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REVIEW / DERLEME

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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Effect of Indigo Naturalis on the Viability of Human Periodontal Ligament Fibroblast Cells: An In Vitro Study

İndigo Naturalis'in İnsan Periodontal Ligament Fibroblast Hücrelerinin Canlılığı Üzerine Etkisi: Bir In Vitro Çalışma

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ÖZ

Amaç: Bu in vitro araştırmada, insan periodontal ligament fibroblast (İPDLF) hücrelerine farklı konsantrasyonlarda İndigo naturalis (İN) uygulanarak hücrelerin canlılık düzeylerinin araştırılması hedeflenmiştir.
Gereç ve Yöntemler: Çalışmamız dahilinde İndigo naturalis DMSO ile çözülerek 5 µg/ml, 10 µg/ml ve 15 µg/ml olacak şekilde üç farklı konsantrasyon hazırlandı. Hücre canlılığını değerlendirmek amacıyla, 96 kuyucuklu plakalara her konsantrasyon için 4 defa tekrarlanacak şekilde hücrelerin ekimi gerçekleştirildi. Hücrelere 24 saat süreyle İndigo Naturalis uygulandı. Canlılık değerlendirmesi için 3-(4,5-dimetiltiyazol-2-il)-2-5-difeniltetrazolyum bromür (MTT) deney protokolü izlendi. Verilerin istatistiksel analizleri SPSS paket programı kullanılarak değerlendirildi. İstatistiksel anlamlılık $p<0.05$ olarak kabul edildi.
Bulgular: İN uygulamasının kontrol grubuna kıyasla test gruplarında anlamlı değişiklik olmadığı görülürken 15 µg/ml ve 5 µg/ml konsantrasyon arasında anlamlı fark tespit edilmiştir.
Sonuç: Bu çalışmanın sınırları içerisinde sağlanan bulgular, İN'nin iPDLF hücrelerinin canlılığı üzerine farklı konsantrasyonlarda benzer etki gösterdiğini ortaya koymaktadır. İN'nin daha farklı konsantrasyonlarda hazırlanarak ve daha uzun süre aralıklarında takip edilerek araştırıldığı çalışmalara gereksinim vardır.
Anahtar Kelimeler: İndigo naturalis, periodontal ligament fibroblastı, hücre canlılığı

ABSTRACT

Objectives: In this in vitro research, it was aimed to examine the viability levels of human periodontal ligament fibroblast (hPDLF) cells by applying different concentrations of Indigo naturalis (IN).
Materials and Methods: Within the scope of our study, IN was dissolved in DMSO and three different concentrations were prepared:, 5 µg/ml, 10 µg/ml and 15 µg/ml. In order to evaluate cell viability, cells were seeded in 96-well plates, repeated 4 times for each concentration. Indigo Naturalis was applied to the cells for 24 hours. 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) experimental protocol was followed for viability assessment. Statistical analyzes of the data were evaluated using the SPSS package program. Statistical significance was accepted as $p<0.05$.
Results: While it was observed that there was no significant change in the test groups with IN application compared to the control group, a significant difference was detected between 15 µg/ml and 5 µg/ml concentration.
Conclusions: The findings obtained within the limits of this study reveal that IN has similar effects at different concentrations on the viability of hPDLF cells. There is a need for studies in which IN is investigated by preparing it in different concentrations and monitoring it for longer periods of time.
Keywords: Indigo naturalis cell viability, periodontal ligament fibroblast.

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GİRİŞ

Periodonsiyum, dişlerin etrafında bulunan ve dişlere destek olan bir yapıdır. Bu yapı; dişeti, sement, periodontal ligament ve alveol kemiğinden oluşur. Periodonsiyum esas olarak dişlerin fonksiyonu esnasında görev alarak dişleri ağızda tutmaya yardımcı olur (Newman ve ark., 2011). Periodontal hastalıklar, periodonsiyumun tahrip olmasına neden olan iltihabi ve ilerleyici hastalıklardır (Tonetti ve ark., 2018). Periodontal hastalıkların tedavisinde ise hedef, hastalığın ilerlemesini durdurmak ve kaybedilmiş dokuları yerine koymaktır (Graziani ve ark., 2017). İdeal periodontal tedavi tahrip olmuş periodonsiyumun rejenerasyonunu kapsamalıdır.

Hücreler, periodonsiyumun rejenerasyonunda sinyal molekülleri ve taşıyı iskelet yapılarla birlikte görev alan esas bileşenlerdir (Taba ve ark., 2015). Periodontal ligamentin periodontal dokuların rejenerasyonunda önemli rol oynayan öncül hücreleri içerdiği bilinmektedir (Melcher, 1976). Periodontal ligamentin temel hücresi olan fibroblastların ise esas görevi kolajen yapım ve yıkım metabolizmasıdır (Benatti ve ark., 2007). Hasar meydana geldiğinde aktifleşerek çoğalır ve yara bölgesine göç ederler, ekstraselüler matriks sentezi yapımına katılarak tamir aşamasında etkin rol oynarlar (Narayanan ve Page, 1983, Melcher, 1976).

Her canlı organizmanın hücresel faaliyetlerinin düzenlenmesinde görevli olan çeşitli sinyal yolları bulunmaktadır. Bunlardan biri olan Wnt sinyal yolu (WSY), embriyo gelişimi sırasında ve erişkin dokuların devamlılığının sağlanmasında çok önemli role sahiptir. Wnt sinyal iletimi, erişkin dönemde rejenerasyon kapasitesi olan hücrelerin adezyonunu ve gen transkripsiyonunu sağlar (Tanır ve Demirezen, 2009). Doku hasarına verilen erken cevap olarak WSY aktive edilir ve bu aktivasyon tüm dokularda hücresel tamirin gerçekleşmesi için gereklidir (Neves ve ark., 2017).

Wnt sinyal iletimi birkaç farklı yol ile meydana gelmektedir. Wnt sinyalleri arasında en önemli olanı kanonik olarak da geçen ß-katenin bağlantılı olan yolaktır (Kikuchi ve ark., 2011). Bu yolakta Wnt ligandının hücre yüzeyindeki reseptörlere bağlanması ile hücre içi sinyalizasyon başlatılır. Bu bağlanmanın gerçekleşmediği, sinyalin inaktif olduğu durumda glikojen sentaz kinaz 3 (GSK-3) enzimi hücre içindeki ß-katenin molekülünün fosforillenerek parçalanmasına yol açar. Wnt ligandlarının varlığında ise GSK-3 aktivitesi inhibe edilerek ß-katenin'in çekirdeğe girmesine izin verilir ve hedef genlerin ekspresyonunu düzenlemek üzere transkripsiyon faktörleriyle etkileşime girer (Minear ve ark., 2010; Whyte ve ark., 2012).

Wnt/ß-katenin sinyal yolunun diş gelişiminde en önemli sistemlerden biri olduğu bilinmektedir (Liu ve Millar, 2010). Wnt aktivasyonunun kemik dokunun osteoblastlar tarafından yapımını sağladığı ve Wnt inhibisyonunun kemik yoğunluğundaki azalma ile ilişkili olduğu çalışmalar tarafından gözlenmiştir (Bonewald ve Johnson, 2008; Hsu ve Kiel, 2012). Yapılan bir in vivo çalışma, yeni sement oluşumunun Wnt/ß-katenin sinyal yolunun düzenlenmesi ile sağlanabileceğini göstermiştir (Han ve ark., 2015). Wnt/β-katenin, sinyal yolağının kemik morfogenetik protein 2 ile ilişkili olarak osteoblastik aktivasyonu düzenlediği bulunmuştur (Zhang ve ark., 2013). Wnt/ß-katenin sinyali, fibroblastlarda proliferasyonu kontrol ederek transkripsiyon faktörleri ve sinyal proteinlerini kodlayan genleri etkilemektedir (Cheon ve ark., 2002; Cheon ve ark., 2004; Klapholz-Brown ve ark., 2007). İnsan periodontal ligament hücreleri Wnt aktivasyonu sağlayacak ortamda bulunduklarında kemik ve sement yapımı ile ilgili gen ve protein ekspresyonunun arttığı gözlenmiştir (Han ve ark., 2015).

İndigo naturalis (İN), *Baphicacavthus cusia, Polygonum tinctorium, İsatis indigotica ve İndigofera tinctoria* gibi indigo taşıyan bitkilerin yapraklarından elde edilen bir Çin bitkisel ilacıdır. Geleneksel Çin doktorları İN'yi ateş düşürücü olarak, kanı detoksifiye etmek amaçlı ve ekimozları çözmek için kullanmışlardır (Sun ve ark., 2020). Ayrıca İN'nin farmakolojik etkileri de incelenmiştir. Modern araştırmalar, İN›nin antienflamatuar, antioksidan, antibakteriyel, bağışıklık düzenleyici ve başka aktiviteleri olduğunu bulmuştur (Gaitanis ve ark., 2018; Naganuma, 2019; Yu ve ark., 2021). Bu özellikleri sayesinde İN; sedef hastalığı, lösemi, ülseratif kolit gibi hastalıkları tedavi etmek amacıyla klinik çalışmalarda kullanılmaktadır (Qi-Yue ve ark., 2020). İN'nin içerdiği indirubin türevlerinin GSK-3 sentezini inhibe ederek WSY'yi aktive ettiği görülmüştür (Leclerc ve ark., 2001).

Bu bilgiler doğrultusunda içeriğinde GSK-3 sentezini inhibe ederek WSY aktivasyonu sağlayan moleküller içeren İN'nin periodontal rejenerasyon açısından olumlu etkileri olabileceği düşüncesi ortaya çıkmıştır. Bu nedenle bu *in vitro* çalışmanın amacı, farklı konsantrasyonlarda İndigo naturalis'e maruz bırakılan insan periodontal ligament fibroblastlarının (İPDLF) canlılık düzeylerini değerlendirmektir.

GEREÇ VE YÖNTEMLER

İndigo Naturalis Ekstresinin Hazırlanması

Hücre kültürü deneylerinde İN gibi suda çözünmeyen doğal bileşikleri çözmek için dimetil sülfoksit (DMSO) sıklıkla tercih edilir. DMSO, birçok organik bileşiği iyi derecede çözebilen ve hücrelere minimal toksisite gösteren bir çözücüdür. Bu nedenle, İN'yi çözmek için DMSO kullanıldı ve hücre ortamında % 0,1 maksimum toksik sınır konsantrasyonu geçmeyecek şekilde hücrelere uygulandı. İN tozu, standardize edilmiş bir tedarikçiden temin edildi. 100 mg/mL konsantrasyonunda çözelti elde etmek amacıyla dimetil sülfoksit (DMSO) içinde çözüldü. Elde edilen çözelti sterilizasyon amacıyla 0.22 µm şırınga filtresi kullanılarak filtreden geçirildikten sonra – 20'C'de saklandı.

Hücre Kültürü

Deneyler, ticari olarak satın alınan İPDLF (CC-7049, LONZA, Basel, İsviçre) hücre hatları kullanılarak yapıldı. İPDLF hücreleri, 37 °C sıcaklıkta; % 1 penisilin/streptomisin, %

1 L-glutamin (CAS No: 56-85-9), % 0,1 amfoterisin B (CAS No: 1397-89-3) ve % 10 fetal sığır serumu (CAS No: 9014-81-7, GibcoTM, Thermo Fisher Scientific, ABD) eklenmiş Dulbecco's Modified Eagle Medium (Biochrom AG, Berlin, Almanya) içerisinde kültür edilerek üretici firmanın talimatları izlenerek çoğaltıldı. İPDLF hücrelerini içeren flasklar % 70-80 yoğunluğa ulaştıktan sonra hücreler, her kuyuda 2 mL besiyeri içeren, kuyu başına hücre yoğunluğu $1,5x10^5$ hücre olacak şekilde 96 kuyucuklu plakalara ekildi ve kuyulara tutunabilmeleri için 24 saat süreyle 37 °C ısıda inkübasyonda bekletildi. İnkübasyon sonrası ortamdaki besiyeri elimine edildi. Hücreleri içeren kuyulara 5, 10 ve 15 µg/ml konsantrasyonunda İndigo naturalis veya 2 mL besiyeri (kontrol grubu) ilave edilerek 24 saat süreyle beklendi. Daha sonra hücre canlılığını ölçmek amacıyla MTT testi uygulama aşamasına geçildi.

MTT Testi

İPDLF hücrelerinde hücre canlılığı 3-(4,5-dimetiltiazol2-il)-2,5-difeniltetrazolyum bromür (MTT) (Glentham Life Sciences, İngiltere) testi uygulanarak araştırıldı. Kuyucuklar fosfat tampon çözeltisi (Wisent, Kanada) kullanılarak yıkandı, her kuyucuğa 132 µL MTT boyama solüsyonu eklenerek 37 °C sıcaklıkta inkübasyon 3 saat için beklendi. Her bir kuyucukta bulunan hücre ortamı uzaklaştırılarak 200 µL DMSO (CAS No: 67-68-5) eklendi ve böylece MTT formazonu açığa çıkarıldı. 10 dk beklendikten sonra, kuyucuklardan alınan 200 µL ortam, 96 kuyucuklu plakalara transfer edildi ve optik dansite (OD) bir plaka okuyucu (Perkin Elmer Enspire multimode, Boston, ABD) yardımıyla 570 nm'de okundu. Okuyucuda izlenen absorbans değerleri kullanılarak aşağıda verilen formül yardımıyla canlılık (%) değerleri hesaplandı.

[(OD örnek – OD hücresiz)/(OD kontrol – OD hücresiz)] x 100

İstatistiksel Analizler

Verilerin istatiksel olarak değerlendirilmesinde SPSS paket programı kullanılmıştır (SPSS Inc., Chicago, IL, ABD). Değişkenlerin dağılımının normal olup olmadığı kontrol edilirken *Kolmogorov Smirnov* testi kullanıldı. Bu değerlendirmenin neticesinde çalışmadan edinilen sayısal değişkenlerin normal dağılım göstermediği tespit edildi. Gruplar arasında çoklu karşılaştırma yapılırken *Kruskal-Wallis* testi, anlamlı farklılık tespit edilmesi durumunda iki grubu kıyaslamak amacıyla *Bonferroni* **düzeltmeli** *Mann-Whitney U* testi kullanıldı. İstatistiksel anlamlılık koşulu için *p* değeri <0,05 olarak değerlendirildi.

BULGULAR

Farklı konsantrasyonlarda İN içeren çözeltilerin 24 saat süreyle uygulanması durumunda İPDLF hücrelerinin canlılık sonuçları Tablo 1'de verilmiştir. Tablo 2'de ise gruplar arası karşılaştırmada elde edilen *p* değerleri verilmiştir. İN uygulamasının kontrol grubuna kıyasla hiçbir konsantrasyonda hücre canlılığına etki etmediği görülürken 15 µg/ml ve 5 µg/ml konsantrasyon arasında anlamlı fark tespit edilmiştir. 5 µg/ml konsantrasyonunda İN uygulamasının, 15 µg/ml konsantrasyonunda İN uygulamasına göre canlılık açısından daha olumlu etki gösterdiği gözlenmiştir.

Tablo 1. İPDLF hücre canlılık sonuçları

	Ort ± SS	Medyan	(Min - Max)
Kontrol[a]	100,00 ± 0,00	100,00	(100,00 - 100,00)
5 µg/ml[b]	122,98 ± 8,82	124,67	(110,76 - 131,81)
10 µg/ml[c]	92,34 ± 22,22	93,47	(64,90 - 117,53)
15 µg/ml[d]	35,58 ± 7,69	35,20	(26,56 - 45,36)
p[*]	0,006		

*Kruskal Wallis testi, SS: Standart Sapma, p<0,05

Tablo 2. Karşılaştırmalı *p* değerleri

P^{a-b}	P^{a-c}	P^{a-d}	P^{b-c}	P^{b-d}	P^{c-d}
0,512	1,000	0,435	0,599	0,003	0,369

#Bonferroni düzeltmeli Mann Whitney-U testi

TARTIŞMA

WSY'nin canlılardaki hücre farklılaşması, hücre proliferasyonu ve hücre göçünde etkinliğiyle birlikte doku yapım ve onarım metabolizmasında çok önemli bir role sahip olduğu bilinmektedir (Neves ve ark., 2017; Tanır ve Demirezen, 2009; Whyte ve ark., 2012). Bu nedenle güncel çalışmalar WSY aktivasyonu yoluyla rejenerasyonu hedeflemektedir. WSY aktivasyonu sağlayan yollardan biri GSK-3 inhibisyonudur (Neves ve ark., 2017). İN'nin içerdiği maddelerden biri olan İndirubinin GSK-3 inhibisyonu yaptığı bilinmektedir (Leclerc ve ark., 2001). Bu çalışma İN'nin periodontal rejenerasyondaki olası etkinliğini değerlendirmek amacıyla yapılan ve İPDLF hücrelerinin canlılığını değerlendiren ilk çalışmadır. Çalışmamız İN'nin İPDLF hücrelerinin canlılığı üzerine olumlu etkisi olacağı hipotezi düşünülerek planlanmıştır.

İN'nin İPDLF hücrelerinin canlılık ve proliferasyon sonuçlarını araştırmak amacıyla MTT testi kullanılmıştır. Bu test, proliferasyona uğrayan hücrelerin enzimlerinin MTT tuzunu mor renkli kristallere dönüştürmesi sayesinde canlılığın spektrofotometrik olarak ölçülmesi esasına dayanır. MTT yöntemi; hızlı ve çok tekrar edilebilir olmakla birlikte güvenilir bir test yöntemidir. Hücre canlılık testleri arasında 'altın standart' olarak kabul edilmektedir (Kumar ve ark., 2018; Mosmann, 1983).

Hücre kültürü çalışmalarında canlılık sonuçlarının araştırılması hedeflenen deney uygulama süreleri çalışma planına göre farklılık arz etmektedir. İPDLF hücrelerinin aktivitesi ve canlılığı ile ilgili çalışmalarda bu süre kısa veya uzun dönem olmak üzere 1 saatten 8 güne kadar değişmektedir (Choe ve ark., 2012; Correia Vde ve ark., 2006). Çalışmamızda hücreler, Heo ve arkadaşları ile Kook ve arkadaşlarının çalışmalarında izlediği gibi 24 saat süreyle muamele edilerek canlılık değerlendirilmesi yapılmıştır (Heo ve ark., 2010; Kook ve ark., 2016).

