Statistik for Odontologer - Eksamensopgave 2019

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48 timers hjemmeopgave.
22. november kl. 15:00 til 24. november kl. 15:00

— Dette er en individuel eksamen. Det er ikke tilladt at arbejde sammen med andre om løsningen af denne opgave i eksamensperioden. —

Eksamensbesvarelsen skal skrives som en rapport, der besvarer de stillede spørgsmål. Der er følgende strukturelle krav til besvarelsen:

- Besvarelsen af hvert spørgsmål **skal** nummereres i overensstemmelse med nummereringen i dette dokument.
- Gentag **ikke** opgaveteksterne/spørgsmålene i dine besvarelser. Det udløser KUs automatiske plagiatkontrol, og din besvarelse vil dermed automatisk blive markeret som at være afskrevet fra dine medstuderende.
- Rapporten må **ikke** indeholde R-kode men skal formuleres i ord, hvor de relevante resultater fra analyserne i RStudio indsættes.
- Indsæt **ikke** store klumper af output fra RStudio som f.eks. `summary`-output fra regressionsmodeller og lignende, men udtræk de relevante talværdier i forhold til opgavespørgsmålet, og diskutér deres betydninger i ord.
- Den komplette R-kode, som du har brugt til dine analyser, **skal** vedlægges rapporten i form af et appendiks. Se løsningsforslag til øvelsesopgaverne fra uge 7 og 8 for hvordan dette kan se ud.
- Alle analyseresultater skal kunne reproduceres ved at køre din vedlagte R-kode på dit personlige datasæt. Besvarelser, der **ikke** kan genfindes som et resultat af en eller flere R-kommandoer eller simple beregninger, godtages **ikke** som en opgavebesvarelse.
- Hver side i afleveringen **skal** i nederste højre hjørne indeholde fortløbende sidenummer samt det totale antal sider. Der er ingen øvre eller nedre grænse for antal afleverede sider.
- Afleveringen (rapporten samt appendiks) uploades som ét samlet dokument i PDF-format til Digital Eksamen.

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Eksamenopgavens struktur

Dette er eksamensopgaven i Statistik for Odontologer efteråret 2019. Hele dokumentet er på 14 sider og består af tre opgaver:

1. Statistisk analyse af et datasæt.
2. Læsning og forståelse af en videnskabelig artikel.
3. Spørgsmål omhandlede generel forståelse.

Hver opgave har forskellige delopgaver, som du skal besvare.

Om opgave 1

I opgave 1 skal du besvare forskellige videnskabelige spørgsmål baseret på dine egne analyser af et **personligt** datasæt. Det er et krav, at din besvarelse er baseret på netop dit personlige datasæt. Du kan indlæse dette i RStudio ved at udføre kommandoen

```
d <- read.csv("http://causal.sund.ku.dk/e19/data6.csv", header=TRUE)
```

Om opgave 2

I opgave 2 skal du læse en videnskabelig artikel publiceret i et internationalt odontologisk tidsskrift og forholde dig til forskellige spørgsmål angående forfatternes konklusioner. De sidste seks sider af denne eksamensopgave indeholder en kopi af den videnskabelige artikel, som du skal forholde dig til i denne opgave.

Om opgave 3

I opgave 3 bliver du præsenteret for nogle generelle spørgsmål, der er uafhængige af spørgsmålene i de to tidligere opgaver.

1 Statistisk analyse af et datasæt

I denne opgave skal du analysere et datasæt. Den videnskabelige problemstilling omhandler effektiviteten af et tandblegningsprodukt i forhold til at reducere misfarvning af tænderne, potentielle bivirkninger, og efterfølgende tilfredshedsvurdering.

Tandblegning både i hjemmet og på en tandlægeklinik er blevet mere og mere populært, og der findes forskellige produkter netop til dette. Et af produkterne består af en maskine med et mundstykke, og ved brug påføres mundstykket først en gel, der blandt andet indeholder hydrogenperoxid, hvorefter man skal sidde med mundstykket i munden i en tidsperiode. I løbet af den tid, hvor maskinen er aktiv, kan man vælge at udsende blå LED-lys direkte på tænderne.

Der er forskellige regulativer om den tilladte koncentration af hydrogenperoxid i hjemmetandblegning rundt i verden. I Danmark må der ikke sælges produkter i håndkøb med koncentration højere end 0.1%, mens den i USA kan være op til 10%. På grund af den måde, som mundstykket er konstrueret på, kan man risikere, at blegningsmidlet også kommer i kontakt med gummerne, hvilket kan forårsage ætsninger og ubehag.

Vi forestiller os et videnskabeligt forsøg, hvor 125 personer er blevet udstyret med et mundstykke med forskellige koncentrationer af hydrogenperoxid, og personerne har valgt, om de ville bruge lys eller ej under behandlingen. Inden behandlingen startede, har man målt deltagerens tandfarve. Efter behandlingen har man igen målt deres tandfarve og afrapporteret deres ændring i farve i forhold til deres måling ved baseline. Til slut har man bedt deltagerne i studiet fortælle, om de har haft ætsninger på gummerne i forbindelse med deres behandling, og til slut om deres tilfredshed med produktet.

