Experimental studies on the pH levels that affect demineralization and remineralization of human tooth enamel

Sherzad Abdulrahman Muhammad[(1)]; Bakhtiar Muhialdin Ahmed[(1)]

Background and Objective: It is thought that demineralization of enamel occurs below pH 5.5. We hypothesize that it also happens at higher levels. Furthermore, the effective pH levels for demineralization and remineralization differ from person to person. The outcome of this study can help in planning new preventive strategies regarding tooth decay. Ths study aimed to detect the pH levels that influence the demineralization and remineralization of enamel.

Materials and Methods: Ninety-five participants of one age group (19-34 years old) were involved in this case-control study and divided into a caries-free group, very low-caries group and low-to-moderate caries group by relying on the DMFT value as a criterion for the diagnosis of dental caries. Forty previously extracted caries-free teeth were exposed to buffered saliva samples of the participants from the three groups at 37 ^{o}C for 24 hours. The concentration of calcium loosed or gained from the teeth was assessed using a spectrophotometer. A correlation test and regression plot were used for the data analysis.

Results: Significant correlations were obtained between the pH levels (4.6 to 7.0) and the demineralization/remineralization of enamel. Moreover, regression plots between the pH and calcium concentrations resulted in new pH points that may play effective roles in the demineralization and remineralization processes.

Conclusion: New pH levels have been obtained regarding demineralization and remineralization of enamel. Responses to fluctuations in these pH levels differ from person to person, which in turn lead to a variation in the incidence and severity of tooth decay.

Keywords: Calcium, Demineralization, pH, Remineralization, Saliva.

[(1)]Department of Basic Science, College of Dentistry, Hawler Medical University, Erbil, Iraq.
Corresponding author: Sherzad A. Muhammad
Email: sherzad7479@outlook.com

Introduction

The devastation of enamel as a result of either bacterial action on carbohydrates (known as caries) or the direct impact of acidic foods and beverages (called erosion) initiates the demineralization of enamel.[1,2] The power of hydrogen pH is one of the effective factors that can contribute to mineral loss from tooth enamel.[3] According to previous studies, tooth enamel begins losing its minerals when it is exposed to pH levels below 5.5.[4,5] The aims of this study are to investigate whether demineralization occurs above pH 5.5, to detect new possible critical pH points concerning tooth decay for three groups of people with different decay experiences, and to specify a safe zone pH scale for each group in which no demineralization (only remineralization) of tooth enamel occurs. Despite the availability of several well-known brands of toothpaste, mouthwash, and other dental care products, people still face tooth decay.[6] This study seeks to answer the question of why people whose sali-

vary pH is more than pH 5.5 and who brush their teeth with toothpaste become victims of tooth decay. Regardless of the availability of tooth care tools along with many years of published studies on dental health, tooth decay is still the most common oral disease across the world.[7]

The human tooth is divided into four parts. The first part, enamel, is the hardest tissue, made mostly from inorganic components in the form of biological hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ crystals.[8] Enamel gradually undergoes wear and this leads to tooth decay because it is the outer part and is exposed directly and frequently to oral environment changes.[9] The other parts are dentin, which is the majority of the root, then pulp, the soft tissue that nourishes the dentin, and the cementum, which holds the tooth in place. Unlike enamel, cementum and dentin continuously form over the life course.[10] Mature enamel combines both organic and inorganic components and comprises almost entirely minerals, about 95% by volume. It has more inorganic content than other hard tissues such as dentin, cementum, and even bone.[11] Dental caries and erosion are results of enamel demineralization, which in turn is due to several factors, principally pH levels around and below 5.5.[4] These are the pH levels at which hydroxyapatite dissolves, releasing calcium.[12] The demineralization-remineralization balance of tooth enamel is strongly affected by the pH of surrounding solutions.[13] When acidity drops below pH 5.5, enamel hydroxyapatite dissolves and undergoes demineralization.

