Aims and Scope

Aurum Journal of Health Sciences (AJHS – A. J. Health Sci.) is an international open access platform for basic, applied, theoretical and clinical studies in health sciences. AJHS publishes double blind peer-reviewed research articles, short reports, case reports, invited reviews and letters to the editor. AJHS is published tri-annually both in printed and electronic version. AJHS is a multidisciplinary journal on health sciences and accepts manuscripts on dental, medical, health services and pharmaceutical studies. The manuscripts linking different disciplines of health sciences will be given a priority in the journal.

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Volume: 2 | No: 2
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Contents

Editorial – Covid-19 Outbreak IX
Gaye Hafez

Letter to Editor 71
Başak Bıyıkoğlu

Research Article

Our Clinical Experience in Upper Lumbar Hernia: Retrospective Evaluation of 47 Patients 73-81
Güray Bulut

Chemical Composition of Essential Oil From Aerial Parts of *Lactuca serriola* L. 83-90
Hüseyin Servi, Ahmet Doğan

Review

Bleaching of Nonvital Teeth: A Review 91-114
Soner Şişmanoğlu

An Overview of Vital Tooth Bleaching 115-139
Soner Şişmanoğlu

BCG Vaccine and New Tuberculosis Vaccines Against *Mycobacterium tuberculosis:* A review 141-153
Özgül Kısa

Instruction for Authors 155-160

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JOURNAL OF HEALTH SCIENCES
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Volume 2 No 2 | May 2020, IX

Editorial – Covid-19 Outbreak

An outbreak that started in Wuhan, China spread rapidly across all continents. Caused by SARS-CoV-2, the Coronavirus Disease 2019 (Covid-19) has become the most important issue in the scientific area due to its high rate of mortality. Competing with time, research centers, institutes, and universities around the world have set to work to develop new projects. As of May 2020, no vaccine has been currently licensed for SARS-CoV-2, but as the studies make progress rapidly, the number of articles on scientific platforms is increasing by each day.

There is an urgent need for treatment strategies to stop the epidemic. Since drug development studies take many years, drug repurposing studies are being conducted for the drugs used in previous SARS and MERS outbreaks. Besides the antiviral agents, many other candidates are also under investigation.

In months, we have understood that the pathology of the disease is complicated. Since there is no pharmacological treatment specific to the disease, being one of the old but effective methods, passive immunization has been approved by the FDA only for critically ill patients. It is well-established from a variety of studies that the human convalescent plasma is an option that had been used safely in previous H1N1, SARS, MERS, and Ebola outbreaks. Despite the lack of clarity on the neutralizing antibody titer of convalescent plasma and the protectiveness of the antibodies, it has been considered as an emergency use until a disease-specific treatment or vaccine is developed.

To share the up-to-date information about Covid-19, many scientific platforms opened all their published and pre-print articles to users for free. In Turkey, Middle East Technical University and TUBITAK have created a web portal where all the current data including clinical trials are accessible. Surmounting a crisis requires the engagement of all fields including healthcare professionals, authorities, patients, and researchers. Therefore, we need reliable research and comprehensive action plans.

Under all these extraordinary circumstances, we have recently published the new issue of our journal: Aurum Journal of Health Sciences. In this issue, we present two research articles and three reviews additional to a *letter to editor*. We would like to thank all the esteemed writers and reviewers contributing to the issue. Stay safe.

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JOURNAL OF HEALTH SCIENCES
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Volume 2 No 2 | May 2020, 71

Letter to Editor

Dear Editor,

In December 2020, when a pneumonia outbreak with an uncertain etiology started in Wuhan, China, the world was unaware of the global crisis becoming shortly. The pathogen was identified from throat swab samples obtained by the Chinese Centre of Disease Control and Prevention on January 7, 2020 and named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV- 2). Later on, the World Health Organization (WHO) named the disease as COVID-19 (referred to COrona VIrus Disease-2019). Most of the infected patients presented mild symptoms like fever, dry cough, sore throat, whereas some cases developed fatal complications such as severe pneumonia, pulmonary oedema and acute respiratory distress syndrome. The transmission routes of COVID-19 include direct transmissions such as sneeze, cough, and inhalation of droplets, and also contact transmission via contact with surfaces and then passed on with hands coming in contact with oral, eye, and nasal mucous membranes. COVID-19 can also be transmitted directly or indirectly by saliva, semen and by the fecal/oral route.

Considering the transmission routes and aerosol generating nature of most of the dental procedures, it is very clear that dentists are among the highest risk categories for transmission of the virus. Dental hospitals and practices carry high risk not only due to the fact typical treatment generates aerosols and airborne microorganisms can remain suspended in the air for an extended period, but also frequent and direct contact with blood, saliva and using of sharp instruments. In accordance with the instructions of Ministry of Health, dentists are providing treatment only for emergency cases. In this aspect, the role of dentists in the time of pandemic seems to be limited to prevention of spread, however, as a clinician and a researcher, I would like to emphasize other aspects of the issue. As dentists, we are very well- educated in order to prevent cross infection, however we cannot deny the growing body of evidence hour by hour regarding clinical symptoms, transmission routes and treatment protocols of Covid-19. It is our main responsibility to follow recent information and adapt our clinical practices. This adaptation consists not only prevention protocols but also possible modifications of the medication regimes prescribed in the treatment protocols, and, being able to evaluate the oral manifestations of the disease.

In spite of the fact that, with the great effort of many researchers from different parts of the world, the blurry image of Covid-19 gets clearer every single day, only limited numbers of manuscripts were published in dental journals. There is a need for research not only to set prevention protocols for dental settings, but also to enhance the knowledge of dentists about the oral manifestations of the infection. Although, peer review process can take time, our aim should be giving the priority to novel coronavirus related articles and provide recent and best data available for the readers.

As it is frequently said these days, we are all in this together…

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Research Article

Our Clinical Experience in Upper Lumbar Hernia: Retrospective Evaluation of 47 Patients

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Abstract: Clinical and radiological examination of upper level lumbar disc hernias (L1-2, L2-3, L3-4) and evaluation of surgical results. 47 patients with upper level lumbar disc hernia (ULLDH) among 282 lumbar disc hernias (LDH) performed in our clinic between April 2015 and April 2017. Age, physical examination, disc distances, radiological findings, preoperative and postoperative findings, complications, recurrence, patient satisfaction were evaluated retrospectively according to Prolo scale (via two 5-point Likert-type scales). Maximum resection principle was applied in the operations. All patients were operated with direct lumbar radiographs and lumbar magnetic resonance imaging (MRI). Lumbar computer tomography and electromyography (EMG) were performed and the diagnoses were supported when necessary. 23 (48.9%) of the cases were male and 24 (51.1%) were female. The average age was 49.9 (25-70). The average period between occurrence of symptoms and attendance to clinic is 3.7 months. The occurrence of L1-2: 3 (6.4%) patients, L2-3: 8 (17%) patients and L3-4: 32 (68.1%) in ULLDH cases (16.6%). Four patients (8.5%) with L2-3 and L3-4 were present. The first operation was not recurred during the 2-year follow-up in our clinic. Spondylodiscitis developed in 1 patient (2.1%) and was improved with medical treatment. In 1 patient (2.1%) preoperative dural injury primer was also repaired. In the early postoperative period, leg pain was disappeared in all cases. According to the Prolo follow-up scale, 31.9% were good and 65.96% were excellent. No bad results had been recorded. The incidence of ULLDH is increasing with the widespread use of MRI. ULLDH, with careful microsurgical technique and maximum disc resection, if operated, surgical success rate increases and complication rate decreases.

Keywords: *Upper; disc; hernia; lumbar; microdiscectomy*

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1. Introduction

Disc herniation is the most common cause of disabling back pain. The upper limits of the tight L4-5, L5-S1. Only 1-11% of disc herniations occur at L1-2, L2-3, L3-4 levels. Disc herniations at this level are called upper level lumbar disc herniations (Hsu et al, 1990; Nadler et al, 1998).

Clinical findings of ULLDH, computer-assisted discs are of particular importance (Nadler et al, 1998). The clinical and radiological findings of ULLDH were discussed in our study.

2. Materials and Methods

In this study, 47 upper-level lumbar disc herniations (16.67%) among 282 lumbar disc herniations operated between April 2015 and April 2017 were reviewed retrospectively.

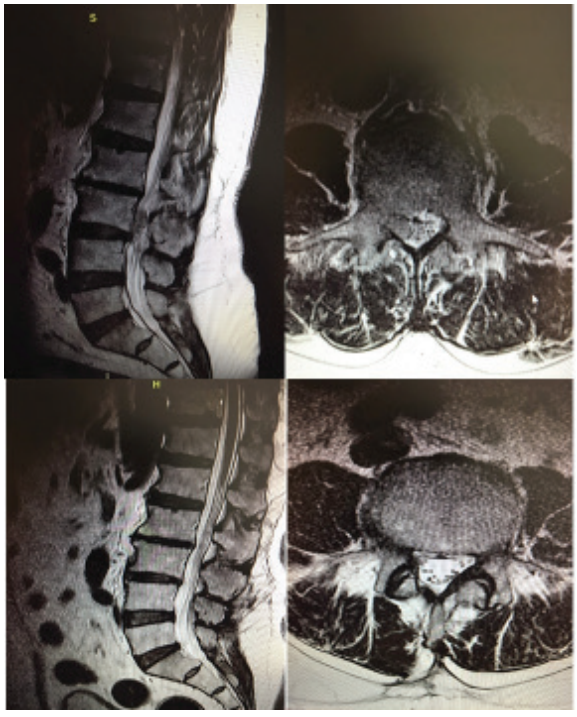
Cases were divided into 3 groups according to age; group 1, 10-29 years of age (4,25%), group 2, 30-49 years of age (48,9%), group 3, 50 years of age and above (46,8%) (Table 1). The cases were evaluated according to their complaints and durations at the time of application, the previous treatments, and the history of physical trauma.

Table 1. Distribution of patients by age ranges

	Number of patients	%
10-29 years	2	4.25
30-49 years	23	48.9
50 years and older	22	46.8

With preoperative neurological examination, muscle strength, loss of sensation and reflex loss were evaluated and femoral stretching test and Lasegue test were performed. In the preoperative radiological examination, lumbosacral radiographs, MRI (Figure 1) and computed tomography (CT) were used. EMG was performed was performed in some cases for neurophysiological examination. All patients underwent microdiscectomy with microsurgical technique. All patients underwent postoperative first-week, first-month, and third-month follow-up examinations. Prolo follow-up scale was used to determine patient satisfaction.

Figure 1. Preoperative and postoperative MRI images of L3-4 right disc herniation



3. Results

In our study, the number of cases with ULLDH was 47 and it accounted for 16.67% of all lumbar disc herniations. Of these, 23 (48.9%) were male and 24 (51.1%) were female (Table 2). Their ages ranged from 25 to 70 years, with a mean of 49.9 years. Twenty-four (51.1%) of the cases were active. We have no cases of acute trauma. There were 6 (12.8%) patients who were traumatized in previous times.

The period between onset of complaints and admission ranged from 7 days to 24 months (4.6 months mean). One patient presented with low back pain, 18 patients with back and right leg pain, 20 patients with waist and left leg pain, 5 patients with back and lower leg pain, and 2 patients with weakness (Table 3). All of the cases had received medical treatment before and 3 (6.4%) received physical therapy. In these cases, 68.1% were at L3-4, 17% at L2-3, 6.4% at L1-2 and 8.5% at two levels (L2-3, L3-4) (Table 4). As a result of neurological examination, 21.2% motor deficit, 63.8% sensory deficit, 53.1% reflex deficits were detected. Lasegue test was found 93.6% and femoral stretching test was positive as 85.1% (Table 5). Lumbosacral X-ray and Lumbosacral MRI were performed directly in all cases. EMG was performed in 5 (10.6%) cases. One of the cases was operated in a different clinic and re-operated at the same level. None of the cases were reoperated due to relapse. There was 1 patient in L1-2 and 3 patients in L3-4.

All patients received one dose of prophylactic antibiotic. The skin was brushed with antiseptic solutions for 5 minutes. Distance was determined by peroperative scopy. All patients were operated by microsurgical technique and maximum disc resection was planned using an operation microscope.

According to the Prolo follow-up scale, 31.9% and 65.96% were excellent results in the postoperative period (Table 6). In the postoperative early period, none of the patients had leg pain. In the late postoperative period, there was significant improvement in motor, sensory deficits and loss of reflex.

Table 2. Sex

Sex	%	Number of patients
Male	48.9	23
Female	51.1	24

Table 3. Symptoms

	%	Number of patients
Low back pain	2.13	1
Low back and right leg pain	38.3	18
Low back and left leg pain	42.55	20
Low back and pain in both legs	10.64	5
Weakness	4.26	2

Table 4. Levels of discectomy performed

	%	Number of Patients
L1-2	6.4	3
L2-3	17	8
L3-4	68.1	32
Two level	8	4

Table 5. Physical examination findings of patients before surgery

	%	Number of patients
Neurological deficit	21.2	10
Reflex changes	53.1	25
Sensory changes	63.8	30
Lasegue test	93.6	44
Femoral tensile test	85.1	40

Table 6. Clinical results according to Prolo follow-up criteria

	%	Number of patients
Excellent	65.96	31
Good	31.90	15
Intermediate	2.13	1
Bad	-	-

4. Discussion

The incidence of ULLDH has been reported as 3.1% to 10.4% (Albert et al, 1993; Demirbas, 2000; Gutterman and Shenkin, 1982). While the frequency of ULLDH was reported as 5% in the series, Albert et al. reported this rate as 10.4% (Albert et al, 1993). In our series, the incidence of ULLDH was found to be 16.6%, which is higher than in the literature. We can explain this especially with routine use and spread of lumbar MRI.

The majority of the cases with ULLDH are male (Eugene and Kim, 1997; Kotilainen et al, 1993; Rastecchini, 1991; Yasuma et al, 1993). In our series, in contrast to the general literature, it is slightly higher in women (51.1%) than men.

ULLDHs are often seen in the 3rd, 4th and 5th decades as disc hernias at the other level. It is thought that is because of being more active in these age groups (Eugene and Kim, 1997; Rastecchini, 1991; Yasuma et al, 1993). In our series, the most common was in the 31-50 years of age group (48.9%). This result is in parallel with the literature. Approximately half of our cases (48,9%) were in this age group.

Trauma is an important predisposing factor in the formation of lumbar disc herniation. The incidence of trauma cases in large series has been reported as 50-70% (Demirbas, 2000). In our series, there were no cases of acute trauma and 12.8% of cases had previously been traumatized. In the Ken Hsu study, the rate of LDH in active workers was 67% (Hsu et al, 1990). In our series, this ratio is 51.1%.

It has been stated that the symptoms are very variable in ULLDH (Albert et al, 1993; Kotilainen et al, 1993). Therefore, there are some difficulties in early diagnosis of ULLDH. Gutterman reported that the rate of pain spreading to the anterior aspect of the leg was 59% (Gutterman and Shenkin, 1982). In our series, this rate is 80.85%. This rate is higher than Gutterman's series. It is necessary and useful to question the pain radiating to the front of the leg, as it is particularly distinctive.

Cauda-equina syndrome is the most important and urgent surgical procedure in lumbar disc hernias. While it is expected to be seen in ULLDH due to anatomical neighborhood, it is reported that it is mostly seen in L4-5 level (Hsu et al, 1990; Kostuik et al,1986; Shapiro, 1993). However, lumbar disc herniation is seen more frequently at L4-5 level (Demirbas, 2000). In our series, there were no patients presenting with Cauda-equina syndrome. We believe that early diagnosis and extensive use of MRI are effective.

The tests used in the first plan for neurological examination are the Lasegue test and the femoral stretching test. The flat leg lifting test is more positive in the upper lumbar disc hernias because it shows the irritation of the roots at the lower lumbar level. The femoral stretching test is more frequent in ULLDH (Jung and Drovak, 1995). Lumbar disc herniations were found to be positive in 85-90% of the patients. The positivity of the Khe Shu femoral stretching test was reported to be 70%. In our series, this rate is higher than the literature (85.1%). Femoral tensile test positivity is not a pathognomonic finding but a useful test in diagnosis (Estridge et al, 1982).

Reflex examination is an objective criterion for LDH examination. The effect of psychosocial status of the patients is not expected in reflex examination. Dysfunction of the patellar reflex is also expected in ULLDH. Kotilainen found the patella reflex to be 70% hypoactive or areflexia (Kotilainen et al, 1993). In our series, reflex deficits rate was found to be 53.2%. 25 cases and 60% (15 cases) of these cases were patella reflex deficits. This rate was lower than in the literature. The reason for this was the early presentation of the cases and the widespread use of MRI.