Datasættet indeholder 125 observationer med følgende fem variable:

- `colorchange`: forskel i tandfarve efter behandlingen sammenlignet med tandfarve før behandlingen. (Dette måles ved hjælp af en "lightness" scala, hvor værdien 0 svarer til sort, og 100 svarer til ren hvid).
- `concentration`: koncentrationen af hydrogenperoxid i tandblegningsproduktet.
- `light`: brug af lys eller ej under behandlingen (`0` = ingen brug af lys, `1` = brug af lys).
- `erosion`: forekomst af ætsning på gummerne efter anvendelse af tandblegningsproduktet (`0` = ingen ætsning, `1`= ætsning).
- `satisfaction`: tilfredshedsvudering af tandblegningsproduktet efter anvendelse (`0` = vil aldrig bruge produktet igen, `1` = vil måske bruge produktet igen, `2` = vil helt sikkert bruge produktet igen).

Du skal starte med at indlæse dit personlige datasæt i RStudio med kommandoen givet på forrige side.

1.1 Datapræsentation

I denne delopgave skal du præsentere dit datasæt. Det skal du gøre, så andre, der læser dit videnskabelige arbejde, får en forståelse for hvilken studiepopulation, du beskæftiger dig med. Besvar følgende spørgsmål.

1. Skriv nummeret, som står i øverste højre hjørne af denne side, som svar på dette spørgsmål. Dette er *ikke* et trickspørgsmål. Det er for at sikre, at dette nummer figurerer et sted i besvarelsen.
2. Lav en præsentation af studiepopulationen ved at lave en „Tabel 1" på baggrund af observationerne i stikprøven. Argumentér desuden for, *hvorfor* du har valgt at strukturere tabellen på den måde, som du har gjort, samt *hvorfor* du har valgt at beskrive de enkelte variable i tabellen på den måde, som du har gjort.

1.2 Overordnet effekt af tandblegningsproduktet

I denne delopgave skal du undersøge, om anvendelsen af tandblegningsproduktet overordnet set har haft en effekt. Det gør du ved at besvare følgende spørgsmål.

1. Brug en relevant statistisk metode til at undersøge, om den gennemsnitlige tandfarve har ændret sig, når man sammenligner farvemålingen efter behandlingen med farvemålingen før behandlingen. Dit svar på dette spørgsmål skal være hvilken statistisk metode, du har anvendt, samt en argumentation for, hvorfor du har valgt netop denne til at besvare det videnskabelige spørgsmål.
2. Beregn og afrapportér p-værdien tilhørende nulhypotesen om, at den gennemsnitlige farve ikke har ændret sig, og forklar i ord, hvad du kan konkludere ud fra dette.
3. Beregn et 95% konfidensinterval for ændringen i tandfarve. Fortolk betydningen af intervallet i ord, og beskriv, hvad det fortæller os om effekten af tandblegningsproduktet.
4. Beregn et 95% referenceinterval for ændringen i tandfarve. Fortolk betydningen af intervallet i ord, og beskriv, hvad det fortæller os om tandblegningsproduktet.
5. Sammenlign de to intervaller udregnet i spørgsmål 3 og 4 ovenfor. Hvis det ene interval er bredere (altså har en større numerisk afstand mellem dets to endepunkter) end det andet, giv da en forklaring på, hvorfor det forholder sig sådan, og om det altid vil være sådan i enhver stikprøve.

1.3 Effekt af koncentration og lys på ændring i tandfarve

I denne delopgave skal du undersøge, om koncentrationen af hydrogenperoxid i tandblegningsproduktet *samt* anvendelsen af lys undervejs har haft en effekt på ændringen i tandfarve. Det gør du ved at besvare følgende spørgsmål.

1. Brug en relevant statistisk metode til at undersøge, hvordan ændringen i tandfarve afhænger af koncentrationen af hydrogenperoxid *samt* brugen af lys under behandling, hvor du tillader,

at sammenhængen mellem koncentration og farveændring afhænger af brugen af lys. Dit svar på dette spørgsmål skal være hvilken statistisk metode, du har anvendt, samt en argumentation for, hvorfor du har valgt netop denne til at besvare dette videnskabelige spørgsmål.

2. Opskriv ligningerne for den estimerede sammenhæng mellem koncentration og farveændring baseret på din model i de to tilfælde, hvor der enten har været lyspåvirkning eller ej.
3. Undersøg den videnskabelige hypotese om, hvorvidt brugen af lys har en betydning for sammenhængen mellem koncentrationen og ændringen i farve. Beskriv nulhypotesen for den tilhørende statistiske hypotese i forhold til parametrene i modellen. Afrapportér p-værdien for denne nulhypotese, og beskriv, hvad du konkluderer ud fra dette.
4. Ud fra modellen estimeret i delspørgsmål 1 ovenfor skal du nu simplificere modellen mest muligt. Du skal komme frem til den mest simple model, der passer bedst til data. Beskriv, hvordan du har foretaget denne simplificering, og hvilken model du er nået frem til.
5. Opskriv ligningen for linjen/linjerne i din *endelige model* fundet i forrige spørgsmål.
6. I din endelig model skal du se særligt på parameteren `(Intercept)`. Hvilken videnskabelig fortolkning har denne parameter i forhold til sammenhængen mellem koncentration og farveændring?
7. Forklar hvilken nulhypotese, der er blevet testet tilhørende den p-værdi, der er afrapporteret for parameteren `(Intercept)`. Giver dette hypotesetest videnskabelig mening, og giver det mening i forhold til den videnskabelige problemstilling?
8. Beregn 95% konfidensintervaller for alle parametrene i din endelige model, og fortolk deres betydning i ord.
9. Brug din endelig model til at prædiktere farveændringen ved en koncentration på 12%. Fortolk dit resultat i ord.
10. Brug din endelige model til at beregne et 95% referenceinterval for farveændringen ved anvendelse af 10% koncentration af hydrogenperoxid. Fortolk dit resultat i ord.

