

Effect of saliva of patients with leukemia on surface hardness and roughness of enamel and dental composite resin during chemotherapy regimen: An – Invitro study

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Abstract

Background: Evaluate the surface roughness and hardness of tooth enamel and dental resin composite restoration immersed into saliva collected from leukemia patient.

Objective: To evaluate the surface roughness and hardness of tooth enamel and dental resin composite restoration immersed on to saliva collected from 4 types of leukemia patient.

Patients and Methods: 30 patients with 4 types of leukemia (Acute lymphoblastic leukemia ALL, Acute myeloid leukemia AML, Chronic lymphocytic leukemia CLL, and Chronic myeloid leukemia CML) were included for taking saliva separately. A total number of 50 enamel specimen and 50 of composite resin disk were prepared and randomly divided into 5 groups of enamel (1,2,3,4, and 5) and 5 groups of composite resin disk (A,B,C,D, and E), surface roughness and hardness were measured and data collected, group A and 1 were immersed in artificial saliva for 7 days as control, group B and 2 immersed into saliva collected from patient with ALL, group C and 3 immersed into saliva collected from patient with AML, group D and 4 immersed into saliva collected from patient with CLL and group E and 5 immersed into saliva collected from patient with CML all group for 7 days then surface roughness and hardness were measured data collected and analyzed and statistically significant regarded at $p < 0.05$.

Results: In group A and 1 artificial saliva there were no significant effect on surface roughness and hardness of both enamel and composite resin disk, while surface roughness and hardness of enamel and composite resin significantly changed after immersing in saliva of ALL, AML, CLL, patients and surface roughness of composite resin highly significantly increased after immersing in saliva of CML patients.

Conclusion: Saliva of ALL, AML, CLL, and CML seems to significantly changes in mechanical properties of enamel and composite, however saliva of CML highly significantly increase surface roughness of composite resin.

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Introduction

One of the malignant hematological disorders is leukemia distinguished by an uncontrolled proliferation of immature cell from blood [1]. There are two types of leukemia, acute and chronic types, subtypes of acute one are (Acute lymphocytic ALL and Acute myloid AML), and subtypes of chronic one are (Chronic lymphocytic CLL and Chronic myloid CML) according to clinical manifestation and cell types, in acute leukemia cells are immature, without function and with rapid growth and multiplication in the bone marrow, and in untreated patients may causing death [2,3]. Chronic form of leukemia (chronic lymphocytic CLL and chronic myloid CML) have appeared at slow rate and in adequate proliferation of mature, differentiated cell. One of the treatment lines for all types of leukemia is chemotherapy.

Among leukemic patients who received chemotherapy, prevalence and ubiquity of dental caries was 37.2% , dental caries may develop due to high dose of chemotherapy which lead to acidic pH and buffering it [4,5]. Saliva plays a role in maintaining oral health and is involved in protection, digestion, lubrication, facilitating oral processing, maintaining a neutral PH, and preventing tooth demineralization [6,7]. In malignancies, development of inflammatory oral pathologies may be due to oxidative stresses, the first line of defense against oxidative stress conciliate by free radicals is saliva, any changes in salivary flow may lead to xerostomia, ulceration and increase dental caries [8,9].

Particular oral examination and dental radiology are necessary in all leukemic patients to help potential orofacial disorder and defect to maintain a healthy oral status during chemotherapy regimens [10].

Dental caries in leukemic patient before, during and after chemotherapy usually should be treated with amalgam, composite or glass ionomer restoration according to extension and location of caries [11]. One of the important factors in keeping oral function, is saliva which consider a constant factor as deterrent in the subsequent occurrence of leukemia disease complication factors, any changes in salivary characteristics that results in different constitution of salivary component which may lead to a change in surface characteristic and morphology of dental enamel, composite resin restoration and implicitly, in the patients quality of life [1,12].