Motor dysfunction in ULLDHs is higher than lower LDHs (Paszlar and Szervas, 1981). This suggests that a dynamic and rapid approach is needed in diagnosis and treatment of ULLDH patients. In our series, motor deficits rate was found to be 21.2%. The sensory deficit was more subjective than reflex and motor deficits and was reported as high rates in the literature (Hsu et al, 1990; Paszlar and Szervas, 1981). In our study, sensory deficit was found to be 63.8%. It was thought that the low incidence of our values according to the literature could be the early application of the cases and the widespread use of MRI.

While the incidence of ULLDH was reported as 1% in the pre-MRI period, these rates increased to 10% with the routine use of MRI (Hsu et al, 1990). Because of the superiority of MRI in the correct diagnosis, we think that it is the first advanced examination to be requested after a good neurological examination. If necessary, this should be supported by CT and EMG.

Williams argued that only free disc fragments were sufficient to avoid damaging the healthy disc. Reported a recurrence rate of 9% in the series (Williams, 1977). Rogers also reported a recurrence rate of only 11% in cases of disc fragmentation (Rogers, 1988). Yaşargil and Caspar argued on the maximization of the disc resection (Caspar et al, 1991; Conrad et al, 1992; Yaşargil, 1977). The recurrence rate is 4%. Maximum resection was performed in our series and no recurrence was observed during follow-up. Based on these results we highly recommend the maximum disc resection.

Prolo follow-up scale is widely used in the evaluation of postoperative recovery of the patients (Demirbas, 2000; Prolo et al, 1986). Good results ranged from 74-93% in various series and no difference was found between levels in ULLDH (Demirbas, 2000). In our series, 31.9% good and 65.96% excellent results were obtained in postoperative controls (Table 5). Improvements in motor and reflex deficits, especially in various publications, can be extended up to 1 year (Gutterman and Shenkin, 1982; Kostuik et al,1986; Paszlar and Szervas, 1981). In our study, there was no significant distribution of the cases according to the levels.

Complications of dural injury, infection, root injury and epidural fibrosis are the most common complications of ULLDH (Carlson, 1991; Caspar et al, 1991; Gutterman and Shenkin, 1982; Yasargil, 1977). Dura injury and

wound infection are observed below 5% (Gutterman and Shenkin, 1982). In our series, spondylodiscitis was seen in 1 patient (2.1%). After medical treatment, the patient recovered. One patient had dura mater injury (2.1%). It was repaired preoperatively. These results were evaluated as better than the literature. Minimal surgical trauma, excessive laminectomy and avoidance of facetectomy, providing good hemostasis without cauterizing the epidural veins as much as possible, protecting the epidural adipose tissue, exercise in the postoperative period and applying the recommendations made are important (Carlson, 1991; Caspar et al, 1991; Gutterman and Shenkin, 1982; Yasargil, 1977).

Conclusion

The incidence of ULLDHs increases with the widespread use of MRI. Because of the superiority of the MRI in the correct diagnosis, we think that it is the first advanced examination to be requested after a good neurological examination. If necessary, it should be combined with CT and supported by EMG.

Contrary to the literature, the rate of women in our series was higher than men. In our series, ULLDHs are more common in the 3rd and 5th decades than in the literature. The history of trauma and active work were not seen as important factors in the patients in our series. Leg pain, especially pain and weakness on the front of the thighs are the most common symptoms of ULLDH. As reflex deficits, we most commonly observed a decrease or absence of patellar reflex. Although Cauda-equina syndrome is not present in our series, it is a very serious neurological condition and requires urgent treatment. After successful surgery, leg pain needs to be relieved in the early postoperative period. Due to the lack of recurrent disc herniation in our series, we recommend maximum disc resection in the operation. Again, we believe that low complication rates in our cases are due to compliance with microsurgical principles.

Ethical Rules

All procedures performed in studies involving human participants were in accordance with the ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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Conflict of Interest

The author declares no conflict of interest.

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Research Article

Chemical Composition of Essential Oil From Aerial Parts of *Lactuca serriola* L.

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Abstract: The volatile oil of the aerial parts of *Lactuca serriola* L. was obtained by the hydro-distillation method for 3 hours with the Clevenger-type apparatus. The chemical composition of oil was determined by GC-MS analyses. Forty-three constituents were identified in oil (84.3%). Heneicosane (8.4%), (*E*)-β-ionone (6.5%), hexadecanoic acid (6.4%), hexahydrofarnesyl acetone (6.3%), tricosane (5.5%), heptacosane (5.5%), phytol (5.0%) and pentacosane (4.1%) were determined as main compounds in the oil. The oil has saturated *n*-alkane derivatives as a dominant group. To the best of our knowledge, this is the first report on the chemical composition of volatile of *L. serriola* from Turkey.

Keywords: *Lactuca serriola; essential oils; n-alkane derivatives*

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1. Introduction

The genus *Lactuca* L. is annual, biennial, and perennial herbs and members of the Lactuceae tribe of the Asteraceae family. The genus has 113 species and is represented by 8 species in Turkey. *Lactuca serriola* L. (Prickly lettuce) is a biennial plant that grows grassy and rocky slopes, field margin, fallow and cultivated fields throughout Turkey (Davis, 1975). *L. serriola* is called as 'Yabani marul' or 'Eşek helvası' in Turkey. Prickly lettuce has been used as a traditional medicine in Turkey for a long time. For example, the decoction of the plant is used to treatment of liver ailments and stomach pain; the infusion of the plant is used to lowering cholesterol and against hemorrhoids; it is used as a sedative if the leaves are eaten raw (Tuzlacı, 2016). Also, *L. serriola* leaves are consumed as a fresh salad in Turkey (Dogan et al., 2004). The plant has milky latex, which contains 'lactucarium'. Lactucone, lactucin and lactucic acids are found in lactucarium. The lactucarium concentration is low in young plants and is high in flowering period. The lactucarium is used internally as a traditional medicine in the treatment of insomnia, anxiety, neuroses,

hyperactivity in children, dry coughs, whooping cough, rheumatic pain. Also, this milky latex is used as medicine due to anodyne, antispasmodic, digestive, diuretic, hypnotic, narcotic and sedative properties (Elsharkawy and Alshathly, 2013). *L. serriola* had sedative-hypnotic, antipyretic, antibacterial, analgesic, anti-inflammatory, antioxidant, anticancer and smooth muscle activities due to sesquiterpene lactones (e.g. lactucin, lactucone), triterpenoid saponin, phenols, vitamins, beta carotene, iron, flavonoids, and sesquiterpene esters (Balogun et al., 2017; Mojab et al., 2010). Balogun et al. (2017) reported that the aqueous and methanol extracts from the leaf of *L. serriola* had antipseudomonal activity (Balogun et al., 2017). Another study, it was found that *L. serriola* methanol extract possessed spasmogenic, spasmolytic, a bronchodilator, and vasorelaxant activities (Janbaz et al., 2013). The antioxidant and allelopathic activities of essential oil of *L. serriola* were previously studied. The main compounds of oil were isoshyobunone (64.2%), isocembrol (17.3%), and alloaromadendrene oxide-1 (7.3%). The oil showed strong antioxidant and allelopathic activities (Abd-ElGawad et al., 2019). Additionally, the anti-inflammatory activity of *L. serriola* essential oil was investigated. Sesquisabinene hydrate (15.1%), thunbergol (8.9%) and globulol (6.5%) were determined as the main compounds in the oil. The oil displayed good anti-inflammatory activity (Elsharkawy et al., 2014). The essential oil composition and anticancer activity of hexane and methanol extracts of aerial parts of *L. serriola* were studied. The main compounds of oil were α-pinene, limonene, germacrene D, *trans*-β-caryophyllene, caryophyllene oxide, and santolina triene. The cytotoxic activity of hexane and methanol extracts was evaluated against A549, HCT116, HepG2, and MCF7 cell lines. The methanol extract had strong activity against HepG2 and MCF7 cell lines. Also, lupeol, lupeol acetate, germincol, α-amyrin, β-amyrin, oleanane, and germanicen were isolated from methanol extract (Elsharkawy and Alshathly, 2013).

According to our literature survey, the essential oil composition of *L. serriola* showed differences rely on geographical regions. There is no report on the volatile oil composition of *L. serriola* in Turkey. The first purpose of this research was to obtain essential oil from aerial parts of *L. serriola*, and the second purpose was to determine the diversity in the essential oil composition of *L. serriola* and to show that essential oil differences are related to geographical regions.

2. Materials and Methods

2.1. *Plant Materials*

The aerial parts of *L. serriola* were collected in İkitelli-Başakşehir, Istanbul, Turkey on 23 July 2017 by Hüseyin Servi Ph.D. The plant was identified by Ahmet Doğan Ph.D. A herbarium specimen was deposited in the Marmara University Herbarium (Voucher no: MARE22155).

2.2. *Volatile Oil Analyses*

The volatile oil of the aerial part (290 g) of *L. serriola* was obtained by the Clevenger apparatus (3 h) with the hydrodistillation method. The oil was kept with *n*-hexane (1 mL).

2.3. *Gas Chromatography-Mass Spectrometry Analysis*

The oil ingredients were determined by GC-MS using an Agilent 5977 MSD system and operated in EI mode. The volatile oil was injected (1 µL) in splitless mode. MS transfer apparatus and injector temperatures were set at 250°C. In GC-MS analyses, the capillary column type was Innowax FSC (60 m x 0.25 mm, 0.25 µm film thickness) and the carrier gas was helium with a flow rate of 1 mL/min. The oven temperature was arranged to 60°C for 10 minutes and increased to 220°C at 4°C/min, where the temperature kept stable for 10 minutes. Then, the temperature was increased to 240°C at 1°C/min. The conditions of the mass spectra were as following; it was saved at 70 eV. Then, in MS chromatograms, the relative percentages of the compounds that separated from the integration of the peaks were calculated.

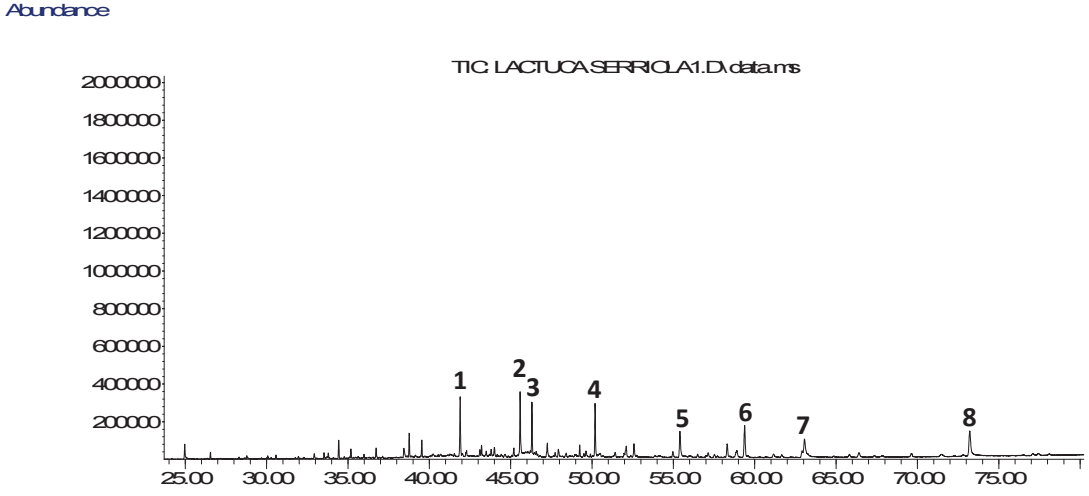
2.4. *Identification of Volatile Oil Components*

The constituents were determined by comparison with GC-MS libraries (Wiley 8th Ed. and NIST 05) and their relative retention indices (RRI) obtained by *n*-alkanes series to the literature.

3. Results and Discussion

The volatile oil yield of *L. serriola* was 0.03% (v/w). Forty-three constituents were identified in oil (84.3%). Heneicosane (8.4%), (*E*)-β-ionone (6.5%), hexadecanoic acid (6.4%), hexahydrofarnesyl acetone (6.3%), tricosane (5.5%), heptacosane (5.5%), phytol (5.0%) and pentacosane (4.1%) were determined as main compounds in the oil. The oil has saturated *n*-alkane derivatives as a dominant group. Other major groups were fatty acid and esters (13.6%) and sesquiterpenoid (9.9%).

Figure 1. GC-MS Chromatogram of *Lactuca serriola* volatile oil



1: (*E*)-β-ionone; **2:** Heneicosane; **3:** Hexahydrofarnesyl acetone; **4:** Tricosane; **5:** Pentacosane; **6:** Phytol; **7:** Heptacosane; **8:** Hexadecanoic acid.

Table 1. The chemical composition of the volatile oil of *Lactuca serriola*

No	RT[1]	RRI[2]	RRI Lit.[3]	Compound	I[4] (%)
1	24.971	1396	1400	Nonanal	1.7
2	28.788	1502	1506	Decanal	0.4
3	30.075	1541	1547	(*E*)-2-Nonenal	0.3
4	30.578	1556	1562	1-Octanol	0.4
5	32.930	1630	1638	β-Cyclocitral	0.5
6	33.532	1650	1655	(*E*)-2-Decanal	0.6
7	33.791	1659	1665	1-Nonanol	0.8
8	34.434	1680	1687	Estragole	1.6
9	35.184	1705	1705	γ-Himachalene	1.1
10	35.988	1733	1737	β-Bisabolene	0.4
11	36.731	1759	1765	(*E*)-2-Undecanal	1.1
12	38.443	1820	1827	(*E,E*)-2,4-Decadienal	1.4
13	38.765	1832	1835	β-Damascenone	2.0
14	39.540	1861	1868	*Trans*-geranyl acetone	1.6
15	**41.897**	**1951**	**1958**	**(E)-β-ionone**	**6.5**
16	42.288	1966	1973	Dodecanol	0.8
17	44.001	2035	2041	Pentadecanal	1.3
18	45.197	2084	2100	Zingiberonol	1.6
19	**45.583**	**2099**	**2100**	**Heneicosan**e	**8.4**
20	**46.310**	**2130**	**2131**	**Hexahydrofarnesyl aceton**e	**6.3**
21	47.735	2191	2179	1-Tetradecanol	0.9
22	47.935	2200	2200	Docosane	1.3
23	48.415	2221	2226	Hexadecanoic acid methyl ester	0.6
24	49.245	2258	2262	Hexadecanoic acid ethyl ester	1.1
25	49.631	2275	2296	Decanoic acid	1.0
26	49.904	2287	2299	Isophytol	0.3
27	**50.189**	**2300**	**2300**	**Tricosan**e	**5.5**
28	50.505	2313	2315	2,4-bis(tert-butyl)-phenol	1.0
29	51.874	2371	2380	Hexylcinnamic aldehyde	0.2
30	52.103	2380	2384	Farnesyl acetone	2.0
31	52.580	2400	2400	Tetracosane	1.6
32	54.972	2486	2492	Dodecanoic acid	1.0
33	**55.405**	**2501**	**2500**	**Pentacosan**e	**4.1**

34	56.494	2532	2538	Linoleic acid ethyl ester	0.6
35	57.142	2551	2592	Diisobutyl phthalate	0.9
36	57.526	2562	2582	Eicosanal	0.5
37	58.308	2585	2594	9-Hexacosene	1.9
38	58.901	2602	2613	Ethyl linolenate	1.8
39	**59.382**	**2614**	**2622**	**Phytol**	**5.0**
40	62.915	2698	2713	Tetradecanoic acid	1.1
41	**63.061**	**2701**	**2700**	**Heptacosan**e	**5.5**
42	66.408	2773	2783	1-Docosanol	1.2
43	**73.216**	**2910**	**2931**	**Hexadecanoic aci**d	**6.4**
				n-alkane derivatives	39.7
				Fatty acid and esters	13.6
				Sesquiterpenoid	9.9
				Diterpene	5.3
				Monoterpenoid	4.1
				Sesquiterpene	1.5
				Others	10.2
				Total	**84.3**

[1]RT: Retention time; [2]RRI: Relative retention time; [3]RRI Lit.: Relative retention time in the literature; [4]The analysis results.

