

The effect of photoactivated Riboflavin on primary dentin remineralization: a pilot study

Beril MURATOGLU ^{1*} , Aysu AYDINOGLU ² , Afife Binnaz HAZAR YORUC ³ , Betül KARGUL ⁴ 

¹ Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Türkiye.

² Department of Metallurgical and Materials Engineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Istanbul, Türkiye.

³ Department of Metallurgical and Materials Engineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Istanbul, Türkiye

⁴ Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Türkiye.

* Corresponding Author. E-mail: beril_muratoglu89@hotmail.com (B.M.); Tel. +00-542-427 85 45.

Received: 20 December 2022 / Accepted: 24 December 2022

ABSTRACT: This study aims to evaluate the effect of photoactivated riboflavin (RF) on demineralized dentin of the primary molars. Fifty primary molars were selected for the study. The specimens were divided into five groups based upon the remineralization of dentin with photoactivated 0.1% RF. In Group I (n = 20), dentin remineralization was done with photoactivated 0.1% RF for 30 s, in Group II photoactivated 0.1% RF for 60 s. In Group III (positive control), dentin remineralization was done with remineralization solution. In Group IV (negatif control) only used distilled water. Surface Microhardness analysis (SMH) was evaluated using Vickers microhardness tester (EmcoTest, Duravision 20 G5, Vickers, DV250539, Mechanical Instrument Hardness Tester, Germany). D-Light Pro offers a unique Detection Mode based on near-UV light only. Data was analyzed with One way ANOVA test, Tukey's test, Student's t-test, Mann-Whitney test, Dunn test and Kruskal-Wallis test were used. The SMH values of test groups were significantly higher than those of the negative control group ($p = 0.01$). The %SMHR values of Group III was significantly higher than Group I and II. The comparison between groups showed no statistically significant differences in %SMHR among Groups I with Group II and IV ($p > 0.05$). According to the findings of this study; we concluded that Riboflavin have a significant effect on demineralized dentine. The remineralization effect of riboflavin on demineralized dentin needs to be relevant to further studies.

KEYWORDS: Riboflavin; Dentin; Remineralization; Microhardness; Primary teeth

1. INTRODUCTION

Dental caries is a preventable disease. On the other hand it is still remains most prevalent disease and are high-priced, currently compose an increased global financial burden, with significant differences between countries [1,2,3]. Dentin is a dental hard tissue and mainly composed of hydroxyapatite (HA), non-collagenous matrix proteins and collagen [4]. The dentin matrix is mainly be formed of type I collagen fibrils with related noncollagenous proteins, forming a three-dimensional matrix that is strengthened by minerals [5]. Dental caries is a dynamic process of rapidly interchangeable periods of pathological demineralization which is a loss in mineral content will occur and physiological protective remineralization [4,6,7].

Dentin collagen has an important role in the remineralization of demineralized dentin [8] Previous studies found that crosslinking treatment of demineralized dentin collagen with cross linking agents depends on time [9]. Understanding the course of action, intensity and content of the demineralisation processes of human dentine in-vivo, will enable the scientists to create predictable study models for in-vitro researches of these processes and in this way look for effective repair or remineralisation strategies that are more easily translatable as clinical interventions [10]. Remineralization of carious dentin can happen either by an inherent including of ions (calcium, phosphate and fluoride) from the oral fluid onto residual crystallites in the demineralized tissue or by treatments that incorporate the same ions from external sources [11].

Riboflavin is an crosslinking antioksidant and water-soluble vitamin B2 extract; involved in body cell growth and function [12].

Riboflavin is a crosslinker and activated by UVA. Riboflavin is a treatment method for keratokonius [13]. Corneal Cross linking is a procedure that can inhibit the progression of keratoconus. Riboflavin is a photosensitizer. Riboflavin

How to cite this article: Muratoglu B, Aydinoglu A, Hazar Yoruc AB, Kargul B. The effect of photoactivated Riboflavin on primary dentin remineralization: a pilot study. J Res Pharm. 2023; 27(3): 1015-1020.

activated by UV light induces covalent bonds, otherwise known as crosslinks, between collagen fibrils. Riboflavin, has proven to support collagen type I crosslinking [14,15]. These crosslinks eventually help patients restore visual acuity [16]. Riboflavin is biocompatible and it has ability to produce free radicals when photo-activated by light which has spectral range from uv to visible light [12,16]. The covalent crosslinks among the neighbouring collagen molecules forms by these free radicals, or reactive oxygen species like O_2 and O_2^- [17].