Demolishing and destroying oral mucosal tissue often involve the oral cavity in patients under chemotherapy regimen. One of the adverse effects of chemotherapy is decreasing immunity of patient which, lead to dysbiosis, infection and changing patients eating pattern and oral hygiene. Oral complications of chemotherapy include oral mucositis, opportunistic infection gingival inflammation and bleeding as well as xerostomia, so that increase susceptibility of caries. Usually low PH and buffering ability are found in patients treated with chemotherapy. Composite resin material provide a simpler and faster line in anterior and posterior restorative dentistry. Beside their advantage physical properties easily

affected by changing salivary characteristic [16]. Saliva with pH lower than 5 is sub saturated in fluoro-apatite and hydroxyapatite, and this create media for demineralization of enamel and subsequent reduce enamel hardness and enamel erosion [17,18]. The study objective was to evaluate the effect of saliva characteristic in leukemic patients on hardness and roughness surface of resin composite restorations and enamel surfaces.

Aims of the study: Evaluate the surface roughness and hardness of tooth enamel and dental resin composite restoration immersed on to saliva collected from 4 types of leukemia patient.

Patients and Methods

Saliva sampling and study population

From nanakaly hospital the ethics committee of study protocol was approved by (183\19.11.2022) in hematology department (erbil city/ Kurdistan). Agreement and assignmet were taken from all patients to participate in this our invitro study study.

A total number of 30 patients with mean age between 20-30 years, diagnosed with leukemia (acute lymphoblastic leukemia ALL, acute myloid leukemia AML, chronic lymphoblastic leukemia CLL and, chronic myloid leukemia CML) were included.

The gingivitis and periodontitis were included in our study criteria, the patients had at least 20 teeth, smoker, alcoholism, and tobacco user patients excluded. For saliva sampling, asked patient to seat in a relaxed position with their head bent forward in order to allow saliva to gather in the anterior region of the oral cavity.

Firstly, patient was directed not to eat, drink liquids and use chewing gum for 2 hours before the saliva was collected, secondly the patient was asked not swallowed and then saliva was collected in a polypropylene tube, as in Figure (1), and immersed in a laboratory water bath at temperature of 37C0 to pretend body temperature, and changed daily.

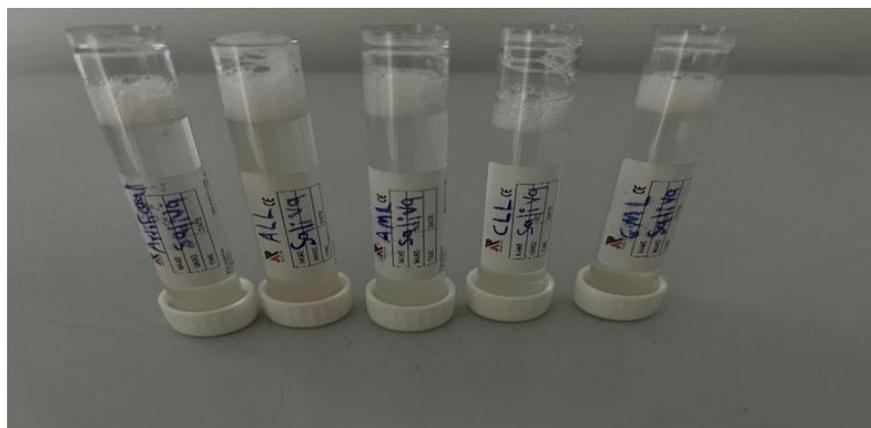


Figure (1): Saliva collection

Composite resin sample preparation

For this study nano hybrid and composite resin shad A2 (PROMEDICA Dental Material GmbH Domagkstr,

Neumuster/Germany) were used, for each type of composite resin we prepared 50 disk shaped specimen (1mm thick and 10 mm in diameter by using plastic mold. The mold

was placed over mylar strip on glass slid after filling the plastic mold with composite resin another mylar strip and glass slide were placed over the mold in order to force out excess composite resin material and voids also to acquired flat and smooth surface of specimens and then polymerized with wide spectrum curing LED unit for 30 second on 2

point in each side of samples after that it was separated from the mold, as in Figure (2) then specimens were finished and polished by finishing points and polishing cups (enhance system, dentsply, canlk.USA) all specimens were immersed in distilled water at 37C for 24 hours.