Lin ve arkadaşlarının yaptıkları bir in vitro çalışmadaki İN konsantrasyon değerleri çalışmamızda seçilen doz konusunda yol gösterici olmuştur (Lin ve ark., 2012). Deney

protokolümüzde 5, 10 ve 15 µg/ml konsantrasyon değerleri kullanılmıştır. Canlılık deneyinde test gruplarında kontrol grubuna göre anlamlı değişiklik bulunmamıştır. Ancak 5 µg/ml konsantrasyonda İndigo naturalis uygulanan gruba kıyasla 15 µg/ml konsantrasyon İN uygulanan hücrelerde canlılık düzeyinde azalma gözlenmiştir.

Güncel araştırmalar İN'nin antienflamatuvar, antioksidan ve onarıcı etkileri olduğunu düşündürmektedir. Literatürde rastlanan bir in vitro çalışmada bulgular; İN'nin, insan nötrofillerinde süperoksit (O_{2^-}) oluşumunu ve elastaz salınımını inhibe ederek antienflamatuvar etki ettiğini göstermektedir (Lin ve ark., 2009). Yapılan bir diğer çalışmada 10 µg/ml İN'nin, insan keratinositlerinde ekzojen reaktif oksijen türevleri (ROS) tarafından indüklenen hücre içi ROS oluşumu ve bir lipit peroksidasyon ürünü olan HNE tarafından indüklenen protein modifikasyonu üzerinde inhibitör bir etkiye sahip olduğu gözlenmiştir (Lin ve ark., 2012). Yine Lin-Ku Yin ve arkadaşlarının yapmış olduğu başka bir in vitro çalışmada IN'nin, claudin-1 ekspresyonunu artırdığı ve insan keratinositlerindeki tight junction fonksiyonunu onardığı sonucuna varılmıştır (Lin ve ark., 2013).

WSY aktivasyonunun fibroblastların etkinliğinin artmasını ve çoğalmasını sağlayarak çeşitli dokularda fibrozis meydana getirebildiği kanıtlanmıştır (Somanader ve ark., 2024). Bu durum miyokard, akciğer, böbrek veya deri dokusunda meydana gelebilmektedir (Griffin ve ark., 2022; Patel ve ark., 2024; Tian ve ark., 2024; Zhang ve Lu, 2024). Heo ve arkadaşları İPDLF hücrelerinde WSY aktivasyonunu araştırmış ve WNT aktivatörü olan lityum klorürün İPDLF hücrelerinde transkripsiyon faktörlerinin uyarılmasıyla birlikte hücrelerin osteojenik soylara farklılaşmasını sağladığını göstermiştir (Heo ve ark., 2010). Kook ve arkadaşları WSY proteinlerinin İPDLF hücrelerinin osteojenik farklılaşmasını ve mineralizasyonunu uyardığını ileri sürdükleri bir çalışmanın ardından başka bir çalışmada ise Hidrojen peroksit (H_2O_2) aracılı oksidatif stresin, İPDLF hücrelerinin hayatta kalmasını ve osteojenik farklılaşmasını azalttığını, oysa bu azalmaların Wnt yolunun aktivasyonuyla önlendiğini kanıtlamıştır (Kook ve ark., 2015; Kook ve ark., 2016). Han ve arkadaşları çalışmalarında WSY aktivasyonunun insan periodontal ligament hücrelerinin proliferasyon ve osteojenik potansiyelinin arttırdığını gözlemlemiştir (Han ve ark., 2012). Daha önce İN'nin içerdiği indirubin türevlerinin GSK-3 sentezinin inhibisyonu yoluyla Wnt aktivasyonu sağladığı kanıtlanmıştır (Leclerc ve ark., 2001). Araştırmamız İPDLF hücrelerinde İN uygulumasını değerlendiren ilk çalışma olmakla beraber araştırmamızın sonucunda yukarda bahsedilen çalışmalarla ilişkili olmayarak test grubunda kontrol grubuna kıyasla anlamlı artış gözlenmemiştir. Ancak bununla birlikte bulgularımız, İN'nin doza bağımlı etkiler sergilediğini düşündürmektedir. Özellikle, daha düşük bir doz olan 5 µg/ml'nin daha yüksek bir doz olan 15 µg/ml'ye göre hücre canlılığı üzerinde daha olumlu etkiler göstermesi, yüksek dozların hücreler üzerinde toksik etkiler oluşturabileceği olasılığını akla getirmektedir. Bu durum, İN'in hücre canlılığı üzerindeki etkilerinin optimizasyonu için düşük dozların daha avantajlı olabileceğini işaret etmektedir. 15 µg/ml konsantrasyonunda gözlenen olumsuz etkiler, İN›nin yüksek dozlarının hücre canlılığını azaltıcı toksik etkiler oluşturabileceğini göstermektedir. Bu bulgu, literatürde İN'nin etkilerini araştıran çalışmalarla tutarlıdır. Bir çok çalışma, İN'nin düşük dozlarda faydalı olabileceğini, ancak yüksek dozlarda toksik olabileceğini bildirmektedir (Xu ve ark., 2024, Sun ve ark., 2021). Çalışmamızın sonuçlarının taramalı elektron mikroskobu ile desteklenmesinin ve hücrelerin proliferasyon ve farklılaşmasına yönelik biyolojik belirteçlerin değerlendirilmesinin gerekli olduğu düşünülmektedir.

SONUÇ

Bu *in vitro* çalışmanın sonucunda 5, 10 ve 15 µg/ml konsantrasyonlarda İN'nin İPDLF hücrelerinin canlılığına olumsuz etki göstermediği öte yandan canlılığı arttırıcı yönde etki ortaya koymadığı sonucuna varılabilir. Çalışmamız İN'nin periodontal ligament üzerine etkinliğini değerlendiren ilk çalışma olduğu için bu alanda ufuk açsa da, farklı periodontal hücreler, geniş doz aralıkları ve çeşitli protein ekspresyonlarının araştırılmasını içeren daha kapsamlı çalışmalara ihtiyaç duyulmaktadır.

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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Investigation of Saliva Parameters and Tissue Factor in Healthy Individuals Who Had Survived COVID-19 Infection

Türkçe başlık: COVID-19 Enfeksiyonunu Geçirmiş Sağlıklı Bireylerde Tükürük Parametreleri ve Doku Faktörünün Araştırılması

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ÖZ

Amaç: Ağız mukozası, COVID-19 enfeksiyonunun yayılması için uygun bir alandır ve tükürük bezlerinde fonksiyon bozukluklarına neden olur. Doku faktörü, tükürükte de bulunan, hemostaz ve pıhtılaşma mekanizmalarında rol oynayan önemli bir düzenleyicidir. Çalışmanın amacı, COVID-19 enfeksiyonunun tükürük bezi fonksiyonu ve ağız sağlığı üzerindeki etkilerini tükürük yoluyla incelemektir.
Gereç ve Yöntemler: Kontrol grubu daha önce COVID-19 enfeksiyonu geçirmemiş 20 kişiden oluşurken, COVID grubu ise en az 6 ay önce COVID-19 enfeksiyonu geçiren 26 kişiden oluştu. Tükürük örneklerinde tükürük akış hızı, tamponlama kapasitesi, pH ve doku faktörü aktivitesi değerlendirildi.
Bulgular: Kontrole göre COVID grubunda tükürük akış hızı, tamponlama kapasitesi ve doku faktörü aktivitesi anlamlı derecede azalırken, pH'ın gruplar arasında istatistiksel olarak anlamlı olmadığı görüldü.
Sonuç: COVID-19 enfeksiyonu sonrasında ağız dokularında çeşitli hasarlar ve buna bağlı fonksiyon bozuklukları gelişmektedir. Bu nedenle COVID-19 geçiren bireylerin ağız hastalıklarına eğilimi daha fazla olabilir.
Anahtar Kelimeler: Tükürük, Akış hızı, Tamponlama kapasitesi, Doku faktörü, COVID-19

ABSTRACT

Objectives: Oral mucosa is a suitable area for the spread of COVID-19 infection and it causes dysfunction in the salivary glands. Tissue factor is an important regulator that plays a role in hemostasis and coagulation mechanisms, also found in saliva. The study aims to examine the effects of COVID-19 infection on salivary gland function and oral health through saliva.
Materials and Methods: The control group consists of 20 individuals who have not had a COVID-19 infection before, while the COVID group consists of 26 individuals who had a COVID-19 infection at least 6 months ago. Salivary flow rate, buffering capacity, pH, and tissue factor activity were evaluated in saliva samples.
Results: Salivary flow rate, buffering capacity, and tissue factor activity decreased significantly in the COVID group compared to the controls, while pH was found to be statistically not significant between the groups.
Conclusions: Various damages and related dysfunctions develop in the oral tissues following a COVID-19 infection. For this reason, individuals who have had COVID-19 might have more tendency to oral diseases.
Keywords: Saliva, Flow rate, Buffering capacity, Tissue factor, COVID-19

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INTRODUCTION

Saliva is a secretion that constantly washes the teeth and oral mucosa and it plays a major role in protecting the integrity of oral tissues and maintaining oral health. Healthy saliva is critical for maintaining oral health and comfort (Wani et al., 2023). When the composition of saliva changes, and/or salivary glands' dysfunction develops, such as a decrease in salivary flow rate and change in saliva pH and buffering capacity, it becomes difficult to chew and swallow food, halitosis occurs, the sense of taste weakens, speech becomes difficult, the use of removable prosthesis becomes difficult, the development of atypical caries accelerates, the frequency of oral infections increases and the rate of dental plaque accumulation increases (Proctor& Shaalan, 2021; Chibly et al., 2022).

Tissue factor is an integral membrane protein that triggers coagulation in case of damage and is inactive in the blood under normal conditions. When activated, it activates the extrinsic pathway of coagulation and initiates the clotting mechanism. Indeed, tissue factor plays crucial roles in immunity and wound healing in addition to coagulation (Unruh&Horbinski, 2020).

SARS-CoV-2 virus, which is the causative agent of COVID-19 infection, binds to the angiotensin-converting enzyme 2 (ACE2) receptors of host cells with glycoproteins on its surface, and these glycoproteins mediate the fusion of the virus with the host cell membrane, allowing the virus to enter the body (Saxena et al., 2020). ACE2 is highly expressed in the oral cavity and is highly abundant in epithelial cells. There is also ACE2 expression in the salivary glands. Therefore, the oral mucosa is a suitable area for the spread of COVID-19 infection, and the salivary glands are one of the foci of infection for SARS-CoV-2 (Xu et al., 2020). When SARS-CoV-2 infects the body, the inflammatory response gets activated and plays an antiviral role. The virus induces an increase in cytokines which may result in a cytokine storm causing damage and dysfunction in cells and organs (Song et al., 2020).

It is known that SARS-CoV-2 replicates in the salivary glands and disrupts the integrity of the gland tissue. Besides, COVID-19 infection causes dysfunction in the salivary glands, resulting in dry mouth, changes in salivary flow and composition, and impaired taste perception (Brandini et al., 2021)

Based on this information, the study aims to examine the effects of COVID-19 infection on salivary gland function and oral health through saliva.

MATERIALS AND METHODS

Ethics committee approval was received from the Marmara University Health Sciences Institute Ethics Committee (protocol no:09.2022.161). Before sample collection, individuals were informed and volunteer individuals were included in the study. Oral anamneses and an interview consisting of questions on oral hygiene habits and oral complaints comprised the initial step of the study.

Groups

Individuals between the ages of 18-65, who do not have a genetic disease, do not have a chronic and/or autoimmune disease, do not use regular medication, and do not have dehydration or salivary gland hypofunction are included in the study.

The control group consists of 20 individuals who have not had a COVID-19 infection before, while the COVID group consists of 26 individuals who had a COVID-19 infection at least 6 months ago.

Assessment of the salivary flow rate, buffering capacity, and pH

Volunteers participating in the study were not allowed to eat, chew gums, or brush their teeth. After rinsing their mouths with distilled water, individuals were placed in a quiet environment and were not exposed to any stress. Individuals were seated with their arms and shoulders free and upright. Individuals first swallowed the saliva available in their mouths. Afterward, the individual spat into the sterile falcon tube for 5 minutes without swallowing and at regular intervals. The amount of saliva collected in the tube was measured and the saliva flow rate was calculated as ml/min (Ahmadi-Motamayel et al., 2013). Salivary buffering capacity was evaluated by using the Ericsson method (Aksit-Bicak et al., 2017). Salivary pH was measured with a Thermo Scientific™ pH meter (USA) for all participants.

Determination of Tissue Factor Activity

Tissue factor activities of saliva samples were evaluated according to Quick's one-step method (Quick, 1953) using healthy plasma, and the activity results were expressed in seconds.

Statistical Analyses

Data was presented as mean ± standard deviation, and the statistical analyses were performed using the Graph Pad 6.0 Prism program. Student t-test was used for normal distribution and Kruskal-Wallis and Mann-Whitney-U tests were used for non-normal distribution to compare COVID and control groups. The significance level was set at 0.05.

RESULTS

In the present study, none of the individuals in the control group had a subjective complaint of dry mouth in oral anamnesis. 17 individuals in the COVID group complained of subjective complaints of dry mouth during COVID-19 infection.

Salivary flow rate decreased significantly in the COVID group compared to the controls ($p<0.05$), while pH was found to be statistically not significant between the groups ($p>0.05$). Buffering capacity decreased significantly in the COVID group compared to the controls ($p<0.01$) (Table 1).

Table 1. Salivary flow rate, buffering capacity, and pH values of the groups.

	COVID group (n=26)	Control group (n=20)
Salivary flow rate (ml/min)	10.58±3.19*	12.75±2.88
pH	7.10±0.51	7.23±0.35
Buffering capacity	5.11±0.66**	5.70±0.17

*p<0.05, **p<0.01 significantly different when compared with the control group.

Tissue factor activity of the COVID group decreased significantly compared to the controls (p<0.01) (Table 2). An increase in clot formation indicates a decrease in tissue activity.

Table 2. Tissue factor activity of the groups.

	COVID group (n=26)	Control group (n=20)
Tissue factor activity (sec.)	166.7±44.18**	136.5±40.40

**p< 0.01 significantly different when compared with the control group.

DISCUSSION

Salivary glands provide a suitable environment for SARS-CoV-2 to settle and multiply. Since ACE2 is expressed in the mouth and found in high amounts in epithelial cells, infection spreads easily in the oral mucosa and causes dysfunction in the salivary glands. Also, SARS-CoV-2 causes replication in the salivary glands and disrupts the integrity of the gland tissue (Brandini et al., 2021). The presence of SARS-CoV-2 in saliva may be associated with viral proliferation and RNA secretion in cells and tissues involved in the production of salivary components, such as periodontal tissue, salivary glands, and upper respiratory tract cells (Matuck et al., 2021). In studies conducted with COVID-19 cases, various damages and dysfunctions were detected in the salivary glands of patients. In a study conducted in Wuhan with 108 patients infected with SARS-CoV-2, 46.3% had dry mouth and 47.2% had amblyopia (Chen et al., 2020). Another study suggested that parotid gland inflammation may develop in COVID-19 patients and this inflammation may be related to intraparotid lymphadenitis or direct spread of the SARS-CoV-2 virus to the parotid tissue (Maegawa&Nishioka, 2022). In another study with fatal COVID-19 cases, viral particles similar to the Coronaviridae family were found by analysis of salivary gland samples taken in biopsy. In addition, a nucleocapsid cluster was also detected, suggesting the destruction of organelles of infected cells and viral replication in salivary gland cells. In histopathological analysis of the study, morphological changes characterized by cytoplasmic and nuclear vacuolization and nuclear pleomorphism were observed in the duct lining epithelium. Also, degenerative changes in the zymogen granules and enlargement of the nucleus of the acinar cells were detected (Matuck et al., 2021).

Some adverse effects of the treatment methods used in patients with COVID-19 infection can be observed in the oral cavity. Even if patients are completely cured of COVID-19 infection due to intensive pharmacotherapy, they may experience problems related to the oral cavity such as soft tissue problems, saliva production, and sensitivity. Some anti-viral treatments used in the treatment of COVID-19 can cause side effects such as stomatitis, ulcers, and dry mouth in these patients (Gutierrez-Camacho et al., 2022)

Salivary flow is crucial as it helps clean bacterial substrates, protects oral surfaces, and controls cavity development. (González-Aragón Pineda et al., 2020.) Hyposalivation, the reduction of unstimulated salivary flow rate, is a common finding in COVID-19 patients (Bergdahl et al., 2000). We also found a decreased salivary flow rate in the COVID group compared to the controls. As a consequence of the medications used in treatment, psychological processes, and inflammatory processes may also lead to hyposalivation in these patients.

A high buffering capacity is one of the desired features for oral and dental health. A decrease in buffering capacity facilitates the formation of oral diseases such as caries (Hatipoglu et al., 2022). In this study conducted after the COVID-19 virus epidemic, whose relationship with oral and dental health is not yet fully known, a significant difference was found between the salivary buffering capacities of those who had and did not have COVID-19 infection. According to our results, those who have had COVID-19 infection have lower levels of buffering capacity compared to those who have not. This result show that the large number of people affected by this virus might be more sensitive about their oral and dental health.

Saliva pH is an important factor in protecting individuals' oral health. Studies have shown that COVID-19 at least affects periodontal tissue and oral flora (Gofur, 2020). In individuals who have had COVID-19, saliva pH value may have changed due to nutritional changes, periodontal inflammations, and similar factors. In our study, the pH value of the individuals who had COVID-19 infection 6 months ago was compared with those who never had it and there was not difference between COVID and control groups. We think that a different result may be found depending on the severity of the disease and the time until treatment.

Tissue factor is present not only in blood but also in tissues and other body fluids, such as saliva (Hu et al., 2022.) It is thought that tissue factor in saliva contributes to hemostasis and the protective function of the oral mucosa in cases of injury in the oral cavity (Yatsenko et al, 2023.). Measurement of tissue factor activity in saliva can be used to evaluate the wound healing potential and bleeding tendency of the oral cavity (van der Vorm et al., 2018). The coagulation cascade can be activated via tissue factor during viral infections (Di Nicolantonio&Mc Carty, 2020; Mackman et al., 2020). According to the results obtained in our study, it can be said that COVID-19 infection causes a decrease in tissue factor activity in saliva. Several factors, such as endothelial dysfunction, inflammatory reactions and the action of proinflammatory cytokines may affect the activation and expression of tissue factor and it may play a role in COVID-19-related thrombosis. It is thought

that this situation may cause problems such as bleeding in periodontal tissues and delayed wound healing. Besides, it might affect neurotropic and mucotropic abilities and the functioning of salivary glands, taste sensations, and oral mucosa integrity, interfering with the dynamic oral environment by exerting influence on microbiota balance (Sabino-Silva et al., 2020).