According to a study from Saudi Arabia, *L. serriola* essential oil was reported to contain sesquisabinene hydrate, thunbergol and globulol as main compounds (Elsharkawy et al., 2014). Isoshyobunone, isocembrol, and alloaromadendrene oxide were detected in higher quantity in the essential oil of *L. serriola* from Egypt (Abd-ElGawad et al., 2019). Another study from Saudi Arabia, α-pinene, limonene, germacrene D, *trans*-β-caryophyllene, caryophyllene oxide, and santolina triene were found as main compounds in the essential oil of leaves of *L. serriola* (Elsharkawy and Alshathly, 2013). The previous reports indicated that *L. serriola* had sesquiterpenoid, diterpene and oxygenated monoterpene as major groups. In the current study, the aerial part essential oil of *L. serriola* had *n*-alkane derivatives, fatty acid, and esters as dominant groups and showed a dissimilar chemical profile from the previous studies. Sesquiterpenoids were a common major group in present study, similar to previous studies. In the present study, hexahydrofarnesyl acetone was found as the main compound in the sesquiterpenoid group. Also, this compound was detected in the Egypt sample (1.77%) (Abd-ElGawad et al., 2019). However, there is quantitative dissimilarity in the main compound of the sesquiterpenoid group of volatile oil from Turkey and Egypt samples. In the current study, phytol was found as the main compound in the diterpene group. But this compound was not determined in previous studies. The difference may be correlated with the geographical region, collection time and specific climate conditions.

Conclusion

The chemical composition of the essential oil of *L. serriola* from Turkey was determined. The current research revealed that *L. serriola* oil was rich in *n*-alkane derivatives and showed variations in the main compounds due to geographical regions compared to previous studies.

Conflict of Interests

The authors declare no conflict of interest.

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Review

Bleaching of Nonvital Teeth: A Review

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Abstract: The teeth frequently become discolored in time due to the endodontic treatment residues in the pulp chamber or hemolytic products accumulated in the dentine tubules after trauma. This condition may cause psychosocial problems for patients. Nonvital bleaching has gained popularity due to its conservative nature and low cost to overcome this unpleasant condition. This article will give an overview of nonvital bleaching techniques, materials and regimens used, bleaching procedure and side-effects.

Keywords: *Discoloration, trauma, bleaching, whitening, nonvital bleaching*

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1. Introduction

Nowadays, people often refer to dentists to have neatly arranged, light-colored, natural-looking teeth. It is even possible to say that aesthetical expectations almost surpass functional needs. However, the dental profession should maintain high ethical standards and do not recommend cosmetic adjustments to suit the patient's demands.

Central and lateral incisors are the most affected teeth as a result of trauma, 69% and 20% respectively (Abbott, 1997). After dental trauma, the teeth may become discolored in time due to the endodontic treatment residues in the pulp chamber or hemolytic products accumulated in the dentine tubules (Abbott and Heah, 2009). The discoloration in the anterior region is a cosmetic problem that needs to be treated since the discolorations in the nonvital teeth are easily distinguishable. Discoloration in the nonvital teeth is usually asymmetrical and concerns a single tooth; however, it can rarely involve several teeth due to trauma. Endodontic materials in the pulp chamber (especially silver-containing pastes) and necrotic byproducts are the main causes of the discoloration. Inadequate irrigation after pulp extirpation is the most frequent iatrogenic cause. Although the exact mechanism of the discolorations caused by pulp

degeneration is not very clear, it is thought to be caused by hemolytic products penetrating to dentin. These products are hemosiderin, hemin, and hematoporphyrin which release iron as the colorant. These substances extending to the dentin tubules can combine with bacterial byproducts and lead to yellow-brown discolorations (Eisenberg, 1975).

Although the discoloration can be treated with restorative treatments, it can also be successfully treated with bleaching. Bleaching is a more conservative option than other restorative options and can be applied easily and safely (Kihn, 2007). In addition, bleaching is cheaper than complicated restorative treatments. Although the mechanism of bleaching is not fully understood (Sulieman, 2004), it is widely accepted that the bleaching agent penetrates hard tissue to oxidize chromophores and reduce discoloration (Joiner, 2004). The success of the nonvital teeth bleaching mainly depends on the etiology of the discoloration, the correct diagnosis of the problem, and the selection of proper bleaching techniques.

Bleaching of discolored, pulpless (nonvital) teeth were first proposed by Truman in 1864 (Truman, 1864). Various agents, such as chloride, sodium hypochlorite, sodium perborate, and hydrogen peroxide have been applied alone or in combination (Howell, 1980). Heat is also often used for the activation of the bleaching agent (Dahl and Pallesen, 2003). In 1961, walking bleach technique was introduced based on keeping sodium perborate and water mixture in the pulp chamber between patient's appointments (Spasser, 1961). This technique was modified by mixing sodium perborate with 30-35% liquid hydrogen peroxide instead of water to increase the bleaching efficiency (Nutting and Poe, 1963). In the 1960s, 10% carbamide peroxide, which its bleaching effect was noticed coincidentally, gained popularity with the nightguard vital bleaching technique in 1989 and started to be used in nonvital bleaching techniques (Haywood and Heymann, 1989).

Today's bleaching systems mainly based on the use of hydrogen peroxide, sodium perborate, or carbamide peroxide with an activation such as heat or light. They can be applied externally or internally to the discolored nonvital tooth to oxidize chromophores in the dentin (Sulieman, 2008).

2. Discolorations

The appearance of the teeth differs according to the light reflecting and absorbing properties of dental tissues consisting of enamel, dentin, and pulp. Although the natural tooth shade is similar to dentin, the transparency and thickness of the enamel are also effective in the appearance (Greenwall, 2001). Dental discolorations are classified as extrinsic, intrinsic and internalized staining (stain internalization). Extrinsic discolorations occur when external chromogens settle on the tooth surface or in the pellicle. Intrinsic discolorations occur either locally due to the chromogens presenting inside the dentin or systematically. It is stated that internalized discolorations begin externally and they spread internally through the defects found in enamel (Sulieman, 2008). The discoloration of nonvital teeth often involves dentin and is of intrinsic origin.

3. The Etiology of Intrinsic Discolorations

The causes of internal discoloration are genetic disorders, drug administration (especially tetracycline), fluorosis, childhood diseases with high fever, dental traumas, iatrogenesis and intracanal medicaments used for endodontic treatment (Plotino et al., 2008) (Table 1). After root canal treatment, the teeth may become discolored due to endodontic materials, pulp remnants, hemorrhage during root canal treatment (Watts and Addy, 2001). The treatment of intrinsic discolorations is more complex than that of extrinsic discolorations. Although the discolored tooth regains its natural color with intracoronal bleaching, the prognosis of the bleaching varies depending on the endodontic sealer type and duration after the endodontic treatment (van der Burgt and Plasschaert, 1986). For instance, stains made by metallic ions are difficult to bleach. Therefore, all residues inside the pulp chamber should be removed with burs, ultrasonic scalers, or air-abrasion before starting intracoronal bleaching procedure. The reasons for intrinsic discoloration with the indication for nonvital bleaching are as follows:

3.1. *Pulp Necrosis*

Intrinsic discolorations due to pulp necrosis are caused by the accumulation of hemorrhagic products in dentine tubules (Grossman et al., 1995; Ho and Goerig, 1989). Pulp inflammations due to bacterial, mechanical or chemical irritations can result in necrosis. Necrotic products penetrating from the pulp complex into the dentin tubules as a result of necrotic processes cause discoloration in the dentin (Attin et al., 2003). The nature of the discoloration depends on the time elapsed after pulp necrosis; i.e. the longer the chromogenic components remain in the pulp chamber, the greater the intensity of discoloration. Such discolorations are generally bleached intracoronally (Rostein, 2002).

Table 1. The etiology of the intrinsic discolorations (Plotino et al., 2008)

Pre-eruptive factors	Post-eruptive factors
• Dental trauma	• Aging
• Genetics (hyperbilirubinemia, amelogenesis imperfecta, cystic fibrosis of the pancreas)	• Endodontic materials, medications, sealers
• Medications (Tetracycline)	• Intrapulpal hemorrhage
• Metabolism (Fluorosis)	• Pulpal necrosis
	• Pulp tissue remnants after endodontic treatment
	• Restorative materials
	• Root resorptions

3.2. *Intrapulpal Hemorrhage*

Pulp extirpation or severe dental trauma can induce hemorrhage by damaging the blood vessels in the pulp tissue. Chromogenic components in the blood also penetrate the dentine tubules and cause discoloration (Arens, 1989; Goldstein and Garber, 1995). Initially observed pink discoloration becomes darker and affects the whole tooth with the hemolysis of the blood cells (Guldener and Langeland, 1993; Watts and Addy, 2001). If pulp necrosis does not occur after trauma, it is reported that this pink discoloration may disappear within a few months due to the revascularization (Andreasen, 1986; Watts and Addy, 2001).

3.3. *Pulp Tissue Remnants*

Discoloration that occurs after endodontic treatment can be seen as a result of excessive bleeding during pulp extirpation or incomplete removal of the pulp tissue. Pulp tissue remnants may remain in the pulp chamber when the endodontic access cavity is insufficiently prepared (Brown, 1965; Faunce, 1983). The discoloration mechanism of these remnants is similar to pulp hemorrhages. With intracoronal whitening, the tooth can be successfully restored to its original shade, but it would be more accurate to perform more careful endodontic treatment at the beginning and to leave no pulp remnants.

3.4. *Endodontic Materials*

Dental discoloration caused by endodontic materials is a common problem for both clinicians and patients and may impair the aesthetic appearance of endodontically treated teeth (van der Burgt et al., 1986a; van der Burgt et al. 1986b,). Endodontic filler, sealer or medication residues left in the pulp chamber cause discoloration (van der Burgt and Plasschaert, 1985; van der Burgt and Plasschaert, 1986; Kim et al., 2000). The discoloration caused by these material residues depends on contact time with the dentin. Therefore, although there seems to be no problem at the beginning, the shade of the tooth darkens over time (Vogel, 1975). To avoid this problem, root canal filling should be completed at the proximal bone level.

Endodontic drugs containing barium, iodine or silver, gutta-percha, and root canal sealers may also cause intrinsic discoloration (Bizhang et al., 2003; Grossman et al., 1995). Discolorations caused by endodontic drugs or root canal pastes may be seen in orange-red, dark red, gray or black colored (Bizhang et al., 2003). Solutions containing phenol, cresatin, and penicillin, streptomycin or chloramphenicol cause slight discoloration in the dentin. The most severe discoloration is caused by N_2 pastes and polyantibiotic pastes containing Terramycin® and tetracycline (particularly Declomycin®) (Gutiérrez and Guzmán, 1968).

The first developed mineral trioxide aggregate (MTA) was gray colored. It is well-known that the gray MTA causes tooth discoloration. The discoloration is seen in 60% of pulpotomy treatments using gray MTA (Hegde and Naik, 2005; Maroto et al., 2006). Therefore, white MTA was developed and white MTA did not show a significant difference in pulp response compared to gray MTA (Holland et al., 2001). The

major difference in chemical composition between white MTA and gray MTA is the concentration of metal oxides such as aluminium-oxide, magnesium-oxide, and iron-oxide, which are considered to be the main causes of discoloration. Nevertheless, tooth discoloration has been reported after the use of white MTA for the treatment of the vital pulp (Belobrov and Parashos, 2011; Boutsioukis et al., 2008). However, this discoloration occurs in the material itself, not in dentin. Therefore, a significant improvement was achieved in dentin shade after the removal of MTA (Belobrov and Parashos, 2011).

4. Bleaching Agents

In dentistry, hydrogen peroxide and its derivatives are preferred as bleaching agents (Goldstein and Garber, 1995). Hydrogen peroxide can be used directly for intracoronal bleaching, as well as materials such as carbamide peroxide, sodium perborate, which disintegrates into hydrogen peroxide in different ratios as a result of chemical degradation. These bleaching agents can be used separately or in combination (Attin et al., 2003). For instance, sodium perborate can be used by mixing with distilled water or by mixing with liquid hydrogen peroxide to increase the bleaching outcome.

4.1. *Hydrogen Peroxide*

Most of the bleaching agents contain hydrogen peroxide as an active ingredient. Hydrogen peroxide plays a role as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules, and hydrogen peroxide anions. These reactive molecules react with long-chained dark chromophores to separate them into smaller, less-colored molecules (Dahl and Pallesen, 2003). Hydrogen peroxide may be administered directly or produced by a chemical reaction from sodium perborate or carbamide peroxide.

4.2. *Sodium Perborate*

Another commonly used bleaching agent is sodium perborate. Sodium perborate is stable when it is dry. However, in the presence of an acid, hot air, or moisture the sodium perborate disintegrates into hydrogen peroxide and free oxygen (Rotstein and Friedman, 1991). Sodium perborate is produced by the reaction of disodium tetraborate pentahydrate, hydrogen peroxide, and sodium hydroxide. Sodium perborate monohydrate, trihydrate, and tetrahydrate forms are present, and the amount of oxygen released depends on its form (Ari and Üngör, 2002). The monohydrate form breaks down better than tetrahydrate and has higher temperature stability (Schubert and Brotherton, 2011).

4.3. *Carbamide Peroxide*

Carbamide peroxide produces hydrogen peroxide and urea which decomposes into carbon dioxide and ammonia (Dahl and Pallesen, 2003). The activity of carbamide peroxide in vital and nonvital teeth varies according to its concentration (Lim, 2004). Carbamide peroxide gel at a concentration of 10% is often used in home bleaching for 4 to 8 hours a day for 2 weeks or more (Sulieman, 2008).

5. Mechanism of Bleaching

The mechanism of tooth bleaching is not fully understood (Sulieman, 2004). It is widely accepted that peroxide penetrates hard tissue and free radicals oxidize to organic chromophores and reduce discoloration (Joiner, 2004).

Bleaching is also known as an oxidation-reduction reaction. According to the chemical theory explaining the bleaching reaction of hydrogen peroxide, peroxides are converted to unstable free radicals. The free radicals formed as a result of the decomposition of hydrogen peroxide (Fasanaro, 1992; Plotino et al., 2008) diffuse into the interprismatic region of the enamel and carry the small molecules that they break down from large organic molecules (chromophores) out to the surface with its foaming properties. These free radicals react with chromophores which cause discoloration in enamel and resulted in simple molecules that reflect less light (Chng et al., 2005; Joiner, 2004; McEvoy, 1989). As the bleaching process is continued, only the hydrophilic colorless structures remain, which is called the saturation point. Bleaching slows down at this point. If the bleaching is continued, the carbon-containing materials and the carbon bonds of the proteins are destroyed. Hydroxyl groups begin to divide and the substrate is divided into much smaller pieces. The remaining substrate rapidly converts into carbon dioxide and water, accelerating the enamel loss (Vilhena et al., 2019).

Benetti et al., (2004) reported that the concentration of peroxide increased during the bleaching time. Factors such as light and heat facilitate this reaction and accelerate bleaching (Dostalova et al., 2004; Ziemba et al., 2005).

Carbamide peroxide can be used in different concentrations. Tooth whitening mechanism with carbamide peroxide differs from hydrogen peroxide (Bulut et al., 2006). First, the carbamide peroxide disintegrates into hydrogen peroxide and urea. 10% carbamide peroxide disintegrates into 6.6% urea and 3.4% hydrogen peroxide. Then urea is broken down into carbon dioxide and ammonia (Christensen, 2003).

6. Nonvital Tooth Bleaching Techniques

Dental radiographs of the tooth should be recorded to assess the quality of root canal obturation and apical tissues before any bleaching procedure. If there is a failure or problem in the canal treatment, retreatment should be done before bleaching. Nonvital bleaching techniques include walking bleach, modified walking bleach, nonvital power bleaching (also known as heat- or light-activated bleaching), and inside/outside bleaching.

6.1. *Walking Bleach*

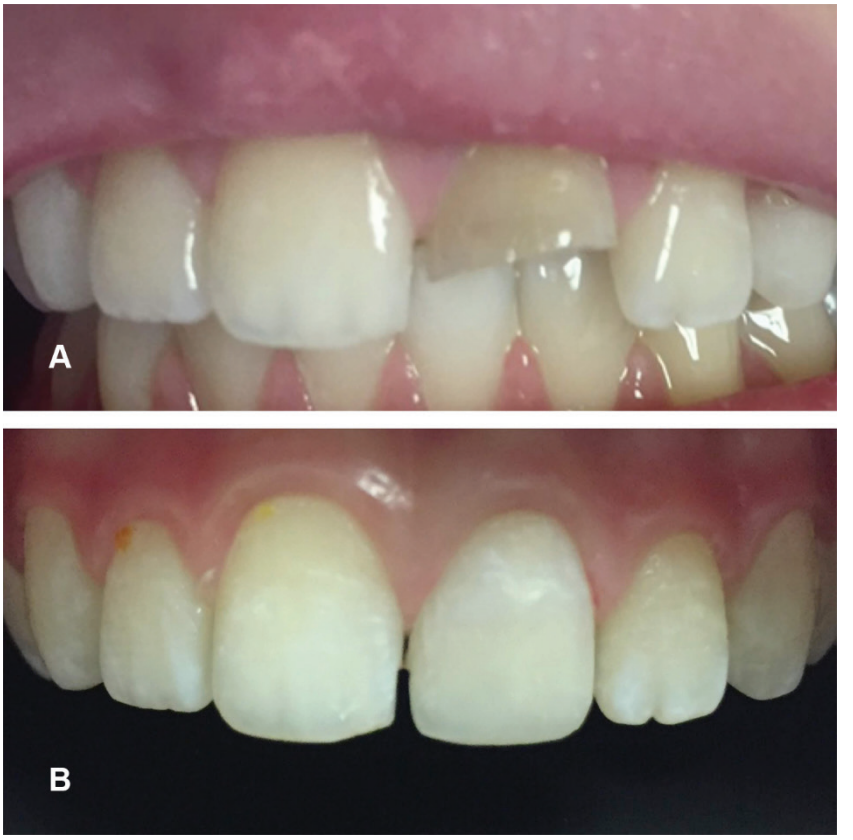
This technique was first described by Spasser and Herbert (1961) and is described as placing the mixture of sodium perborate and water in the pulp chamber. Next appointment, the procedure would be repeated until the desired shade reached. The application protocol of this technique is given in Table 2 and a case representing the walking bleach technique is presented in Figure 1.