Figure (2): Composite disk preparation

Teeth selection and preparation

A total 25 extracted premolars were selected (upper or lower right or left premolar extracted due to orthodontic indication) to be used in this study, teeth washed by tap water and tooth brush to remove any debris and all teeth were cleaned using hand scalers and scalpels, and then their root were separated from crown at level of cemento enamel junction CEJ by a low-speed 0.28 mm diamond disk under water cooling as in Figure 3. The obtained crown sectioned

mesio distally to obtain a total of 50 piece of tooth (buccal and lingual part) by using 0.28mm diamond disk, each piece of crown (buccal and lingual) embedded in cold cure acrylic resin in custom made mold, enamel surface (buccal and lingual) surface facing upward in acrylic block. The test surface of each sample was then serially polished with 400-600 grit aluminum oxide abrasive paper in water-cooled mechanical grinder. All prepared samples placed in distilled water for 24 hours at 37C0.



Figure (3): Tooth specimen preparation

Sample grouping

All prepared samples (composite resin disks and teeth) divided in to 4 groups; 2 groups of composite resin disk and 2 group of teeth.

The surface hardness and surface roughness of each sample was measured before immersing samples into saliva by knoop hardness machine and surface roughness machine and data collected.

The teeth samples were divided as follow:

Group 1: 10 samples of teeth putted in side artificial saliva as control groups.

Group 2: 10 samples of teeth putted in side saliva of patient with ALL (acute lymphocytic leukemia)

Group 3: 10 samples of teeth putted in side saliva of patient with AML (acute myeloid leukemia)

Group 4: 10 samples of teeth putted in side saliva of patient with ALL (chronic lymphocytic leukemia)

Group 5: 10 samples of teeth putted in side saliva of patient with ALL (chronic myeloid leukemia)

The composite resin disks were divided as follow:

Group A: 10 specimen of composite resin disk putted in artificial saliva as control groups.

Group B: 10 specimen of composite resin disk putted in saliva of patient with ALL (acute lymphocytic leukemia)

Group C: 10 specimen of composite resin disk putted in saliva of patient with AML (acute myeloid leukemia)

Group D: 10 specimen of composite resin disk putted in saliva of patient with ALL (chronic lymphocytic leukemia)

Group E: 10 specimen of composite resin disk putted in saliva of patient with ALL (chronic myeloid leukemia)

All samples remain in saliva for 7 days [2], and each day a new saliva samples was taken and saliva media was changed.

The exposed samples to saliva were draw out from storage receiver and washed by distilled water to detach any trace, and then dried with a cotton and air syringe.

Surface roughness test

Evaluation of surface roughness was done by using arugosimeter (Hommel-Etamic W10; villingen- Schwenningen Germany). Roughness reading were execute at speed of 0.05mm\second and cut-off of 0.25mm, in three different point reading were performed which pass through the center of specimen then specimen rotated around 1200 figure 4, before and after placement on to saliva and mean of three reading were obtained and collected for analyzing.



Figure (4): surface roughness testing machine

Knoop hardness test

Surface hardness was measured on top surface in three different point of each specimen by using knoop micro hardness tester (HMV-G2oST shimadzn Corp; Tokyo, Japan) under load 25g for 5 second. Three indentations in each specimen (enamel and composite disk) were made, and mean of microhardness value were obtained before and after placement inside saliva. Indentation was carry out in 3 points, in middle, right and left side of specimen with 100 Mm from, the average of three reading were collected for analyzing.