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O$$

The left-to-right direction is demineralization due to increased acidity. When calcium (Ca^{2+}), phosphate (PO_4^{3-}), and hydroxyl (OH^-) ions accumulate, demineralization slows down until the moment when saliva reaches saturation. When the pH rises toward alkalinity, the re-sedimentation of minerals will prevail, and the reaction alters from right to left, leading to remineralization.[7]

Methods

Informed approval was obtained from all participants. Methods were approved by the specialized ethics committee at the College of Dentistry, Hawler Medical University, Erbil, Iraq, and the protocol was written in accordance with the standards established by the Declaration of Helsinki. Subjects included in the study were 95 adults of one age group (19-34 years of age) and selected from people attending the polyclinics of the College of Dentistry. Furthermore, they were educated, using regular dental hygiene and of similar diet and drinking water sources. The decayed, missing, and filled permanent teeth (DMFT) index was used to assess tooth decay and its severity according to the World Health Organization WHO guidelines for dental caries prevalence in the Middle East.[14] Epidemiologists are still collecting data based upon the DMFT index, as other indices and newly developed tools for epidemiological caries assessment are yet to evolve sufficiently.[15]

The collection of unstimulated saliva was conducted after the patients' agreement. People with no systematic diseases were shortlisted. Exclusion criteria involved oral infections, ulcers, extensive periodontal diseases except for caries, smoking, pregnancy, and the use of chronic diseases medications.[16, 17] Three groups were organized, relying on the DMFT index as a diagnostic criterion; caries free (CF); DMFT = zero, very low caries (VL); DMFT 1-4, and low-to-moderate caries (LM) DMFT ≤ .5 The CF group accounted for about 9.6% (55.56% male and 44.44% female) (DMFT mean= zero), VL group, 50% (66.18% male and 33.82% female) (DMFT mean = 2.83), and LM group, 40.4% (85.29% male and 14.71% female) (DMFT mean = 7.33) of the whole sample. High caries incidence was not recorded in this age group.

Collection of saliva

The unstimulated saliva was collected from patients there ages (19 to 34) without any systemic diseases and have normal salivary rate (0.3-0.4 ml/min) [18], they had ceased eating, drinking, using cosmetic lipstick, chewing gum, and practicing oral hygiene for one hour before saliva collection; the collection time lasted 2 hours (9-11 am), the saliva samples were then stored at 4 ^oC until the day of the experiment.[19] Rinsing of the mouth with distilled water for 1 minute preceded the collection of approximately 5 ml of saliva carried out 5 minutes

later; subjects were instructed to spit into disposable test tubes.[20] The difference in the natural saliva composition between people may result in different critical pH points at which neither demineralization nor remineralization occurs.

Collection of teeth

Teeth extracted for orthodontic reasons were collected from the orthodontic clinics of the College of Dentistry, Hawler Medical University after patients' approval. Permanent teeth with no active or initial caries lesions, fractures, developmental defects, and staining or enamel defects were included.[21] The freshly extracted human teeth were cleaned from debris and stored in sterile 10% formalin, a method of disinfection/ sterilization, until they were tested.[22] Forty teeth completely free of decay were included in the study.

The buffers

All buffer solutions used to adjust the pH of saliva samples were prepared by mixing the recommended volumes, in ml, of citric acid (0.1 mol/l) and disodium hydrogen phosphate (0.2 mol/l).[23]

Procedure

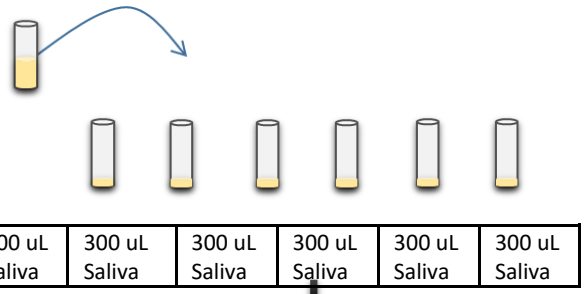
This study was managed using a case-control study design that divided the subjects into three groups. Simple random selection was used to allocate groups. Six human premolar teeth for each subject were selected randomly per session.[24] The whole tooth was painted with acid-resistant nail varnish except for a window of 2x3 mm at the enamel surface to prevent any possible mineral loss from other surfaces than enamel.[25] For each subject, 6 test tubes were prepared, each containing 0.3 ml saliva and 0.3 ml buffer. Buffers used to control the saliva pH were of pH 4.6, 5.0, 5.6, 6.0, 6.6, and 7.0 as the old and the new possible critical pH points might lie within this range, buffers were added to tubes 1 to 6, respectively. A painted tooth was placed in each test tube upside down so that the enamel was covered by the saliva-buffer mixture. During each trial, eighteen teeth were exposed to saliva samples from the 3 groups of people: CF, VL, and LM. It was verified that the exposed area was not damaged after each trial, using the same criteria for testing when approving the health of the tooth, otherwise, a new area was selected and the tooth repainted. Saliva was mixed with the buffer solution in a ratio of 1:1.