1.4 Sammenhæng mellem ætsning og brugertilfredshed

I dette delspørgsmål skal du undersøge, hvordan tilfredshedsvurderingen blandt brugerne af tandblegningsproduktet afhænger af tilstedeværelsen af ætsninger på gummerne undervejs i behandlingsforløbet. Det gør du ved at besvare følgende spørgsmål.

1. Brug en relevant statistisk metode til at undersøge, om ætsning på gummerne i behandlingsforløbet har en effekt på den efterfølgende tilfredshedsvurdering. Dit svar på dette spørgsmål skal være hvilken statistisk metode, du har anvendt, samt en argumentation for, hvorfor du har valgt netop denne til at besvare det videnskabelige spørgsmål.
2. Baseret på din analyse i forrige spørgsmål, skriv en konklusion af, hvorvidt ætsning på gummerne har en statistisk signifikant effekt på tilfredshedsvurderingen. Hvilken nulhypotese har du testet, hvad er den tilhørende p-værdi, og hvad konkluderer du?

3. Beregn sandsynligheden for helt sikkert at ville anvende produktet igen givet, at der har været ætsninger på gummerne samt det tilhørende 95% konfidensinterval for denne sandsynlighed. Forklar i ord, hvordan dette konfidensinterval skal fortolkes.
4. Udregn den relative risiko for aldrig at ville anvende tandblegningsproduktet igen, når man sammenligner dem, der oplevede ætsning på gummerne i forhold til dem, der ikke oplevede ætsning på gummerne. Forklar i ord, hvordan den relative risiko skal fortolkes.
5. Udregn odds ratioen for måske at ville anvende tandblegningsproduktet igen, når man sammenligner dem, der oplevede ætsning på gummerne i forhold til dem, der ikke oplevede ætsning på gummerne. Forklar i ord, hvordan denne odds ratio skal fortolkes.

1.5 Sammenhæng mellem koncentration og ætsning

I dette delspørgsmål skal du undersøge, hvordan koncentrationen af hydrogenperoxid i tandblegningsproduktet påvirker den efterfølgende risiko for ætsning på gummerne. Det gør du ved at besvare følgende spørgsmål.

1. Brug en relevant statistisk metode til at undersøge, hvordan risikoen for ætsning på gummerne afhænger af koncentrationen af hydrogenperoxid i tandblegningsproduktet. Dit svar på dette spørgsmål skal være hvilken statistisk metode, du har anvendt, og en argumentation for, hvorfor du har valgt denne metode til at besvare det videnskabelige spørgsmål.
2. Opskriv udtrykket for den statistiske model, hvor du indsætter værdierne af de estimerede parametre.
3. Brug modellen til at udregne odds ratio for at få ætsning på gummerne samt et 95% konfidensinterval for denne odds ratio. Skriv en fortolkning i ord af, hvordan denne odds ratio og dens tilhørende konfidensinterval skal fortolkes.
4. En repræsentant for firmaet, der producerer det pågældende tandblegningsprodukt, påstår, at der ingen sammenhæng er mellem koncentrationen af hydrogenperoxid og risikoen for ætsning på gummerne. Er du enig i dette udsagn, og hvad kan du konkludere ud fra din model? Forklar din nulhypotese, beregn og afrapportér den tilhørende p-værdi, og skriv i ord, hvad du kan konkludere.
5. Brug din model til at prædiktere odds for at få ætsning på gummerne, når koncentrationen af hydrogenperoxid er 6%. Skriv en fortolkning i ord af, hvordan din prædikterede odds skal forstås.
6. Brug din model til at prædiktere sandsynligheden for at få ætsning på gummerne, når koncentrationen af hydrogenperoxid er 6%. Skriv en fortolkning i ord af, hvordan din prædikterede sandsynlighed skal forstås.
7. Beregn ud fra din model den koncentration af hydrogenperoxid, hvor sandsynligheden for at få ætsning på gummerne overstiger 80%.

2 Læsning og forståelse af videnskabelig artikel

I denne opgave skal du læse en videnskabelig artikel og besvare nedenstående spørgsmål. Artiklen er inkluderet som de sidste seks sider af denne eksamensopgave.