Statistical Analysis

The obtained data were collected and analyzed by using SPSS version 20 by post hoc and ANOVA test of variance, P-Value <0.05 regarded as statistically significant.

Results

As in Table (1) showed that there was no significant change in surface roughness in group 1 before and after immersing specimens on to artificial saliva but slightly significant increase enamel surface roughness in group 2,3,4, and 5 after immersing specimens on to saliva of leukemia patients (ALL, AML, CLL, and CML) which is p<0.005.

Table (1): Mean and standard deviation of enamel surface roughness before and after immersing on saliva

Groups	Sample size	Mean+ SD	Mean+ SD	P- Value
	N	Before immersing	After immersing	
Artificial saliva 1	10	0.100 (0.031)	0.105 (0.033)	0.0
Saliva of ALL 2	10	0.198 (0.053)	0.201 (0.074)	0.00
Saliva of AML 3	10	0.188 (0.081)	0.198 (0.053)	0.00
Saliva of CLL 4	10	0.187 (0.067)	0.197 (0.054)	0.00
Saliva of CML 5	10	0.176 (0.064)	0.203 (0.067)	0.00

As in Table (2) showed that there were no significant reduction or decrease in enamel surface micro hardness in group 1 before and after immersing specimens on to artificial saliva which is P>0.05 but there was statistically significant reduction of enamel

surface micro hardness in group 2,3,4, and 5after immersing specimens on to saliva of 4 types of leukemia patients (ALL, AML, CLL, and CML) which is p value <0.005 (p=0.007).

Table (2): Mean and standard deviation of enamel surface micro hardness before and after immersing on saliva

Groups	Sample size	Mean+ SD		P- Value
		Before immersing	After immersing	
Artificial saliva 1	10	335.06 (13.65)	334.09 (13.5)	0.1
Saliva of ALL 2	10	323.28 (16.70)	316.93 (16.35)	0.00
Saliva of AML 3	10	320.60 (14.21)	311.77 (11.22)	0.00
Saliva of CLL 4	10	322.83 (17.19)	306.69 (13.06)	0.00
Saliva of CML 5	10	335.07 (13.64)	319.27 (17.77)	0.00

As in Table (3) showed that there was no significant increase in surface roughness of composite resin disk in group A after immersing in artificial saliva but there was significant increase in surface roughness in group B, C, and D after immersing specimen

in saliva of patients with ALL, AML, and CLL and highly significant increase in surface roughness of group E after immersing specimen in saliva of patient with CML which is P- value 0.0007.

Table (3): Mean and standard deviation of composite surface roughness before and after immersing on saliva

Groups	Sample size	Mean+ SD		P- Value
		Before immersing	After immersing	
Artificial Saliva A	10	0.476 (0.095)	0.477 (0.090)	0.0
Saliva of ALL B	10	0.474 (0.097)	0.501 (0.138)	0.00
Saliva of AML C	10	0.507 (0.153)	0.555 (0.157)	0.00
Saliva of CLL D	10	0.474 (0.124)	0.505 (0.169)	0.00
Saliva of CML E	10	0.299 (0.191)	0.474 (0.097)	0.000

According to Table (4) there was no significant difference in mean value of microhardness of composite disk in group A, before and after immersed specimen in artificial saliva but there was significant

reduction in micro hardness of composite disk in group A, B, C and D that immersed in saliva of leukemia patient of (ALL, AML, CLL, and CML) P value < 0.05.

Table (4): Mean and standard deviation of composite resin disk surface micro hardness before and after immersing on saliva

Groups	Sample Size	Mean+ SD		P- Value
		Before immersing	After immersing	
Artificial Saliva A	10	58.4 (2.1)	58.2 (1.6)	0.0
Saliva of ALL B	10	61.2 (1.9)	55.3 (2.6)	0.00
Saliva of AML C	10	56.01 (2.1)	48.5 (2.1)	0.00
Saliva of CLL D	10	65.02 (3.7)	56.4 (1.8)	0.00
Saliva of CML E	10	57.2 (1.8)	49.5 (1.6)	0.00

Discussion

One of the base line treatments of leukemia for all four types are chemotherapy.