The final pH of each test tube content was measured using an Electrolyte analyzer-Genius GE 300 (Pareco Tech-Accra, Ghana). Calcium concentration was determined in mg/dL before incubation by means of a spectrophotometer (BIO-TEK, BIO-TEK Instruments, Milan, Italy). In an alkaline medium, calcium combines with O-Cresol Phthalein Complexone (OCPC) to form a purple-colored complex. The intensity of the color formed is directly proportional to the amount of calcium present in the salivary sample.[26] All test tubes were sealed tightly to avoid the evaporation of the mixture and the effect of open environmental circumstances that might allow air and microorganisms to enter the medium, and after 24 hours of incubation at 37^{o}C, the calcium test was performed again to assess the amount of calcium loss/gain as an indication of demineralization and remineralization of enamel.

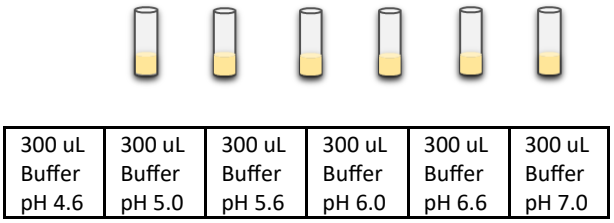
The difference in calcium concentration before and after incubation was recorded, this difference is the amount of dental calcium loss/gain. Statistical analyses were performed by using SPSS program (version 19.0; SPSS Inc., Chicago, IL, USA). Correlation tests and regression plots were used in the analysis. $P \leq 0.05$ was accepted as significant.

For each individual, the practical steps were as follows:

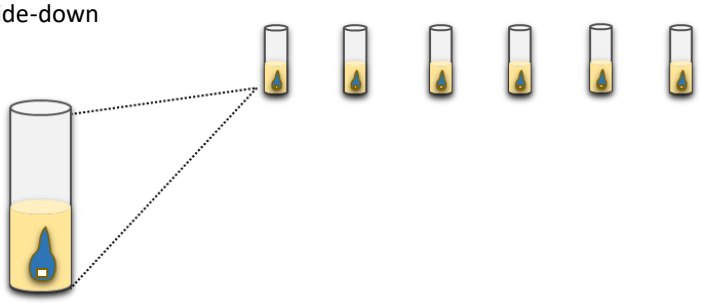
Step 1:
Each person gave 5 ml unstimulated saliva.



Step 2: Buffers were added.



Step 3: A caries-free tooth was placed upside-down in each test tube.



Step 4: The final pH for each test tube was measured using the electrolyte analyzer Genius GE 300.

Step 5: Calcium concentrations (mg/dL) were determined for each test tube (before incubation).

Step 6: All tubes were sealed tightly and incubated in a water bath at 37 °C for 24 hours.