The aim of this in vitro study is to evaluate the effect of photoactivated 0.1% Riboflavin on primary molar dentin remineralization using surface microhardness measurements (SMH).

2. RESULTS

The mean and standard deviations (SD) of SMH of dentin values at baseline, demineralization and after different photoactivation time of photoactivated 0.1% RF treatment and level of significance for all groups were shown in Table 1. After photoactivated 0.1% RF, the mean SMH values of all test groups were significantly higher than those of the negative control group ($p = 0.01$), and Group I (30 s. photoactivated 0.1% RF treatment) showed the highest mean SMH values among test groups.

Table 1. Surface microhardness test results of dentin specimens.

Study Group	Baseline Mean (SD)	After Demineralization Mean (SD)	After Riboflavin Treatment Mean (SD)	p
Group I 30s photoactivated RF	75.68 ± 7.79	38.14 ± 4.02	42.34 ± 4.16	0.001
Group II 60s photoactivated RF	55.3 ± 5.88	27.66 ± 3.56	32.39 ± 3.87	0.001
Group III Positive control	48.43 ± 6.22	25.9 ± 3.73	33.47 ± 3.9	0.001
Group IV Negative control	59.14 ± 6.65	30.31 ± 4.32	32.29 ± 4.66	0.01

^aSignificance difference $p < 0.05$. SD: standard deviation

The percentage recovery of SMH (%SMHR) among groups were shown in Table 2. There was statistically significant difference of % SMHR values between all groups at after photoactivated 0.1%RF treatment ($p=0,001$) (Table 2). The lowest percentage recovery of SMH in negatif control, whereas the highest mean value %SMHR was found in positive control which is 34.76 ± 20.62 (Table 2).

Table 2. The %SMHR results after photoactivated 0.1%RF treatment of dentin specimens.

Study Groups	%SMHR Mean (SD)	p
Group I 30s photoactivated RF	11.49 ± 6.95	0,001*
Group II 60s photoactivated RF	16.62 ± 11.93	
Group III Positive control	34.76 ± 20.62	
Group IV Negative control	6.77 ± 9.35	

^bKruskal Wallis and Dunn test. ($p < 0.05$), SD = standard deviation.

Nevertheless, the comparison between groups showed no statistically significant differences in %SMHR among Groups I with Group II and IV ($p > 0.05$), as shown in Table 3. Meanwhile, on analysis for multiple comparisons between various groups, statistically significant difference was seen in values of %SMHR at the photoactivated 0.1%RF treatment stage between Group I with Group III; Group 2 with Group III and IV; and Group III with Group IV (Table 3).

Table 3. Analysis of intergroup comparisons of the %SMHR

%SMHR	Group I 30s photoactivated RF	Group II 60s photoactivated RF	Group III Positive control	Group IV Negative control
Group I 30s photoactivated RF		0,151	0,001*	0,072
Group II 60s photoactivated RF			0,005*	0,001*
Group III Positive control				0,001*

3. DISCUSSION

Regarding in restorative dentistry towards a minimally invasive techniques has totally changed the manner in which dentinal caries is handled [21]. In this study, the demineralization and remineralization process was used to imitate caries formation that occur in oral cavity [19].

Dentin lesions has become after 7 days of demineralizing period in primary teeth, which is the most appropriate time for this study [19,20]. Demineralized dentin is mainly composed of collagen fibrils [22]. The organic matrix of dentin contains noncollagenous proteins (NCPs), which play a very important role in the biomineralization of dentin [23]. Dentin caries lesions results in reduced mineral contents, means that has been diminished mechanical properties [24]. In an attempt to evaluate the conditions found in artificial caries-affected dentin, several methods of producing caries-like lesions in vitro have been developed [25].