In conclusion, various damages and related dysfunctions develop in the oral tissues after COVID-19 infection. Therefore, individuals who have had COVID-19 might have more tendency to oral diseases such as decreased salivary flow, ulcerations, and gingivitis as a result of impaired immune system and/or susceptible oral mucosa (Dziedzic &Wojtyczka, 2021). It is recommended that patients pay attention to their oral health after treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Comparison of Inferior Alveolar Nerve Block and Infiltration Anesthesia Techniques for Pain Control in Primary Mandibular Molar Extractions in pediatric patients

Türkçe başlık: Çok Hastalarda Süt Mandibular Molar Diş Çekimlerinde Ağrı Kontrolü İçin İnferior Alveolar Sinir Bloğu ve İnfiltrasyon Anestezisi Tekniklerinin Karşılaştırılması

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ÖZ

Amaç: Bu çalışma, çocuk hastalarda süt mandibular molar diş çekimleri sırasında ağrıyı yönetmede inferior alveolar sinir bloğu (IASB) ve bukkal infiltrasyon anestezisinin etkinliğini karşılaştırmayı amaçladı.
Gereç ve Yöntemler: Bu prospektif gözlemsel çalışma Marmara Üniversitesi Diş Hekimliği Fakültesi'nde, rutin süt molar diş çekimi için sevk edilen çocukları kapsıyordu. Katılımcılar IASB ve infiltrasyon anestezi gruplarına ayrıldı. Çalışmanın birincil sonuç ölçütü, görsel analog skala (VAS) kullanılarak ölçülen ağrı şiddetiydi. Bu çalışmanın kovaryantları anestezi grubu, kalan diş kökü uzunluğu ve rezorpsiyon evresiydi. Tanımlayıcı istatistikler demografik ve klinik özellikleri özetledi. Mann-Whitney ve Kruskal-Wallis testleri, gruplar ve rezorpsiyon evreleri arasındaki VAS skorlarını karşılaştırırken, Pearson korelasyon ve çoklu doğrusal regresyon analizleri, ağrı şiddetinin ilişkilerini ve belirleyicilerini değerlendirdi.
Sonuçlar: Katılımcıların ortalama yaşı 8.3 yıl (SD = 1.8) idi. Cinsiyetler, anestezi grupları veya rezorpsiyon evreleri arasında VAS skorlarında anlamlı bir fark bulunmadı. Korelasyon analizi, ağrı şiddeti ile kalan diş köklerinin yüzdesi arasında anlamlı bir ilişki göstermedi. Çoklu doğrusal regresyon analizi, hiçbir belirleyicinin ağrı şiddetini anlamlı şekilde açıklayamadığını gösterdi.
Sonuç: İnfiltrasyon anestezisi, çocuklarda süt mandibular molar diş çekimleri sırasında ağrıyı yönetmede en az IASB kadar etkilidir. Bulgular, daha basit uygulanabilirliği ve daha az komplikasyonları göz önüne alındığında, bukkal infiltrasyon anestezisinin IASB'na geçerli bir alternatif olarak kullanılabileceğini desteklemektedir.
Anahtar Kelimeler: İnfiltrasyon anestezisi, rejyonal anestezi, mandibular sinir, süt dişi

ABSTRACT

Objectives: This study aimed to compare the effectiveness of inferior alveolar nerve block (IANB) and infiltration anesthesia in managing pain during primary mandibular molar extractions in pediatric patients.
Materials and Methods: This prospective observational study at the Marmara University Faculty of Dentistry included children referred for routine primary molar tooth extraction. Participants were divided into two groups: the IANB group and the infiltration anesthesia group. The primary outcome measure was pain severity, which was measured using the visual analog scale (VAS). The covariates for this study were anesthesia group, remaining tooth root length, and the stage of resorption. Descriptive statistics summarized demographic and clinical characteristics. Mann–Whitney and Kruskal–Wallis tests compared VAS scores between groups and resorption stages, while Pearson correlation and multiple linear regression analyses evaluated relationships and predictors of pain severity.
Results: The mean age of participants was 8.3 years (SD = 1.8). No significant differences in VAS scores were found between genders, anesthesia groups, or resorption stages. Correlation analysis showed no significant relationship between pain severity and the percentage of remaining tooth roots. Multiple linear regression indicated that none of the predictors significantly affected pain severity.
Conclusions: Infiltration anesthesia is at least as effective as IANB for managing pain during primary mandibular molar extraction in children. The findings support the potential use of infiltration anesthesia as a viable alternative to IANB, given its simpler application and fewer complications.
Keywords: Infiltration anesthesia, regional anesthesia, mandibular nerve, primary tooth

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INTRODUCTION

Pain-free dentistry is crucial for treating children, and local anesthesia plays a vital role (Chopra et al., 2016). Controlling pain during dental procedures is essential, and every dentist prioritizes optimal pain control and discomfort reduction (Moaddabi et al., 2023). While the injection of local anesthesia can cause anxiety in both children and adults, it is necessary to ensure comfort during subsequent dental treatments. In children, the injection process is a major source of fear, especially when combined with tooth extraction, making tooth extraction one of their most dreaded dental procedures (Tirupathi & Rajasekhar, 2020). Providing less painful local anesthesia has significant benefits, as it reduces the need for surgery under general anesthesia (Tirupathi & Rajasekhar, 2020).

In adults, the bone anatomy of the posterior mandible restricts effective diffusion of the anesthetic solution. Inferior alveolar nerve block (IANB) is commonly used for anesthesia in treating mandibular primary or permanent molar teeth, particularly in mixed dentition cases (Foster et al., 2007; Klingberg et al., 2017; Shabazfar et al., 2014). Although IANB provides broad-area anesthesia, it is often painful and has a relatively high failure rate (Kaufman et al., 1984). There are specific risks associated with this technique, including potential damage to the lingual and/or inferior alveolar nerves (Pogrel, 2007). Other drawbacks include intravascular injections, hematoma, muscle injury and trismus (Peedikayil & Vijayan, 2013; Shabazfar et al., 2014; Wright, 2011). Soft tissue anesthesia following IANB often exceeds treatment timeframes, which can increase the risk of burns and bite injuries, especially in children and mentally disabled patients (Chi et al., 2008).

Since its adoption in dentistry, articaine has been noted for its high lipid solubility attributed to the presence of a thiophene ring that increases its ability to penetrate bone and soft tissue (Arrow, 2012). Several studies with adult subjects have reported reduced procedural pain during maxillary molar extractions using a single buccal infiltration of 4% articaine without requiring an additional palatal injection (Bataineh & Al-Sabri, 2017; Lima-Junior et al., 2009; Uckan et al., 2006). Research by Corbett et al. showed higher anesthesia success rates for first permanent molars with a buccal infiltration of articaine (70.4%) than for those with an IANB with lidocaine (55.6%) in adult volunteers, suggesting that a buccal infiltration may be sufficient instead of an IANB (Corbett et al., 2008). Although subsequent similar findings have been published in adults, there is insufficient evidence in the pediatric population, and further research is necessary (Jung et al., 2008; Poorni et al., 2011). To the best of the authors' knowledge, no studies have evaluated root resorption in this context. Tirupathi et al.'s systematic review concluded that more evidence is needed regarding the analgesic efficacy of 4% articaine for primary molar extraction in children before justifying its use (Tirupathi & Rajasekhar, 2020).

Clinical guidelines for local analgesia in pediatric dentistry prioritize safe and comfortable application tailored to the needs of children and adolescents (Kuhnisch et al., 2017). This study aimed to demonstrate an IANB technique and infiltration anesthesia technique for primary mandibular molar extraction in children. The effectiveness of IANB versus infiltration anesthesia in managing pain during primary mandibular molar extraction was compared, with the null hypothesis stating no significant difference in pain severity during extraction as measured by the visual analog scale (VAS) between the two methods.

MATERIALS AND METHODS

Study Design

This prospective observational study was conducted at the Marmara University School of Dentistry, Department of Oral and Maxillofacial Surgery. Ethical approval was obtained from the Marmara University School of Medicine Ethics Committee, ethical approval number 9.2024.126, and the study adhered to the principles outlined in the Declaration of Helsinki. Children referred to the Oral and Maxillofacial Surgery Department for routine primary molar tooth extraction were incuded in the study. Children who continued to visit this clinic and met the inclusion criteria were invited to participate in the study. The parents and the children were thoroughly informed about the entire procedure, and written consent was obtained from the parents, while verbal assent was obtained from the children.

Inclusion and Exclusion

Children aged 6-12 years with medically healthy conditions, without allergies to medications or local anesthetic solutions, and who were able to communicate in Turkish were included. Those requiring local anesthesia for the extraction of one or more mandibular posterior teeth due to irreversible pulpitis, failed pulp therapy, recurrent caries, or orthodontic reasons were also included. Each tooth requiring simple extraction under infiltration anesthesia was considered independently because it needed its own anesthesia. If multiple teeth on one side required extraction under the IANB, only the tooth that best met the inclusion criteria was considered.

Teeth with acute apical infection, purulent drainage from the gingival sulcus or surrounding tissues, or excessive mobility were excluded. Patients with acute dentoalveolar infection, multiple decayed teeth, allergies to anesthetics, who had medical conditions endangering general health, who refused to participate and who had taken analgesics within 12 hours before the dental appointment were also excluded from the study.

Sample Size Calculation

The sample size was calculated based on the study by Jorgenson et al. (2020). The effect size was determined as $d = (24 - 14.62)/8 \approx 1.1725$. Using an alpha error probability (α) of 0.05 and a power (1-β error probability) of 0.8, the required sample size was calculated using a two-tailed t

test for the difference between two independent means. The final sample size for each group was adjusted to 30, considering potential dropouts.

Anesthesia Procedure

The anesthesia technique was performed as follows: Initially, a topical anesthetic was applied to reduce discomfort associated with needle insertion into the mucosal membrane. Lidocaine 10% pump spray (AstraZeneca AB, Södertalje, Sweden) was applied to the area to be anesthetized with a sterile swab and left in place for two minutes. For local anesthesia, 80 mg/2 ml articaine hydrochloride and 0.01 mg/2 ml epinephrine (Maxicaine, Vem İlaç, İstanbul, Turkey) were used for either buccal infiltration or IANB. The choice of anesthesia was decided by performing radiographic examination of the patients and also the mobility of the tooth was examined during the intraoral examination. Accordingly, infiltration anesthesia was preferred in teeth with a resorption degree greater than the middle third of the root as estimated from the radiograph, and inferior alveolar block anesthesia was preferred in teeth with less remove one. In both groups, the anesthetic solution was administered using a 2.5 cc syringe with a 27-gauge needle (Bahrololoomi & Rezaei, 2021). The injection rate was approximately 1 ml/min for both techniques. IANB was performed using the conventional direct method previously described (Kammerer et al., 2012). If the child reported any pain or discomfort during anesthesia control, an additional injection was given and treatment was done but excluded from the study. Behavioral guidance techniques such as positive reinforcement, and nonverbal behavior guidance were used before anesthesia and during tooth extraction. And the VAS scale applied by the treating physician.

Fourth – or fifth-year dental students who had previously undergone training in extraction and anesthesia carried out the procedures.

Outcome Measures

The data collected in this study included descriptive variables such as age, gender, systemic condition, and the number of extracted teeth. The primary outcome measure was pain severity during tooth extraction, which was assessed using the colored visual analog scale (VAS) with facial expressions. Pain was evaluated immediately after the procedure by asking the children to rate their pain on the VAS, which is a 10 cm scale ranging from 0 (no pain) to 10 (worst pain possible) (Fig. 1). The covariates for this study were anesthesia group (infiltration vs. IANB), the length of remaining tooth root (metric), the percentage of the remaining tooth root (%), and the stage of resorption (stage I, II, or III). The length of the remaining tooth root was measured using a ruler, as shown in Fig. 2.

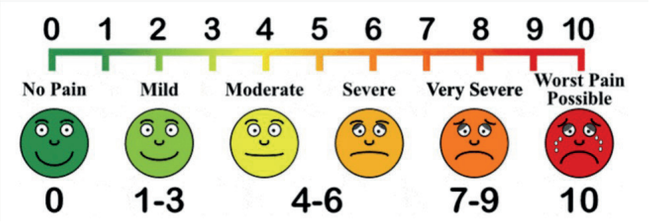

Figure 1: The visual analog scale (VAS) used for pain assessment, ranging from 0 (no pain) to 10 (worst pain possible). The scale includes descriptors and facial expressions to help children rate their pain levels more effectively.

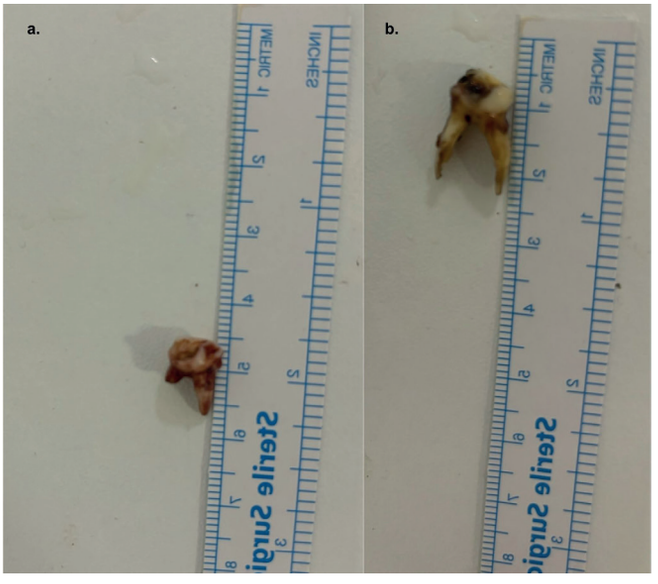

Figure 2: Measurement of the remaining tooth root length using a ruler. (a) Example of a primary molar with minimal remaining root. (b) Example of a primary molar with substantial remaining root.

Statistics

Categorical data were analyzed for frequencies and percentages. The distribution of the data was tested using the Kolmogorov–Smirnov test. Descriptive statistics were calculated based on the normality of the data, including the mean and standard deviation for normally distributed data and the median with 95% confidence intervals for nonnormally distributed data. Various statistical tests, such as the Mann–Whitney test, Kruskal–Wallis test, Pearson's correlation test, and multiple linear regression analysis, were applied to examine specific relationships and factors affecting VAS scores. For statistical reasons, each tooth was considered a separate unit. Statistical analyses were performed using Prism 10 software (GraphPad Inc., Boston, USA), with a significance level set at $p<0.05$.

RESULTS

The demographic and clinical characteristics of the study participants are summarized in Table 1. The mean age of the participants was 8.3 years, with a standard deviation (SD) of 1.8 years. Of the total participants, 33.3% were female (n=10), and 66.7% were male (n=20). Regarding tooth number, 23.3% of the teeth were identified as tooth number 74 (n=7), 26.7% as tooth number 75 (n=8), 16.7% as tooth number 84 (n=5), and 33.3% as tooth number 85 (n=10). Participants were divided into two groups based on the anesthetic technique used: 40.0% received IANB anesthesia (n=12), while 60.0% received an infiltration (n=18). The remaining root length had a mean of 6.4 mm with an SD of 2.5 mm, and the percentage of the remaining root length was 58.6% with an SD of 22.7%. Resorption stages were categorized as follows: 46.7% in Stage I (n=14), 40.0% in Stage II (n=12), and 13.3% in Stage III (n=4).

Table 1. Descriptive statistics of the study population.

	n	%	Mean	SD
Age			8.3	1.8
Systemic status				
Healthy	30	100		
Gender				
Female	10	33.3		
Male	20	66.7		
Tooth number				
74	7	23.3		
75	8	26.7		
84	5	16.7		
85	10	33.3		
Group				
Infiltration	18	60.0		
IANB	12	40.0		
The remaining root length			6.4	2.5
Percentage of remaining root length (%)			58.6	22.7
Resorption stage				
Stage I	14	46.7		
Stage II	12	40.0		
Stage III	4	13.3		

IANB, Inferior alveolar nerve block; SD, Standard deviation

The Mann–Whitney test showed no significant difference in the VAS score between genders (P = 0.520). The median VAS score was 4.00 for females (n=10) and 4.50 for males (n=20), with a median difference of – 0.500 (Fig. 3a). Similarly, no significant difference was found in the VAS score between the IANB group and the infiltration group (p = 0.100). The median VAS score was 5.00 for the IANB group (n=12), and 4.00 for the infiltration group (n=18) with a median difference of – 1.00 (Fig. 3b). The Kruskal–Wallis test comparing VAS scores across the three stages also revealed no significant differences (p = 0.285). These results indicate no statistically significant variation in medians among the stages (p > 0.05) (Fig. 3c).

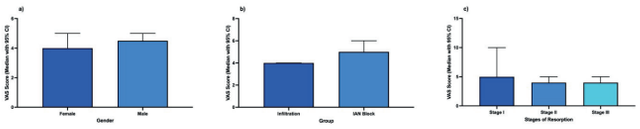

Figure 3. Comparison of visual analog scale (VAS) scores. (a) VAS scores by sex, showing the median and 95% confidence intervals (CIs) for females and males. (b) VAS scores by anesthesia group (Infiltration vs. IANB), showing the median and 95% CI. (c) VAS scores by stage of resorption (stage I, II, and III), showing the median and 95% CI.

The Pearson correlation coefficient (r) between pain severity and the percentage of remaining tooth roots was 0.31, with a 95% confidence interval ranging from – 0.052 to 0.61. The R squared value was 0.099. The two-tailed p value was 0.091, indicating no significant correlation (Fig. 4).

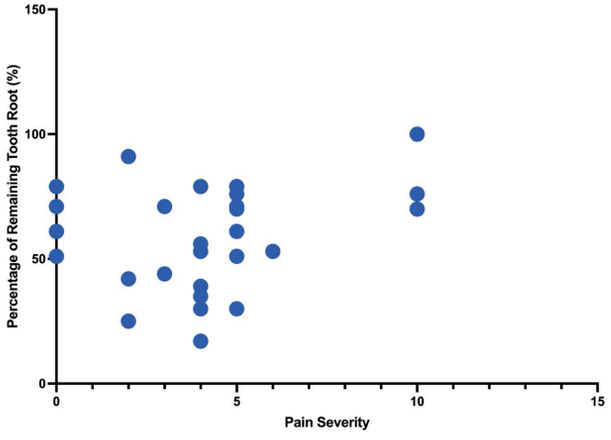

Figure 4. Scatter plot showing the correlation between pain severity (VAS score) and the percentage of the remaining tooth root.