1. I artiklens tabel 2 undersøges det, hvordan de 4 mål for en tands farvenuance har ændret sig efter at tænderne har ligget i te i 7 dage. I dette spørgsmål vil vi udelukkende koncentrere os om variablen ΔL. Gruppe AS svarer til ingen blegning, mens CR, LS, SC og OP svarer til 4 forskellige hjemmeblegningsprodukter. På baggrund af forsøgsopstillingen, hvad ville du så umiddelbart forvente at se for ΔL, hvis man sammenlignede de 5 grupper umiddelbart efter de 7 dage, hvor tænderne har ligget i te?

2. Lav på baggrund af artiklens tabel 2 et 95% konfidensinterval for ændringen af lysintensiteten, ΔL, for produktet CR. Giv en fortolkning af dette konfidensinterval i ord.

   [Hjælp: Man kan i dette tilfælde udregne standardfejlen som spredningen delt med $\sqrt{n}$, hvor n er antallet af tænder, der har været brugt til at udregne den gennemsnitlige ændring og den tilhørende spredning.]

3. I artiklen laver forfatterne en ensidet variansanalyse (se fx teksten lige før afsnit 3, og starten af afsnit 3). Forklar i ord, hvad nulhypotesen hørende til den ensidede variansanalyse undersøger.

4. Forfatterne finder, at den ensidede variansanalyse giver en p-værdi > 0.05. Hvis man regner efter bliver p-værdien mere præcist 0.7842. Beskriv, hvad du konkluderer om de 5 grupper ud fra dette resultat.

5. Betragt nu tabel 3. Forklar i ord, hvordan resultatet "12.42 (4.47)" for behandling CR skal fortolkes, og hvorfor det er interessant at blive præsenteret for det resultat.

6. Vi bruger nu det samme resultat fra tabel 3 som i spørgsmål 5 ovenfor. Hvis du selv skulle analysere effekten af CR til "Time 3", hvilket statistisk test ville du benytte, og hvorfor?

7. Betragt nu figur 2 i artiklen. Er det muligt på baggrund af figur 2 at konkludere, om de fire produkter CR, LS, SC og OP er bedre til at blege tænderne end gruppe AS? Begrund dit svar.

8. Betragt stadig figur 2 i artiklen. Hvis du anvendte en model, der modellerer Δa som funktion af tid og gruppe ville du så tro, at du ville finde en interaktion (vekselvirkning) mellem tid og gruppe? Begrund dit svar.

9. I artiklen abstract skriver forfatterne *"After 56 days, no significant differences were found among the mouthwash products with respect to color change"* Er du enig i denne påstand? Hvorfor / hvorfor ikke?

3 Generelle spørgsmål

Denne sidste opgave omhandler generel forståelse af forskellige statistiske begreber. Spørgsmålene i denne opgave har ingen relation til beregninger eller resultater fra de to foregående opgaver.

1. Du har på forhånd valgt, at dit signifikansniveau skal være på 7%, og du laver et statistisk hypotesetest, hvor nulhypotesen er, at middelværdien i populationen for en variabel er lig med nul. Du får en p-værdi på 5%. Hvad konkluderer du?

   [Vælg her kun én mulighed blandt nedenstående, og skriv blot enten a, b, c, d eller e som din besvarelse og ikke selve teksten.]

   a. Du forkaster nulhypotesen og kan derfor konkludere, at den sande middelværdi er forskellig fra nul.
   b. Du kan ikke forkaste nulhypotesen og derfor kan du ikke konkludere, at den sande middelværdi ikke er forskellige fra nul.
   c. Du forkaster nulhypotesen og kan derfor konkludere, at den sande middelværdien er lig med nul.
   d. Du kan ikke forkaste nulhypotesen, og derfor kan du konkludere, at middelværdien er lig med nul.
   e. Du kan ikke konkludere noget ud fra ovenstående muligheder.

2. Du har udregnet et 95% konfidensinterval for en middelværdi, og resultatet er $[0.1; 0.4]$. Hvad kan du konkludere ud fra dette?

   [Her kan du vælge én eller flere muligheder blandt nedenstående, og din besvarelse skal være én eller flere af a, b, c, d, e eller f og ikke selve teksten.]

   a. Sandsynligheden for, at den sande middelværdi i populationen er større end 0, er større end 95%.
   b. Sandsynligheden for, at den sande middelværdi i populationen er lig med 0, er mindre end 5%.
   c. Nulhypotesen om, at den sande middelværdi i populationen er lig med 0, er sandsynligvis forkert.
   d. Der er 95% sandsynlighed for, at den sande middelværdi i populationen er mellem 0.1 og 0.4.
   e. Der er 95% sandsynlighed for, at intervallet fra 0.1 til 0.4 indeholder den sande middelværdi i populationen.
   f. Hvis du gentog eksperimentet igen og igen, vil vi forvente, at den sande middelværdi vil ligge mellem 0.1 og 0.4

3. Personer, der regelmæssigt drikker et særligt sukkerholdigt produkt, har vist sig at have en øget risiko for huller i tænderne på 25% i form af en relativ risiko, når man de sammenligner risikoen for huller i tænder blandt dem, der drikker produktet, med et tilfældigt udsnit af befolkningen, der ikke drikker dette produkt. Risikoen for huller i tænderne blandt dem, der drikker produktet er 10%.

   a. Udregn risikoen for huller i tænderne blandt dem, der *ikke* drikker produktet.
   b. Udregn forskellen i risiko for huller i tænderne i procentpoint, når man sammenligner dem, der drikker produktet, med dem, der ikke drikker produktet.