Riboflavin is used to improve the chemical, physical and antimicrobial properties of demineralized dentin [22]. Meanwhile, the clinical applicability of riboflavin activated using UVA is a concern [13]. The usage of riboflavin and UVA irradiation to crosslink corneal collagen in vivo has been approved by the U.S. Food and Drug Administration in 2016. The absorbance UV/VIS spectrums of riboflavin: two in the UV (230, 260 nm), one at 370 nm and one at 450 nm [24,25].

Therefore the tungsten/halogen lamp curing units which are frequently used in dental offices, the blue light can also be used for photoactivation [13]. The alternatives to the UVA light source, like conventional blue-light halogen-lamp curing units, can activated riboflavin, by means of its ready availability and its ease and using safely in dentistry [13]. The use of UVA has reported that it is been using effectively as a photoactivator of riboflavin for crosslinking but for dental use security measures and its practicality should be noted [13,17].

As well as its ideal curing functions, D-Light Pro also has a unique Detection mode (DT) that uses near-UV light only. Thus, UV light at 400 nm used in the present study would match the riboflavin 370 nm peak. The use of 30 s of blue light to activate riboflavin in the current study was identical to that used by Fawzy et al. [13] and Abunawareg [26]. As well as its ideal curing functions, D-Light Pro also has a unique Detection mode (DT) that uses near-UV light only.

RF has been shown to enhance the microhardness of demineralized dentin by promoting chemical modifications of the collagen. The photoactivated RF treatments induced chemical modifications in the dentin collagen and revealed remineralizing effects. In the present study, the microhardness of dentin was increased by 30 and 60 s photoactivated-RF treatment. The 60 s photoactivated-RF yielded the most pronounced remineralization under the in vitro conditions chosen. Therefore, the use of RF can be preferred within the concept of supporting the increase of dentin microhardness. Whereas, the 30 and 60 min UVA-activated riboflavin resulted in a significant increase in mechanical properties when compared to the untreated dentin samples. Additionally, the 30 and 60 min UVA-activated riboflavin treatment concluded in chemical modifications in the dentin collagen observed by spectroscopic analysis [27].

Nevertheless, as we had controlled all factors, that could affect the examination of SMH values, like sample preparation it was found that the difference of SMH values between control and all test groups at baseline and after riboflavin treatment was significantly different ($p > 0.05$) [19].

To improve the long-term strength of resin-dentin interfaces, strategies to reduce protease inhibitors activity on dentin, as collagen cross-linking agents, such as riboflavin, glutaraldehyde, proanthocyanidin, grape-seed extract, tannic acid, carbodiimide, have been suggested [28].

Recent studies suggest that the organic matrix degradation process starts with revealing of cleavage sites of collagen fibers and also have show that the proteolytic enzymes are inactivated when pH decreases [29,30]. Dentin matrices comprise proteolytic enzymes, the most well-known group being matrix metalloproteinases (MMPs) that were inactive in mineralized dentin [31]. Cross linking is nonspecific for the inactivation of proteolytic enzymes [26].

Moreover, in this study we suggested that the dental visible blue curing light could be regarded to be a efficient and safer substitute compared to the UVA activated RF [18].

4. CONCLUSION

In conclusion, within the limitations of the experiments performed, photoactivated-RF treatment increased the microhardness of demineralized dentin, possibly because of increased crosslinking of the dentin collagen matrix.

5. MATERIALS AND METHODS

5.1 Specimen preparation

The extracted human primary molars were selected for this study following approval by the Research Ethics Committee at the Dental School, University of Marmara (2018-235).

All experiments in this study involved caries free primary molars without any previous restorations that were extracted for surgical reasons. The specimens were kept in 0.1% thymol at 4°C prior to the experiment to prevent any microbial growth and were used within one month of extraction.

Each tooth was embedded in epoxy resin and the occlusal dentin surface was exposed (Struers® EpoFix Kit) for microhardness measurements. Dentine blocks (5x4x5 mm) were prepared from coronal dentine by sectioning with a slow speed precision blade saw (Discoplan-TS, Struers TS-Method™) with a diamond wafering blade. The blocks were placed in a polisher (Struers -Rotapol 35, 50-500 rpm, Copenhagen, Denmark), followed by ultrasonically cleaning in deionized water for 15 min to remove the residues. Each dentine sample was partitioned into 3 parts using nail varnish (Nail enamel, Flormar, Turkey).