The multiple linear regression analysis for pain severity, using the least squares method, yielded an R-squared value of 0.2864, indicating that 28.64% of the variance in pain severity is explained by the model. The ANOVA results showed that the overall model was not statistically

significant (F(8, 21) = 1.053, P=0.4301). None of the individual predictors, including age, sex, extracted tooth number, group, the longest remaining tooth root, or the percentage of remaining tooth root, were significantly different (all P>0.05). The parameter estimates showed the following results: intercept (Estimate = 17.86, SE = 9.97, 95% CI = – 2.873 to 38.59, t = 1.791), age (95% CI = – 1.164 to 0.5404, t = 0.7612), sex (female) (95% CI = – 3.284 to 2.445, t = 0.3044), number of extracted teeth (95% CI = – 0.3717 to 0.1048, t = 1.165), group (IANB) (95% CI = – 2.538 to 3.523, t = 0.3381), longest remaining tooth roots (95% CI = – 1.059 to 3.801, t = 1.173), and percentage of remaining tooth roots (95% CI = – 0.4255 to 0.1341, t = 1.083). The residuals passed normality tests, confirming that the model's residuals followed a normal distribution (Table 2).

Table 2. Multiple linear regression analysis of pain severity

	Estimate	SE	95% CI	t
Intercept	17.86	9.97	-2.873 to 38.59	1.791
Age			-1.164 to 0.5404	0.7612
Gender (female)			-3.284 to 2.445	0.3044
Extracted tooth number			-0.3717 to 0.1048	1.165
Group (IANB)			-2.538 to 3.523	0.3381
The longest part of the remaining tooth root			-1.059 to 3.801	1.173
Percentage of remaining tooth root			-0.4255 to 0.1341	1.083

SE, Standard error; CI, Confidence interval; IANB, Inferior alveolar nerve block

DISCUSSION

In this prospective, observational clinical study, IANB anesthesia and infiltration anesthesia were compared in a routine clinical setting for the extraction of primary mandibular posterior teeth. Notably, to the best of the authors' knowledge, this study is the first to evaluate root resorption in the context of these anesthesia techniques. According to our results, infiltration anesthesia is at least as effective as IANB for this purpose. Consequently, we cannot reject the null hypothesis, as our findings indicate no significant differences in pain severity between the two anesthesia techniques under the conditions of this study. Our results are consistent with those of studies by Corbett et al. (2008) and Poorni et al. (2011) in adult populations, who reported similar findings. These findings are also consistent with those of Jorgenson et al.'s study (2020) in the pediatric population, which did not evaluate the degree of root resorption.

IANB remains the most commonly used anesthesia technique for surgical and restorative treatments in the posterior mandible (Foster et al., 2007; Shabazfar et al., 2014). However, it involves more complications than does buccal infiltration anesthesia (Choi et al., 2009; Jung et al., 2008; Takasugi et al., 2000). Buccal infiltration anesthesia is relatively less technique-sensitive in its application, highlighting the need for further studies to explore its use as an alternative to IANB anesthesia. In this context, our findings contribute to the ongoing discussion about optimizing local anesthesia techniques in pediatric dentistry.

Articaine is frequently preferred as an anesthetic due to its low allergic and toxic potential (Kammerer et al., 2014; Santos et al., 2007). Compared to other local anesthetic agents, it has high lipid solubility due to the thiophene ring, allowing it to penetrate bone and soft tissue more effectively, making it more efficient for infiltration injections (Arrow, 2012). It is also possible for the anesthetic agent to diffuse through the medullary bone via the accessory foramina of the mandible (Etoz et al., 2011; Madeira et al., 1978; Stein et al., 2007). These properties suggest that buccal infiltration anesthesia with articaine can be a viable option considering the thick cortical bone in the posterior mandible. The lack of difference in VAS scores between the two types of anesthesia observed in this study may be related to these properties of articaine. Furthermore, the multiple linear regression results, where root resorption was controlled as a covariate, did not seem to affect this outcome. Pearson analysis also revealed no significant correlation between the remaining root length and pain score.

In pediatric patients, one of the techniques used for pain assessment involves scales ranging from 0 to 10 (Bijur et al., 2001; Cohen et al., 2008). While this method can be influenced by patients' fear and pain expectations, it remains a reliable and widely used method (Ezoddini Ardakani et al., 2010; Kammerer et al., 2017). However, it should be noted that self-reported pain perception is subjective and can be confounded by various factors, such as the sensation of pressure, patient anxiety, and the effectiveness of the operator's behavioral management skills. This subjectivity is a limitation of this study.

The authors acknowledge that a crossover study design, including appropriate randomization and blinding, would be the ideal choice to increase internal validity (variability among patients). However, the research team decided against this approach, considering that it would further reduce participation in the study. Anesthetics were administered by a school of dentistry students with similar clinical experience rather than by a single dentist, and the study was not a split-mouth study.

In our study, the first primary molar and second primary molar teeth were examined without considering the group difference. What was important for us was the amount of resorption. However, due to their different positions in the jaw, the relationship of the teeth to the inferior alveolar nerve may be different, and the amount of numbness may also be different. For this reason, it would be more accurate to examine the results in separate groups as the first primary molar and second primary molar teeth.

These factors are considered significant limitations.

CONCLUSIONS

The findings of this study indicate that the IANB and infiltration anesthesia techniques are effective, with no significant differences in pain severity between the two methods. The lack of a significant correlation between root resorption and pain severity further supports the robustness of these results. Given the possible complications associated with IANB and the relatively simple application of infiltration anesthesia, our results suggest that infiltration anesthesia could be a viable alternative in pediatric dentistry. However, it is essential to conduct further research to confirm these findings and address other important factors, such as patient comfort, anxiety levels, and long-term outcomes.

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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Comparison of Colour Stability of Lithium Disilicate, Indirect Resin Composite and Zirconia: An In Vitro Study

Lityum Disilikat, İndirekt Rezin Kompozit ve Zirkonyanın Renk Stabilitesinin Karşılaştırılması: İn Vitro Çalışma

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ABSTRACT

Objectives: The study was aimed to examine the colour stability of lithium disilicate, indirect resin composite and zirconia.

Materials and Methods: One hundred and twenty samples were split into three groups (n=40) of materials lithium disilicate, indirect resin composite and zirconia (10 mm diameter and 1mm thick discs). Discs were cemented with three different dual cured resin cements. Thermocycling of 10000 cycles for 168 hours was applied to 20 specimens from each group. The samples then emerged in staining solutions (coffee and tea) for 7 days. Colour changes were measured at four different stages to determine ΔE using spectrophotometer.

Results: The study results revealed a statistically significant difference between the materials before and after cementation and thermal cycle ($p<0.05$). Regarding staining solutions, the results also showed a statistically significant difference between three materials ($p<0.05$). No statistically significant difference was found between coffee and tea in terms of colour changing effects ($p<0.05$).

Conclusions: It was observed that the thermal cycle, cementation process and staining solutions influenced the color stability of all materials. The material with the highest color stability was determined to be IPS e.max. Colour stability is one of the main elements for the success of a dental treatment. Thus, staining resistance is an essential property for the longevity of aesthetic restorations.

Keywords: Lithium disilicate, Indirect resin composite, Zirconia, Colour stability, Staining.

ÖZ

Amaç: Çalışmanın amacı lityum disilikat, indirekt rezin kompozit ve zirkonyanın renk stabilitesini incelemektir.

Gereç ve Yöntemler: Yüz yirmi örnek, lityum disilikat, indirekt rezin kompozit ve zirkonya (10 mm çap ve 1 mm kalınlığında diskler) materyallerinden oluşan üç gruba (n=40) ayrıldı. Diskler üç farklı dual cure rezin simanıyla simante edildi. Her gruptan 20 örneğe 168 saat boyunca 10000 döngülük termal siklüs uygulandı. Daha sonra örnekler 7 gün boyunca boyama solüsyonlarında (kahve ve çay) ortaya çıktı. Renk değişimleri spektrofotometre kullanılarak ΔE'yi belirlemek için dört farklı aşamada ölçüldü

Bulgular: Çalışma sonuçları, simantasyon öncesi ve sonrası materyaller ile termal siklus arasında istatistiksel olarak anlamlı bir fark olduğunu ortaya koydu ($p<0.05$). Boyama solüsyonlarına ilişkin sonuçlar, üç malzeme arasında istatistiksel olarak anlamlı bir fark olduğunu da gösterdi ($p<0,05$). Kahve ve çay arasında renk değiştirme etkileri açısından istatistiksel olarak anlamlı bir fark bulunmamıştır ($p<0,05$)

Sonuç: Termal siklus, simantasyon işlemi ve boyama solüsyonlarının tüm materyallerin renk stabilitesi üzerinde etkisi olduğu görüldü. Materyaller arasında en yüksek renk stabilitesine sahip materyal IPS e.max olarak tespit edildi. Renk stabilitesi, bir diş tedavisinin başarısı için ana unsurlardan biridir. Bu nedenle, renklenmeye karşı direnç estetik restorasyonların uzun ömürlülüğü için önemli bir özelliktir.

Anahtar Kelimeler: Lityum disilikat, dolaylı reçine kompozit, zirkonya, renk stabilitesi, leke oluşumu

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INTRODUCTION

The colour stability throughout the materials functional lifetime is essential mainly for the durability of treatment and cosmetic reasons (Prashanthi et al., 2015). Staining resistance is an essential property for the longevity of removable fixed dentures, crowns and direct restorations in aesthetic areas (Wilson et al., 1997).

Discoloration of dental composite restorations can be caused by both exogenous and endogenous reasons (Kolbeck et al., 2006). The endogenous reasons include the discoloration of resin matrix and the link between resin matrix and fillers (Wilson et al., 1997). They usually occur when the materials are aged under various chemical and physical conditions including thermal changes and humidity (Cook et al., 1987).

There are three main types of composite resin discolorations. The first type is extrinsic discoloration which is caused by the build-up of plaque (Satou et al., 1989); the second type is intrinsic discoloration which is caused by the aging of the material and third type is the alteration of the surface colour which is caused by the superficial degradation, the reaction of the staining agents on the inner side of the superficial composite resin layer (Turgut & Bagis, 2011).

Numerous studies investigated the staining of composites by coffee, tea and other beverages. Um and Ruyter evaluated the colour stability of resin based veneering materials using boiled coffee and tea at 50°C (Um & Ruyter, 1991). Their study indicated that discoloration of materials occurred by the absorption of the colourants into the organic phase of the veneering materials. Similarly, Dietschi et al. compared the colour stability of 10 new generation light cured composites by using numerous colouring solutions including coffee, E1010 food dye, vinegar and erythrosine. Their specimens were exposed to post curing thermocycling and polished before staining procedures. The study revealed that erythrosine caused the most colour change (Dietschi et al., 1994). Moreover, Scotti et al. investigated the colour stability of acrylic resins by simulating the oral condition through mixing the specimens in synthetic saliva combined with coffee, tea or chlorhexidine at 37°C and concluded that the synthetic saliva and coffee caused more colour change (Scotti et al., 1997).

In the current literature, there is a wide range of studies examining rhe colour stability of different restorative materials. Yet, limited number of studies are available that compares those materials after exposed to different procedures. The purpose of this study is to examine the colour stability of lithium disilicate (IPS e.max), indirect resin composite (Gradia) and zirconia by using dual cure resin cements. The null hypothesis is that there is no statistically significant difference in colour stability between these materials after exposed to different procedures (before and after cementation, after thermal cycle, after processed with staining solutions).

MATERIALS AND METHODS

One hundred and twenty samples were split into three groups (n=40) of materials lithium disilicate, indirect resin composite and zirconia. All samples were fabricated as mentioned in Turgut & Bagis (2011, 2013), Alabdulwahhab et al., (2015) as discs of 10 mm diameter and 1mm thickness (Fig. 1)

Figure 1: The final shape of the disc materials (Zirconia, E-max and Gradia)

Heat-pressed method was preferred by using IPS e.max press Programat EP3000 press furnace (Ivoclar Vivadent, Schaan, Liechtenstein) for the fabrication of lithium disilicate glass-ceramic material (IPS e.max Press HT and LT, A1 shade, Ivoclar Vivadent, Schaan, Liechtenstein). Zirkonzahn translucent blocks (Zirkonzahn, der Ahr, Gais, Italy) were used for fabrication zirconia samples. The zirconia samples were manufactured with 5-axis wet-grinding and dry-milling technology in one compact unit by using CAD/CAM Ceramill Motion2 (Amanngirrbach, Koblach, Austria). Indirect resin composite samples of Gradia (GC Europe NV, Leuven, Belgium) were manufactured by placing into metal ring between two glass slides and fixed with an elastic band. Then samples were stapled with a stapler machine for 15 minutes to achieve the accurate dimension and inserted inside the light-cured machine (Lumamat100, Ivoclar Vivadent, Schaan, Liechtenstein) for 12 minutes to polymerise the discs.

Before cementation one surface of all discs were sandblasted from 10 mm distance for 15 seconds with 30 µm alumina sands (Al_2O_3) by a sandblasting unit (Renfert, Hilzingen, Germany). IPS e.max and Gradia samples were cemented with dual cure resin cements (Variolink N (Ivoclar Vivadent AG Schaan, Liechtenstein) of transparent "0" shade and Nexus Third Generation NX 3 – Nexus 3 (SDS Kerr, California, USA) of ''clear'' shade. Zirconia samples were cemented with dual cure resin cements Multilink N (Ivoclar Vivadent AG Schaan, Liechtenstein) of transparent shade and Nexus Third Generation NX 3 – Nexus 3 (SDS Kerr, California, USA) of ''clear'' shade. All disc-shaped specimens were placed over the glass slab to create a cement layer with approximately 100πm thick underneath the ceramic disc (Hernandes et al., 2016.).

Light cure device of the wavelength range of 385 to 515 nm (Bluephase N; Ivoclar Vivadent AG Schaan, Liechtenstein) was applied for 1 minute for every sample of each material to achieve optimum polymerization (Fig. 2).

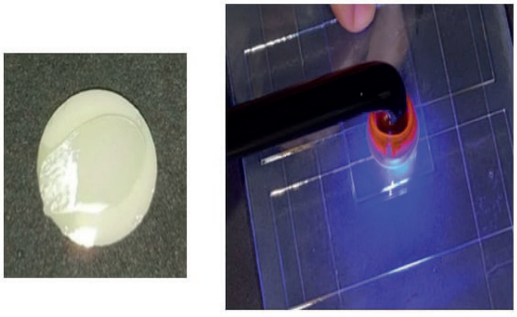

Figure 2: Mylar Strip technique

Thermocycling (SD Mechatronik Thermocycler, Feldkirchen-Westerham, Germany) was applied to 20 specimens from each group of 10000 cycles for 168 hours (7 days). The procedure was performed with temperature switching from 5°C to 55°C with dwell time of 20 seconds. Three groups containing 5 specimens from each material were immersed in 200 mL of black tea and coffee for 7 days. The specimens were then left in the solution until the temperature reached 37°C and then they were placed in paper cups. This process was repeated every 24 hours to prevent any possible chemical changes.

The colour stability was checked with the spectrophotometer (Vita Easy Shade, Vita Zahnfabrik, Bad Sackingen, Germany) in four stages: Before and after cementation, after thermocycling and after staining (Fig. 3). All colour measurements were performed three times for each specimen and the average of the three readings were calculated. The total colour difference (ΔE*) was calculated using the standart equation. Descriptive statistics was performed by using SPSS (Statistical Package for Social Sciences) for Windows 21.0 program. T-test and One-way ANOVA test were used to compare the quantitative data between groups, as the data showed normal distribution, and the Tukey HDS test was used to identify the groups causing the statistical differences. The results were evaluated at the significance level of $p<0.05$.

Figure 3: Spectrophotometer (VITA Easyshade V) measurement

RESULTS

Table 1 shows the results of the colour measurements for control group, table 2 shows the colour measurements before and after cementation and after thermal cycle and table 3 shows the colour measurements after staining. One-way ANOVA test was used to determine the difference between IPS e.max, Gradia and Zirconia before and after cementation and after thermal cycle (Table 4). Before thermal cycle the study results revealed a statistically significant difference between IPS e.max and Zirconia or Gradia ($p<0.001$). Moreover, after thermal cycle there were also a statistically significant difference between IPS e.max and Zirconia or Gradia ($p<0.001$). After staining solutions, the results showed another statistically significant difference between IPS e.max and Zirconia or Gradia ($p<0.001$). Lastly, the t-test was used to determine the difference between coffee and tea, and no statistically significant difference was found ($p>0.05$).