Chemotherapy has many of side effect, one of them usually impairs salivary gland function, which the features of saliva (quality and quantity) are altered, increased levels of

amylase and peroxides are observed [19]. In the present studies we investigate the effects of leukemia patient's saliva (ALL, AML, CLL, and CML) on surface roughness and hardness of enamel and dental composite resin restoration. Protein and ions provide main constitutions of saliva, although pH of saliva is the strongest determination for saturation level leading to de mineralization or remineralization of teeth under clinical condition., the interaction of phosphate ions and the hydroxyl ion at acidic pH of saliva in the tooth- biofilm interface by forming complex such as hydrogen phosphate and water, thus saliva becomes unsaturated with regard to phosphate ions which lead to dissolution of hydroxy apatite crystallin enamel in an attempt to re saturate saliva [20,21]. As in our study saliva of leukemia patient could alter the mechanical properties of enamel and composite resin restoration. Also, chemotherapy affect basal and stimulated saliva in different manner. The pH of basal saliva presented without changes while the stimulated saliva pH become acidic [22]. Also the level of urea also increases, moreover electrolytic changes also detected in leukemic patient treated by chemotherapy. This alteration in mechanical properties include surface roughness and hardness may be due to alteration in salivary pH and level of urea, and this is agreed with results obtained by Losif *et al* 2022. Increasing polymer erosion usually occurred in low salivary pH (acidic saliva) which lead to damaging of composite resin restoration [22]. Surface roughness was measured in this research since it is important mechanical properties in many dental restorative materials which change due to the influence

of pH change of saliva [22,23]. In this research there was non-significant changes in surface roughness and surface hardness of both enamel teeth specimens' group and composite resin disk groups which immersed on to artificial saliva. But there was significant increase in surface roughness of enamel teeth and composite resin disk group which immersed on to leukemic saliva for all types (ALL, AML, CLL, and CML) this results agreed with result by Pribadi and Soetojo 2011. Changing physical and mechanical properties of dental materials is influenced by multiple factors most important one is saliva [24]. Saliva contains composition micro elements dissolved salts, enzymes, proteins, amino acids vitamins, mucus as well as other substance and high percent of water. Hydrolysis and enzyme reaction mechanisms of salivary element can influence the chemical humiliation and destroying of dental materials [2,20]. According to Masetar *et al* 2021 acute lymphoblastic leukemia ALL s saliva have strong influence on the surface changes of the composite resin [2]. As this research investigation showed that surface roughness of composite resin increased in all types of leukemia, this is may be related to change in salivary constitution during chemotherapy regimen which lead to changing some components of the saliva which are able to significantly changing and deteriorating the surface of the composite [25]. In one study the ratio of Na, K, Mg, P, and Ca elements and Ca\P weigh ratio analyzed of the primary and permanent teeth hard tissue which observe the radiation therapy lead to irregular increase and decrease of these element and lead to significant effects on enamel

microhardness [2,26]. Researchers in recent years, have focused on the effects of direct radiation and chemotherapy on dental hard tissue [27,28,32]. In present study there is significant reduction in enamel and dental composite resin disks hardness after immersing specimen on saliva of patient with ALL, AML, CLL, and CML these changes may be due to changes in salivary electrolytes, elements and low pH (acidic saliva). Because demineralization was responsible for reduction of microhardness value of enamel and composite resin. Important contemplating factors in selecting a restoration material are mechanical properties [29,33]. The strangeness of restorative materials must be sufficient to with stand the stress of mastication because they are used to build up and restoring of tooth structure [30,32,33]. In this study microhardness of composite resin disk significantly reduce as, Elad et al. concluded that low pH acidic environment had significantly effect on hardness of CAD/CAM materials when exposed for 7 day [31]. However there were no more studies about the effect of salivary of four types of leukemia patients on hardness and roughness of enamel and composite resin.