5.2 Surface treatment and remineralization agents

From a total of 40 specimens of primary teeth randomly divided into four groups, Group I: photoactivated 0.1% RF treatment for 30 s 400 nm, Group II: photoactivated 0.1% RF treatment for 60 s 400 nm, Group III: positive control (remineralization solution), Group IV: negative control (deionized water). The demineralizing solution consisted of 1.5 mM of CaCl₂, 0.9 mM of KH₂PO₄, 50 mM of acetic acid and 0.02% of NaN₃ with the pH value adjusted at 4.5 using NaOH) at 37°C for 3 days. Following this, the samples were rinsed thoroughly with deionized water. The remineralizing solution consisted of 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCl and adjusted to a pH of 7.0 with 1 M KOH. The demineralizing and remineralizing solutions were freshly prepared for each process and kept separately in the containers for each group throughout the study [19,20] The other experimental groups were treated with the 0.1% Riboflavin solution irradiated by 400 nm photoactivation (D-Light® Pro-GC Europe).

5.3 Surface Microhardness (SMH) analysis

Surface Microhardness analysis (SMH) was measured at the baseline, after the demineralization and after remineralization for each group using a Vickers microhardness tester (EmcoTest, Duravision 20 G5, Vickers, DV250539, Mechanical Instrument Hardness Tester, Germany).

Vickers microhardness (VHN) of the dentin was measured in a moist state immediately after removing from distilled water. The specimens were placed under the Vicker indenter of a microhardness tester and subjected to a load of 50 g and dwell time of 10 s at each test point. Three measurements were made for each sample and the mean value was established as the VHN and the mean of 3 measurements was calculated for each sample. The percentage recovery of the surface microhardness (%SMHR) was then calculated.

SMHR- Surface MicroHardness Recovery (S= initial microhardness value, S1= after demineralization, S2= after treatment)

$$SMHR \% = \frac{100(S_2 - S_1)}{S_1 - S}$$

Figure 1. The formulation of surface Microhardness Recovery

5.4 Statistical analysis

The one-way analysis of variance (ANOVA) was used to compare the SMH values at baseline, before, and after pH-cycling. Data were analyzed with SPSS statistical software (SPSS 22 package program). Shapiro-Wilk was used to evaluate normality. It was determined that the groups had a normal distribution ($p < 0.05$). The data analysis was set at 0.05 for significant level in all tests. Tukey's multiple comparison tests were used to test the difference of mean SMH and percentage recovery of SMH among groups. In the subsequent stages of analyses Dunn test and Kruskal-Wallis test were used.

Acknowledgements: I funded all requirements for the research.