Results

The correlation between greater saliva pH and calcium loss/gain concentrations of tooth enamel was significant for all the three groups; CF, VL and LM, as the Pearson correlation coefficient values were 0.898, 0.913 and 0.973 respectively Table 1, 2 and 3. The significant correlation between pH and calcium concentration, particularly at pH levels 5.6, 6.0, 6.6, and 7.0, is of high importance as the demineralization of these levels has not been studied by other researchers. Previous studies have focused mainly on pH 5.5 and lower. Detection of new effective pH points helps in specifying the remineralization zone (safe zone) for each group. Regression plots between the pH of the saliva-buffer mixture and the calcium concentration reveal differences in the safe zone between groups regarding susceptibility to tooth decay; the discovered critical pH point, the pH point at which neither demineralization nor remineralization occurs, for the CF group is 6.11, with the largest remineralization zone (safe zone) that covers pH ≥ 6.11. The VL group possesses a critical pH point of 6.48, so it occupies a smaller safe zone that includes pH ≥ 6.48. Finally, the LM group has the highest critical pH point at 6.72, so it has the smallest safe zone, represented by pH ≥ 6.72 Figures 1, 2, and 3.

Table 1. Correlation test reveals the relationship between pH and calcium loss/gain for enamel in the CF group

		pH	Calcium
Average pH	Pearson correlation Sig. (2-tailed) N	1 6	0.898* 0.015 6
Calcium concen-tration mg/dL	Pearson correlation Sig. (2-tailed) N	0.898* 0.015 6	1 6

* Correlation is significant at the 0.05 level (2-tailed).

Table 2. Correlation test reveals the relationship between pH and calcium loss/gain for enamel in the VL group

		pH	Calcium
Average pH	Pearson correlation Sig. (2-tailed) N	1 6	0.913* 0.011 6
Calcium concentra-tion mg/dL	Pearson correlation Sig. (2-tailed) N	0.913* 0.011 6	1 6

* Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation test reveals the relationship between pH and calcium loss/gain for enamel in the LM group

		pH	Calcium
Average pH	Pearson correlation Sig. (2-tailed) N	1 6	0.973** 0.001 6
Calcium concentra-tion mg/dL	Pearson correlation Sig. (2-tailed) N	0. 973** 0.001 6	1 6

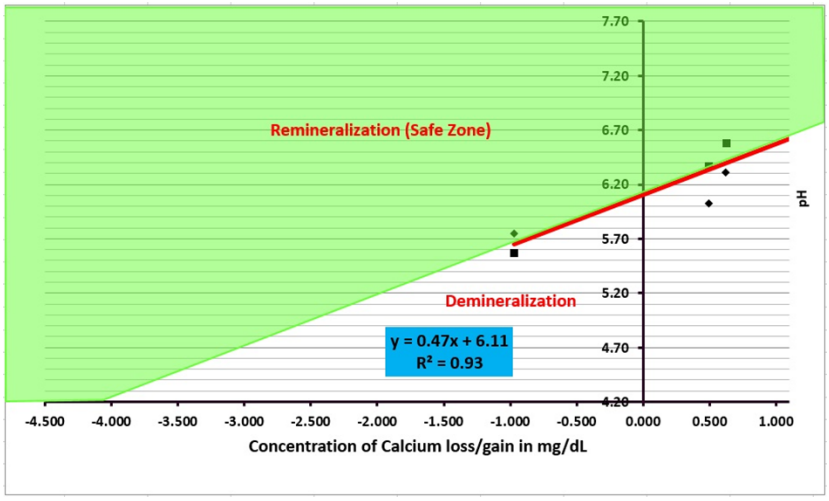


Figure 1. A regression plot for the effect of pH on enamel de-remineralization in the CF group