Table 2. The application of the walking bleach technique

1. After the shade determination and initial photography taken, discolored tooth was isolated by rubber-dam or gingival barrier.
2. The existing restoration is removed and the endodontic access cavity is modified to ensure that no pulp horns, pulp remnants, or endodontic material residues remained. Because, these remnants will cause recurrence.
3. It should be ensured that all the restorative material is removed until the dentin surface is reached. Also, if there are superficial stains, they should also be removed using non-aggressive methods such as air-abrasion.
4. To prevent cervical root resorption, the root canal filling should be removed up to the proximal bone level with Gates-Glidden bur. This level is approximately 2-3 mm apical of cemento-enamel junction (CEJ) and can be determined with the help of periodontal probe. Then, the root canal filling should be sealed coronally with calcium hydroxide layer and glass ionomer cement.
5. After the root canal system sealed, the bleaching agent is applied to the pulp chamber. For the classical walking bleach technique, sodium perborate is mixed with distilled water to produce a semi-thick, viscous paste as a whitening agent. In combination technique (also called as modified walking bleach) 30% hydrogen peroxide is used instead of distilled water. After the paste inserted to pulp chamber the excess water is removed by a cotton pellet. Nowadays, 10% carbamide peroxide gel is preferred as a bleaching agent in general. The pulp chamber is sealed with a temporary restoration such as glass ionomer cement.
6. The patient was recalled depending on the bleaching material used and the treatment repeated if the desired shade was not reached.

The walking bleach technique was modified by placing a mixture of 30% hydrogen peroxide and sodium perborate in the pulp chamber. This technique is called modified or a combination walking bleach technique (Nutting and Poe, 1963). Hydrogen peroxide mixed with sodium perborate increases its effect and provides a better outcome. This process is faster and therefore results can be obtained after 1 week (Rotstein, 2001). In today's walking bleach technique, 10% carbamide peroxide is inserted into the pulp chamber with a syringe instead of sodium perborate mixture and the patient is examined every 3 to 5 days (Sulieman, 2008).

Figure 1. A case representing the walking bleach technique (A) Initial photography of trauma induced discoloration and fracture (B) 6-month follow-up photography of the case postbleaching with 10% carbamide peroxide



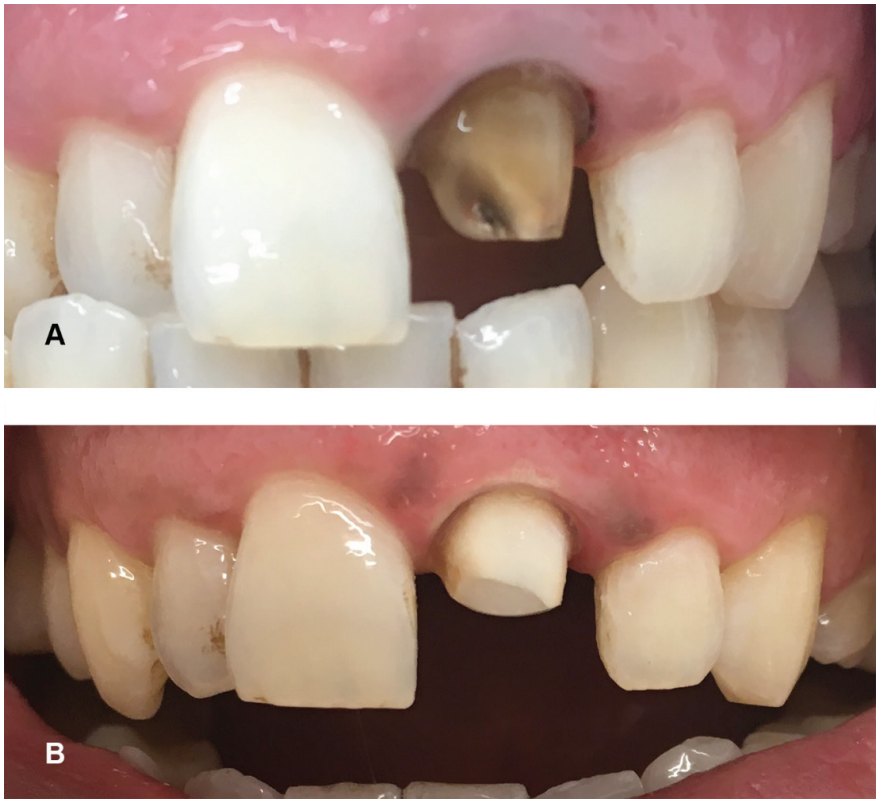
6.2. *Nonvital Power Bleaching*

This technique is the least preferred technique due to the use of high temperature causing an increased risk of cervical root resorption. The hydrogen peroxide gel is applied to the pulp chamber at a concentration of 30-35% and activated by light or heat. The temperature is usually around 50-60°C and the heat activation should be interrupted after 5 minutes for 5 minutes to cool down (Rotstein, 2001). The tooth is reevaluated after 2 weeks to determine whether additional treatment is required and, if necessary, the walking bleach technique is applied (Sulieman, 2008) (Table 3). A case representing nonvital power bleaching is presented in Figure 2.

Table 3. The application of the nonvital power bleaching technique

1. The tooth is prepared as in the walking bleach technique.
2. The hydrogen peroxide gel (30-35%) is placed in the pulp chamber as a bleaching agent and activated by heat or light. The tooth is exposed to the activated bleaching gel for 5 minutes with the temperature usually between 50-60°C. Then, the tooth is allowed to cool down for 5 minutes and the bleaching agent is washed away for 1 minute. The tooth is dried and the walking bleach technique is applied.
3. Another variation of this technique is applied using 35% hydrogen peroxide without a heat activation. In this technique, the whitening agent is applied both to the pulp chamber and to the facial surface of the tooth. As with vital tooth whitening treatment, activation is provided with the light source. After applying 3 sessions of 5 minutes, the tooth is washed with water and the pulp chamber is closed with temporary restoration. The patient is recalled after 2 weeks to assess if further treatment is necessary or is ready for the definitive restoration.

Figure 2. A preprosthetic case representing the nonvital power bleaching using 38% hydrogen peroxide with light activation (modified nonvital power bleaching) (A) Initial photography of endodontically treated tooth (B) Postbleaching photography after 2 weeks



6.3. *Inside/Outside Bleaching*

Settembrini et al., (1997) proposed a bleaching technique called inside/outside bleaching technique using a combination of intracoronal and extracoronal bleaching techniques with carbamide peroxide. In this technique, patients are responsible for the daily at-home use of the bleaching agent and therefore the bleaching effect is directly dependent on their compatibility. The inside/outside bleaching technique uses carbamide peroxide at varying concentrations of 5%, 16%, 22% or 35%. The application protocol of this technique is given in Table 4 and a case representing inside/outside bleach technique is presented in Figure 3.

Figure 3. Inside/outside bleaching case with 10% carbamide peroxide (A) Initial photography of the patient with a trauma induced discoloration (B) 7-month follow-up photography of the case

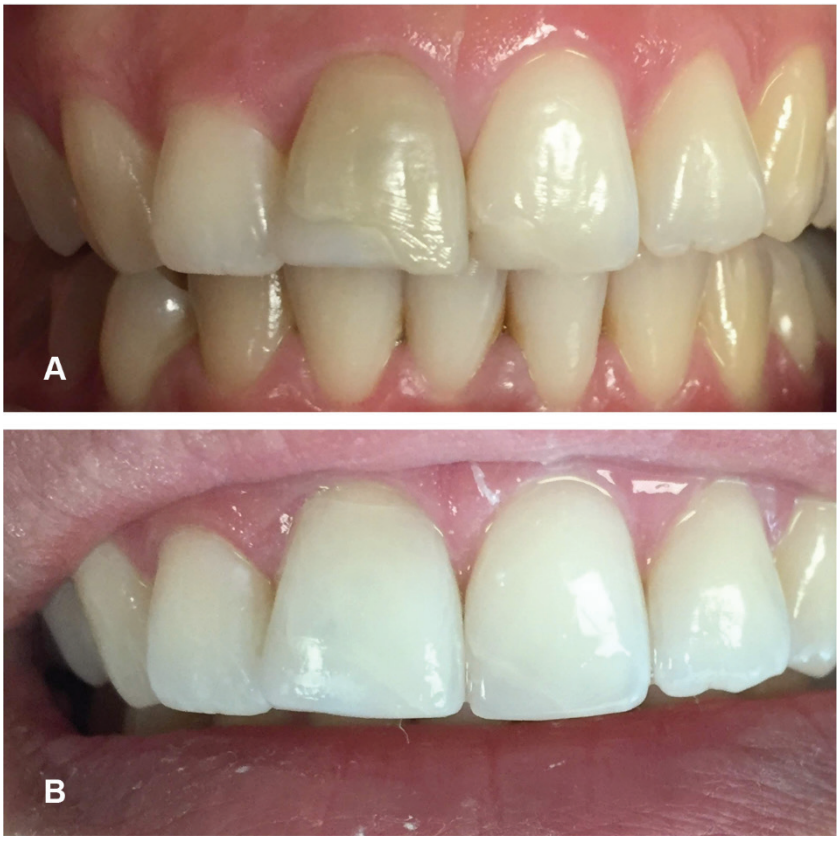


Table 4. The application of the inside/outside bleaching technique

1. A custom fitting tray is prepared for the patient and the pulp chamber is covered only with a cotton pellet. The patient removes the cotton pellet with a toothpick and inject the bleaching gel into the cavity to start bleaching session. The patient also applies bleaching gel to the corresponding portion of the bleaching tray and inserts the bleaching tray to the mouth. After the insertion, the patient removes the excess gel with a cotton bud or a toothbrush.
2. After 2-hours of bleaching session, the patient cleans the cavity with a provided syringe and inserts a clean cotton pellet. Also, after every meal, the cotton pellet is renewed in the same way.
3. The patient must protect the cavity from food impaction by placing a cotton pellet to the cavity, and should not eat anything during the bleaching session.
4. In general, it is reported that results are obtained after 5-8 applications and depending on the frequency of the application. Therefore, the patient is recalled for assessment after 3-7 days.
5. When the desired shade is reached, the pulp chamber is closed with temporary restoration, and the definitive restoration is placed after 2-week of delay.

This technique is simply a combination of at-home bleaching technique and intracoronal bleaching technique. The advantage of this technique in bleaching nonvital teeth is that the bleaching agent is applied both intracoronally and extracoronally. In addition, vital tooth bleaching (at-home bleaching) and nonvital bleaching can be combined simultaneously with the inside/outside bleaching technique (Carrillo et al., 1998). A lower concentration, generally the use of 10%, carbamide peroxide is thought to reduce the risk of cervical root resorption. The main disadvantage of this technique is that patient compliance is necessary and that hand ability is required to place the bleaching agent into the pulp chamber (Sulieman, 2008).

7. Neutralization

Bleaching of endodontically treated teeth with carbamide peroxide, sodium perborate or hydrogen peroxide is a commonly used method for regaining natural color. However, it has been reported that this procedure is followed by cervical root resorption (Friedman et al., 1988; Harrington and Natkin, 1979). Some researchers have claimed that bleaching materials penetrate periodontium and initiate an inflammatory process following cervical root resorption (Fuss et al., 1989). Therefore, the neutralization effects of the following agents were investigated.

7.1. *Calcium Hydroxide*

Today, intracoronal bleaching is routinely performed as a low-risk treatment method to correct the aesthetics of nonvital teeth. The major side effect of nonvital bleaching is cervical root resorption, which

can occur due to inflammation in the periodontium. The risk of cervical root resorption can be reduced by adequate cervical sealing and by avoiding high doses of bleaching agents (Zimmerli et al., 2010). Harrington and Natkin (1979) reported that the leakage of bleaching agents through dentin tubules can directly damage periodontal tissues and initiate an inflammatory response in the cervical region. Similarly, Lado et al**.,** (1983) reported that bleaching agents could penetrate the dentin tubules and denature dentine in the cervical region.

Calcium hydroxide has been used in the treatment of cervical root resorption (Fuss et al., 1989). The mechanism that makes calcium hydroxide effective is not yet known. It has been suggested that the diffusion of ions from the root canal increases the pH of dental tissues, thereby inhibiting osteoclastic activity and activates alkaline phosphatases (Tronstad et al., 1981). On the other hand, the study by Lambrianidis et al**.,** (2002) suggested that the use of calcium hydroxide as a barrier during intracoronal bleaching did not have a significant effect on preventing acidic pH on the external root surface. However, further studies are needed for the clarification of the exact mechanism.

7.2. *Sodium Ascorbate*

Many methods have been proposed to improve the bond strength of restorative materials to dental tissues after bleaching treatment. The first method that comes to mind is "delayed bonding". Although it varies according to the bleaching agent used and its concentration, it is generally recommended that delaying the bonding for 1 to 2 weeks to recover bond strength (Lago and Garone-Netto, 2013; Miranda et al., 2013). Benni et al., (2014) state that the use of ethanol or acetone-based bonding agent can also improve bond strength to bleached enamel.

Another frequently used method is the application of antioxidant agents to the enamel surface after bleaching. Sodium ascorbate, α-tocopherol (vitamin E), grape seed extract (proanthocyanidins), lycopene, epigallocatechin gallate (green tea) are among the known antioxidants (Abraham et al., 2013; Guler et al., 2013; Khamverdi et al.,2013). In recent studies, sodium ascorbate has been used as an antioxidant to remove oxidative compounds, especially free radicals (Gutteridge, 1994; Rose and Bode, 1993; Soeno et al., 2008). Soeno et al., (2008) reported that ascorbic acid acts as an antioxidant agent, and ascorbic acid and ferric chlorite increase the bond strength to dentine. Turkun et al**.,** (2009) reported that adhesion to dentin increased by 35% after applying sodium ascorbate as an antioxidant to bleached dentin.

8. Recurrence and Efficiency

Increasing rates of recurrence have been reported ranging from 10% to 49% in the literature with the time elapsed after bleaching (Friedman, 1997; Friedman et al., 1988; Glockner et al., 1999; Holmstrup et al., 1988). Lise et al., compared two different intracoronal bleaching techniques (walking bleach and inside/outside bleaching) and reported that both techniques were effective after 1-year clinical follow-up with no recurrence (Lise et al., 2018). Deliperi detected recurrence in 15 of 25 teeth with intracoronal bleaching as a result of a 5-year clinical follow-up, however, he added that the recurrence had a maximum of 6 shades on the VITA color scale (Deliperi, 2008) (Table 5). In another study conducted by Deliperi and Bardwell (2005),

the researchers reported a similar recurrence rate of approximately half of the 26 bleached teeth with up to 4 shades. Glockner et al., reported 79% of clinical success as a result of a 5-year clinical follow-up. In addition, they reported that if only the endodontic access cavity was prepared and the remaining tooth tissues were intact, the success rate increased to 91% (Glockner et al., 1999). According to Amato et al., (2006), the success rate of intracoronal bleaching after 16 years was 62.9%. In the same study, no cervical root resorption was observed in radiological examinations (Amato et al., 2006). Recurrence is relatively common in intracoronal bleaching. For this reason, some researchers recommend overbleaching the teeth to compensate recurrence (Bersezio et al., 2017) (Figure 4).