Table 1. Colour measurements for control group before and after thermal cycles

	Zirconia (n=10)		E-max (n=10)		Gradia (n=10)	
	BT	AT	BT	AT	BT	AT
S1	1.6	0.6	2.1	3.4	2.6	0.9
S2	1.6	0.5	2.4	3.8	2.4	0.2
S3	1.6	0.9	1.5	3.0	2.6	0.6
S4	2.0	1.5	2.6	2.8	2.0	0.7
S5	1.7	1.4	2.0	2.9	2.1	0.5
S6	1.7	1.3	2.2	2.7	2.4	1.3
S7	1.9	0.3	2.6	3.4	2.2	0.8
S8	1.7	1.0	2.3	2.6	2.3	1.3
S9	1.8	0.5	2.9	2.7	2.4	0.4
S10	1.8	1.1	2.8	3.4	2.3	1.9
Mean	1.7	0.9	2.3	3.1	2.3	0.9

S: Sample, BT: Before thermal cycle, AT: After thermal cycle

Table 2. Colour measurements before and after cementation and after thermal cycle

	Zirconia (n=60)						E-max (n=60)						Gradia (n=60)					
	Multilink N (30)			Nexus 3rd (30)			Variolink N (30)			Nexus 3rd (30)			Variolink N (30)			Nexus 3rd (30)		
	BC (10)	AC (10)	AT (10)	BC (10)	AC (10)	AT (10)	BC (10)	AC (10)	AT (10)	BC (10)	AC (10)	AT (10)	BC (10)	AC (10)	AT (10)	BC (10)	AC (10)	AT (10)
S1	1.8	2.0	0.8	1.9	2.9	2.4	2.7	3.6	3.5	3.0	3.9	4.2	2.1	2.1	2.3	2.4	1.5	1.5
S2	2.1	2.1	2.1	2.2	3.0	0.8	2.6	1.8	2.7	2.9	4.0	3.7	1.8	2.6	0.8	2.0	1.6	0.9
S3	1.8	2.3	1.4	2.1	2.9	0.3	3.3	3.1	3.5	2.7	4.4	4.1	2.0	2.0	0.9	1.6	1.5	2.7
S4	1.8	2.6	0.9	1.9	2.1	1.4	2.9	2.2	2.3	2.9	4.5	2.3	1.8	2.5	1.0	2.3	1.1	1.0
S5	1.8	2.0	1.3	2.3	2.7	2.9	2.9	2.4	2.8	2.2	4.8	3.9	1.5	2.2	2.3	2.3	1.6	2.6
S6	2.1	2.5	0.5	2.0	3.6	2.9	2.4	3.9	2.4	3.0	6.0	2.8	2.1	2.5	1.3	1.8	1.0	2.1
S7	1.9	1.6	1.9	1.8	2.8	2.0	2.6	2.2	3.6	3.8	4.0	2.8	2.1	2.1	2.2	2.1	1.6	1.0
S8	1.7	1.4	1.7	1.9	3.0	2.8	2.9	1.3	2.3	2.8	4.8	3.7	1.8	2.5	2.3	1.9	1.6	2.9
S9	2.2	2.0	0.9	1.8	2.8	1.7	3.3	0.9	3.3	2.2	4.4	3.4	2.2	2.0	0.8	2.2	3.1	1.6
S10	2.0	2.3	0.2	2.3	2.7	2.9	3.0	1.8	2.8	1.6	4.9	4.7	2.0	2.2	0.7	2.3	3.2	2.1
Mean	1.9	2.1	1.2	2.2	2.9	2.0	2.9	2.3	2.9	2.7	4.6	3.6	1.9	2.3	1.5	2.1	1.8	1.8

S: Sample, BC: Before Cementation, AC: After Cementation, AT: After Thermal cycle

Table 3. Colour measurements after staining

	Zirconia (n=30)						E-max (n=30)						Gradia (n=30)					
	Control Group		Multilink Group		Nexus Group		Control Group		Variolink Group		Nexus Group		Control Group		Variolink Group		Nexus Group	
	cof	TE	cof	TE	cof	TE	cof	TE	cof	TE	cof	TE	cof	TE	cof	TE	cof	TE
S1	2.2	1.2	1.7	1.0	1.9	1.9	1.9	3.0	1.9	1.8	1.6	1.2	2.4	1.2	2.3	2.1	1.5	1.6
S2	2.1	1.7	1.8	1.6	2.0	2.3	0.3	1.7	1.4	1.8	2.4	2.4	5.6	0.8	1.8	1.4	1.4	2.1
S3	2.2	1.3	2.8	1.2	1.0	1.4	2.4	2.5	2.0	1.9	1.9	1.4	4.1	1.3	2.1	1.6	2.1	2.5
S4	2.0	2.2	2.5	2.6	1.8	1.7	1.6	2.6	1.9	1.8	1.6	2.3	3.4	1.1	2.1	0.5	1.0	2.0
S5	2.1	0.4	1.9	1.8	2.1	2.3	1.8	2.5	0.6	1.6	1.9	2.7	3.9	2.5	1.6	1.6	1.3	2.0
Mean	2.1	1.4	2.1	1.6	1.8	1.9	1.6	2.5	1.6	1.8	1.9	2.0	3.9	1.4	2.0	1.4	1.5	2.0

S: Sample, TE: Tea, cof: Coffee

Table 4. Statistical differences between the materials

			Difference of mean	P value	95% Confidence Interval	
					Lower limit	Upper limit
BC	Zirconia	E-max	-0.81500	.000	-1.0589	-0.5711
		Gradia	-0.04500	.897	-0.2889	0.1989
	E-max	Zirconia	0.81500	.000	0.5711	1.0589
		Gradia	0.77000	.000	0.5711	1.0139
	Gradia	Zirconia	0.04500	.897	-1.989	0.2889
		E-max	-0.77000	.000	1.0139	-0.5261
AC	Zirconia	E-max	-0.97500	.004	-1.6779	-0.2721
		Gradia	0.44500	.288	-0.2579	1.1479
	E-max	Zirconia	0.97500	.004	0.2721	1.6779
		Gradia	1.42000	.000	0.7171	2.1229
	Gradia	Zirconia	-0.44500	.288	-1.1479	0.2579
		E-max	1.42000	.000	-2.1229	-0.7171
AT	Zirconia	E-max	-1.65000	.000	-2.2442	-1.0558
		Gradia	-0.65000	968	-0.6542	0.5342
	E-max	Zirconia	-0.6000	.000	10.558	2.2442
		Gradia	1.59000	.000	0.9958	2.1842
	Gradia	Zirconia	0.06000	.968	-0.5342	0.6542
		E-max	-1.59000	.000	-2.1842	-0.9958

BC: Before cementation, AC: After cementation, AT: After thermal cycle

DISCUSSION

The main factors for selecting restorative materials include patients' esthetical demands, expectations, and manufacturing techniques (Harryparsad et al., 2014). The null hypothesis of our study was rejected. The study results revealed a statistically significant difference between materials after exposed to different procedures. IPS e.max showed more stable values before and after cementation, after thermal cycle and after staining solutions. Acar et al., (2016) found that lithium disilicate (IPS E-max) was the most colour stable material compared to hybrid nano-ceramic and nanocomposite. Sayed also found that IPS e.max veneers exhibited the best colour stability when compared with nano hybrid and Vita Enamic veneers (Sayed et al., 2016). Lee and Choi (2018) found high translucency of lithium disilicate ceramics exhibited greater colour changes after aging. On contrary, Hamza et al., (2018)found that the colour stability of IPS e.max and IPS Empress were not affected by aging process. They also found that resin cement systems also influence the colour change of prosthetic restorations. Moreover, other studies found that thermocycling is associated with volumetric contraction and expansion of materials which can cause degradation (Gürdal et al., 2002; Shimizu et al., 2008).

However, colour may also be affected by the other factors such as water or material ageing (Shimizu et al., 2008). Papadopoulos found that indirect resin composites (Adoro, HFO, Gradia) showed a yellow shift after accelerated aging and indirect resin composites (Gradia, Solidex) demonstrated a colour change after water immersion (Wagner et al., 1995; Gürdal et al., 2002; Nakamura et al., 2002; Papadopoulos et al., 2010). Several researchers found that the factors such as discoloration that may happen over time when exposed to different foods and drinks such as tea, coffee, cola, chlorohexidine or bleaching agent (Malekipour et al., 2012; Khaledi et al., 2014; Rosentritt et al., 2015). The current study also found that cementing the materials with different resin cements had an impact on the colour stability of the ceramic materials.

It has been reported that a 7-day conditioning period of ceramic materials will take up a significant staining within the first week of exposure to solutions (Borges et al., 2011; Al-Shalawi et al., 2017). Many studies also found that different beverages have varying degrees of staining on different types of materials (Reis et al., 2003; Lamba et al., 2012; Nikzad et al., 2012). According to Kelly and Benetti (2011), IPS e.max ceramics are more translucent, thus less likely to stain. This study also evaluated the impact effect of coffee and tea on the colour stability. According to the study results, IPS e.max was the most colour stable material. However, there was no statistically significant difference between Zirconia and Gradia in colour change after staining.

Sayed et al., (2016) found that coffee had the highest impact on the colour change of restorative materials. This may be explained by the easier absorption of the coffee into the material (Al Kheraif et al., 2013). Similarly, Raeisosadat et al., (2016) assessed the colour stability of three commonly used resin-based materials and revealed that all the materials were more significantly affected by the coffee when compared to the tea. Bagheri et al. (2005) argued that coffee includes a yellow colour which causes the materials with low polarity to easily stain. In this study there was no statistically significant difference between coffee and tea in terms of colour changing effects.

CONCLUSION

Within the limitations of this study the following conclusions may be drawn:

IPS e.max has the best colour stability after cementation and thermal cycle.

IPS e.max has the best colour stability after exposed to staining solutions.

There is no difference between coffee and tea in terms of colour changing effects.

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CASE REPORT / OLGU SUNUMU

Lung Cancer with Oral Cavity Metastasis: Two Case Reports

Oral Kavite Metastazı Görülen Akciğer Kanseri: İki Olgu Sunumu

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ÖZ

Malign tümörlerin önemli biyolojik ayırt edici özelliklerinden olan invazyon ve metastaz; kansere bağlı morbidite ve mortalitenin en önemli nedenlerindendir. Oral kavite de görülen metastatik tümörler oral malign karakterli tümörlerin %1-1.5' ini oluşturmaktadır ve bu bölgeye metastaz yapan en yaygın primer odaklar arasında meme, akciğer, böbrek, kemik, prostat ve kolon kanserleri gelmektedir. Çene kemiklerinde en sık tutulum alanı mandibula posterior iken yumuşak doku tutulumları en sık dişetine olmaktadır. 63 yaşında erkek hasta mandibula anterior dişlerin çekimi sonrası gelişen, alveol kemiğe invaze, yüzeyi düzensiz ve ülsere alanlar içeren kitle şikayetiyle başvurmuştur. İnsizyonel biyopsi ile değerlendirilen kitlenin histopatolojik incelemesinde saydam hücrelerden baskın karsinom tanısı almıştır. Primer odağın araştırılması için onkoloji servisine yönlendirilmiş ve PET-CT taraması ile akciğer kanseri teşhisi koyulmuştur. Anamnezinde bir yıl önce akciğer kanseri teşhisi koyulan ve uzun kemik metastazları bulunan 62 yaş erkek hasta sol mandibulada rezidüel diş kökünden kaynaklandığını düşündüğü ağrı şikayetiyle başvurmuştur. Yapılan CBCT incelemesinde sol mandibula ramus bölgesinde inferior alveolar kanalı içine almış, kortikal ve trabekül yapıda güve yeniği tarzında osteolitik alanlar izlenmiştir. Akciğer kanseri ve uzak organ metastazları bulunan hasta onkoloji bölümüne yönlendirilmiş ve palyatif tedavisine devam etmiştir. Her iki hasta da 3 ve 6 ay takip sonunda yaşamını yitirmiştir. Metastazların varlığı kötü prognoza işaret eder ve evrelere göre çeşitli tedaviler uygulanabilir. Oral kavite de meydana gelen değişimler dikkatle takip edilmeli, gerekli durumlarda histopatolojik inceleme ile tanıya gidilmelidir.

Anahtar Kelimeler: Oral kavite, oral kanser, akciğer kanseri, histopatoloji, metastaz

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ABSTRACT

Invasion and metastasis, which are important biological hallmarks of malignant tumors, are among the most important causes of cancer-related morbidity and mortality. Metastatic tumors in the oral cavity constitute 1-1.5% of oral malignant tumors and the most common primary foci metastasizing to this region are breast, lung, kidney, bone, prostate and colon cancers. The most common site of involvement in the jaw bones is the mandible posterior, while soft tissue involvement is most common in the gingiva. A 63-year-old male patient presented with a mass invading the alveolar bone with irregular and ulcerated areas after extraction of mandibular anterior teeth. Incisional biopsy was performed and histopathological examination of the mass revealed a diagnosis of carcinoma predominantly composed of clear cells. He was referred to the oncology service for investigation of the primary focus and lung cancer was diagnosed by PET-CT scan. A 62-year-old male patient who was diagnosed with lung cancer one year ago and had long bone metastases in his anamnesis presented with pain in the left mandible, which was thought to be caused by the residual tooth root. CBCT examination revealed moth-eaten osteolytic areas in the cortical and trabecular structure involving the inferior alveolar canal in the ramus region of the left mandible. The patient with lung cancer and distant organ metastases was referred to the oncology department and palliative treatment was continued. Both patients died after 3 and 6 months of follow-up. The presence of metastases indicates a poor prognosis and various treatments can be applied according to the stages. Changes occurring in the oral cavity should be followed carefully and diagnosis should be made by histopathological examination when necessary.

Keywords: Indigo naturalis cell viability, periodontal ligament fibroblast.

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INTRODUCTION

Lung cancers and related mortality rates are increasing worldwide. According to GLOBOCAN (Global Cancer Statistics) 2020 data, approximately 2.20 million new cases and 1.79 million deaths were reported in both genders (Sung et al., 2021; Bade et al., 2019). It is more common in men than women andthe use of tobacco products is among the most important etiological factors (Nasim et al., 2019; Schabath & Cote, 2019). Significant diversity in lung cancer incidence and demographic distribution is observed among nations, with tobacco smoking rates and economic development stage influencing these trends. Although cancer data in emerging nations are less trustworthy, the recent increase in smoking prevalence in China, Indonesia, Eastern Europe, and Northern and Southern Africa is predicted to increase lung cancer incidence in developing regions (Bray et al., 2018; Torre et al., 2015). Up to 80% of current smokers currently live in low – or middle-income nations, and less developed regions account for more than half of lung cancer fatalities (Torre et al., 2016; Torre et al., 2015).

Invasion and metastasis, which are important biological distinguishing features of malignant tumors, are among the most important causes of cancer-related morbidity and mortality (Kumar et al., 2010). Many cases metastasized to the jaw bones and oral soft tissues have been reported in the literature (Lopes et al., 2023; Kirschnick et al., 2022; Gupta et al., 2022; Hirshberg et al., 2008; D'Silva et al., 2006). The most common primary foci metastasizing to the oral cavity include breast, lung, kidney, bone, prostate and colon cancers (Hirshberg et al., 2014; Bodner et al., 2006; D'Silva et al., 2006). The majority of primary tumors metastasizing to the oral cavity are tumors of epithelial origin and are mostly seen in the 4th and 7th decade. The primary source of metastases to the oral cavity in males is lung cancer, whereas breast cancer has been reported in females (Hirshberg et al., 2014; Hirshberg et al., 2008; Bodner et al., 2006; D'Silva et al., 2006).

Metastatic tumors in the oral cavity constitute 1-1.5% of oral malignant tumors. Approximately 30% of oral cavity metastases are seen as the first sign of malignancy (D'Silva et al., 2006). Lesions can be found in bone, soft tissues or both. The most common site of involvement in the jaw bones is the mandible posterior, while soft tissue involvement is most commonly in the gingiva (Özbayrak, 2020; Pekiner & Özbayrak, 2016; Curien et al., 2007). In the early stages, soft tissue lesions may be confused with reactive lesions such as peripheral giant cell granuloma, pyogenic granuloma and epulis fissuratum (Özbayrak, 2020). Oral cavity metastases can be observed radiographically in many different ways. Sometimes they mimic periodontal diseases with cysts and sometimes they may give radiographic findings like osteolytic areas and moth-eaten appearance (Kumar & Manjunatha, 2013).

Diagnosis of metastases represents a poor prognosis. Surgical excision, radiotherapy and chemotherapy can be applied in oral cavity metastases after treatment of the primary tumor. If the primary lesion recurs or involves metastases to other sites, symptomatic treatments such as pain reduction and maintenance of function can be performed (Rao et al., 2014; Kumar & Manjunatha, 2013; Hirshberg et al., 2008; D'Silva et al., 2006). In these case reports, two different cases of lung cancer with oral cavity metastasis resulting in loss of patients are presented.

CASE REPORT

Case 1

The complaint of a 63-year-old male patient admitted to our clinic was a painless but bleeding mass in the mandibular anteiror region, which gradually grew following tooth extraction about 2 months ago. The patient's anamnesis revealed heavy smoking although he had quit smoking 2 years ago. A radiologically bone-invading mass with irregular and clinically ulcerated areas was observed in the anterior edentulous region of the mandible (Figure 1,2). Incisional biopsy of the lesion was performed under local anesthesia. Histopathological examination revealed a diagnosis of clear cell-dominant carcinoma and the primary focus was requested to be investigated (Figure 3A,B). The patient was referred to the Oncology Department to determine the primary focus and to investigate metastatic spread and PET-CT revealed lung involvement with clavicular and scapular metastases (Figure 4). The diagnosis of lung cancer was made as a result of histopathological and radiological evaluations. The patient who received radiotherapy and chemotherapy for lung cancer and distant organ metastases died after 3 months.

Figure 1: A lesion with irregular and ulcerated areas on the alveolar crest in the anterior edentulous region of the mandible following tooth extraction.

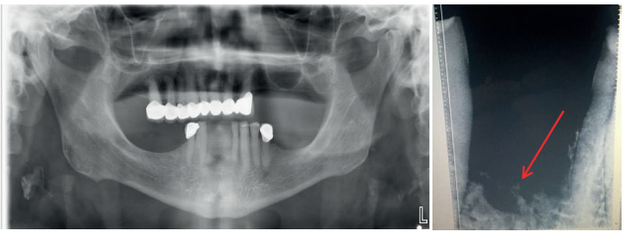

Figure 2 : Invasion of soft tissue lesion to bone on panoramic and periapical radiography.

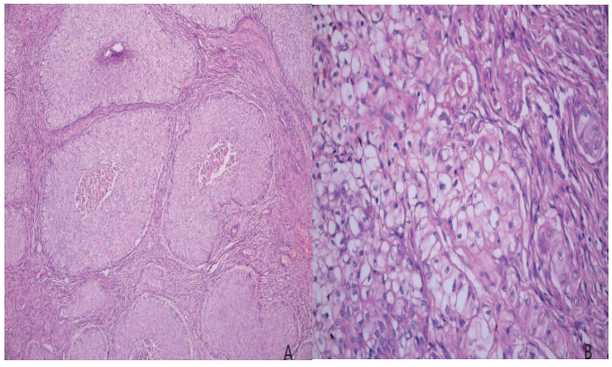

Figure 3: A) Areas of comedo necrosis (HxE, x100) B) Atypical epithelial cells with clear cytoplasm (HxE, x400)

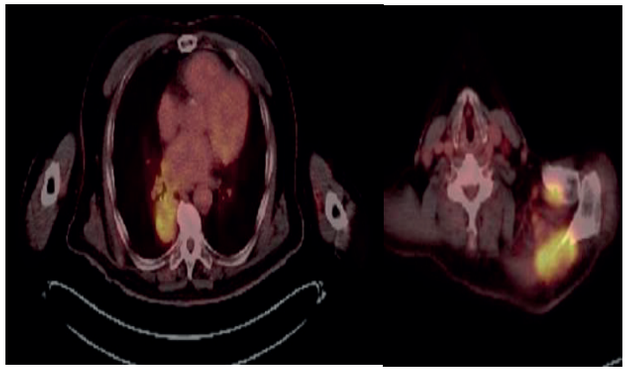

Figure 4: Lung involvement and clavicular and scapular metastases in PET-CT.

Case 2

The patient was admitted to our clinic with the complaint of severe pain in the posterior region of the left mandible, which was thought to be caused by the residual tooth root in the edentulous area (Figure 5) . In the anamnesis of the patient, it was learned that he smoked one packet of cigarettes a day for 30 years and received radiotherapy and chemotherapy for lung cancer one year ago. A history of metastasis to the coccyx bone, femur bone and kidney tissues due to lung cancer was also present. Clinical examination did not reveal any asymmetry or swelling except pain. Panoramic radiography and Cone Beam Computed Tomography (CBCT) examination revealed moth-eaten osteolytic areas in cortical and trabecular structure involving the inferior alveolar canal in the ramus region of the left mandible. The patient with lung cancer and distant organ metastases was referred to the oncology department and palliative treatment was continued. After 6 months of follow-up, the patient died.