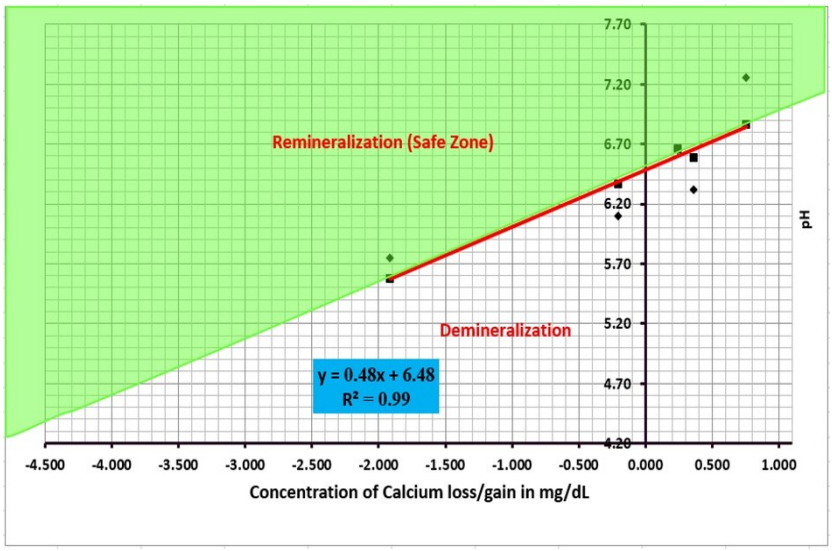


Figure 2. A regression plot for the effect of pH on enamel de-remineralization in the VL group

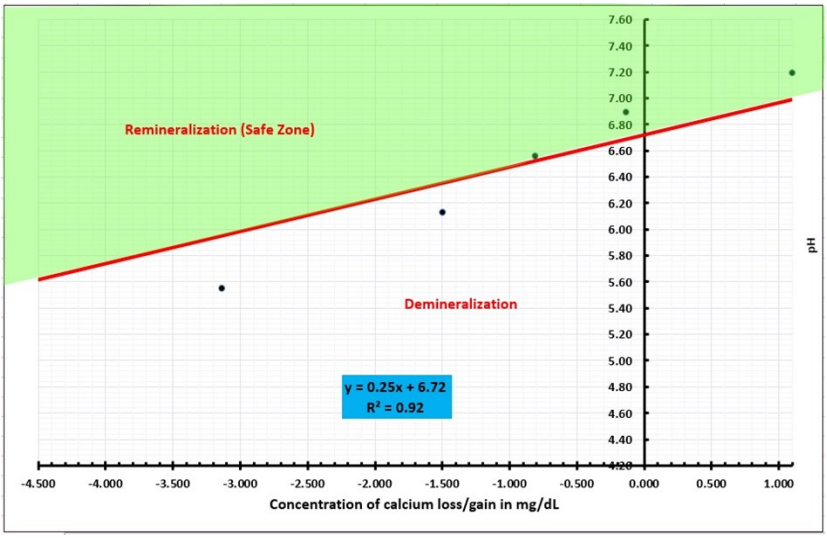


Figure 3. A regression plot for the effect of pH on enamel de-remineralization in the LM group

Discussion

Despite the availability of dental care tools, people still experience tooth decay. This study deals with one of the most important factors that contributes to tooth decay, the pH factor, since the interpretation and understanding of tooth decay and its repair mechanisms are largely associated with this factor. The hypotheses upon which we rely to understand the causes of tooth loss regarding the role of pH may need to be revised after the outcome of this study. Tooth longevity depends on the demineralization and remineralization processes of the enamel; moreover, researchers state that demineralization occurs at acidic pH levels, around pH 5.5 and lower. If so, and since saliva's pH is relatively high, around pH 7, and teeth are not exposed to such low levels for long terms, the incidence of tooth decay is supposed to be minimal, particularly for people who use means of tooth care regularly, but the continued prevalence of decay confirms the opposite. Almost all previous studies state that the demineralization of tooth enamel takes place below pH 5.5, and they consider 5.5 the critical pH.[27,28] Other researchers declare that the critical pH does not have a fixed value, and it is within the pH range 4.3 to 5.[24,29] A few researchers discuss the consequences of exposure to pH levels such as 4 to 6.9, but they do not detail whether the effect is the same for all people or whether demineralization could be the only process happening in this pH range.