Figure 4. Endodontic treatment related discoloration (A) and (B) removal of endodontic material to the proximal bone level (C) overbleached tooth (D) 11-month follow-up photography

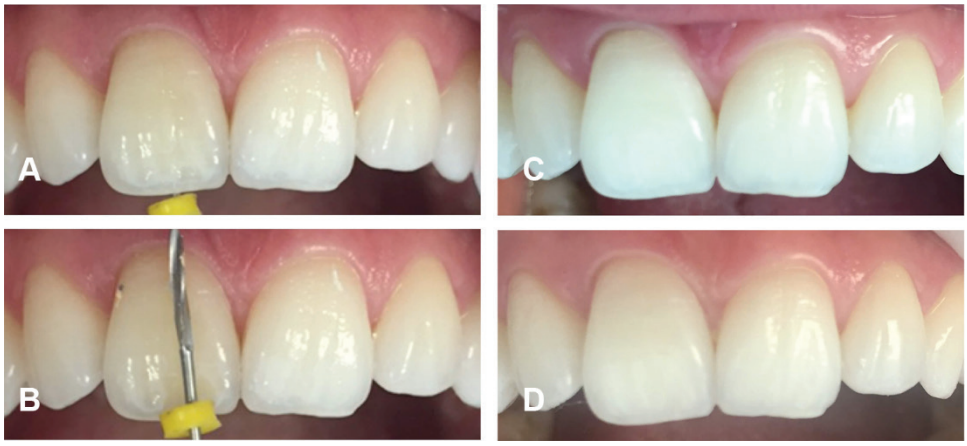


Table 5. Reordering of the Vita Shade Guide by value (light to dark by manufacturer)

Tab	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
Rank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Bersezio et al., evaluated the effectiveness of walking bleaching with different bleaching agents (35% hydrogen peroxide and 37% carbamide peroxide). They also investigated the effect of intracoronal bleaching on patients' psychosocial and aesthetic self-perceptions. As a result of their study, it was reported that effective and satisfactory results were obtained for patients after intracoronal bleaching (Bersezio et al., 2017). Gupta et al., reported 95% of patient satisfaction as a result of intracoronal bleaching performed on 41 patients with discoloration due to the trauma (Gupta and Saxena, 2014).

Color parameters L*, a*, b* established by the Commission Internationale de L'Eclariage (CIE) in 1978 (CIELAB). This system includes a lightness variable (L*) and chromatic coordinates (red/green, a* and yellow/blue, b*) by following human color perception. With this system, the difference between two different color measurements can be determined quantitatively (ΔE). Studies reported that 5 units ΔE (according to CIELAB color space) change is required for successful results of bleaching treatment (Bersezio et al.,

2017). Hence, it can be interpreted that intracoronal bleaching treatment provides effective bleaching (Bersezio et al., 2017; Deliperi, 2008; Poyser et al., 2004). On the other hand, it was reported in a study that dentists were more critical in evaluating the bleaching outcome compared to patients. As a result of a 5-year clinical follow-up of intracoronal bleached teeth, the success rate was rated as 75% and 98% according to the dentists and patients, respectively (Glockner et al., 1999).

9. Cervical Root Resorption

Although cervical root resorption is the most serious adverse effect of intracoronal bleaching (Feiglin, 1987; MacIsaac and Hoen, 1994), the underlying mechanism is not yet fully understood. The first cases of cervical root resorption were reported by Harrington and Natkin (1979). According to Heithersay (1999a), cervical root resorption was seen only 3.9% of the intracoronal bleaching cases. He also reported that cervical root resorption was due to orthodontic treatment (24.1%), dental trauma (15.1%) and surgical procedures such as periodontal or transplantation (5.1%), respectively. However, in combination with intracoronal bleaching and dental trauma, the cervical root resorption rate increases even more (Heithersay, 1999b). Several long-term follow-up studies have reported that cervical root resorption can be seen even years after intracoronal bleaching (Abou-Rass, 1998; Aldecoa and Mayordomo, 1992; Anitua et al., 1990; Harrington and Natkin, 1979; Holmstrup et al., 1988; Lado et al., 1983).

It is thought that the cervical root resorption due to intracoronal bleaching is caused by the penetration of the bleaching agent to the periodontium. The presence of several predisposing factors that increase the penetration of the bleaching agent is mentioned (Baratieri et al., 1995; Friedman et al., 1988; Niederman et al., 1998). Dietschi (2006) recommends the use of low-concentration bleaching agents or sodium perborate mixed with distilled water with thin dentin walls. In this way, the penetration possibility of the bleaching agent into the periodontium would be reduced. In addition, an increased incidence of cervical root resorption has been reported in patients undergoing intracoronal bleaching at a young age (Abou-Rass, 1998; Aldecoa and Mayordomo, 1992; Anitua et al., 1990; Friedman et al., 1988; Harrington and Natkin, 1979; Holmstrup et al., 1988; Lado et al., 1983), due to having relatively larger dentine tubules.

Heat activation is known to increase the efficacy of bleaching agents. It is reported that hydrogen peroxide applied to the pulp chamber by thermocatalytic technique can penetrate the outer surface of the tooth (Al-Nazhan, 1991; Dahlstrom et al., 1997; Farmer et al., 2006; Friedman et al.,1988; Friedman, 1989; Gimlin and Schindler, 1990; Goon et al., 1986; Lado et al., 1983; Latcham, 1986; Latcham, 1991; Madison and Walton, 1990; Montgomery, 1984; Szajkis et al., 1986). Therefore, the thermocatalytic technique is not preferred today due to the high risk of cervical root resorption (Attin et al., 2003; Friedman, 1997; Madison and Walton, 1990). On the contrary, cervical root resorption was not observed for sodium perborate – hydrogen peroxide solution with walking bleaching technique (Madison and Walton, 1990). Nowadays, carbamide peroxide is frequently preferred as an intracoronal bleaching agent (Bersezio et al., 2017; Ganesh et al., 2013; Shaheen et al., 2017; Valera et al., 2009). According to Lee et al. (2004), 35% carbamide peroxide showed the least extraradicular diffusion, followed by sodium perborate and 35% hydrogen peroxide, respectively. In addition, carbamide peroxide was found biocompatible than the hydrogen peroxide (Llena et al., 2019).

Another important predisposing factor of cervical root resorption observed after intracoronal bleaching is cervical sealing (Plotino et al., 2008). However, cervical root resorption rate can be reduced to 1.9% at 16-19 years follow-up (Amato et al., 2006; Heithersay et al., 1994), with a proper cervical sealing (Heller et al., 1992). Dietschi (2006) reports that cervical root resorption is not observed after 20 years of intracoronal bleaching with 30% hydrogen peroxide due to correct cervical sealing. To ensure proper cervical sealing, it is necessary to reduce the root canal filling to 3 mm apical of the cemento-enamel junction (CEJ) and then a layer of calcium hydroxide covered with a cement material such as zinc phosphate or glass ionomer applied prior to intracoronal bleaching (Rotstein et al., 1992). Ideally, cervical sealing should be determined according to the visible crown length of the discolored tooth. For this, it would be appropriate to remove the existing root canal filling up to the proximal bone level to seal interproximal dentin tubules (Carrillo et al., 1998; Steiner and West, 1994) (Figures 5 and 6).

Figure 5. Proximal view of a nonvital anterior teeth

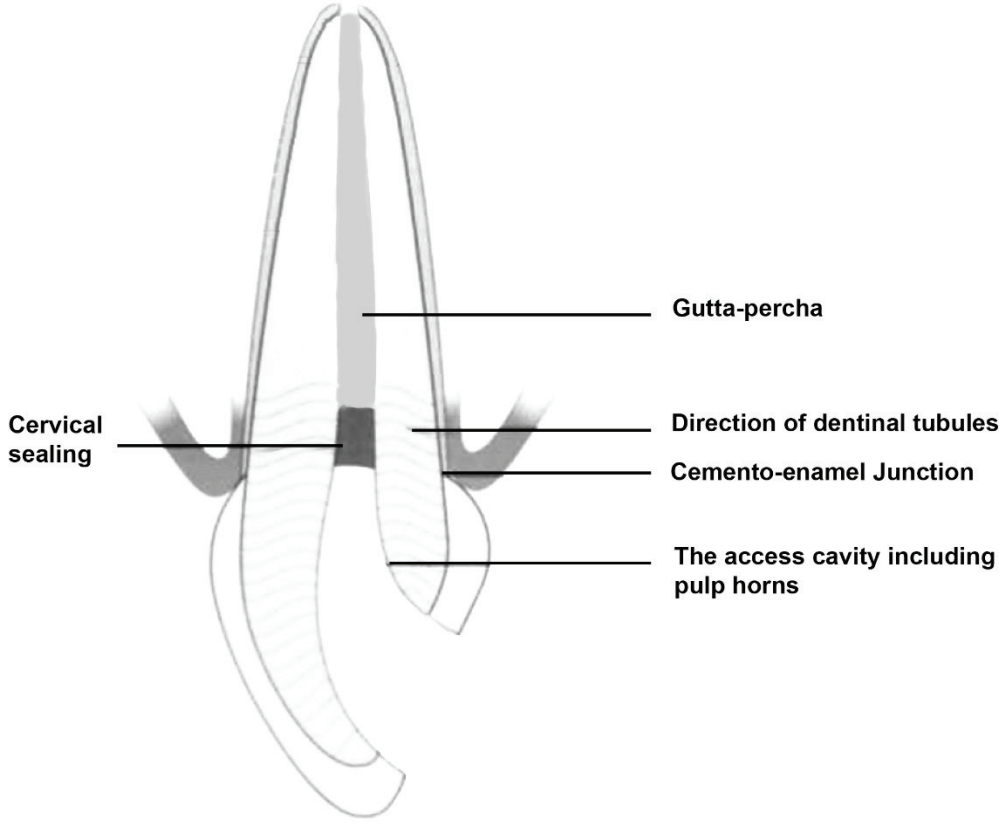
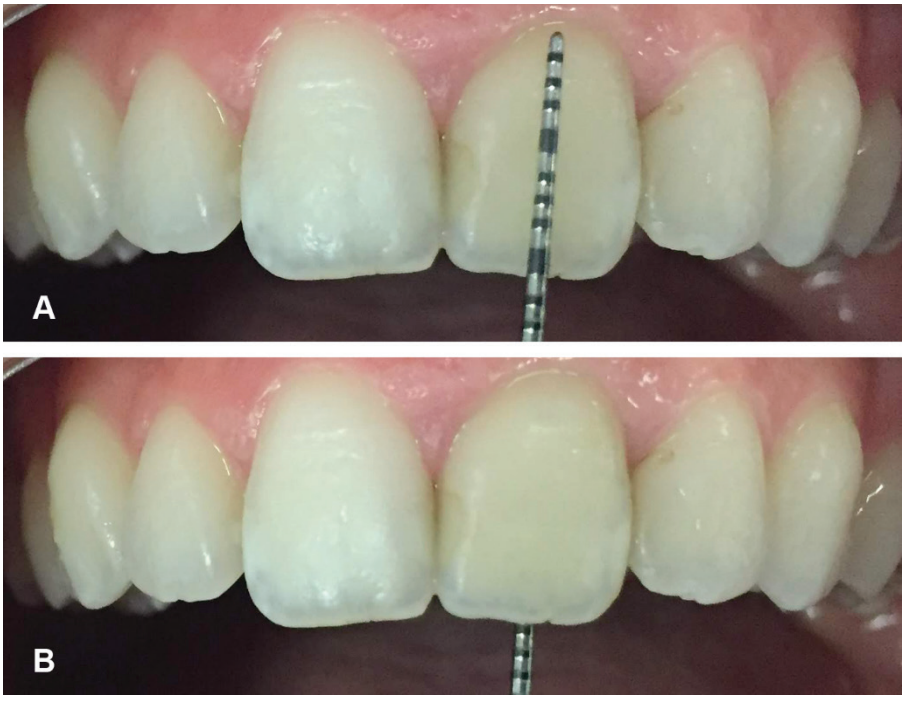


Figure 6. Determination of the cervical sealing level (A) Measuring the clinical crown length with the periodontal probe (B) Checking that the endodontic material is removed to the specified level



Cervical root resorption is generally clinically asymptomatic (Plotino et al., 2008). Therefore, the bleached teeth should be examined radiographically in the first five years following the intracoronal bleaching. Early diagnosis of cervical root resorption can be treated, whereas extraction is the only option, in severe root resorption (Goon et al.,1986; Latcham, 1986).

Conclusion

The discolorations in nonvital teeth, particularly in the anterior region cause aesthetic problems for patients and can affect everyday life. Although 100% success is not achieved in all intracoronal bleaching and certain rates of recurrence are encountered, it is a conservative treatment that can be preferred at least for delaying invasive restorative treatments.

Cervical root resorption is observed in nonvital bleached teeth, especially in the presence of dental trauma history. Therefore, it should be ensured that adequate cervical sealing is performed firstly. On the other hand, the thermocatalytic technique and high-dose hydrogen peroxide applications should be avoided. Recurrence appears to be inevitable after Intracoronal bleaching, hence more clinical trials are needed on color stability for better understanding.

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Conflict of Interests

Author declares no conflict of interests.

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Review

An Overview of Vital Tooth Bleaching

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Abstract: Bleaching of tooth discolorations became more attractive with the increasing importance of aesthetics. Therefore, in recent years, bleaching treatment has become one of the fastest-growing parts of aesthetic dentistry. Bleaching can generally be carried out with hydrogen peroxide or carbamide peroxide both at-home and in-office. Bleaching systems have been offered to the public as a more conservative and economical approach for improving dental appearance. However, the dental profession should maintain high ethical standards and not recommend cosmetic adjustments to the tooth color to suit the patient's demand. Therefore, in this article, vital tooth whitening applications are discussed.

Keywords: *Discoloration; tooth color; bleaching; whitening; vital*

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1. Introduction

Cosmetic dentistry is a very important part of dental restorative applications. Nowadays, individuals are not only content with healthy teeth but also want to have a perfect smile (Joiner, 2004). With the increase in aesthetic concerns, individuals often apply to dental clinics for a whiter smile. The majority of these individuals are not satisfied with the color of their teeth, and whiter teeth are thought to be related to health and beauty and are preferred. In a study of patient satisfaction with their tooth color, researchers reported indifference up to 50%, 30% were dissatisfied, and 10% were very dissatisfied with their tooth color (Odioso et al., 2000). Composite resin and porcelain veneers, crowns, composite resin restorations, mechanical abrasion, and bleaching are among the preferred treatments for tooth discoloration. In a survey conducted by Clinical Research Associates, 91% of dentists reported that they used bleaching treatment in their clinics with a success rate of 79% (Christensen and Christensen, 1995). Furthermore, it has been reported that some of the adolescents aged between 14 and 19 have bleached their teeth and

have the idea of having bleaching again (Boeira et al., 2016). Vital bleaching procedures for the treatment of discoloration are a more conservative and cost-effective approach compared to restorative treatments (Barghi, 1998; Dutra et al., 2004). Bleaching treatment can be carried out by the dentist in-office or at-home by the patient under the control of the dentist (Haywood, 1992; Haywood and Heymann, 1989; Sulieman et al., 2005). High concentrations of hydrogen peroxide or carbamide peroxide are used to provide fast, safe and very effective bleaching in-office (Garber, 1997; Haywood, 1992; Lee et al., 1995; Sulieman et al., 2003).

2. Discoloration

Tooth discolorations are categorized as extrinsic and intrinsic discolorations according to their origin (Nathoo, 1997; Watts and Addy, 2001; Zantner et al., 2006). Extrinsic discoloration is caused by the consumption of chromogenic foods and beverages, tobacco products, medicaments such as chlorhexidine (Haywood and Heymann, 1989; Watts and Addy, 2001). Acquired pellicle is a formation that is prone to discoloration (Viscio et al., 2000), and extrinsic discolorations are often observed in areas adjacent to gingival margins and interdental papillae, which are difficult to reach by insufficient brushing (Freedman et al., 2012). Therefore, while scaling and polishing treatments remove most of the extrinsic discolorations (Walsh, 2000; Yap and Wattanapayungkul, 2002), bleaching treatments are applied to remove stubborn stains (Duckworth, 2006; Joiner et al., 2002).

Intrinsic discoloration is caused by the passage of chromogenic substances into the enamel and dentin tissue during odontogenesis or during tooth eruption (Swift and Perdigão, 1998; Watts and Addy, 2001). Dental fluorosis due to the high levels of fluoride exposure, the use of tetracycline antibiotics, hereditary diseases and traumas affecting tooth development are among the main causes. After the eruption, pulp necrosis, discoloration due to some restorative materials and iatrogenesis are also considered in this category. Thereby, intrinsic discolorations can occur for many different reasons and are also divided into two according to the formation period: pre-eruptive and post-eruptive intrinsic discolorations.

