks and eight weeks) and some subjective evidence of the enamel surface of samples with different degree in different period times.

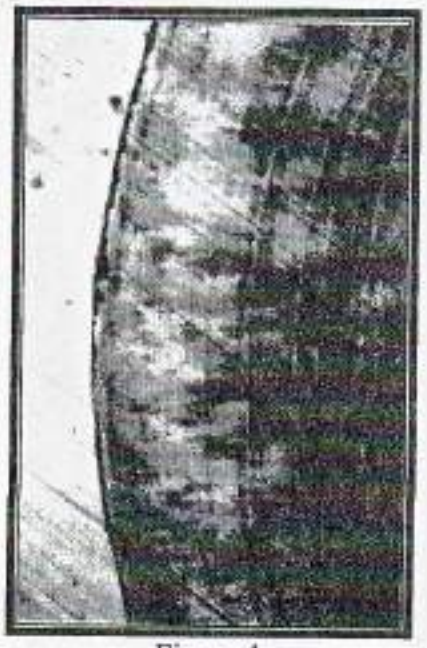

Figure:1

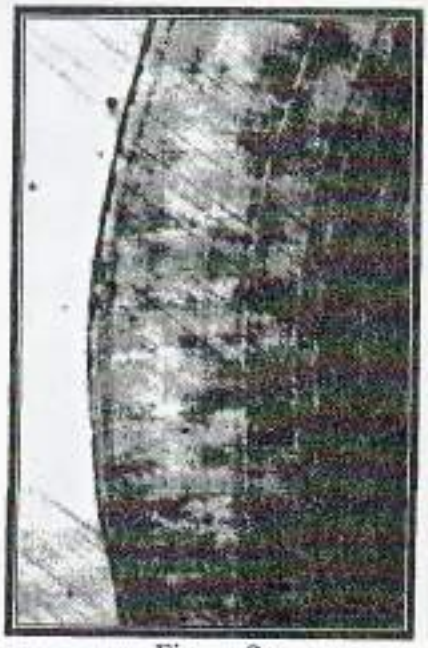

Figure:2

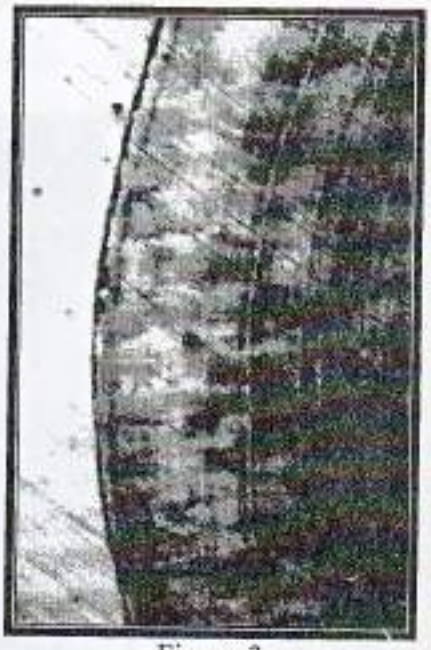

Figure:3

Figures (4-9) show different degree of destruction of perikymata of enamel it less during 2 weeks period times but increased in destruction during 4 weeks period times and become worst destruction during 8 weeks period times. This destruction can compare with normal perikymata which consist of normal groves and crest running in parallel lines as in figures (1 and 2).

Figure:4

Figure:5

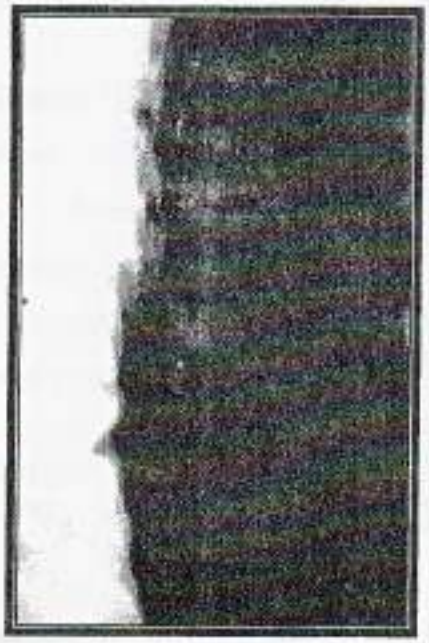

Figure:6

Figure:7

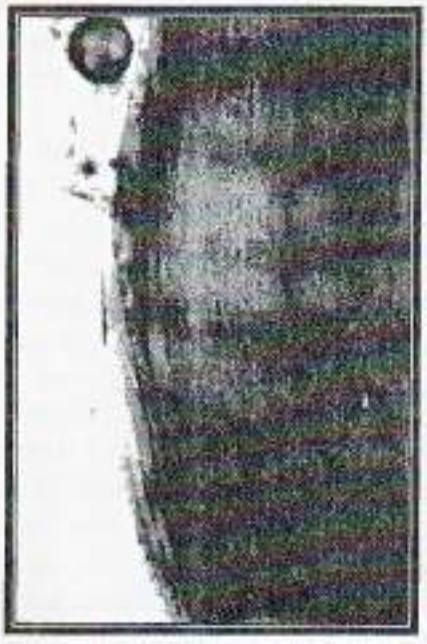

Figure:8

Figure:9

Figures (10-12) also show destruction of perikymata of enamel during 2 weeks period times and increased in destruction during 4 weeks period times but during 8 weeks period times there is remineralization of enamel as showing in figure (12).