Although the exposure of the tooth surface to acids with pH < 4 is not a common biological occurrence,[30] we hypothesize that exposure to pH ranges from 5 to 7 is possible because saliva has different pH ranges in different people.[31] New results have been attained in the current study about what occurs when tooth enamel is exposed to saliva samples from three groups of people, six saliva samples (pH 4.6, 5, 5.6, 6.0, 6.6, and 7.0) from each group, and these results may go beyond the existing theories and contribute to understanding why decay still occurs everywhere at different levels. The significant correlations between the increase of saliva pH from 4.6 to 7.0, and calcium loss/gain from the exposed tooth enamel in all three groups of people supports that the loss of calcium is not limited to low acidity levels, such as pH 5.5 and lower.[27] Furthermore, the results indicate that tooth enamel would lose calcium even at higher pH levels, such as 5.6, 6.0, 6.6, and around 6.7. This outcome is in line with the findings of Abou Neel et al. (2016).[30] This scientific gain may add substantial details in that the calcium release from the enamel at pH levels higher than 5.5 could vary from person to person, and people with different DMFT values have dissimilar critical pH points that contribute to decay incidence. It is worth mentioning that some critical pH points are located within the normal range of saliva, which is pH 6.1-7.[5,32] This means that some people may experience demineralization even in the normal range of salivary pH. This study clarifies that the variation in the incidence and severity of tooth decay from person to person could be dependent mainly on the chemical composition of saliva which protects against dental caries and erosion, and these effects are the result of saliva's being saturated with respect to tooth minerals such as calcium or phosphate ions,[33] as well as total protein, urea, bicarbonate, and phosphate, which play a role in salivary buffering capacity,[34-36] so different saliva samples show different responses. In these experiments, when teeth are exposed to saliva samples, cross points are generated as a result of a relationship between the change in pH and the saturation of saliva with respect to tooth minerals. These points change from person to person depending on the degree of saturation, the more calcium and phosphate that are present in saliva, the lower its critical pH.[29] This is especially true as the same caries-free, painted teeth were reused to conduct the experiments for all three groups. Tooth enamel structure and its resistance to the environmental acidic pH levels may also play a role.[17]

Remineralization is the only active process above the new detected critical pH points for all groups in this study, and it helps by means of returning minerals, specifically calcium and phosphate, to the tooth enamel to compensate for mineral loss. Along the pH scale from 4.6 and up to 7.0, the demineralization process is fading and finally disappears to be replaced by the remineralization process when the pH approaches 7. The results reveal a direct relationship between increasing DMFT values and increased critical pH points. On the other hand, the DMFT index increase is offset by decreases in the remineralization pH scale (safe zone). Corresponding to these results and regarding the role of pH in oral diseases, the difference in the severity of tooth decay between individuals is due to the difference in critical pH and how large the remineralization pH scale (safe zone) is that extends from the critical pH and up. Colin Dawes (2003) states that the critical pH is not a static value and depends on the salivary calcium and phosphate concentrations that may support these results.[29] Caries-free people have low critical pH values around 6.11, from which their safe zone starts toward the neutral pH level. Therefore, those people are less susceptible to demineralization because this drop in pH may not initiate calcium release. Consequently, there is less possibility of tooth decay because these people possess the greatest safe zone through which remineralization is the only predominant activity. In contrast, the LM group has the smallest safe zone due to its members' high critical pH at 6.72, from which their small safe zone starts up. They are the group that is most susceptible to enamel demineralization as the normal human salivary pH is around 7.[37] The VL group has a modest safe zone that begins from 6.48, and this explains their very low DMFT value. So, the best capability to avoid decay lies with the CF group, while the lowest is with the LM group. The limitation of our study includes that it is an in vitro method depends on the use of extracted teeth only, for this reason, it is preferred to apply the study for clinical purposes during childhood as the primary teeth will be available due to the natural loss.

Conclusion

We conclude that individuals have different responses to fluctuations in the pH levels of the mouth, which leads to a variation in the incidence and severity of tooth decay, and this may be attributed to the difference in the composition of saliva. Hence, the chemistry of saliva may play a major role in the demineralization-remineralization balance, and this role may be an indirect impact of another internal or external factor. However, highly accurate results can be obtained by using non-stored fresh saliva samples, and the method could be used to assess the power of saliva in the resistance to tooth decay which in turn helps to avoid the infection of more teeth. Further investigation of the relationship between calcium loss/gain from tooth enamel, the chemistry of saliva, and diet is required to find the missing points regarding the role of salivary components.

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Conflicts of interest

The authors have no known conflict of interest to disclose.

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