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Research Article

Efficacy of Mouthwashes Containing Hydrogen Peroxide on Tooth Whitening

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The aim of this study was to analyze the efficacy of mouthwashes containing hydrogen peroxide compared with 10% carbamide peroxide (CP) gel. Fifty enamel-dentin samples were obtained from bovine incisors and then stained in a tea solution. The stained samples were randomly divided into five groups according to the whitening product applied ($n = 10$): AS: no whitening (negative control), with the samples stored in artificial saliva; CR: Crest 3D White mouthwash; LS: Listerine Whitening mouthwash; SC: Scope White mouthwash; and OP group: 10% CP Opalescence PF (positive control). Color measurements were carried out with a spectrophotometer before staining, after staining, and on the 7th, 28th, and 56th day of the whitening period. The data were analyzed using two-way analysis of variance followed by a Tukey post hoc test. The color change (ΔE) was significantly greater in all the groups compared to that of the AS group. After 56 days, no significant differences were found among the mouthwash products with respect to color change ($P > 0.05$). The whiteness of the teeth treated with the mouthwashes increased significantly over time. Nevertheless, the color change achieved with the mouthwashes was significantly lower than that achieved with the 10% CP at-home bleaching gel.

1. Introduction

Patients today demand more than a healthy mouth and a perfect smile. The color and aesthetics of teeth are very important to patients, as they influence self-esteem and professional relationships [1]. Tooth bleaching has become one of the most popular and common esthetic dental procedures for whitening discolored teeth in modern esthetic dentistry. This process is a relatively simple and conservative option compared to other forms of treatment, such as veneers and crowns [2]. Tooth bleaching refers to any procedure that does not use restorative materials and that changes the color and appearance of teeth that were discolored due to intrinsic and extrinsic staining [3]. Fundamental vital tooth bleaching techniques can be generally classified as at-home (dentist-supervised nightguard bleaching), in-office or power bleaching (professionally administered) and over-the-counter (OTC) or mass-market products [4–6].

OTC products are a low-cost alternative for white discolored teeth without dentist supervision [7, 8]. Different OTC agents are available in supermarkets and pharmacies and on many websites [8]. These products generally contain lower levels of a whitening agent and are self-applied to teeth by means of gum shields, strips, paint-on brushes, toothpastes, and mouthwash products. They commonly require two daily applications for up to 2 weeks [6].

Mouthwashes are very popular oral hygiene agents. They act to chemically control cariogenic biofilms and have remineralizing therapeutic properties. Due to the increased concern of patients' in recent years about dental esthetics, the number of mouthwash products containing hydrogen peroxide (HP) has risen significantly [9]. HP penetrates the tooth and produces free radicals, which attack and break apart the chromophore bonds of large, long chain, dark-colored molecules; this eventually breaks down the molecules and chromophore bonds, resulting in changes in tooth color [6]. However, in some cases, HP may not whiten teeth substantially due to the method of application and the length of time it is in contact with the teeth [3].

A few studies have evaluated the effectiveness of mouthwashes and whitening agents. Despite the increased number and sales of whitening products and mouthwashes, there is

Table 1: Details of mouthwashes products and bleaching gel used in this study.

Brand name (code)	Manufacturer	Material ingredient
Listerine Whitening mouthwash (LS)	Johnson & Johnson Healthcare Products, Skillman, NJ, USA	Water, alcohol (8%), hydrogen peroxide, tetrapotassium pyrophosphate, pentasodium triphosphate, citric acid, poloxamer 407, flavor, sodium saccharin, and sucralose
Scope White mouthwash (SC)	Procter & Gamble, Cincinnati, OH, USA	Water, glycerin, alcohol (5%), 1.5% hydrogen peroxide, hexametaphosphate, poloxamer 407, sodium citrate, flavor, sodium saccharin, and citric acid
Crest 3D White Multi-Care whitening mouthwash (CR)	Procter & Gamble, Cincinnati, OH, USA	Water, 1.5% hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, sodium saccharin, and citric acid
Opalescence PF 10% (OP)	Ultradent Products Inc., South Jordan, UT, USA	Glycerin, water, xylitol, carbamide peroxide, flavor, carbomer, PEG-300, sodium hydroxide, potassium nitrate, EDTA, and sodium fluoride

little evidence of their effectiveness. The tooth whitening of mouthwashes may also differ, depending on the constituents of the product. Therefore, the purpose of this study was to evaluate at different periods of immersion the whitening effect of three mouthwashes containing HP compared with the whitening effect of 10% carbamide peroxide (CP) used in at-home tray bleaching gels. The null hypotheses were that (1) the mouthwashes would not have any effect on the color change of teeth, (2) the immersion time in the mouthwashes would not influence tooth-whitening results, and (3) there would be no significant differences among the mouthwashes used.