2.1. *Pre-Eruptive Intrinsic Discoloration*

Dental Fluorosis

Dental fluorosis is characterized by a high concentration of fluoride exposure to enamel during tooth development, resulting in decreased mineral content and consequently increased porosity on the enamel surface. Dental fluorosis can be in forms ranging from a white cloudy manifestation to a discolored perforation. The severity of dental fluorosis varies depending on the period of exposure and intensity of the fluoride. Today, Thylstrup and Fejerskov (TF) index is used in the classification and treatment planning of dental fluorosis (Sherwood, 2010; Thylstrup and Fejerskov, 1978). The extent of the dental fluorosis is classified from 1 to 6 according to the TF index. Patients with a TF score of 3 or less may be treated with bleaching to mask the localized, chalky appearance of fluorosis with no cavitation. Thus, the surrounding healthy enamel is bleached and localized fluorosis is rendered relatively vague. However, in cases where enamel loss is evident, TF score 4 and above, resin infiltration or other restorative treatment options should be considered (Akpata, 2001).

Antibiotics

The most common antibiotic-related tooth discoloration is due to tetracycline. Tetracycline interacts with the calcium at hydroxyapatite crystals and forms a tetracycline-calcium phosphate complex. The oxidation of tetracycline molecules in the tetracycline-calcium phosphate complex results in tetracycline-induced tooth discoloration (Azer et al., 2011). Clinically, teeth with tetracycline discoloration may present light yellow to dark gray colored bands (Haywood, 1991). These bands indicate the period of tooth development corresponding to tetracycline exposure. Bluish-grayish bands may be observed in the discoloration of minocycline, a derivative of tetracycline. Minocycline discoloration can be misdiagnosed with pulpal hemorrhages in severe discoloration cases (Sánchez et al., 2004).

Tetracycline can cross the placenta barrier and therefore affect both primary and permanent dentition. Discoloration in permanent teeth is less intense but more common compared to primary teeth. Tetracycline exposure, even as short as 3 days, may cause tooth discoloration from intrauterine 4th month to 9 years old (Sánchez et al., 2004). Yellowish-brown discolorations can be treated more successfully than gray-bluish discolorations in terms of bleaching treatment (Freedman et al., 2012; Haywood, 1991; Haywood, 2000). In addition, selective etching of brown discoloration bands before the bleaching may increase the effectiveness (Freedman et al., 2012).

Some researchers have reported amoxycillin-clavulanic acid-related tooth discoloration and stated that this coloration is dose dependent (Garcia-Lopez et al., 2001).

2.2. *Post-Eruptive Intrinsic Discoloration*

Post-traumatic pulpal hemorrhage is the most common cause of post-eruptive intrinsic discoloration. Blood enters the dentin tubules and decomposes so that the degradation products cause discoloration. Pulp extirpation or necrosis can also lead to the formation of chromogenic degradation products (Arens, 1989). In addition, the physiological abrasion of the enamel together with the increase in secondary dentin and dentin sclerosis due to aging, affect the light transmittance of the tooth, thereby the tooth appears more yellow (Watts and Addy, 2001).

3. History of The Tooth Bleaching

Whiter teeth have been people's quest for ages. The first record for tooth bleaching is based on Assyro-Babylonian (Akkadian) cuneiform tablets (Aschheim, 2014). Since the beginning of the 19th century, dentists have begun to perform cosmetic procedures such as bleaching and tooth contouring. However, during these periods, bleaching was controversial among dentists due to its technical sensitivity and the poor prognosis of the whitening effect. In the second half of the 19th century oxalic acid was used to whiten vital teeth (Haywood, 1992). From the beginning of the 20th century, oxalic acid was replaced by pyrozone (ether peroxide) (Atkinson, 1892) and hydrogen peroxide as an oxidizing agent. Initially, hydrogen peroxide was administered to patients in liquid form (Fisher, 1911). In 1990, hydrogen peroxide was made available to dentists in gel form (Bartlett, 2001) and it

was applied in a much safer way. Nowadays, hydrogen peroxide used for in-office bleaching approach is presents in gel and powder-liquid forms with concentrations ranging from 25% to 40% (Haywood, 2000; Ontiveros, 2011).

4. Mechanism of Bleaching

In recent years, hydrogen peroxide and carbamide peroxide have been used as bleaching agents. Carbamide peroxide can be used in different concentrations. Bleaching with carbamide peroxide is different from hydrogen peroxide bleaching. First, the carbamide peroxide disintegrates into hydrogen peroxide and urea (Joiner, 2004). Carbamide peroxide with the concentration of 10% disintegrates into 6.6% urea and 3.4% hydrogen peroxide. Then, urea is broken down into carbon dioxide and ammonia.

Hydrogen peroxide can be used in different concentrations. Although it is known that hydrogen peroxide is easily diffused through enamel due to its lower molecular weight, it is not known exactly how it whitens teeth (Bowles and Ugwuneri, 1987). According to the chemical theory explaining the bleaching reaction of hydrogen peroxide, active hydrogen peroxide disintegrates into water (H_2O) and oxygen (O_2) and perhydroxyl radicals (HO_2). Bleaching is also known as an oxidation-reduction reaction. Peroxides are converted to unstable free radicals (Hannig et al., 2003; Kashima-Tanaka et al., 2003). The free radicals formed as a result of the decomposition of hydrogen peroxide, diffuse into the interprismatic regions of the enamel and carry the small molecules that it detaches from large organic molecules to the surface due to its foaming properties. These free radicals react with organic molecules causing discoloration and result in simple molecules that reflect less light (Sulieman, 2004).

Another theory for the mechanism of the peroxide reaction is carbon-carbon bond cleavage causing the ring-opening of the chromophores. Yellow double-bonded carbon compounds are converted to almost colorless hydroxyl compounds (Haywood, 2001). Studies have shown that hydrogen peroxide modifies these long-chained chromophores to more translucent molecules and provides bleaching in tooth discoloration. Chromophores are divided into two: long-chain organic compounds with double bonds and metal-containing compounds. Bleaching of organic compounds with hydrogen peroxide involves oxidation of double bonds. This causes the simpler molecules to reflect less light, making the darker regions lighter. Bleaching of the metallic compounds is more difficult, hence invasive restorative treatments may be a better treatment option for such teeth (Carey, 2014).

As the bleaching process continues, a point is reached where only hydrophilic colorless structures remain. This point is called the saturation point and bleaching slows down at this point. If the bleaching is further continued, the carbon-containing dental tissues and the carbon bonds of the proteins are destroyed. At this stage, excessive bleaching disrupts tooth enamel without whitening and results in irreversible alterations on the enamel structure and mineral loss (Vilhena et al., 2019).

5. Composition of Bleaching Agents

The current bleaching materials contain hydrogen peroxide or carbamide peroxide as the active ingredient (Joiner, 2004; Joiner and Thakker, 2004). Along with the active ingredient, inactive ingredients, such as thickening agents, carriers, surfactants and pigment dispersants, preservatives, and flavorings are also present in bleaching materials (Gokay, 2005; Greenwall, 2001; Hannig et al., 2003; Joiner and Thakker, 2004; Kashima-Tanaka et al., 2003).

Thickening Agents

The most commonly used thickening agent in bleaching gels is carbopol (carboxypolymethylene), which is a high molecular weight polyacrylic acid polymer (Joshi, 2016). Thickening agents increase the viscosity of the bleaching material and increase its retention to the applied surface. On the other hand, it slows the release of active oxygen in hydrogen peroxide up to four times (Rodrigues et al., 2007), reducing the need for replacement of the bleaching material during the process (Gokay et al., 2005; Greenwall, 2001; Joiner and Thakker, 2004).

Carriers

Glycerin and propylene glycol are generally used in bleaching gels (Joshi, 2016). Glycerin increases the viscosity of the bleaching material and provides ease of use, but causes dehydration (Greenwall, 2001). Dehydration results in a temporary loss of tooth translucency. Propylene glycol does not cause dehydration and retains moisture. It also contributes to the dissolution of other ingredients in the bleaching material (Joiner and Thakker, 2004).

Surfactants and Pigment Dispersants

Surfactants as surface wetting agents provide a much better spread of bleaching material to the tooth surface (Feinman et al., 1991). Therefore, surfactant agents increase the effectiveness of the bleaching material (Gerlach et al., 2002). Pigment dispersants also keep pigments to remain in the bleaching gel (Alqahtani, 2014).

Preservatives

Various preservatives are used in the composition of bleaching materials. Sodium benzoate and methyl propyl paraben are added to prevent bacterial growth in the bleaching gel (Joiner and Thakker, 2004). On the other hand, preservatives such as citric acid, citroxain, phosphoric acids or sodium stannate also prevent the breakdown of hydrogen peroxide, until its use. These preservatives increase the stability and durability of the bleaching gel while keeping the pH of the gel mildly acidic (Joshi, 2016).

Flavorings

Flavorings such as banana, melon, peppermint are added to increase patient acceptance of the bleaching material (Alqahtani, 2014; Joiner and Thakker, 2004).

Additives

Various substances are added to the bleaching materials to eliminate the side effects of the bleaching treatment (Sulieman, 2008).

Amorphous Calcium Phosphate-Casein Phosphopeptide

Amorphous calcium phosphate-casein phosphopeptide (ACP-CPP) provides rapid desensitization by causing depletion of phosphate and calcium ions to exposed dentin tubules (Giniger et al., 2005). It has been reported that the incorporation of ACP-CPP into the bleaching gel significantly reduces sensitivity and increases bleaching efficiency (Joshi, 2016).

Fluoride

It is known that fluoride blocks the dentin tubules and relieves sensitivity by decelerating the dentinal fluid flow (Petersson, 2013). Fluoride also enhances the microhardness of enamel (Attin et al., 2007; Basting et al., 2003) and hence fluoride-containing bleaching gels have been reported to cause less demineralization without affecting the bleaching efficiency (Chen et al., 2008). Furthermore, in 2013, the present author reported that fluoride pretreatment before 35% hydrogen peroxide bleaching resulted in lower surface roughness compared to no fluoride-treated controls (Sismanoglu et al., 2013). It has also been reported that the fluoride incorporated into the bleaching gel positively contributes to the consequent restorative treatments regarding the bond strength (Chuang et al., 2009).

Potassium Nitrate

Potassium nitrate shows an anesthetic-like effect by preventing the repolarization of the depolarized nerve (Poulsen et al., 2006; Tarbet et al., 1981). This reduces post-operative sensitivity without altering the bleaching effect. Matis et al., (2007) stated that ACP-CPP-containing bleaching gel provides similar sensitivity reduction with potassium nitrate-containing bleaching gel, but showed less bleaching effect. It has been reported to be effective even in light-activated bleaching (Browning et al., 2008; Haywood et al., 2001; Tam, 2001).

6. Patient Selection

Almost every patient may desire whiter teeth, but in each case, the aesthetic expectations of the patient may not be adequately met or there is no guarantee that successful results would be achieved (Joshi, 2016). The indications for bleaching at-home and in-office are basically the same, but the clinician should decide the appropriate approach by the patient's needs (Sulieman, 2008). The patient's expectations, lifestyle, the amount of time can be allocated for bleaching treatment, dental sensitivity, baseline shade, and etiology of the discoloration are important factors in choosing the appropriate bleaching approach for the patient (Sulieman, 2005). In the presence of caries, periapical lesion or hypersensitivity, priority should be given to remedy these problems rather than bleaching (Sulieman, 2004). Furthermore, bleaching

is contraindicated in pregnancy, since the effects of bleaching materials on the fetus are still unknown (Sulieman, 2005). The indications and contraindications of vital tooth bleaching are presented in Table 1.

Clinical trials, case reports, systematic reviews, and clinical experience provide the clinician with information on which discoloration would respond to bleaching treatment (Joiner and Thakker, 2004; Sulieman, 2004, 2005). Yellow-toned teeth, which generally do not have developmental pathology, can be effectively bleached, whereas brown stains may be more stubborn. For instance, brown stains caused by tobacco consumption generally respond to longer bleaching regimes (Kugel et al., 2002). In addition, there is also a relationship between sclera and shade of teeth. If the teeth to be bleached are lighter than the sclera, bleaching success would be less (Greenwall, 2001)**.** It has not been established that gender affects the bleaching outcome (Gerlach and Zhou, 2001), but effective bleaching is achieved for younger individuals compared to older ones (Joshi, 2016). Although white fluorosis spots are not suitable for bleaching, they become less pronounced as a result of bleaching the surrounding enamel (Haywood, 2000; Sulieman, 2004). More interventional restorative or resin infiltration treatments should be considered for individuals with moderate to severe fluorosis. Moreover, bleaching of extensive tetracycline discoloration is quite difficult and may require the application of months-long bleaching regimens (Haywood, 2000; Sulieman, 2004). Therefore, direct or indirect restorative treatments are preferred to cover tetracycline discoloration bands.

7. Types of Vital Teeth Bleaching

Treatment of discolorations begins with the removal of extrinsic stains by polishing until then the bleaching can be performed. There are basically three different bleaching approaches: in-office or power bleaching (Feinman et al., 1987), at-home or dentist-supervised nightguard vital tooth bleaching (Haywood and Heymann, 1989), and bleaching with the over-the-counter (OTC) products (Kihn, 2007; Sagel et al., 2000). Bleaching systems and materials can be classified according to the active ingredient, and the application/delivery method. The American Academy of Cosmetic Dentistry classifies bleaching systems according to their application/delivery methods (Joshi, 2016).

Whitening Toothpaste

These toothpastes contain higher amounts of abrasive particles and detergents than whitening agents compared to the standard toothpastes and remove extrinsic stains on the tooth surface (Lima et al., 2008). The enzymes contained in their chemical formulae cause the decomposition of organic molecules in the pellicle. It should not be ignored that abrasive particles can cause a permanent loss of enamel on tooth surfaces (Joiner et al., 2008). Some toothpastes may contain relatively low concentrations of carbamide peroxide or hydrogen peroxide as a whitening agents instead of abrasive particles. The whitening agent must be kept separate from dentifrice until its use to keep the agent stable. Dual-chambered tube technology enables this separation. It is stated that tooth color can be bleached one to two shades with whitening toothpastes. In addition, a silica toothpaste containing blue covarine has attracted great attention in recent years. With this toothpaste, which is a good example of the adaptation of metamerism to dentistry, teeth are perceived as whiter.

Whitening Mouthwashes

The whitening mouthwashes contain a low concentration of hydrogen peroxide (2%) and sodium hexametaphosphate to prevent tooth discoloration. Prolonged use may irritate the oral mucosa and tooth sensitivity (Carey, 2014).

Whitening Strips

Recently, whitening strips are developed to make the bleaching gel easier to apply as an alternative to other bleaching systems (Alqahtani, 2014; Sagel et al., 2000). These bleaching strips contain 150-200 mg of bleaching gel homogeneously distributed on the surface of flexible polyethylene material. The concentration of hydrogen peroxide ranges from 5.3% to 6.5% (Donly et al., 2007), and patients are advised to use this system for 30 minutes twice a day for 14 days long. Bleaching strips are very popular because they are easy to apply, cost-effective and have a considerable bleaching effect (Alqahtani, 2014; Gerlach and Barker, 2004).

Paint-on Systems

These products are based on the application of a suspension containing hydrogen peroxide or carbamide peroxide to the tooth surface with a brush (Kishta-Derani et al., 2007). However, their bleaching activity is considerably low. This is most likely due to the short contact time of the bleaching agent (Lo et al., 2007).

Tray-Based Tooth Whiteners

These whitening products are offered directly to the consumer without any control of the dentist, such as other cosmetic products. OCT products appeared in the United States in the early 2000s. These products whiten teeth at lower costs than professional treatments (Demarco et al., 2009). However, these products are generally dispersed by standard/uniform trays and can cause gingival irritation because they are applied without custom fitting trays (Demarco et al., 2009).

At-Home Bleaching (Nightguard Vital Tooth Bleaching)

Although the bleaching effect of carbamide peroxide was discovered randomly in the late 1960s by B. Klusemeir, an orthodontist (Joshi, 2016), the nightguard vital tooth bleaching approach at home was first described in 1989 by Haywood and Heymann (1989). Although this method has undergone many changes to date, it is essential that bleaching agents containing carbamide peroxide are used for 2 to 6 weeks in custom fitting trays for a period of 6 to 8 hours a day.