Figure 5: Clinical image of the left mandibular posterior edentulous area at the time of the patient's presentation to the clinic.

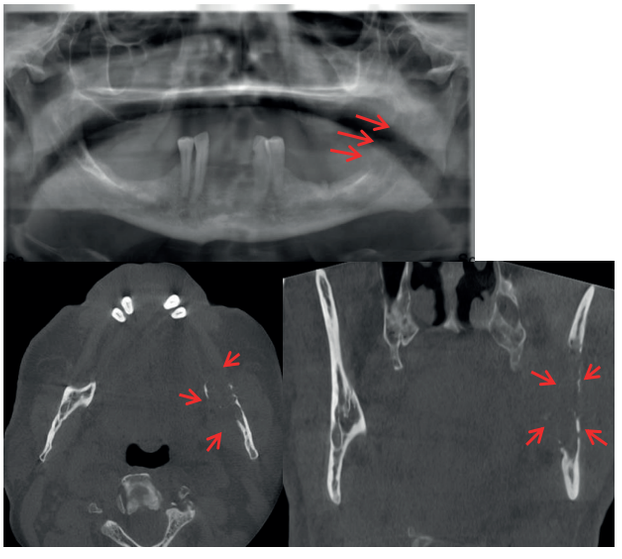

Figure 6: A) On panoramic radiography, irregular areas of cortical bone extending to the ramus in the left posterior edentulous region of the mandible. B) Moth-eaten osteolytic areas in cortical and trabecular structure involving the inferior alveolar canal in the ramus region of the left mandible on CBCT axial and sagittal images.

DISCUSSION

Metastasis to the oral cavity is uncommon, accounting for 1-2% of all oral cancers (Ito et al., 2017; Hirshberg et al., 2014). Oral metastases may be falsely identified as a benign primary oral disease at the time of identification due to its rarity and lack of particular physical features. Some researchers claim that lung, breast, kidney, prostate, thyroid, and stomach cancers are the most common malignancies metastasizing to the oral cavity

(Hirshberg et al., 2014; Tatlidil & Gözübüyük, 2011; Pires et al., 2004), whereas others claim that lung cancer is the most common malignancy metastasizing to the oral cavity, followed by breast, kidney, and liver cancers (Pires et al., 2004). Moreover, lung, breast, kidney, prostate, renal and thyroid carcinomas are the most common primary foci metastasizing to the oral cavity in literature (Lopes et al., 2023; Kirschnick et al., 2022; Gupta et al., 2022; Hirshberg et al., 2014; Hirshberg et al., 2008; D'Silva et al., 2006; Bodner et al., 2006). These lesions, which can be seen at any age, are more common between the ages of 40s and 70s (Hirshberg et al., 2014).

Hirshberg et al (Hirshberg et al., 2014) emphasized that oral cavity metastases are the first clinical sign of the primary tumor with a rate of 30%. In the first case presented, oral cavity lesion appeared as the first clinical sign and primary source was detected after. In the second case, primary lung cancer was diagnosed one year ago and distant organ metastases were known, but oral cavity metastasis was diagnosed as a result of clinical and radiological examination. Both patients were in their 60s. Metastatic lesions in the oral cavity may manifest with clinical features such as pain, paresthesia, swelling, and the presence of progressively growing soft tissue lesions, but it has also been reported that these lesions may be asymptomatic (Shen et al., 2009). The first case presented with painless swelling that progressed rapidly following tooth extraction, while the second case had only severe pain. Oral cavity metastases are more frequently encountered in the mandible (Özbayrak 2020; Kaplan et al., 2019; Curien et al., 2007). Both cases presented herein were found in the mandible. Metastasis in the oral cavity may be a sign of widespread metastases with a poor prognosis. This may occur in advanced stages of cancer and may result in death in a short time. In both cases, primary lung cancer resulted in death in a short period of time despite the presence of distant organ metastases. The incidence of metastasis formation differs from the occurrence of primary tumors, owing to variances in biologic behavior (Hirshberg et al., 2014). It has been reported that malignancies in the kidney, liver, and lung are more likely to spread to the maxillofacial area. And initial cancers in the lungs tend to metastasize to the jawbones and soft tissue (Shen et al., 2009).

The process of metastatic dissemination is complicated, encompassing both tumor features and host response characteristics. For these reasons, the prevalence of metastasis and the specific location of metastases in distant organs varies greatly between tumor types. However, several patterns in metastasis to the oral soft tissues and bone have been recognized and documented in the past. Metastasis from prostate carcinomas was found to be predisposed to the jawbones, and those from the breast were twice as common in the jawbones as in the soft tissue; in contrast, those from the lung and kidney were more common in the soft tissues (Allon et al., 2014, Hirshberg et al., 2014). Both cases presented in our study had bone involvement.

It is worth noting that the majority of oral metastases occur in the mandible, with maxillary metastases being less prevalent. The circulation and the lymphatic system are two potential pathways for metastasis to the oral cavity. Considering the mandible and maxilla do not have lymphatic capillaries, the only feasible pathway for metastasis to the jaw is through blood vessels (Ito et al., 2017). Metastatic foci in the bones are mostly found in the red marrow (Ito et al., 2017; Yin et al. 2005); however, in adulthood, the mandible includes red marrow primarily in the ascending ramus and angles, whereas the maxilla exclusively contains fatty marrow.

Occasionally, metastatic lesions in the jaw may present with vague pain and be misdiagnosed as pathological entities of dental origin, such as pulpal/periapical disease (Kumar & Manjunatha, 2013). Recently, a case of metastatic breast cancer masquerading as a periodontal abscess in the mandible has been reported (Khalili et al., 2010). In a few cases, tooth extraction preceded the discovery of metastasis. The role of trauma to the oral mucosa, particularly from ill-fitting dentures, sharp teeth or restorations, poor oral hygiene and trauma from tooth extraction, in the causation of oral metastasis needs further investigation (Poulias et al., 2011).

It is crucial to highlight that the majority of jaw bone metastases are most likely to go unnoticed. When an oral metastasis is discovered, it can lead to the detection of a hidden primary tumor elsewhere in the body. The presence of this primary tumor can be detected using advanced imaging methods. Because the majority of patients have micrometastases, the prognosis is poor. End-stage disease leads to loss to follow-up or, in some cases, patient death. For the first case in our study a radiologically bone-invading mass with irregular and ulcerated areas was observed in the anterior edentulous region of the mandible following tooth extraction in the alveolar crest. The lesion was first assumed to be a benign reactive lesion, but an excisional biopsy revealed the presence of a clear cell-dominant carcinoma with a primary tumor on the patient's lung. All things considered, these tumors are of significant clinical importance since they might represent the first hint of a malignancy that has not yet been detected at a distant main site or the first proof that a recognized tumor has spread from its primary location.

CONCLUSION

The detection and supportive treatment of oral cavity metastases following the diagnosis of primary cancer is critical for maintaining patient comfort and function. In circumstances when the underlying malignancy is unknown, clinical, radiological and histological assessments can aid in the early diagnosis of the disease. Dentists should carefully check any changes in the oral cavity, and every alteration in cancer patients should be closely examined.

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CASE REPORT / OLGU SUNUMU

Tumoral Calcinosis: Clinical and Radiological Findings

Tümoral Kalsinozis: Klinik ve Radyografik Bulgular

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ABSTRACT

Tumoral calcinosis (TC) is an infrequent hereditary ailment described by localized ectopic calcification around the joints in affected regions. Reports of TC patients manifesting dental findings are scarce. Dental radiographs of a TC diagnosed patient show pulp stones, obliterated pulp cavities and short roots. In this case report, a 21-year-old female patient with TC was clinically and radiologically evaluated.
Keywords: calcification, calcinosis, dental radiography, pulp calcification, tooth abnormalities

ÖZ

Tümoral kalsinozis (TK), etkilenen bölgelerdeki eklemler etrafında anormal kalsifikasyonla belirgin, nadir görülen kalıtsal bir bozukluktur. TK hastalarının dental bulgularını içeren raporlar sınırlıdır. TK tanısı konmuş bir hastanın radyografisinde kısa kökler, pulpa taşları ve oblitere olmuş pulpa boşlukları belirgindir. Bu olgu sunumunda, 21 yaşındaki TK tanılı kadın hasta klinik ve radyolojik bulgular yönünden değerlendirilmiştir.
Anahtar Kelimeler: dental radyografi, diş anomalileri, kalsifikasyon, kalsinoz, pulpa kalsifikasyonu.

INTRODUCTION

Tumoral calcinosis (TC) is a progressive ailment described by the accumulation of calcium salts and crystals in periarticular soft tissues. TC lesions are predominantly noted on the extensor surfaces of the limbs, shoulder, the trochanter of the hip, the posterior aspect of the elbow, the humeral head, the acromio-clavicular joint, the inferior angle of the scapula, as well as the hands and feet (Witcher et al., 1989). The disease is infrequent, and there is no consensus on its etiological factors and pathogenic mechanisms. It typically presents between the ages of 10 and 30, being extremely rare in early childhood. There have been a total of 16 reported cases during infancy (Hammoud et al., 2005).

The expression TC was introduced by Inclan in 1943, describing slow-growing calcified masses commonly observed in proximity to major joints such as the elbow, shoulder, and hip (Inclan, 1943). Duret provided the first description of TC in 1989 (Duret, 1899). The initial study carried out in Turkey was published by Hacıhanefioğlu in 1978 in the English literature (Hacıhanefioğlu, 1978).

Familial TC cases have been predominantly documented in the Black population, with no observed gender bias (Gal et al., 1994). Familial tumoral calcinosis is more commonly observed in Africa and New Guinea, whereas it is rare in the populations of Europe and North America (Marinho et al., 1999). Also, TC cases are nearly absent in Asian region (Hunter et al., 1973).

TC is divided into two distinct groups: the primary form that occurs without an underlying disease (also known as familial TC), and the secondary form, which is linked to underlying conditions such as hypervitaminosis D, chronic kidney failure, scleroderma, sarcoidosis, primary hyperparathyroidism, milk-alkali syndrome, and malignancy. Within familial TC a subclassification is employed, distinguishing between a normophosphatemic variant described by sadnormal levels of serum calcium and phosphate, and a hyperphosphatemic subtype displaying normal serum calcium levels but elevated phosphate concentrations. (Olsen & Chew, 2006; Polykandriotis et al., 2004).

The clinical symptoms of TC include multiple or single hard, painless swellings in periarticular areas without redness, reducing the patient's capacity for joint movement. These swellings can sometimes lead to ulceration with chalky discharge and secondary infection on the overlying

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skin. Additionally calcified masses that are observable and palpable, in conjunction with dental and ocular abnormalities, may also be detected (Olsen & Chew, 2006; Polykandriotis et al., 2004; Foster et al., 2014).

There are few reports describing dental and oro-facial symptoms in familial TC (Dumitrescu et al., 2009; Krstevska et al., 2012; Naikmasur et al., 2008). Numerous manifestations affecting the facial and intraoral soft tissues have been documented in individuals with TC. These manifestations include angular cheilitis; maculopapular rash, erythematous patches, and calcified masses localized in the facial region. Additionally, there are erythematous lesions evident in diverse regions of the tongue, buccal mucosa, palate and adherent gum with papillary hyperplasia. Also, alterations in periodontal conditions are evident (Gal et al., 1994). Radiographically, most researchers have documented pulp stones, short and bulbous roots, and incomplete or complete obliteration of the pulp cavity in the impacted teeth (Witcher et al., 1989; Naikmasur et al., 2008; Burkes et al., 1991; Krsteyska et al., 2012; Favia et al., 2014). The observations resemble dentin dysplasia, featuring short and bulbous teeth with irregular calcifications (Ramnitz et al., 2016; Krstevska et al., 2021). The existence of abnormal calcification within the pulp space may impede the progress of root canal treatments (Stewart, 1995).

In this case presentation, the detailed clinical and radiological findings of a patient diagnosed with TC, who presented to our clinic with pain and mobility complaints, have been evaluated, and treatment options have been discussed.

CASE REPORT

A 21-year-old female patient presented to the Marmara University Faculty of Dentistry with a complaint of pain and advancing teeth mobility. Before coming to the faculty, the patient had previously visited a private dental clinic due to decayed teeth and pain. At this clinic, the patient underwent a tooth extraction procedure to alleviate the pain. When the patient applied to the faculty, her teeth were missing, decayed and mobile. The initial panoramic radiography, which was taken at the faculty, is shown in Figure 1.

Figure 1: Patient's panoramic image; The X-ray was taken when the patient first applied to the faculty

Considering the diagnosis of TC, an examination was conducted on other family members, but no additional findings were identified. It has been learned that the mother and father are carriers of the TC. The serum concentrations of calcium and phosphorus in the patient were systematically documented. In our patient, the serum concentrations of calcium and phosphorus exceeded the established reference range (serum calcium, 10,6 mg/dl and serum phosphorus, 7,3 mg/dl) (Reference values: Serum calcium 8.6-10 mg/dl, Serum phosphorus 2.5-4.5 mg/dl).

In 2018, an X-ray of the right knee joint, requested by the Department of Orthopedics and Traumatology, observed a calcified mass (Figure 2). The patient underwent knee surgery later on.

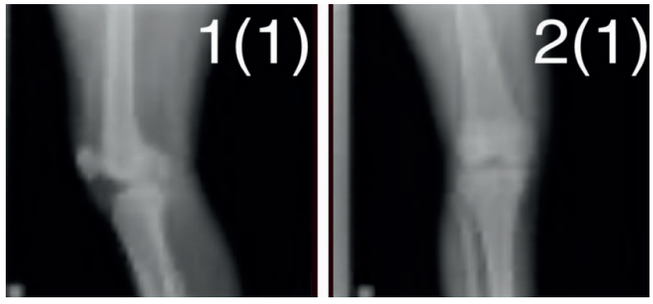

Figure 2: Calcified soft tissue mass at the right knee joint

As a result of the physical examination, no symptoms of TC were identified. During the intraoral examination, no abnormalities in the soft tissues were observed. The mucosa exhibited a normal color and structure. Normal salivary flow was observed from the major salivary glands. Additionally, no intraoral swelling was observed.

Teeth numbered 14, 16, 24, 25, 26, and 46 have been extracted in the patient due to caries or mobility (Figure 3). Teeth numbered 32, 31, 41, and 42 exhibited mobility. Teeth numbered 31 and 41 had severe mobility. The patient has a history of extraction due to mobility in non-carious teeth. The patient indicated in the taken medical history that the teeth crumble and fall apart like powder. In the medical history obtained, the patient mentioned that a previously filled tooth was causing pain and mobility, and they pulled it out painlessly with their own hands.

Figure 3: Patient's intraoral image; edentulous space and normal appearing clinical crowns

The patient exhibits general sensitivity to hot and cold, along with mobility in specific teeth. Especially tooth number 31 exhibited severe bone loss and mobility (Figure 4). Another noteworthy observation included the whole obliteration of both pulp chambers and root canals in all teeth. (Figure 5). Irregular areas on the root surfaces of some teeth were detected in periapical radiographs, such as in teeth numbered 33 and 34 (Figure 6).

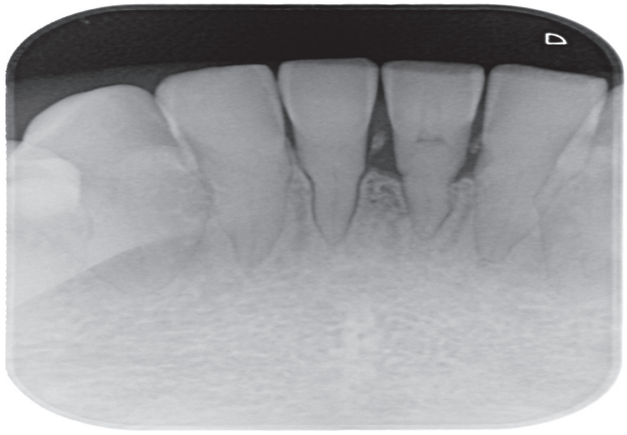

Figure 4: Periapical radiographs of the patient exhibiting bone loss

Figure 5: Periapical radiographs showing obliteration of pulp chambers and root canals

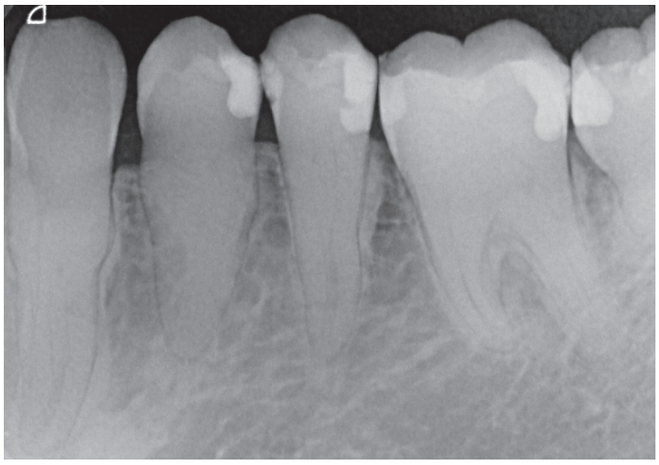

Figure 6: Periapical radiographs showing irregular areas on the root surfaces and nlargement of the coronal third of the roots

Normal appearing clinical crowns (Figure 3), the lengths and shapes of the patient's roots were also normal. There were no observed abnormally short or conical-shaped roots. Accessory roots and root dilacerations were not detected in the teeth.

A detailed medical history was taken from the patient at our faculty, and a clinical and radiological examination was performed. At our faculty, the patient's teeth with excessive mobility and bone loss were extracted. The patient's decayed teeth were filled. The patient's teeth numbered 14, 16, 24, 25, and 26 were extracted, and fillings were performed on teeth numbered 15, 17, 22, 23, 27, 32, 35, 36, 37, 47, and 48. The patient was referred to the prosthetics department for the edentulous spaces.

DISCUSSION

TC is an infrequent hereditary autosomal disorder distinguished by the existence of a cystic mass in the periarticular area containing calcified materials. It is characterized by various clinical manifestations. Possible causes encompass a congenital anomaly in phosphate metabolism, renal osteodystrophy, hypercalcemia due to sarcoidosis, or potentially an idiopathic origin (Davies, 2002). The predominant age group affected is children and adolescents, particularly those between 6 and 25 years old (Yochum & Rowe, 2005). Similarly, the patient mentioned in this case presentation was 21 years old.