2. Materials and Methods

2.1. Preparation of the Samples. Fifty extracted bovine incisors were selected for this study and cleaned with a periodontal hand scaler. They were stored in 0.5% chloramine-T solution and used within 2 weeks of extraction. Teeth with spots and fractures were excluded from the study. Enamel-dentin sections (dimension, 5 × 5 mm; thickness, 3 mm) were obtained from the midcoronal regions of teeth using water-cooled diamond disks (Impect PC10; Equilam Lab Equip, Diadema, SP, Brazil). The dentin surfaces were polished to standardize the thickness of each sample. Using molds, each enamel-dentin sample was individually mounted in transparent acrylic resin to expose the enamel surface. Each sample was polished for 10 s across the buccal surface with the use of a prophylaxis paste, applied with a polishing brush under manual pressure at a low-speed contra-angle. Then, each sample was washed with distilled water for 10 s. The prepared samples were immersed for 7 days in a tea mixture to allow the bleaching effectiveness of the 4 products to be compared on a set of stained samples. The tea solution was prepared by brewing 3.5 g of black tea in 100 mL of boiling distilled water for 10 min (Çaykur, Altınbaş Tea, Rize, Turkey). Then, the samples were washed in distilled water for 60 s.

2.2. Whitening or Bleaching Procedure. The stained samples were randomly assigned to five groups ($n = 10$) as follows: an AS group (negative control): the samples were immersed in artificial saliva; a CR group: the samples were immersed at 37°C in 30 mL of a whitening mouthwash (Crest 3D White) for 4 min daily [10] for 56 days; an LS group: the procedure was the same as that in the CR group but with a different whitening mouthwash (Listerine Whitening); an SC group: the procedure was the same as that in the CR group but with a different whitening mouthwash (Scope White); and an OP group (positive control): a 1.5–2 mm layer of bleaching gel (Opalescence PF 10% CP) was spread on the enamel surface for 4 h daily for 14 days. The samples were immersed at 37°C in artificial saliva [11] for the rest of the day. The constituents of the mouthwashes and those of the bleaching gel used in this study are presented in Table 1.

2.3. Color Evaluation. Before color measurement, the samples were dried with absorbent paper. A trained examiner conducted the color measurements of each sample against a white background in standardized D65 daylight using a digital spectrophotometer (VITA Easyshade Advance, Zahnfabrik, Bad Säckingen, Germany). The spectrophotometer was calibrated according to the manufacturer's instructions by using the calibration plate. The following spectrophotometric data were recorded for each sample: L^*, a^*, and b^* coordinate values created by the Commission Internationale de l'Éclairage. The L^* value represents the degree of lightness in a sample and varies from black (0) to white (100). The a^* and b^* values represent the degree of red ($+a^*$)–green ($-a^*$), yellow ($+b^*$)–blue ($-b^*$) in the samples, respectively. Each sample color was measured at 5 time points: at baseline; after staining; and at 7 days (time 1), 28 days (time 2), and 56 days (time 3) after immersion in the respective mouthwashes. The difference between the two colors was calculated by the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$.

Color differences after staining were calculated using baseline color parameters. The ΔE, ΔL, Δa, and Δb values after 7, 28, and 56 days were calculated using color parameters after staining. In the OP group, these parameters were calculated at 7, 14 (recorded as 28 days in this study), and 56 days of the treatment period.

2.4. Statistical Analysis. PASW Statistics software 18 (SPSS Inc., Chicago, IL, USA) was used to analyze data. The data

Table 2: Means and standard deviations (SD) of color parameters after being stored in tea solution.

Group	ΔE	ΔL	Δa	Δb
AS	12.80 (3.01)	−11.84 (3.45)	4.54 (1.32)	7.28 (3.10)
CR	11.65 (4.12)	−8.35 (2.23)	3.37 (1.87)	5.85 (3.79)
LS	13.62 (4.56)	−9.79 (3.03)	4.14 (1.14)	8.97 (4.03)
SC	14.25 (6.10)	−10.98 (5.01)	5.30 (3.13)	6.75 (2.18)
OP	12.90 (5.10)	−10.86 (5.23)	3.40 (2.30)	5.79 (3.07)

Table 3: Means and standard deviations (SD) of color changes (ΔE) after whitening.

Group	ΔE (SD)		
	Time 1	Time 2	Time 3
AS	1.23 (0.45)^{Aa}	1.98 (0.76)^{Aa}	2.28 (1.35)^{Aa}
CR	5.82 (1.43)^{Ba}	7.85 (2.71)^{Ba}	12.42 (4.47)^{Bb}
LS	8.51 (1.45)^{Ba}	11.35 (3.93)^{Cb}	14.70 (3.39)^{Bc}
SC	7.84 (1.72)^{Ba}	10.88 (3.03)^{Cb}	14.34 (4.87)^{Bc}
OP	12.18 (4.37)^{Ca}	21.35 (2.73)^{Db}	20.28 (5.63)^{Cb}

Different uppercase letters represent statistically significant difference among groups. Different lowercase letters represent statistically significant difference at the time intervals evaluated ($P < 0.05$).

obtained after staining were analyzed with a one-way ANOVA to prevent possible differences in color among the groups. After the whitening process, the color parameters (ΔE, ΔL, Δa, and Δb) were analyzed with a two-way ANOVA (mouthwashes and time). Multigroup comparisons were conducted with the Tukey test at a 95% confidence interval.