Usually, 10-15% carbamide peroxide is recommended for this bleaching technique. The bleaching materials are in the form of a transparent gel or white pat. Carbopol-containing bleaching materials are preferred because carbopol increases viscosity and prolongs the oxidation process (Gokay et al., 2005; Greenwall, 2001; Joiner and Thakker, 2004; Rodrigues et al., 2007). The 10% carbamide peroxide agent is composed

of 3.5% hydrogen peroxide and 6.5% urea. The presence of urea gives the agent a longer shelf life and slows the release of hydrogen peroxide. Many studies have shown that it is safe and effective to perform bleaching agents containing carbamide peroxide in accordance with dentist recommendations (Haywood and Heymann, 1991). However, patients' cooperation on long-term tray usage is poor.

In-Office Bleaching

For in-office bleaching, hydrogen peroxide is generally used in concentrations ranging from 25% to 40%. The bleaching process is completely under the control of the dentist and can be stopped when the desired shade is reached. Hydrogen peroxide is a material with caustic effects and therefore protective measures such as rubber-dam or gingival barrier should be taken during its application (Sulieman, 2008). Penetration of hydrogen peroxide to the pulp chamber is also possible, but considering its long-term effect, it does not cause any adverse effects on the pulp (McEvoy, 1995).

The in-office bleaching approach can be used in patients who do not have enough time for the at-home bleaching approach, who have gag reflexes or who do not like the taste of home bleaching gels. Another advantage is that the immediate results obtained for the in-office bleaching motivate the patient to continue with bleaching at-home to maximize the outcome. Therefore, the in-office bleaching was frequently combined with at-home bleaching.

The bleaching process can be activated with the help of heat and light to increase the speed and effectiveness of the bleaching treatment. Hence, in-office bleaching approach is also called as "power bleaching". Nowadays, heat application has been abandoned due to possible harmful effects on pulp. Manufacturers produce many light devices specific to tooth bleaching. With the help of these devices, the disintegration ratio of hydrogen peroxide increases and the decomposition of chromophore molecules through oxidation is accelerated, thereby the time required for bleaching is decreased. One of the uses of lasers in dentistry is tooth bleaching. The most commonly used lasers in this field are diode, CO_2 and argon lasers. Using photons of a specific wavelength close to the absorption spectrum of the bleaching agent (480 nm and 520 nm) would increase the chemical reaction rate and reduce the bleaching time (Downs et al., 2011). Torres et al. (2009) reported that the laser activates highly reactive hydrogen peroxide molecules, enabling them to rapidly ionize.

The most used power bleaching sets include hydrogen peroxide gel, light-cured gingival barrier material, neutralizing gel and/or neutral fluoride gel. Although the use of rubber-dam is recommended for the isolation of soft tissues, gingival barriers are frequently used in the power bleaching process as the rubber-dam may cause application problems at the gingival third of the teeth. Following the application of the gingival barrier, the hydrogen peroxide gel is applied to the surfaces to be bleached according to the manufacturer's instructions forming approximately 3-mm thick layer (Figure 1). Although the process varies from brand to brand, it is usually repeated 3 times in about 10-15 minutes. Each repetition is termed as "passes" (Joshi, 2016). After each passes, the whitening gel is cleaned over the teeth with suctioning and wiping using clean gauze. If the bleaching gel leaks despite the gingival barrier and comes into contact with soft tissues during bleaching, it may cause blanching and irritation. If this happens, the irritated area

should be washed with plenty of water and the neutralizing agent supplied with the bleaching kit should be applied. Generally, these neutralizing agents include vitamin E, which is an antioxidant. The bleaching process can be resumed after the reapplication of the gingival barrier.

All bleaching agents that are not at neutral pH reduce the microhardness and modulus of elasticity and increase the surface roughness of the enamel. This increase in roughness creates a favorable environment for extrinsic discoloration (Azer et al., 2009; Pinto et al., 2004). Therefore, bleached enamel surfaces should be polished. Subsequent to bleaching, a neutral fluoride gel can be applied to teeth. It is noteworthy, the teeth appear lighter than it is due to the postbleaching dehydration, and slight rebound occurs on rehydration. This should be considered by the clinician, and color evaluation should be better after 1-2 days (Haywood, 1996).

Combination Treatments

The combined use of both in-office bleaching and at-home bleaching approaches is often preferred by dentists. Especially in the treatment of tetracycline discolorations or cases of discoloration with different etiology, such an application provides successful results. Patient cooperation is another important factor in the success of bleaching treatment (Odioso et al., 2000). In particular, for the patients with weak motivation, initiating the bleaching treatment with in-office bleaching before at-home bleaching would increase the motivation towards treatment and affect their cooperation positively.

Another combination is the use of whitening toothpaste after bleaching treatments. Such a combination would be useful to prevent as much of the rebounds as possible after bleaching to maintain color stability.

8. Adverse Effects of The Bleaching

The most common side effects of tooth bleaching are recurrence of the discoloration, hypersensitivity, irritation of gingival tissue and oral tissues, alterations on enamel and restorative material surfaces (Li and Greenwall, 2013). Apart from these, tray-based tooth whiteners without dentist orientation may also cause temporomandibular joint problems (Gerlach et al., 2009).

8.1. *Recurrence of The Discoloration*

To assess and understand the reversal of the whitening effect, patients' tooth shade should be recorded before the bleaching process. According to all clinical and laboratory research results, tooth bleaching is effective and safe with the latest generation of vital whitening products (Li, 2003; Luk et al., 2004). Reversal after in-office bleaching has been reported at a rate of 41% per year according to Clinical Research Associates (2004). For at-home bleaching, a return of 26% in 18 months and stated that the original concentration of bleaching agent is not relevant to the reversal rate (Meireles et al., 2009).

After the completion of in-office bleaching or after the removal of the tray at at-home bleaching, patients often notice a large bleaching effect due to the effect of dehydration. It is better to perform the final color evaluation 1-2 days after bleaching (Haywood, 1996). To prevent reversal, it may be

advisable to use whitening toothpastes, and it may be recommended for patients to undergo annual at-home bleaching.

All bleaching agents result in a reduction in enamel microhardness and modulus of elasticity after bleaching and result in surface roughness. This increase in roughness creates an environment conducive to extrinsic coloration (Azer et al., 2009; Pinto et al., 2004). Therefore, after whitening, tooth surfaces should be polished.

8.2. *Hypersensitivity*

Hypersensitivity is the most commonly reported adverse effect of bleaching. The frequency of hypersensitivity observed in patients using 10% carbamide peroxide is ranging between 11 to 93% (Leonard et al., 2002) and the average initial reporting time of hypersensitivity is after 5 days (Tam, 1999). This side effect is usually mild and temporary pain; often causes significant discomfort in the patient (Rosenstiel et al., 1996). The cause of sensitivity after bleaching is explained by different mechanisms such as hydrodynamic theory or morphological changes in enamel (increased surface porosity, precipitation, superficial irregularities). However, recent studies have indicated that direct activation of neuronal receptors may be the main reason for the occurrence of this sensitivity. It has been reported that with the addition of agents, which inhibit the activation of neuronal receptors such as potassium nitrate to the bleaching materials, it is possible to reduce the severity of this sensitivity without resulting in a decrease in bleaching efficiency (Markowitz, 2010). Potassium nitrate, sodium fluoride or ACP-CPP can be incorporated into bleaching materials as desensitizing agents or applied to the tooth surface prior to bleaching (Tay et al., 2009).

8.3. *Alteration on The Enamel Surface*

Micro- and nano-mechanical investigations have shown that bleaching agents reduce enamel hardness, modulus of elasticity and fracture resistance. This decrease occurs regardless of peroxide concentration, pH or exposure time (De Abreu et al., 2011). Therefore, the decrease in stiffness and modulus of elasticity is thought to be due to the protein denaturation (Ushigome et al., 2009). In a study, it was stated that carbamide peroxide causes more protein denaturation compared to hydrogen peroxide and urea content in carbamide peroxide may cause this situation (Elfallah et al., 2015). Besides, it is reported that the mineral loss as a result of bleaching does not cause harm to the tooth (Goo et al., 2004). Furthermore, mineral loss due to 12-hour bleaching treatment is similar to mineral loss caused by a few minutes of soft drink or juice exposure (Lee et al., 2006). When these studies are taken into consideration, alterations caused by bleaching in the enamel can be considered insignificant (Alqahtani, 2014).

Each tooth has its final level of lightness, which is called "inherent lightness potential" (Matis et al., 2000). This point represents the endpoint of the bleaching process for that tooth. If bleaching is continued after this point, no more whiteness can be achieved, besides irreversible damage to the enamel may occur. Studies have reported that this endpoint can be reached regardless of the active ingredient and concentration used for bleaching over 6 weeks.

In some studies, whitening products have been reported to increase surface alterations in enamel. When compared with untreated control groups, the bleached enamel surface undergoes morphological changes (Alqahtani, 2014). However, these changes have been reported to be reversible (Demarco et al., 2011). After bleaching, dentists advise their patients, especially against smoking and some chromogenic beverages (Cavalli et al., 2004; McGuckin et al., 1992; Titley et al., 1988). Coffee, tea, fruit juices, red wine, and sodas are chromogen beverages that have the potential to stain or discolor the bleached enamel surface. The bleached enamel surface may be very sensitive to discoloration, especially in acidic solutions (Berger et al., 2008). Mouth rinsing or brushing can be performed immediately after the consumption of foods and beverages to prevent discoloration. Patients can use straws while consuming beverages. In patients who smoke and drink beverages that cause the excessive coloration, it may be necessary to repeat the whitening process very often.

8.4. *Effects on Pulp Tissue*

The risk of dentin pulp complex being affected by dental materials depends on the permeability of the components of these products through enamel and dentin (Hanks et al., 1993). The diffusion of H_2O_2 has been shown to increase with increasing concentration and duration of administration (Matis et al., 2000). H_2O_2-induced free radicals cause oxidative stress in pulp cells. As a result, further tissue damage is prevented by releasing endogenous antioxidant agents such as peroxidase and catalase from pulp cells (de Souza Costa et al., 2010). Although the cytotoxic effect of H_2O_2 on the pulp is proven, pulpal cells are sufficient to eliminate this effect and initiate odontoblastic differentiation (Soares et al., 2015; de Souza Costa et al., 2010).

8.5. *Soft Tissue Effects*

The contact of bleaching agents with oral tissues causes chemical burns. If this contact is short-term, it is seen as whitening of the tissue and this whiteness disappears within a few hours. Ulceration may occur for longer periods of contact. In such cases, topical vitamin E administration should be recommended to accelerate healing (Li and Greenwall, 2013).

8.6. *Effects on Restorative Materials*

In most of the studies in the literature, composite resins have been investigated as restorative materials. In a laboratory study, a 3-week application of 10% carbamide peroxide has been shown to alter the surface roughness of composite resins (Basting et al., 2005). However, surface microhardness was not significantly changed (Basting et al. 2005). *In situ* studies also showed no change in surface microhardness as a result of the application of 15% carbamide peroxide to composite resins for 4 weeks (Li et al., 2009; Yu et al., 2008). In fact, the bleaching of these composite resins have also been observed (Li et al., 2009). The authors commented that this bleaching may be related to surface changes and oxidation of chromophores. On the other hand, in another laboratory study, the effect of a 2-week administration of 10% carbamide peroxide on the surface microhardness was examined under two different storage temperatures (Yu et

al., 2011). No significant difference was observed in the surface microhardness for specimens stored at room temperature (25°C), while a significant softening was observed for the specimens stored at body temperature (37°C) (Yu et al., 2011).

There are several controversies among studies on subsurface microhardness. Yu et al., (2011) reported that the subsurface microhardness of bleaching-induced composite resins was stable at different environment temperatures. However, Hannig et al., reported that composite resin subsurface microhardness may be affected up to 2-mm after bleaching (Hannig et al., 2007). Therefore, it is certain that further studies are needed on this subject.

In many studies, it has been reported that application of carbamide peroxide does not cause adverse effects on flexural strength and fracture toughness of composite resins (Cho et al., 2009; Hatanaka et al., 2013; Yu et al., 2010). In addition, in-office bleaching with higher concentrations did not alter the tensile bond strength of composite resins (Cullen et al., 1993; Yap and Wattanapayungkul, 2002). As a result, if bleaching treatment is applied in the presence of composite resin restorations, polishing of these restorations after the treatment would be appropriate.

Bleaching treatment is known to cause the release of monomer and some other substances on dental composites. Durner et al., (2011) reported that bleaching with hydrogen peroxide degrades three-dimensional polymer networks in composites when compared to non-bleaching controls, leading to an increase in the release of unpolymerized monomers and other substances.

According to the information obtained from laboratory studies, it has been shown that mercury release is increased in dental amalgams in contact with carbamide peroxide. Bleaching agents have been shown to improve the solubility of glass-ionomer and other cements (El-Murr et al., 2011; Yu et al., 2009; Yu et al., 2015). Polyacid modified resin-based composites, resin-modified glass-ionomer cements, and zinc-oxide cements are exhibited increased softening and fluoride release in contact with high-concentration bleaching agents. Moreover, cracks have also been reported in some studies (Yu et al., 2015).

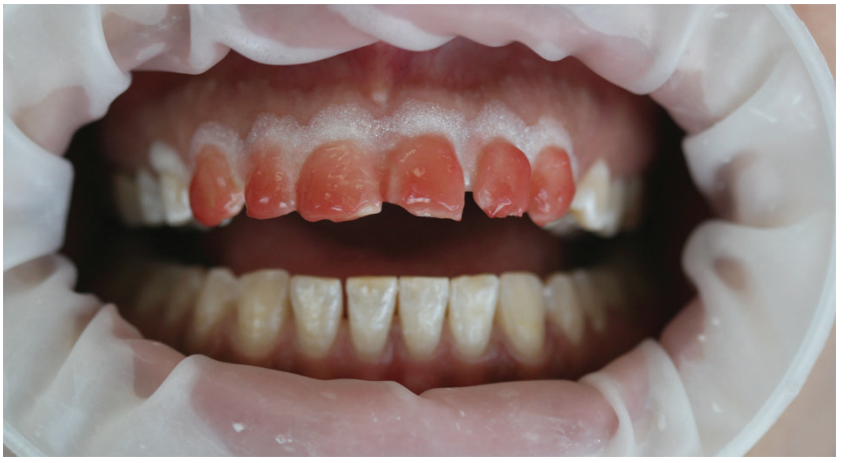
The decrease in the bond strength of adhesive restorations after the application of hydrogen peroxide has been proven by many researchers. The reason for this decrease is the changes in enamel structure, inhibiting the infiltration of the resin by the breakdown of hydrogen peroxide and inhibiting the resin polymerization (Cvitko et al., 1991; García-Godoy et al., 1993; Toko and Hisamitsu, 1993). It has been reported that the resin tags in the bleached enamel are much shorter and fewer compared to the unbleached controls (Titley et al., 1991). Therefore, it was stated that bond strength reduction reversed if the procedure is delayed for 2 weeks (Da Silva Machado et al., 2007)**.** Unlu et al., (2008) recommended delayed bonding for at least 24 hours after bleaching with 10% carbamide peroxide and for at least 1 week with 35% hydrogen peroxide bleaching. However, many studies indicate that a 1-week delay is not sufficient to obtain optimal bonding results (Bulut et al., 2006; Türkün and Kaya, 2004; Türkün et al., 2009). In addition, studies have reported that the use of a number of antioxidant agents (green tea extract, sodium ascorbate) as a pretreatment to reverse decreased bond strength. However, according to current knowledge, delayed bonding for 2 weeks seems to be a more appropriate option.

Bleaching-induced composite resins were found to be more susceptible to extrinsic discoloration due to the surface softening (Yu et al., 2009). Furthermore, it has been reported that 10% carbamide peroxide can remove extrinsic stainings such as juice, tea, and chlorhexidine on the composite resin surface (Fay et al., 1999).

Table 1. Indications and contraindications of vital tooth bleaching (Sulieman, 2008)

Indications	**Contraindications**
Generalized staining	Higher patient expectation
Age-related discoloration	Caries and periapical lesion
Smoking and dietary discoloration	Pregnancy
Fluorosis	Cracks and exposed dentine, sensitivity
Tetracycline staining	Existing crowns or large restorations
Trauma-related discoloration	Elderly patients with visible recession

Figure 1. The bleaching gel applied to vestibular toot surfaces approximately 3-mm in thickness



9. Considerations

Patients are often eager for other aesthetic dental or orthodontic procedures after tooth bleaching. However, as mentioned earlier, the bond strength of adhesive restorations or brackets is low in bleached teeth (García-Godoy et al.,1993; Toko and Hisamitsu, 1993). Although different methods have been tried to prevent this decrease in bond strength; the most commonly used method is delay bonding

of adhesive treatments after bleaching (Da Silva Machado et al., 2007). Lai et al., (2002) showed that the decrease in bond strength of composite resins after tooth bleaching can be reversed by the use of antioxidants. Ascorbic acid and its salts are products of low toxicity and used as antioxidants in the food industry. Ascorbic acid has a mean pH of 4, while sodium ascorbate has a pH of 7. Therefore, sodium ascorbate is a more suitable product for dental applications (Hansen et al., 2014; Vohra and Kasah, 2014). In addition, it has been shown that 5 minutes of application is sufficient for its antioxidant effect (Freire et al., 2009). However, the delayed bonding option is still the best choice if the patient does not have time constraints.