Author contributions: Concept B.M., B.K.; Design B.M., B.K.; Supervision B.K.; Resources B.M., B.K., A.A., A.B.H.Y.; Materials B.M.; Data Collection and/or Processing B.M., A.A.; Analysis and/or Interpretation B.M., A.A., A.B.H.Y., B.K.; Literature Search B.M., B.K.; Writing B.M., B.K.; Critical Reviews B.M., B.K., A.A., A.B.H.Y.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- [1] Manton DJ Child dental caries a global problem of inequality. *E Clinical Medicine* 1 2018: 3-4. <https://doi.org/10.1016/j.eclinm.2018.06.006>
- [2] Meier, T, Deumelandt, P, Christen, O, Stangl, G I, Riedel, K, Langer, M. Global burden of sugar-related dental diseases in 168 countries and corresponding health care costs. *J Dent Res.* 2017; 96(8): 845-854. <https://doi.org/10.1177/0022034517708315>
- [3] Banerjee A, Frencken JE, Schwendicke F, Innes NPT. Contemporary operative caries management: consensus recommendations on minimally invasive caries removal. *Br Dent J.* 2017; 223(3): 215-222. <https://doi.org/10.1038/sj.bdj.2017.672>
- [4] Chen R, Jin R, Li X, Fang X, Yuan D, Chen Z, Yao S, Tang R, Chen Z. Biomimetic remineralization of artificial caries dentin lesion using Ca/P-PILP. *Dent Mater.* 2020 ;36(11):1397-1406. <https://doi.org/10.1016/j.dental.2020.08.017>
- [5] Bertassoni LE, Habelitz S, Kinney JH, Marshall SJ, Marshall GW Jr. Biomechanical perspective on the remineralization of dentin. *Caries Res.* 2009; 43(1):70-7. <https://doi.org/10.1159/000201593>
- [6] Hashem M. Antimicrobial capacity and physico-chemical characteristics of adhesive resin containing riboflavin after photodynamic therapy. *Photodiagnosis Photodyn Ther.* 2021; 33: 102145. <https://doi.org/10.1016/j.pdpdt.2020.102145>
- [7] Kranz S, Heyder M, Mueller S, Guellmar A, Krafft C, Nietzsche S, Tschirpke C, Herold V, Sigusch B, Reise M. Remineralization of artificially demineralized human enamel and dentin samples by zinc-carbonate hydroxyapatite nanocrystals. *Materials (Basel).* 2022; 14;15(20): 7173. <https://doi.org/10.3390/ma15207173>
- [8] Firouzmandi M, Shafiei F, Jowkar Z, Nazemi F. Effect of silver diamine fluoride and proanthocyanidin on mechanical properties of caries-affected dentin. *Eur J Dent.* 2019; 13(2): 255-260. <https://doi.org/10.1055/s-0039-1693237>
- [9] Liu R, Fang M, Xiao Y, Li F, Yu L, Zhao S, Shen L, Chen J. The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin. *J Mater Sci Mater Med.* 2011; 22(11): 2403-11. <https://doi.org/10.1007/s10856-011-4430-4>
- [10] Miller CA, Ashworth E, Deery C, El Sharkasi L, Moorehead RD, Martin N. Effect of demineralizing agents on organic and inorganic components of dentine. *Caries Res.* 2021; 55(5): 521-533. <https://doi.org/10.1159/000518463>
- [11] Ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. *Crit Rev Oral Biol Med.* 1991; 2(3): 283-296. <https://doi.org/10.1177/10454411910020030101>
- [12] Al-Kheraif AA, Mohamed BA, Khan AA, Al-Shehri AM. Role of Riboflavin; Curcumin photosensitizers and Ozone when used as canal disinfectant on push-out bond strength of glass fiber post to radicular dentin. *Photodiagnosis Photodyn Ther.* 2022; 37: 102592. <https://doi.org/10.1016/j.pdpdt.2021.102592>
- [13] Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, Neo J. Riboflavin as a dentin crosslinking agent: Ultraviolet A versus blue light. *Dent Mater.* 2012; 28(12): 1284-1291. <https://doi.org/10.1016/j.dental.2012.09.009>