3. Results

The mean values of the color parameters for each group after staining are given in Table 2. After 7 days of immersion in the tea solution, L^* values decreased from baseline recordings, whereas a^* and b^* values increased. One-way ANOVA revealed no significant difference among groups for each color parameter ($P > 0.05$).

The means and standard deviations of the ΔE values after whitening are shown in Table 3. At the end of the whitening process, there was no statistical difference in the color change (ΔE) among the mouthwash groups ($P > 0.05$). The color change (ΔE) in the OP group was significantly higher than those in the other groups. The results of the two-way ANOVA showed that the immersion time and mouthwashes (groups) and their interaction had a significant effect on the color change (ΔE) ($P < 0.01$). The ΔE values of all the groups were significantly different than the ΔE value of the AS group at 7, 28, and 56 days.

The means of the ΔL, Δa, and Δb values after whitening are shown in Figures 1, 2, and 3, respectively. In all groups, except for the AS group, the ΔL, Δa, and Δb values changed significantly over time ($P < 0.05$). After the 56-day treatment period, no significant difference for the Δa values was found among the whitening products ($P = 0.16$), but the ΔL and Δb values were significantly different ($P < 0.05$). ΔL values

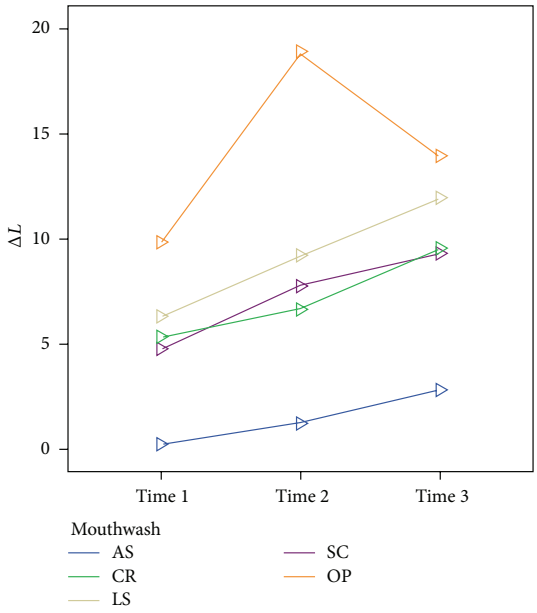

Figure 1: Mean ΔL values after whitening at 7, 28, and 56 days.

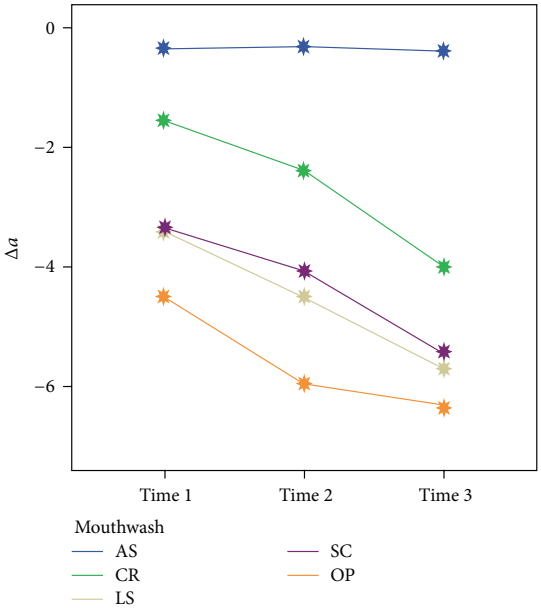

Figure 2: Mean Δa values after whitening at 7, 28, and 56 days.

decreased in the OP group after 14 (28 in Table 2) days, whereas they increased in the other groups over time. The redness and yellowness of all the samples decreased over time.

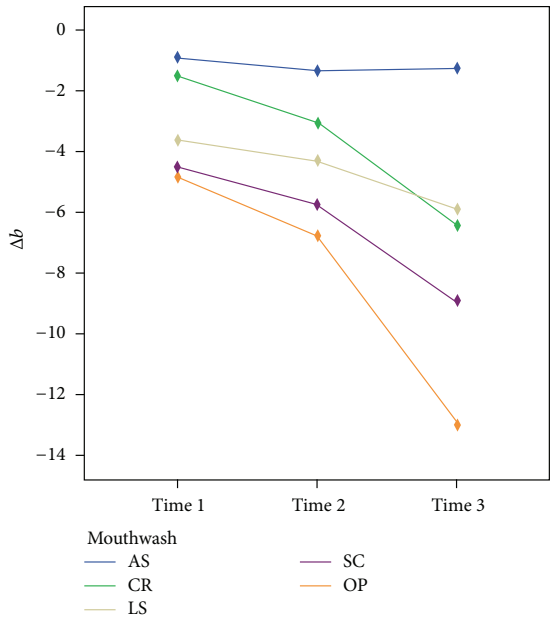

Figure 3: Mean Δb values after whitening at 7, 28, and 56 days.

4. Discussion

This in vitro study evaluated color changes of stained teeth treated with commercially available mouthwashes containing HP compared with those of teeth whitened in a 10% CP gel. The findings of the two-way ANOVA revealed that the immersion period and the mouthwashes had a major influence on color changes. Thus, the null hypothesis that the mouthwashes would have no effect on the whitening of stained teeth was rejected.