Conclusions

According to results of this in vitro study showed that artificial saliva have no or mild effect on the surface of dental composite and surface of enamel roughness and micro hardness but saliva from leukemia ALL, AML, CLL, and CML lead to the surface micro hardness and surface roughness of both dental composite and enamel.

Recommendations

However more research in the assessment of salivary change during and after chemotherapy regimen in leukemic patients and it is effect on tooth structures and properties of dental restoration are needed in order to have clear conclusions.

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Ethical clearance: ethical approvals were obtained from the Oncology Department in Nanikaly Hospital \ Erbil Kurdistan, and College of Dentistry \ Hawler Medical University.

Conflict of interest: Nil

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تأثير لعاب مرضى اللوكيميا على صلابة وخشونة السطح للمينا ورائج الاسنان (كومبوست ريسن) المركب اثناء العلاج الكيميائي, دراسة مختبرية

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الملخص

خلفية الدراسة: تقييم خشونة السطح و صلابة مينا الاسنان و ترميم استعادة مركب رائج الاسنان المغمور في اللعاب الذي جمعه من مرضى اللوكيميا.

اهداف الدراسة: لتقييم خشونة السطح و صلابة مينا الأسنان و ترميم مركب رائج الأسنان المغمور في اللعاب الذي تم جمعه من 4 أنواع من مرضى سرطان الدم.

المرضى والطرائق: تم تضمين 30 مريضاً يعانون من اربعة انواع من اللوكيميا (سرطان الدم الليمفاوي الحاد, سرطان الدم النخاعي الحاد, ابيضاض الدم اللمفاوي المزمن و سرطان الدم النخاعي المزمن) للاخذ لعاب بشكل منفصل تم تحضير اجمالي عدد 50 عينة من المينا و 50 من قرص الرانتج المركب و قسمت بشكل عشوائي الى 5 مجموعات من المينا و 5 مجموعات من قرص الرانتج المشترك, تم قياس خشونة السطح و الصلابة و تم جمع البيانات, تم غمر المجموعة أ و في اللعاب الصناعي لمدة 7 أيام كعنصر تحكم, تم غمر المجموعة ب و 2 عن لعاب مرضى سرطان الدم النخاعي الحاد, المجموعة ج و 3 تم غمرهم في لعاب مرضى سرطان الدم النخاعي الحاد, و تم غمر المجموعة د و 4 في لعاب مرضى ابيضاض الدم الليمفاوي المزمن و المجموعة ي و 5 تم غمرها في لعاب مرضى سرطان الدم النخاعي المزمن, و تم غمر جميع المجموعات لمدة 7 أيام ثم تم قياس خشونة السطح و الصلابة, و تم جمع البيانات و تحليلها و النظر فيها احصائي عند $P < 0.05$.

النتائج: في المجموعة ع و 1 (اللعاب اللصطناعي) لم يكن هناك تأثير علي خشونة السطح و صلابة كل من المينا و قرص الرانتج المركب بينما تغيرت خشونة السطح و صلابة المينا و الرانتج المركب بشكل ملحوظ بعد الغمر في لعاب المرضى المصابين بسرطان الدم, بينما خشونة سطح الرانتج المركب زادت بشكل ملحوظ جداً بعد الغمر في لعاب سرطان الدم النخاعي المزمن.

الاستنتاجات: يبدو ان لعاب مرضى اللوكيميا يتغير بشكل كبير في الخواص الميكانيكية للمينا و المركب, ومع ذلك لعاب مرضى مصابين بسرطان الدم المزمن يزيد بشكل كبير من خشونة سطح الرانتج المركب.

الكلمات المفتاحية: اربعة انواع من سرطان الدم, العلاج الكيميائي, خشونة السطح و الصلابة, المينا, الرانتج المركب لاسنان

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