Clinical symptoms typically involve periarticular, subcutaneous, and soft tissue calcifications occurring in areas prone to recurrent trauma. In this case, calcified masses were also observed in the right knee joint. An alternative manifestation of TC is dental anomalies. Existing literature, it has been observed that the majority of dental manifestations of TC are documented in individuals with hyperphosphatemia (Dumitrescu et al., 2009; Burkes et al., 1991; Favia et al., 2014; Hunter et al., 1973).

Clinically; enamel hypoplasia (Hunter et al., 1973; Favia et al., 2014), morphological changes in crowns (Burkes et al., 1991; Naikmasur et al., 2008) or normal coronal tooth structure (Witcher et al., 1989) have been identified. In this case, similar to the findings of Witcher et al., normal coronal tooth structure was observed.

Gal et al. conducted an investigation into head and neck symptoms among a cohort of six patients, revealing the presence of calcified masses in varying distributions across facial regions such as the cheeks, nose, jaw, and lips. Notably, one patient exhibited papillary hyperplasia of the lips, and all individuals displayed early periodontal alterations typified by marginal gingivitis. The soft and hard palates of all six patients exhibited erythematous changes, while three patients showed such alterations on the buccal mucosa and five patients on the tongue. It is noteworthy that calcified deposits were uniformly found on the cheeks, nose, jaw, and lips in all six cases, and importantly, none of these patients manifested atypical calcifications in periarticular tissues (Gal et al., 1994). In this case, however, the soft tissues were normal, and the gums were healthy. There were no calcified deposits in the neck and head region. Three cases of TC have been reported in the temporomandibular region (Shirasuna et al., 1991; Noffke et al., 2000; Zanetti et al., 1994) and one case has been reported in the premaxilla (Marinho et al., 1999).

Dental observations encompass thistle-shaped dental pulps, short and bulbous teeth, shortened roots, root dilacerations, obliteration of pulp chambers and root canals, pulp stones and taurodontism (Burkes et al., 1991; Dumitrescu et al., 2009). In this case as well, obliteration of the pulp chamber and root canal is similarly detected but unlike the previous case, there were no root dilacerations.

Yılancı et al. observed short bulbous roots, coronal expansion in the upper third of the root, the presence of oval radiopacities in this region, obliteration of the pulp, periapical radiolucencies in non-carious teeth and root dilacerations. They also noted that the most commonly impacted teeth were incisors and premolars (Yılancı et al., 2017). Burkes et al. recorded that the most notable alterations were discerned in the premolars of both the maxillary and mandibular arches (Burkes et al., 1991).

In the literature, significant bilateral mandibular tori and bone sclerosis of the mandible, maxilla, and skull have been recorded in one patient (Krstevska et al., 2012). Additionally, cases have been recorded in the literature involving progressive enlargement of gingival and alveolar tori, hypoplasia in the maxillary and mandibular regions, skeletal Class II malocclusion, and the presence of a deep bite (Krstevska et al., 2012; Favia et al., 2014). In this case, there were no torus enlargement, and malocclusion was not present.

Favia et al. reported multiple impacted teeth related to TC (Favia et al., 2014). In this case, no impacted teeth were observed. The findings in this case resemble those reported by Polykandriotis et al., as well as the observations in the publications of Burkes et al. (Polykandriotis et al., 2004; Burkes et al., 1991).

Dentists should be cognizant of the distinctive features of pulpal obliteration and the presence of roots resembling thistles, as dental radiographic observations may serve as the initial indication of the disease, especially in patients lacking other systemic manifestations (Yamamoto et al., 2016; Topaz et al., 2004; Ichikawa et al., 2007).

The uncommon occurrence of enamel abnormalities in TC may elevate the risk of caries; meanwhile, more frequently encountered pulp calcifications hinder the endodontic treatment of carious lesions (Favia et al., 2014). As a result, the implementation of dental sealants for the preventation of caries is recommended as a more effective treatment choice.

CONCLUSION

TC is an uncommon disorder of mineral metabolism observed among adolescents and juvenile adults, distinguished by the pathological accumulation of calcific masses surrounding major joints. Laboratory analyses commonly unveil hyperphosphatemia alongside normocalcemia, normal parathyroid hormone levels, and alkaline phosphatase within the standard range. Dental observations bear similarities to dentin dysplasia, encompassing the obliteration of both root canals and coronal pulp chambers to varying degrees. The obliteration of pulp chambers and root canals presents a challenge in endodontic treatment procedures. Dentition may be periodontally affected, leading to early permanent tooth loss. It is crucial for the dentist to be aware of the dental indications of TC when treating a juvenile patient with completely or partial obliteration of pulp chambers or root canals.

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CONFLICTS OF INTEREST

The authors confirm that there are no conflicts of interest.

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REVIEW / DERLEME

Minimally Invasive Access Cavities

Minimal İnvaziv Giriş Kaviteleri

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ABSTRACT

Preparing an appropriate access cavity is critical to successful root canal treatment, allowing adequate visibility and access to the canals. Traditional endodontic access cavities often require significant removal of dental tissue, which raises concerns about tooth fracture resistance. In response, a minimally invasive approach has been introduced that emphasizes the preservation of dental tissue. The choice between minimally invasive and traditional access cavities requires careful consideration. The impact of these cavities on teeth remains controversial. Tools such as CBCT, a microscope, and ultrasonic tips are required to treat these access cavities effectively. Further and long-term studies are necessary to draw definitive conclusions regarding the advantages and disadvantages of minimally invasive access cavities, the risk of complications and malpractice, whether they provide adequate root canal cleaning, the tooth's fracture resistance, and overall treatment success. In this review, the specified factors have been evaluated based on findings from various studies, and a general perspective has been presented.
Keywords: Conservative access cavity, traditional access cavity, endodontics, access cavity, root canal morphology.

ÖZ

Kanallara yeterli erişim ve görünürlüğü sağlayan uygun bir giriş kavitesi açmak, başarılı bir kök kanal tedavisi uygulamak için önemlidir. Geleneksel endodontik giriş kavitelerinin önemli miktarda dental doku kaybına neden olması, dişin kırılma direnciyle ilgili endişeler doğurmuştur. Buna karşılık, diş dokusunun korunmasını öneren minimal invaziv yaklaşım ortaya çıkmıştır. Minimal invaziv giriş kaviteleri ve geleneksel giriş kaviteleri arasındaki seçim dikkatli bir değerlendirme gerektirir. Çünkü bu kavitelerin diş üzerindeki etkisi tartışmalıdır. Bu kavitelerin efektif bir şekilde tedavide kullanılabilmesi için CBCT, mikroskop ve ultrasonik uçlar gibi aletlerin kullanılması gerekir. Minimal invaziv giriş kavitelerinin avantaj ve dezavantajlarına, komplikasyon ve malpraktis oluşma riskine, yeterli bir kök kanal temizliği sağlayıp sağlamadığına, dişin kırılma direncine ve genel olarak tedavi başarısına dair kesin sonuçlara ulaşmak için daha fazla ve uzun dönem çalışmaların yapılması gereklidir. Bu derlemede, belirtilen faktörler farklı çalışmalardaki bulgulara göre değerlendirilmiş ve genel bir bakış açısı sunulmuştur.
Anahtar Kelimeler: Konservatif giriş kavitesi, geleneksel giriş kavitesi, endodonti, giriş kavitesi, kök kanal morfolojisi.

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INTRODUCTION

A successful root canal treatment is essential for the patients to maintain the health and function of their tooth for many years. Preparing a suitable access cavity is one of the most essential steps in achieving a successful root canal treatment (Ballester et al., 2021). Traditional endodontic access cavities require the removal of caries and restorations within the tooth. The configuration of the access cavity depends on the tooth type and pulp morphology. The entire pulp chamber roof must be removed to access the canals, along with any cervical dentin projections. Adequate access is fundamental to the progress of treatment, as it is crucial to ensure that all canal orifices are visible from the cavity to avoid treatment failure. Meeting these requirements is necessary under the traditional approach. However, this method results in significant tooth tissue loss, leading to reduced fracture resistance (Tang et al., 2010).

An approach that aims to preserve dental tissue as much as possible is gaining traction in dentistry. Endodontics has adopted the minimally invasive trend, which is becoming popular across dentistry, with a system that advocates for creating smaller access cavities. This method emphasizes preserving dentin around the tooth's cervical region and the pulp chamber's roof while minimizing dental tissue loss. Preserving dental tissue is advised to reduce the likelihood of tooth fractures. The minimally invasive endodontic approach prioritizes the preservation of as much pericervical dentin as possible due to its benefits for tooth resistance. This area includes 4 millimeters below and 4 millimeters above the bone (Plotino, 2021).

The purpose of this review is to explore the classifications of minimally invasive access cavities in the literature, define the commonly accepted cavity types, introduce the necessary armamentarium, and evaluate the impact of minimally invasive access cavities on key aspects of endodontic treatment in comparison to traditional access cavities.

1. Minimally Invasive Access Cavities

In dentistry, minimally invasive access cavities represent a conservative approach to tooth preparation, particularly in restorative dentistry and endodontics. The objective is to minimize the removal of healthy tooth structures while providing access to the affected area. Traditionally, dental procedures involve larger access cavities to ensure adequate visibility and access to the dental pulp or affected region (Ballester et al., 2021). However, the concept of minimally invasive access cavities promotes a more conservative method, focusing on preserving as much healthy tooth structure as possible. These cavities are typically smaller and strategically positioned to maintain the tooth's integrity and strength (Chan et al., 2022).

Minimally invasive access cavity preparations have become a significant concept in endodontics, primarily preserving pericervical dentin while achieving the necessary treatment outcomes. Pericervical dentin is crucial in distributing stress across the tooth, contributing to long-term stability and fracture resistance (Plotino, 2021). The underlying assumption is that preserving this dentin can enhance the tooth's fracture resistance (Silva et al., 2018).There are some concerns regarding the impact of traditional access preparations on tooth survivability and long-term strength. In contrast, minimally invasive access preparations prioritize the preservation of natural tooth structure. The idea is to minimize the loss of healthy tissue because unnecessary removal can weaken the tooth and endanger its prognosis.

However, it is crucial to take a critical approach when considering the concept of minimally invasive endodontics. While preserving tooth structure is advantageous, evaluating the potential drawbacks and limitations is essential. Each case should be assessed individually, considering factors such as the extent of the pathology, the overall health of the tooth, and the accessibility of the root canal anatomy. Selecting the most appropriate treatment requires careful consideration of these factors.

1.1. Terminology and Classification

In this field, standardized terminology has yet to be fully established. Different articles and textbooks have proposed various names and classifications, also the anatomical borders of these cavities are not clearly defined (Silva et al., 2018). However, for clarity, it is essential to standardize the terminology. In this review, access cavities are examined under seven headings.

1.1.1. Traditional Access Cavity

In posterior teeth, the entire pulp chamber roof is removed, and a straight-line access is created to reach the canal orifices (Fig. 1). In anterior teeth, the pulp chamber ceiling, pulp horns, and lingual shoulder of dentin are removed to achieve straight-line access to the canal openings. Removing the lingual shoulder is crucial for establishing this straight-line access .

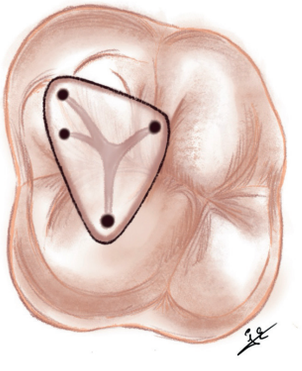

Figure 1: Illustration of traditional access cavity on maxillary first molar.

1.1.2 Conservative Access Cavity

This approach emphasizes preserving the remaining healthy tooth structures compared to traditional endodontic

cavities. In posterior teeth, the access cavity starts at the central fossa of the occlusal surface and extends only as far as is necessary to locate the canal orifices (Ballester et al., 2021). Achieving complete straight-line access is not essential. The cavity walls are prepared to converge towards the occlusal surface, allowing visibility of the pulp chamber and canal orifices while preserving part of the roof (Fig. 2). Alternatively, the walls can be prepared with a divergent design (Shabbir et al., 2021). Clinicians can visualize the chamber area and floor by tilting the mirror. In anterior teeth, a small triangular or oval-shaped access cavity is created, enhancing the possibility of preserving the pulp horns and pericervical dentin, which refers to the tooth structure located 4 mm above and 4 mm below the alveolar bone crest (Chan et al., 2022; Ingle et al., 2019).

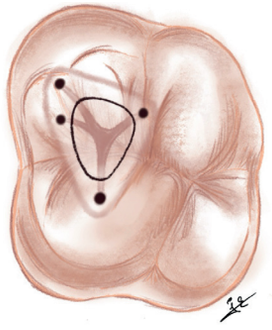

Figure 2: Illustration of conservative access cavity on maxillary first molar.

1.1.3. Ultra-Conservative Access Cavity

Also known as “ninja” access cavities, this method focuses on extreme preservation of the pulp chamber ceiling by creating a highly constricted cavity with sharply convergent walls. The process begins similarly to the conservative approach, creating access through the central fossa, but without further extensions (Fig. 3). In anterior teeth, where the lingual surfaces may exhibit attrition or deep concavities, access is made through the incisal edge parallel to the tooth’s axis (Chan et al., 2022; Ingle et al., 2019).

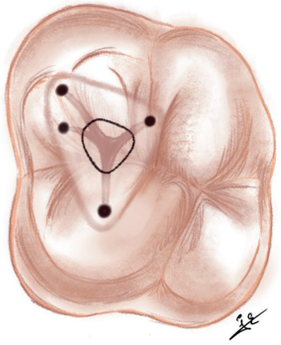

Figure 3: Illustration of ultra-conservative access cavity on maxillary first molar.

1.1.4. Truss Access Cavity

The method is also called an orifice-directed dentine conservation access cavity (Chan et al., 2022). This technique involves creating multiple small cavities to reach the canal openings in teeth with multiple roots while maintaining the dentinal bridge that separates them. For example, three distinct cavities can be created in the molars of the upper jaw (Fig. 4). Likewise, a total of two separate access cavities can be created for mandibular molars, one for the mesial canals and one for the distal canals. Even separate access cavities can be created for each channel.

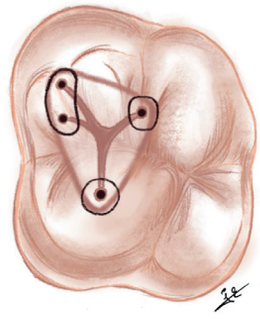

Figure 4: Illustration of truss access cavity on maxillary first molar.

1.1.5. Caries-Driven Access Cavity

This approach removes all decayed tissues while preserving the healthy tooth structure (Fig. 5). Access to the canals is achieved by eliminating the decayed areas only (Plotino, 2021; Chan et al., 2022).

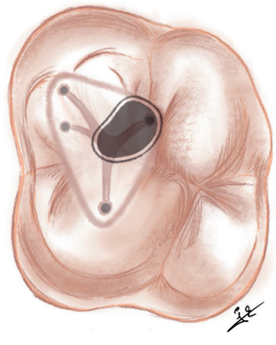

Figure 5: Illustration of caries-driven access cavity on maxillary first molar.

1.1.6. Restorative-Driven Access Cavity

In restored teeth without cavities, the pulp chamber is accessed by entirely or partially removing the existing restorations while preserving the remaining tooth structure (Fig. 6). This method is advantageous because it utilizes the loss of structure due to the restoration of canal access, eliminating the need to enlarge the pre-existing cavity (Plotino, 2021).

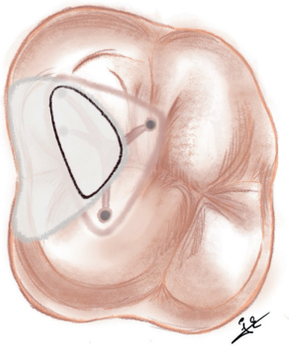

Figure 6: Illustration of restorative-driven access cavity on maxillary first molar.

1.1.7. Computer-Assisted Cavities

Computer-assisted access cavity preparations utilize software and 3D imaging to aid clinicians in creating a precise pathway to the root canal while preserving the tooth's integrity. These tools are also particularly useful in managing calcified structures. Two categorization can be made for these cavities (Shabbir et al., 2021).

Guided Access Cavity

Guided access cavities require intraoral scanners and imaging techniques to fabricate a customized stent that provides precise guidance for the drill. This approach is conservative and goal-oriented. After intraoral scanning, cone beam computed tomography (CBCT) scanning is carried out. Following that, a virtual drill path are developed on the computer screen by combining intraoral scans and CBCT data. On the basis of virtual planning, templates for guidance, access cavities, and sleeves are created (Zehnder et al., 2016). However, it may involve longer planning times and limited accessibility, particularly posterior teeth. There is also a risk of overheating during drilling, and artifacts may affect the accurate location of canals (Shabbir et al., 2021).

Dynamic-Navigated Access

This method employs real-time navigation by using CBCT, and software to guide the drilling process. Unlike guided access cavities, dynamic-navigated access does not require extensive planning, but it is more expensive due to the need for multiple intraoral attachments. It is a freehand approach (Shabbir et al., 2021).

1.2. Armamentarium

This section describes the tools required to apply minimally invasive access cavities in clinical practice.

1.2.1. Burs

The primary tool used to access the pulp chamber of all teeth is a diamond, slightly tapered bur with a rounded end. This bur can be either cylindrical or conical in shape. Various sizes are available, depending on the dimensions of the tooth. Typically, a size #10–12 burs is used for accessing smaller teeth or calcified pulp chambers, while size #12–14 burs are used for larger teeth or those with substantial pulp chambers. The unique, rounded end of the bur helps create smoother cavities. High-speed ball burs can cause excessive tooth tissue removal, which is not desirable for minimally invasive access cavities but a multi-blade carbide stainless-steel bur, specifically a ball bur in sizes #12 or #14, is recommended for use with low-speed handpieces. These burs can be used to remove caries and pulp tissue, after using diamond burs. Endodontic access burs can be used to remove pulp horns and reduce the possibilty of excess removal of tooth tissue, and can also be helpful when working with difficult cases where canal orifices located deep within tooth. Low-speed Munce discovery burs, Clark's EG3 micro-access burs, Endo-Z burs and Endoguide burs are suggested in different studies (Plotino, 2021; Freitas et al., 2021; Moore et al., 2016; Roperto et al., 2019).