One of the most prevalent drinks in the world is tea, following water. The reported health benefits of tea have made the beverage increasingly popular, but patients are concerned about its effects of staining on teeth [12]. In vitro studies have demonstrated the staining effects of coffee, red wine, and tea [13, 14]. In the present study, tea staining was preferred because tea has been proven to have a higher capacity for staining teeth than other solutions, such as coffee or chlorhexidine [15, 16].

The surfaces of the samples were not flattened before the experiment in order to simulate clinical situations. This situation might have led to greater variations among the samples in the adsorption of color molecules and measurement of color because of irregularities in the surface textures of the samples [17].

As reported in previous studies, bovine incisors were selected to assess the tooth color change because of the ease of standardization and obtaining samples [17, 18]. The use of human teeth in in vitro investigations is limited due to ethical restrictions [19]. Extracted human teeth generally have restorations or caries that interfere with the color analysis of teeth. On the other hand, bovine teeth provide an adequate flat surface, making it easier to obtain standardized measurements [20]. As the chemical composition and structure of bovine teeth are similar to those of human teeth, bovine tooth hard tissues are often used as substitutes for human teeth in research [21]. A previous study reported that the staining of bovine and human teeth was similar, as were the effects of whitening [18].

Whitening mouthwashes have a low concentration of HP and sodium hexametaphosphate, potassium pyrophosphate, and sodium citrate. These ingredients work to whiten teeth either by bleaching or by removal and control of stains. Hydrogen peroxide diffuses through the organic matrix of tooth and produces free radicals that lead to successful whitening [22, 23]. However, the efficacy of whitening mouthwashes may be decreased by the fact that they are in contact with the teeth for a short period of time compared with bleaching gel for use at home. The results of this study showed that the amount of time the stained teeth were immersed in the mouthwash was a significant factor for tooth whitening.

Sodium hexametaphosphate has multiple binding sites and antitartar properties that help prevent staining of teeth. Also, known as polypyrophosphate, sodium hexametaphosphate chemically removes existing stains and provides long-lasting inhibition of new-stain chromogen adsorption to the tooth surface [24]. In the present study, mouthwashes containing sodium hexametaphosphate did not have an effect with respect to color change that was statistically significant compared to the other tested mouthwashes.

The literature is somewhat contradictory with respect to the effectiveness of whitening mouthwashes. A previous study reported that different peroxide-based whitening mouthwashes did not have a bleaching effect on stained teeth after a 21-day application period [25]. On the other hand, Torres and colleagues reported that the color change achieved with whitening mouthwashes used for 12 weeks was similar to that achieved with 10% CP used for 14 days [26]. In a recent study, de Jaime and colleagues examined the efficacy of a mouthwash containing HP compared with 10% CP and reported that one mouthwash (Colgate Plax Whitening) was able to whiten stained enamel, but they reported that the amount of color change was significantly lower than that obtained with 10% CP used for 28 days [10]. In the present study, the color changes (ΔE) of all the samples treated with the mouthwashes were significantly lower than those of the samples treated with the 10% CP at all of the evaluated time intervals, but they were significantly higher than those of the negative control group. In this study, all of the mouthwashes were used according to the recommendations of their manufacturers. Comparing this study to other studies is challenging due to a number of possible factors, including study protocol differences, staining level of samples, sample preparation, mouthwash application protocol, and in vivo and in vitro conditions.

Home bleaching treatment caused teeth whitening that was significant compared with the whitening caused by use of the mouthwashes. This difference often depended on the changes of ΔL and Δb parameters obtained with home bleaching gel, which were statistically significant compared

to the mouthwashes. For Δa, there were no significant differences among the whitening products at the end of treatment period. However, the ΔL values of the OP group decreased after 14 days; this reduction was likely caused by color regression that the organic substances of the artificial saliva might contribute to [27]. Li and colleagues reported that most of the color regression was stimulated by the L^* value [28]. Despite completion of the home bleaching treatment, the Δa and Δb values continued to decline, which may be explained by the presence of the remaining oxygen radicals in tooth structure or some alterations within the tissues of the teeth. The changes in the color parameters of tested mouthwashes were statistically similar after the completion of whitening treatment.

The 10% CP was chosen as the positive control group because previous studies confirmed that it was a safe and effective technique; this has been reported in the majority of publications on home bleaching in the last 20 years [29]. A previous study also reported that the most acceptable way to whiten teeth was the at-home bleaching method [30]. Research has demonstrated that the whitening effect achieved by home bleaching was stable and long lasting [31]. Home bleaching gel (10% CP) contains 3.5% HP, and this percentage is greater than that found in the mouthwashes examined in this study. Although home bleaching HP/CP gel in a tray has limited contact with teeth and gums, mouthwash is in contact with all the oral mucosa [26].

5. Conclusions

Within the limitations of this in vitro study, each of the tested mouthwashes increased the whiteness of teeth over time, bleaching the stained teeth. However, none of the mouthwashes were as effective as 10% CP at-home bleaching gel. No significant differences were found among the mouthwashes with respect to color changes (ΔE). When the samples were exposed to whitening products, the ΔL values and Δb values showed significant differences according to the product used.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this study.

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