Figure:10

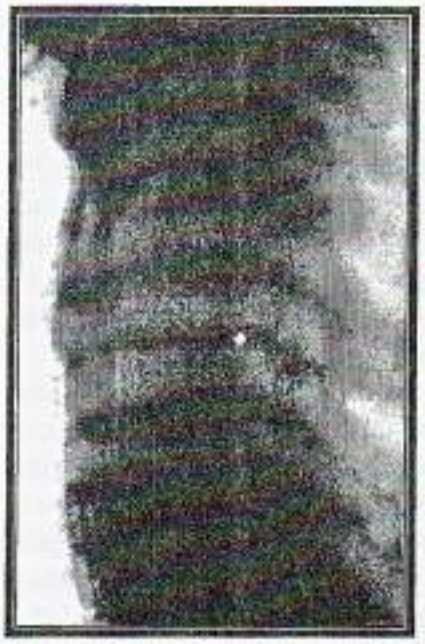

Figure:11

Figure:12

Discussion:

Dentists are interested in learning about the properties of the adhesive systems they used in order to optimize their ability to handle them properly and efficiently.

The composite adhesives, nowadays, have huge controversy among orthodontists. These various thoughts may be due to psychological accommodation and routine skills for the material used. But the various opinions for the tested orthodontic adhesives show the agreement and disagreement with the interested authors.

This study clearly revealed the existence or absence of the differences in microhardness among the three composite adhesive systems .Vickers hardness test was used in investigate the microhardness of the enamel surface after debonding of brackets. The property ;of hardness is of major importance in the comparison of adhesive materials. Hardness is a measure of the resistance of a material to indentation, it is measured as a force per unit area of indentation [17]. Vickers hardness test is the most common method for testing the hardness of small areas and hard materials. Many researchers have studies adhesion to enamel, and at present, acid etching is probably the best method of bonding resins to enamel [19]. Unfortunately, demineralization of enamel surface around brackets specially at the gingival part of teeth are typical complications of orthodontics treatment [2,19] Microhardness measurement have been used together with other methods such as micro radiography, Vicker hardness test has been reported by many researchers to determine the microhardness of the tooth [11,20]. Microhardness is also useful tools to investigate the tooth surface properties. An optimal load is important to measure the hardness of an object by the microhardness test. The optimal load was considered to hardness of depend on the physical property of an object, when the hardness of dental tissues is compared, the measurement should be made under the same load. The optimal load was considered to be 100g. However, a value of the load should be small to

avoid plastic strain of an object when hardness of dental hard tissue is measured with the Vicker indenture [21].

The microhardness of the enamel has been reported to vary greatly from one tooth to another and in different area on the same tooth as in the present study on the labial surface of the upper and lower premolars before bonding (treatment) procedure as controlled teeth. This fact was supported by study which recorded range of hardness (242-440) in the result of true local differences in the hardness of the enamel and not attributed solely to the mechanical difference in obtaining satisfactory indentation [22]. Other study found that the microhardness of enamel near dentin was about (330) and in the outer shell of enamel, a maximum hardness value of 2050 but in mid-way through enamel (400-670) by using sectioning procedure from enamel of cusped teeth [23].

The slight variety in grand mean value of microhardness measurement between upper and lower control sample was (434) and (433).This seems to agree quite well with other finding [24]. Microhardness of table enamel of the maxillary teeth were slightly greater than those of the lingual enamel, while the opposite result was recognized in the average values of microhardness of labial enamel of the mandibular teeth. Also those result appear to be agreement with other study which showed that the maxillary molars and premolars had significantly less decalcification than their mandibular opponents after bonding and bonding [25].

From this variety in range of microhardness number in enamel surface either due to the direction of enamel prism or structural orientation and location across the surface. This fact of variety in range of microhardness in control group of samples reported by many authors finding [22,23,26].

The present study which find that (Advantage No-mix) and (Reliance orthodontic product) adhesive material exhibit more demineralization after eight weeks period times, although both of them have anti-cariogenic agent in there composition due to contain fluoride. There is significant linear relationship between (V.H.N) and demineralization process (loss of minerals) and this explanation supported by other studies [27,28]. There is non significant difference at level ($P>0.05$) between (Advantage No-mix) and (Orthodontic bonding system) after 2-4 weeks period times bonding in reduction of microhardness values in both upper and lower teeth. While high significant difference at level (P0.001) between of them in microhardness values after 8 weeks period times bonding in both upper and lower teeth. Incorporated fluoride in there composition of adhesive material provide better result include increase hardness and reduce solubility of enamel surface.

The orthodontic bonding system adhesive material not only inhibit demineralization but also demonstrate the ability to rematerialize enamel due to action of anti cariogenic agent which found in composition of this adhesive material.

We can concluded that the clinical significant of the present study is that enamel demineralization is associated with fixed orthodontic appliance. Therapy the result showed that the hardness was highest with group III (Orthodontic bonding system) followed by group I (Reliance orthodontic products) and group II (Advantage No-mix).

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