- [14] Agrawal VB. Corneal collagen cross-linking with riboflavin and ultraviolet - a light for keratoconus: results in Indian eyes. *Indian J Ophthalmol*. 2009;57(2):111-114. <https://doi.org/10.4103/0301-4738.44515>
- [15] Wollensak G, Aurich H, Wirbelauer C, Sel S. Significance of the riboflavin film in corneal collagen crosslinking. *J Cataract Refract Surg*. 2010; 36(1): 114-120. <https://doi.org/10.1016/j.jcrs.2009.07>
- [16] Hatami-Marbini H, Jayaram SM. Effect of UVA/Riboflavin collagen crosslinking on biomechanics of artificially swollen corneas. *Invest Ophthalmol Vis Sci*. 2018; 1;59(2):764-770. <https://doi.org/10.1167/iovs>
- [17] Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol*. 2003;135(5):620-627. [https://doi.org/10.1016/s0002-9394\(02\)02220-1](https://doi.org/10.1016/s0002-9394(02)02220-1)
- [18] Snibson GR. Collagen cross-linking: A new treatment paradigm in corneal disease - A review. *Clin Exp Ophthalmol*. 2010;38(2):141-153. <https://doi.org/10.1111/j.1442-9071.2010.02228.x>
- [19] Kasemkhun P, Rirattanapong P. The Efficacy of non-fluoridated toothpastes on artificial enamel caries in primary teeth: An in vitro study. *J Int Soc Prev Commun Dent*. 2021;3;11(4):397-401. https://doi.org/10.4103/jispcd.IJSPCD_64_21
- [20] Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. *Dent Mater J*. 2013;32(1):138-143. <https://doi.org/10.4012/dmj.2012-243>
- [21] Betancourt F, Kiss A, Krejci I, Bortolotto T. ToF-SIMS analysis of demineralized dentin biomodified with calcium phosphate and collagen crosslinking: Effect on marginal adaptation of class V adhesive restorations. *Materials (Basel)*. 2021; 12;14(16): 4535. <https://doi.org/10.3390/ma14164535>
- [22] Tay FR, Pashley DH. Guided tissue remineralisation of partially demineralised human dentine. *Biomaterials*. 2008; 29(8): 1127-1137. <https://doi.org/10.1016/j.biomaterials.2007.11.001>
- [23] Uemura R, Miura J, Ishimoto T, Yagi K, Matsuda Y, Shimizu M, Nakano T, Hayashi M. UVA-activated riboflavin promotes collagen crosslinking to prevent root caries. *Sci Rep*. 2019; 4;9(1): 1252. <https://doi.org/10.1038/s41598-018-38137-7>
- [24] Abuelenain DA, Abou Neel EA, Abu-Haimed T. Effects of dentin modifiers on surface and mechanical properties of acid-etched dentin. *Int J Adhes Adhes*. 2018; 81: 43-47. <https://doi.org/10.1016/j.jadhadh.2017.11.006>
- [25] Koziol J. Studies on flavins in organic solvents-1*. Spectral characteristics of riboflavin, riboflavin tetrabutylate and lumichrome. *Photochem Photobiol*. 1966; 5(1): 41-54. <https://doi.org/10.1111/j.1751-1097.1966.tb05759.x>
- [26] Abunawareg M, Abuelenain DA, Elkassas D, Haimed TA, Al-Dharrab A, Zidan A, Pashley D. Role of dentin cross-linking agents in optimizing dentin bond durability. *Int J Adhes Adhes*. 2017, 78: 83-88. <https://doi.org/10.1016/j.jadhadh.2017.06.009>
- [27] Liu X, Zhou J, Chen L, Yang Y, Tan J. UVA-activated riboflavin improves the strength of human dentin. *J Oral Sci*. 2015; 57(3): 229-234. <https://doi.org/10.2334/josnusd.57.229>
- [28] Gajjela RS, Satish RK, Sajjan GS, Varma KM, Rambabu T, Vijaya Lakshmi BH. Comparative evaluation of chlorhexidine, grape seed extract, riboflavin/chitosan modification on microtensile bond strength of composite resin to dentin after polymerase chain reaction thermocycling: An in vitro study. *J Conserv Dent*. 2017; 20(2): 120-124. <https://doi.org/10.4103/0972-0707.212241>
- [29] Balalaie A, Rezvani MB, Mohammadi Basir M. Dual function of proanthocyanidins as both MMP inhibitor and crosslinker in dentin biomodification: A literature review. *Dent Mater J*. 2018; 30;37(2): 173-182. <https://doi.org/10.4012/dmj.2017-062>
- [30] Firouzmandi M, Vasei F, Giti R, Sadeghi H. Effect of silver diamine fluoride and proanthocyanidin on resistance of carious dentin to acid challenges. *PLoS One*. 2020; 17;15(9): e0238590. <https://doi.org/10.1371/journal.pone.0238590>
- [31] Seseogullari-Dirihan R, Mutluay MM, Vallittu P, Pashley DH, Tezvergil-Mutluay A. Effect of pretreatment with collagen crosslinkers on dentin protease activity. *Dent Mater*. 2015; 31(8): 941-947. <https://doi.org/10.1016/j.dental.2015.05.002>

This is an open access article which is publicly available on our journal's website under Institutional Repository at <http://dspace.marmara.edu.tr>.