1.2.2. Canal Preparation Instruments

In traditional endodontic approaches, Gates Glidden burs and Peeso reamers are commonly used to enlarge the coronal portion of the canals, providing straight access. However, these instruments are known for being aggressive, potentially over-enlarging the canals and leading to complications such as furcation strip perforation or canal misalignment. According to the principles of minimally invasive dentistry, excessive canal enlargement is detrimental to the overall prognosis of the tooth. However, the future of endodontics looks promising with the use of nickel-titanium (NiTi) instruments. Made from NiTi alloy, these instruments are flexible and maintain their shape within the canal, offering greater resistance to metal fatigue (Plotino, 2021).

1.2.3. Operating Microscope and Loupe

An operating microscope (OM) is crucial for minimally invasive endodontic access cavity procedures. Most studies comparing traditional access cavities with minimally invasive approaches have shown that operating microscopes and loupes are essential tools. The OM provides better vision and ergonomics for the operator, allowing clear visualization of the pulp chamber and easier identification of important landmarks. In minimally invasive endodontics, operating microscopes and loupes significantly enhance the clinician's chances of success.

Loupes are highly recommended, specifically those with a magnification of 4.5 to 5. Nearly 80% of endodontic cases can be resolved using loupes. However, certain situations, such as locating calcified root canals, removing broken instruments, or extracting fiber posts, may require a microscope. Additionally, LED lights can be mounted on loupes to improve visibility, which is especially beneficial in challenging cases (Plotino, 2021).

1.2.4. Ultrasonic Instruments

Ultrasonic instruments play a crucial role in minimally invasive cavity preparation by effectively cleaning and removing obstructions such as pulp stones or calcifications. These instruments allow for the removal of calcifications without disturbing the canal walls or floor (Plotino, 2021). Additionally, they are valuable for locating additional canals. Ultrasonic tips offer a less aggressive method for identifying the canal orifice than traditional dental drills or handpieces. Combining an operating microscope with ultrasonic instruments enhances the success and predictability of root canal treatment, providing better control and visibility during the procedure (Chan et al., 2022).

1.2.5. CBCT

The advent of CBCT has significantly advanced the practice of minimally invasive endodontics, ushering in a new era of detailed imaging and precision (Chan et al., 2022). CBCT is particularly useful in retreatment and endodontic surgery cases, offering detailed imaging to detect apical pathosis, pulp canal calcifications, and complex tooth anatomy. It is invaluable for identifying missed canals, ledge formation, perforations, and fractured files. In such cases, CBCT can assist in designing the access cavity, and unique guides made of silicon metal can be prepared for completely calcified canals, like those used in implant procedures. This ensures more accurate preparation of access cavities, paving the way for more precise and effective treatments in the future.

Intraoperative 3D navigation, adapted from implant-guided surgery, allows clinicians to monitor the procedure in real time using CBCT images. Implementing this method in endodontics would offer significant advantages, enabling more precise and controlled treatment (Plotino, 2021).

1.3. Effects of Different Cavities on Root Canal Treatment

This section explores and compares the effects of traditional and minimally invasive access cavities on root canal therapy from various perspectives.

1.3.1. Resistance to Fracture

Changes in tooth biomechanics can result from tissue loss caused by procedures such as caries removal, fractures, and access cavity preparation. Endodontic access alone reduces tooth stiffness by only about 5% (Allen et al., 2018). Subsequent canal instrumentation and obturation cause only a slight decrease in fracture resistance. According to studies, minimally invasive access cavities have minimal impact on tooth biomechanics. However, traditional access cavities involve more extensive preparation. Excessive preparation, including the loss of the marginal ridge, has been shown to reduce fracture resistance significantly (Tang et al., 2010). The depth of the cavity, the width of the isthmus, and the configuration of the cavity are critical factors that influence tooth stiffness and fracture risk.

Most studies on minimally invasive access cavities focus on whether these cavities make teeth more resistant to fractures and cracks. It is generally accepted that traditional access cavity preparations result in a more significant loss of tooth tissue. Since minimally invasive access cavities preserve more tooth structure, they are expected to offer higher fracture resistance. For example, a study by Krishan et al. (2014) demonstrated that mandibular premolar and molar teeth with conservative access cavities had higher fracture resistance. However, no post-endodontic restorations were performed on these teeth.

Two other studies (Plotino et al., 2017; Franco et al., 2020) compared teeth with minimally invasive and traditional access cavities after post-endodontic restorations. They found that teeth with minimally invasive access cavities had higher fracture resistance. Other studies investigating both mandibular and maxillary premolars and molars (Xia et al., 2020; Saberi et al., 2020; Moore et al., 2016; Corsentino et al., 2018; Özyürek et al., 2018) showed no significant difference in fracture resistance between the two approaches.

Overall, minimally invasive access cavities in posterior teeth show no significant difference or result in slightly better outcomes than traditional access cavities. A study on the fracture resistance of anterior teeth (Krishan et al., 2014) found no discernible difference between the two approaches. A comprehensive systematic review (Silva et al., 2018) concluded that no conclusive scientific evidence supports using minimally invasive access cavities to increase fracture resistance. This underscores the need for further research and the ongoing scientific discourse.

A study comparing Truss access cavities with traditional access cavities (Saberi et al., 2020) found that teeth with Truss access cavities exhibited increased fracture resistance. However, another study on the same subject (Barbosa et al., 2020) found no significant difference in fracture resistance between the two types of cavities. It is important to note that both studies may not have accurately replicated in vivo conditions.

Regarding ultra-conservative access cavities, one study (Plotino et al., 2017) reported no significant difference in fracture resistance between conservative and ultra-conservative cavities. However, the study also suggested that ultra-conservative access cavities showed increased fracture resistance compared to traditional access cavities. Conversely, another study (Silva et al., 2020) found no significant difference in fracture resistance between teeth with ultra-conservative and traditional access cavities.

The conflicting results among these studies may be due to several factors, including sample sizes, the type of teeth used, the presence or absence of post-treatment restorations, the decreased hardness of teeth in elderly individuals, and variations in crown-root morphologies and testing parameters. One study (Silva et al., 2020)

recommended using CBCT to address these variations. The same study also indicated limited evidence to support increased fracture resistance with ultra-conservative access cavities.

1.3.2. Preservation of Remaining Tooth Structure

Minimally invasive access cavity preparation has been proposed to preserve critical structural dentin. Lin et al. (2020) examined tissue loss from the incisal/occlusal part of the tooth to the cementoenamel junction. The results revealed that teeth with traditional access cavities required the most dentin removal, whereas those with minimally invasive cavities showed less tissue loss.

Another study by Jain et al. (2020) found that teeth treated with dynamically navigated access preparations experienced less substance loss than those with freehand access preparations, particularly in anterior teeth with calcified canals. This was attributed to the more accurate identification of the canal entrance.

Additionally, a study on extracted human intact maxillary incisors, mandibular premolars, and molars (Krishan et al., 2014) demonstrated that coronal dentin was preserved across all three types of teeth. In contrast, a study focusing on the remaining dentin in mandibular premolars during canal instrumentation (Barbosa et al., 2020) found an increased unprepared canal surface area. However, another study on mandibular incisors (Rover et al., 2017) reported no significant difference in dentin preservation. The consensus is that minimally invasive access cavities reduce the amount of coronal dentin loss, contributing to the preservation of important tooth structure.

1.3.3. Canal Orifice Detection

One of the significant challenges in minimally invasive endodontics is the difficulty in locating the canals due to the limited visibility of the pulp chamber (Plotino, 2021). A study conducted on maxillary molars (Rover et al., 2017) divided the canal location process into three stages: the first stage involved locating the canals without magnification; in the second stage, a microscope was used; and in the third stage, both a microscope and ultrasonic troughing were employed. The study found that the first and second stages of conventional access cavities significantly increased the likelihood of identifying the canal orifice. However, there was no significant difference after the third stage.

Another study (Saygili et al., 2018) observed that ultraconservative access cavities, even when used with an operating microscope and ultrasonic tips, reduced the detection rate of additional canals. However, a study simulating a clinical environment (Mendes et al., 2020) demonstrated that when a skilled clinician used an operating microscope and ultrasonic tips, the design of the access cavity—whether traditional or minimally invasive—did not affect the detection of middle mesial canals in mandibular molars. In summary, when an operating microscope and ultrasonic tips are utilized, the design of the access cavity does not significantly impact canal detection. Further research in this area is necessary.

1.3.4. Microbial Cleaning

Effective cleaning of the root canal system requires the removal of all pathological factors to ensure the success of the treatment (Hargreaves et al., 2016). Recent studies have examined the impact of minimally invasive endodontic cavities on root canal instrumentation and disinfection, focusing on the amount of untouched canal area and the bacterial load after debridement.

Some studies on mandibular molars (Krishan et al., 2014; Barbosa et al., 2020) found that teeth with minimally invasive access cavities had more untouched canal area than those with traditional access cavities. These studies also reported a higher percentage of residual pulp in the pulp chamber, which could negatively impact the effectiveness of disinfection. However, other studies (Krishan et al., 2014; Rover et al., 2017; Augusto et al., 2020; Vieira et al., 2020) showed no significant difference in untouched canal area among maxillary and mandibular molars, maxillary premolars, and mandibular incisors. Additionally, the number of positive samples for *E. faecalis* was higher in the group with minimally invasive access cavities after preparation. Overall, the effectiveness of disinfection and instrumentation in minimally invasive cavities remains controversial. The minimally invasive design does not appear to provide any advantage in canal instrumentation or cleaning.

1.3.5. Residual Pulp Tissue and Debris

One of the primary goals of mechanical preparation in endodontics is the thorough removal of pulp tissue, debris, and bacteria to prevent treatment failure. A study by Allen et al. (2018) conducted in a simulated clinical environment with maxillary premolars compared traditional and ultraconservative access cavities. The results indicated that ultraconservative access cavities left more debris in the root canal, which complicated the cleaning process and extended the procedure time. However, a study on mandibular incisors (Rover et al., 2017) found that the shape of the cavity did not significantly affect the amount of remaining pulp tissue and debris. Similarly, another study on maxillary molars (Rover et al., 2017) found no significant differences in accumulated hard tissue debris after preparation. In contrast, a study by Neelakantan et al. (2018) revealed that orifice-directed dentine conservation access cavities on mandibular molars resulted in less thorough cleaning of the pulp chamber. However, this did not affect the cleanliness of the root canals or isthmuses.

1.3.6. Canal Transportation

Canal transportation refers to the unintended shifting of the canal foramen from its original position during endodontic procedures (Ingle et al., 2019). The transportation rates of teeth with minimally invasive

access cavities have been a significant research focus. A study on maxillary molars (Rover et al., 2017) found that canal transportation occurred 7 mm from the apical end in the palatal canal of teeth with minimally invasive access cavities. In comparison, canal preparation was more centralized in the palatal canal of teeth, with traditional access cavities at 5 and 7 mm from the apical end. Another study by Alovisi et al. (2018) suggested that traditional access cavities better preserve the original root canal structure compared to minimally invasive access cavities, possibly due to the absence of obstacles in the coronal area, which reduces the need for frequent pecking motions during instrumentation.

Conversely, a study on mandibular molars (Barbosa et al., 2020) that compared conservative and truss access cavities with traditional access cavities found no significant differences in canal transportation. Similarly, two other studies on molars (Augusto et al., 2020; Marchesan et al., 2018) also reported no significant differences. However, a study on premolars by Xia et al. (2020) indicated that apical transportation after instrumentation was significantly greater in minimally invasive access cavities compared to traditional access cavities in premolars with two dental roots.

1.3.7. Instrument Fracture

Instrument fractures are common due to excessive or improper use of dental instruments. Contributing factors include deviations from normal canal morphology, using damaged instruments, inadequate irrigation, excessive force applied to the instrument while working inside the canal, and insufficient access cavity preparation (Ingle et al., 2019).

A study by Silva et al. (2021) investigated the effect of ultraconservative access cavities on the cyclic resistance of NiTi instruments, specifically RECIPROC R25 and RECIPROC Blue R25. The study found that when accessing lower molars with ultraconservative access cavities, both file systems exhibited lower cyclic fatigue resistance than traditional access cavities. This suggests a need for further research to evaluate the efficiency of alternative file systems in ultraconservative access cavities. In contrast, other studies (Krishan et al., 2014; Moore et al., 2016; Rover et al., 2017; Alovisi et al., 2018; Marchesan et al., 2018) examining the impact of minimally invasive access cavities on the fracture resistance of teeth did not report an increased occurrence of instrument fracture. Using flexible NiTi instruments in minimally invasive access cavities has shown the potential to reduce the risk of instrument fracture.

1.3.8. Canal Filling Quality

The goal of obturation is to create a watertight seal along the entire length of the root canal system, from the orifice to the apical end (Torabinejad et al., 2021). This seal prevents the spread of bacteria within the canal and beyond the root, thereby preventing infection.

Research on mandibular incisors (Rover et al., 2017) revealed that the formation of voids was more likely in teeth with minimally invasive access cavities when the single cone and warm vertical compaction techniques were used. Similar results were observed in a study on mandibular premolars (Silva et al., 2020). These findings suggest that the warm lateral compaction method may yield superior results. However, a study comparing traditional, conservative, and truss access cavities in mandibular molars (Silva et al., 2020) found no significant difference in voids within root fillings. Similarly, two other studies on maxillary premolars (Barbosa et al., 2020) and maxillary and mandibular first premolars (Xia et al., 2020) also reported no significant differences in void formation.

1.3.9. Restoration After Root Canal Treatment

Restoring endodontically treated teeth is crucial for the long-term survival of the treated teeth, mainly when restorations are inadequate or missing. It is important to recognize that treated teeth differ structurally from healthy ones, making effective coronal restorations vital to prevent treatment failure. These restorations act as a barrier against microbial infiltration. Even with excellent endodontic treatment, inadequate sealing, especially in the marginal integrity of post-endodontic restorations, can lead to treatment failure. Leakage can occur due to insufficient sealing of temporary restorations (Hargreaves et al., 2016).

Cuspal coverage and a post are generally not required for teeth with only an occlusal endodontic cavity, allowing for direct restoration. If a marginal ridge is lost, a post is usually unnecessary. However, cuspal coverage may be advisable in posterior teeth, depending on the quantity and quality of the remaining tooth structure (Plotino, 2021). The most conservative cavity design can preserve vital tooth tissue, potentially allowing for direct or indirect restorations without cuspal coverage, even without a marginal ridge (Plotino et al., 2017). However, losing all marginal ridges significantly reduces tooth stiffness, necessitating full cuspal coverage and often a post. In most cases, partial adhesive restorations with cuspal coverage are effective. When significant structural loss occurs, a crown with post and cuspal coverage is generally the better option.

1.3.10. Aesthetics

When a tooth loses vitality, it undergoes biochemical changes that affect dentin's light refraction, leading to a shift in its natural color. Insufficient root canal cleaning and the presence of sealer or filling material in the pulp chamber can further contribute to discoloration. Evaluating the impact of minimally invasive techniques on aesthetics, particularly in anterior teeth, is essential. Minimally invasive endodontic access cavities, which involve partial removal of the pulp chamber roof while preserving the pulp horns, present challenges. This approach can prevent the complete removal of pulp remnants and hinder the proper placement of

bleaching agents. Despite the use of ultrasonic tips and magnification, operators often struggle to eliminate residual filling material before placing restorations in ultraconservative access cavities, which can lead to prolonged procedures, patient and dentist fatigue, and the risk of tooth discoloration due to residual material, compromising aesthetics (Marchesan et al., 2018).

In the same study (Marchesan et al., 2018), carbamide peroxide bleaching was applied to discolored maxillary central incisors with minimally invasive access cavities. However, the teeth did not regain their previous brightness. In contrast, teeth with traditional access cavities could restore their original brightness. This suggests that opening minimally invasive access cavities in the aesthetic zone may pose some aesthetic challenges.

1.3.11. Treatment Duration

Various factors, including canal morphology and the tooth's position, can influence the duration of endodontic treatment. The design of the access cavity also impacts treatment time, and several studies have focused on this.

Research has consistently shown that minimally invasive access cavities increase canal preparation time (Marchesan et al., 2018; Tüfenkçi et al., 2020; Silva et al., 2020). One study (Silva et al., 2020) noted that cleaning the pulp chamber is more challenging in teeth with ultraconservative access cavities, leading to a longer overall treatment time. Another study (Marchesan et al., 2018) found that minimally invasive access cavity preparation required 2.5 times more canal preparation than traditional cavities. A different study (Tüfenkçi et al., 2020) suggested that the increased treatment time is due to the direct access to the canals provided by traditional access cavities. In contrast, minimally invasive access cavities necessitate more pecking motions to reach the apical foramen, extending the overall treatment duration.

1.4. Decision-Making Criteria

The debate over reducing access cavity width in endodontic procedures remains unresolved, highlighting the need for further research to understand the advantages and disadvantages. Preserving as much tooth tissue as possible is particularly important for post-endodontic restoration, especially in younger patients who may be more susceptible to future caries or fractures. The choice between minimally invasive and traditional approaches should be guided by clinical conditions, which often require deviation from the ideal due to factors like extensive caries or damaged restorations.

When creating an access cavity, extensive caries or damaged restorations may necessitate deviations from the ideal design, limiting the number of cases suitable for minimally invasive access cavities. Criteria for deciding on the approach include minimal occlusal damage, preserved marginal ridges, and the long-term viability of the restoration. The caries-driven approach focuses on retaining non-carious tissue, which may not always provide sufficient access to the canals. In contrast, the restorative-driven approach partially retains existing restorations during access cavity creation. If the restoration is intended to be temporary, the size of the access cavity need not be restricted. However, for long-term restorations, a minimal cavity opening is preferable.

Consideration of canal anatomy is crucial, especially in challenging cases with complex morphologies. Unlocatable canals can negatively impact treatment outcomes, underscoring the importance of thoroughly examining pulp anatomy before beginning treatment. Additional factors such as patient mouth opening, tooth position, and ease of access also play a significant role in decision-making. In complex cases, a minimally invasive approach might complicate the procedure, making traditional access cavities a more practical choice.

Traditional access cavities are often more suitable for retreatment cases, where minimizing restorative material removal and addressing missed canals from the initial treatment are priorities. Advanced equipment, including an operating microscope and ultrasonic tips, can improve the outcomes of minimally invasive access cavities, making them a viable option for experienced dentists with the necessary tools and clinical expertise (Ballester et al., 2021).

CONCLUSION

This review has explored the concept of minimally invasive access cavities in endodontics. In conclusion, using minimally invasive access cavities offers potential benefits in preserving tooth structure and minimizing tissue loss. However, the decision between minimally invasive and traditional access cavities should be made on a case-by-case basis, considering factors such as clinical conditions, canal anatomy, ease of access, and retreatment. Dentists with adequate tools and clinical experience can successfully perform minimally invasive access cavities, while traditional access cavities may be more appropriate in specific situations.

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