Light activation in combination with the in-office bleaching approach is a controversial topic in the literature. In recent systematic review and meta-analysis studies, the effect of light activation with in-office bleaching on the effectiveness of bleaching and tooth sensitivity was evaluated (Bernardon et al., 2010; Buchalla and Attin, 2007; He et al., 2012). In these studies, it was reported that light activation does not contribute to bleaching efficiency. Contrarily, increased tooth sensitivity and potential pulp irritation with the combination of light activation and use of high concentrations of hydrogen peroxide (25-35%) was reported (He et al., 2012).

In-office bleaching usually takes two or three sessions for effective and stable results. It is known that clinicians prefer to leave a gap of 1 week between sessions. Although this is not evidence-based, it is a common choice to reduce tooth sensitivity and prevent pulp damage. According to a recent study on this subject, it was found that there was no significant difference between 2-days and 7-days of waiting periods in terms of tooth sensitivity and bleaching efficiency (De Paula et al., 2015). Thereby, it is a safe way to wait at least 2 days between consecutive bleaching sessions.

Conclusion

Tooth bleaching combines both aesthetic and conservative approaches for the removal of tooth discoloration. Both the knowledge and experience of the clinician are critical for a thorough understanding of the etiology of discoloration and the selection of the proper bleaching approach. Successful treatment of these discolorations would increase patient satisfaction and motivation. However, the dental profession should maintain high ethical standards and not recommend cosmetic adjustments to suit the patient's demand.

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Conflict of Interests

Author declares no conflict of interests.

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Review

BCG Vaccine and New Tuberculosis Vaccines Against *Mycobacterium tuberculosis:* A review

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Abstract: *Mycobacterium tuberculosis* (*M. tuberculosis*) causes tuberculosis (TB) which is a serious infectious disease. Bacteria are spread from person to person through tiny droplets released into the air via sneezing and coughing. Despite global efforts to control TB, the disease is the second most common cause of death after Acquired Immune Deficiency Syndrome (AIDS). Currently, Bacillus Calmette-Guérin (BCG) vaccine is used to prevent tuberculous meningitis and miliary disease, particularly in young children, but its protective efficacy is variable in adults. Therefore, there is an urgent need for the development of alternative TB vaccines. Recently, new TB vaccine development efforts have been advanced in different clinical studies. Most of these vaccines are live-attenuated or recombinant mycobacterium, live viral vector-based, and protein/adjuvant vaccines. This review explains the recapitulation of the current status of new TB vaccines updated with scientific literature references.

Keywords: *Mycobacterium tuberculosis; new vaccines; clinical studies; BCG; review*

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1. Introduction

Tuberculosis is an infectious and disseminated granulomatous disease that is caused by a member of a mycobacteria group called *Mycobacterium tuberculosis* complex (MTBC). When Dr. Robert Koch identified and described the *M. tuberculosis* bacilli, Albert Calmette and Camille Guérin simultaneously discovered Bacillus Calmette–Guérin (BCG) vaccine (Daniel et al., 2006). Although TB is curable with medications and is preventable with the BCG vaccine, it is one of the most dangerous infectious diseases worldwide. Even if the incidence rates of TB considerably dropped recently, TB has been still accepted as a global problem by the World Health Organization (WHO, Global Tuberculosis Report, 2018).

According to WHO 2018 report, it is estimated that TB disease developed in 10 million people (range 9.0–11.1 million) in 2017. Asia region has been most severely affected by TB. Over 95% of TB deaths occur in low or middle-income countries as India, Indonesia, China, the Philippines, and Pakistan. Approximately, 9% of TB cases were among people with Human Immunodeficiency Virus (HIV). In 2017, there are 1.3 million deaths among HIV-negative people, whereas 300.000 deaths were seen among HIV-positive people due to TB disease. On the other hand, there are 1.7 billion people with latent TB infection who have a risk of developing active TB infection along their lifetime (WHO, Global Tuberculosis Report, 2018).

In 2015, WHO declared the End TB Strategy Plan to end TB by 2035. The strategy aimed to reduce TB deaths by 95% and TB incidence by 90% compared with 2015 (WHO, The End TB Strategy 2015). If effective TB vaccines and new medications to decrease TB disease are not developed, it may not be able to achieve this goal (Schrager et al., 2019).

Despite the use of *Mycobacterium bovis* BCG vaccine and global efforts to prevent disease TB continue to remain a serious disease around the world. The more effective new TB vaccines are required to protect people from all kinds of TB forms (Montagnani et al., 2014). Moreover, these persons may have another disease or infection as HIV infection or diabetes mellitus. Research on the novel TB vaccines to prevent TB started over the past decade. Recent research and developments in the new TB vaccine will be summarized in this review.

2. Bacillus Calmette-Guérin (BCG) Vaccine

BCG vaccine containing an attenuated strain of *M. bovis* is the only licensed vaccine that has been used to prevent TB for more than 90 years. (WHO Global Tuberculosis Report 2012; Zwerling et al., 2011). At present, WHO recommends a single dose of BCG for neonatal inoculation in the countries with high TB prevalence and incidence (WHO, Biologicals, BCG vaccine). First tested in the year 1921, BCG had highly variable protective efficacy, ranging from 0–80% in the adult population in different settings. BCG vaccination has been indicated to be effective at preventing from disseminated TB disease and meningeal TB in children, but it fails to exhibit adequate protection against pulmonary TB, particularly among young adults in high-endemic regions. (Hussey et al., 2007).

3. Development in TB Vaccines

Currently, there are numerous TB candidate vaccines at different stages in clinical trials. These candidate vaccines may be classified into conventional vaccines, prophylactic vaccines, booster vaccines, therapeutic/post-exposure vaccines and vaccines to prevent reinfection (Table 1) (Gopal et al., 2013; Soundarya et al., 2019; Usman et al., 2017). Some of these candidates are focused to replace BCG or as a boost BCG induced immunity. There are advantages and challenges of each vaccine type (Schito et al., 2015). The conventional TB vaccine BCG which is an attenuated *M. bovis* strain is an only licensed vaccine for TB currently. Also, recombinant BCG (rBCG) developed by using recombinant BCG technology is included in this category. The rBCG has been constructed either by

adding certain genes to BCG or removing specific genes from the natural mycobacterial genome. For that purpose, RDI and RD2 loci that are known as immunodominant *M. tuberculosis*-specific antigens are integrated into BCG. In the rBCG30 is over-expressed the gene Ag85B. Compared to BCG, rBCG30 secretes more over-expressing Ag85B and induces the greater Th1 immune response that inhibits intracellular mycobacteria.

Priming and boosting vaccines should be administered before exposure to TB bacilli. While a priming vaccine is normally applied to newborn infants, a boosting vaccine which is targeted at adolescents and adults are administered to enhance the immune response. In other words, to generate bigger and long-lasting immunity booster vaccines are given. Therapeutic or post-exposure vaccines could be given to already infected individuals or those potentially exposed during TB treatment (Kaufmann et al., 2017; Soundarya et al., 2019). The essential purpose of a therapeutic vaccine is to potentiate chemotherapy and to increase the rate of bacterial clearance (Soundarya et al., 2019). Vaccination for preventing reinfection is recommended during or after the treatment for TB (Orme et al., 2015).

Table 1. Application of different vaccine types against TB

Different Vaccine Types and Application	
Conventional	BCG, rBCG
Prophylactic	VPM 1002, MTBVAC
Booster	Ad5 Ag85A, ChAdOx1 MTB85A
Therapeutic	RUTI, ID93/GLA-SE
Prevent Reinfection	BCG Revaccination, H1/H56:IC31 (in trials)

BCG, Bacillus Calmette-Guérin; rBCG, recombinant BCG.

4. TB Vaccine Candidates in Clinical Phase

At present, many new TB vaccines have been developed in clinical studies. The current state of new TB vaccine candidates is shown in Table 2 and Figure 1.

Figure 1. Clinical phases of current TB vaccine candidates that adapted from AERAS website. Available www.aeras/org/pages/global-portfolio (accessed date: 21 October 2019). BCG, Bacillus Calmette-Guérin; MIP, *Mycobacterium indicus pranii*; TB, tuberculosis.

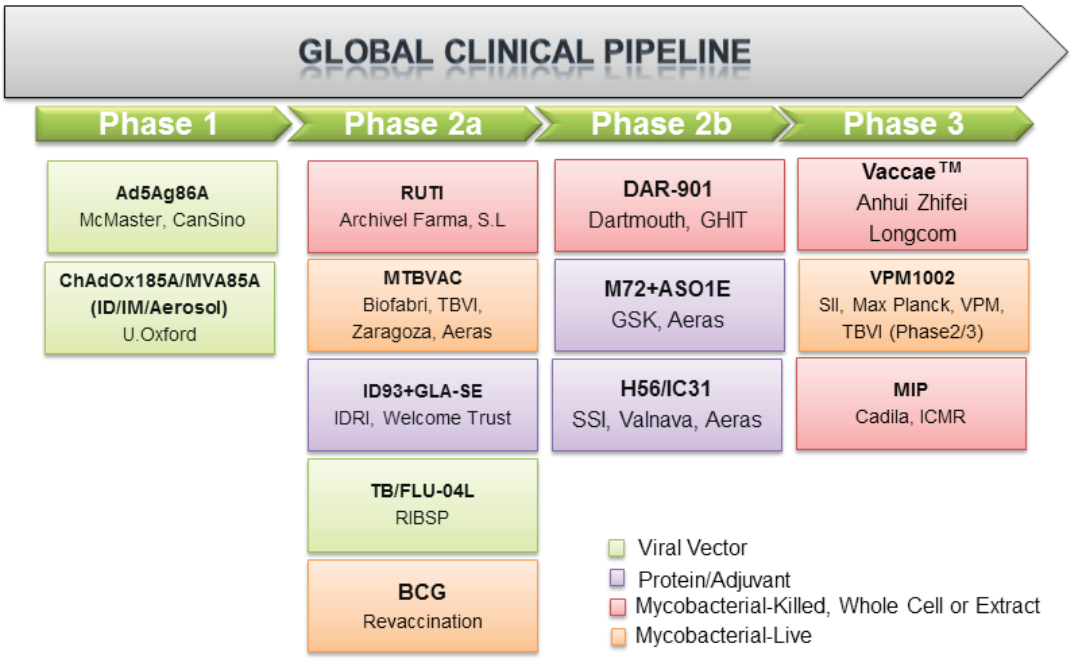

Table 2. Current novel TB vaccines in clinical trials against TB

TB vaccine name	Vaccine Type/Strategy	Clinical trial status	Reference
Ad5 Ag85A	Viral-vectored vaccine/Prime boost	Phase I	Tang et al. 2016
ChAdOx1 85A + MVA85A	Viral vector/Prime boost	Phase I	Tameris et al. 2013
RUTI	Mycobacterial-whole cell or Extract / Fragmented MTB	Phase IIa	Vilaplana et al. 2010
MTBVAC	Live-attenuated vaccine/Priming vaccine	Phase IIa	Clark et al. 2017
ID93 + GLA-SE	Adjuvanted subunit/Prime-Boost	Phase IIa	Penn-Nicholson et al. 2018
TB/FLU-04L	Viral vector/Prime booster	Phase IIa	Safrit et al. 2018
DAR-901	Mycobacterial-whole cell or Extract/ Prime booster	Phase IIb	Panga et al. 2016
M72F:AS01	Protein and adjuvant/prime boost	Phase IIb	Van Der Meeren et al. 2018
Hybrid 56 + IC31	Adjuvanted subunit/Prime-Boost	Phase IIb	Luabeya et al. 2015
Mycobacterium vaccae	Mycobacterial-whole cell or Extract / Boost, Post infection, immunotherapy	Phase III	Hawkridge et al. 2011
VPM1002	Recombinant live/Priming vaccine	Phase III	Nieuwenhuizen et al. 2017
MIP (*Mycobacterium indicus pranii*)	Heat-killed whole *Mycobacterium indicus pranii*	Phase III	Kamal et al. 2016

4.1. *Phase-I TB Vaccine*

Ad5Ag85A

The Ad5Ag85A vaccine is primed with non-replicating viral adenovirus serotype 5 (Ad5) vector expressing *M. tuberculosis* antigen 85A (Usman et al., 2017). Preliminary studies showed that the Ad5Ag85A vaccine candidate protected for TB when it is intranasally administered like BCG booster vaccine in mice. In phase-I trial of this vaccine, safety, immunogenicity and tolerability of vaccine evaluated and found to be safe and well-tolerated. Also this vaccine was capable of inducing polyfunctional $CD4^+$ and $CD8^+$ T cells immunity in BCG-primed young people (Tang et al, 2016). However, neutralization of the antigen with Ad5 antibodies existed is the major difficulty in performing clinical trials with the Ad5 vector-based vaccine (Orme et al., 2015, Khoshnood et al., 2018). In the circumstances, the antigen is removed from the tissues without providing the necessary immunity and memory cells after vaccination (Soundarya et al., 2019).

MVA85A

The viral-vector subunit TB vaccine MVA85A expresses immunodominant mycobacterial antigen 85A (Ag85A) (Dockrell et al., 2016). BCG boosted with the MVA85A vaccine induces cellular immunity. In preclinical studies, the MVA85A vaccine indicated that it can improve protective efficacy against TB in mice. In the phase-I clinical studies, intradermal and aerosol formulations of the vaccine are experimented (Khoshnood et al. 2018). When the vaccine is administered as an aerosol, cellular immunity has been induced better than the intradermal route. The immune responses generated against this vaccine are variable in studies performed in different individuals groups. Furthermore, this vaccine did not demonstrate a protective effect against TB in a phase-IIb clinical trial (Tameris et al., 2013). Currently, phase-I clinical trials are continued with both intradermal and aerosol formulations of the vaccine in the United Kingdom (Frick et al., 2015).

4.2. *Phase-IIa TB Vaccine*

RUTI

RUTI, is a liposomal vaccine produced with detoxified, fragmented *M. tuberculosis* cells. This vaccine is being developed as a therapeutic vaccine for adults. RUTI is designed to shorten the chemotherapy treatment for both LTBI and TB disease. A strong cellular and humoral immune response are generated against *M. tuberculosis* bacilli after the vaccine administration. A phase-I trial of RUTI study demonstrated specific immune responses against *M. tuberculosis* antigens (Vilaplana et al., 2010). In a phase-II trial RUTI was found safe and was indicated that is trigged polyantigenic responses after a short course of chemotherapy administration (Cardona et al., 2006). In recent a study, efficacy of RUTI was found as similar to the BCG vaccine (Vilaplana et al., 2011).

MTBVAC

MTBVAC is constructed by attenuation of the *M. tuberculosis* clinical isolate Mt103. Both the more reliable and impressive vaccine is generated by the deletion of phoP and fadD26 virulence genes existed on the genome of *M. tuberculosis* clinical isolate Mt103 (Clark et al., 2017; Khoshnood et al., 2018). In extensive preclinical studies, MTBVAC showed safety comparable to BCG, with superior immunogenicity and efficacy against TB bacilli in mouse and guinea pig models. When MTBVAC is applied as a booster vaccine to BCG, it supplied a longer-lasting immunity in a guinea pig model (Arbues et al., 2013; Clark et al., 2017). In a phase-Ia study, MTBVAC demonstrated the immunogenicity in without BCG-vaccine adults living in TB non-endemic regions (Spertini et al., 2015). Then, a dose-escalation study is ongoing in newborns in an endemic TB area of South Africa. Dose defining safety and immunogenicity study of MTBVAC, a phase-IIa is planned in 99 newborns non-exposed to HIV in TB-endemic regions of sub-Saharan Africa. Additionally, to evaluate dose-escalation safety and immunogenicity of MTBVAC vaccine is planned as randomized, double-blind, controlled phase-IIa study in 120 healthy South African adults with and without LTBI (Schrager et al., 2019).

ID93/GLA-SE

ID93 is a fusion protein composed of four immunogenic *M. tuberculosis* proteins. Each protein is separated into different categories: Rv2608 is within the PPE protein family outer membrane-associated (Rv2608), while Rv3619 and Rv3620 are the EsX protein family of secreted virulence factors. The other protein Rv1813 is up-regulated under hypoxic conditions. Rv1813 is associated with the latent growth of *M. tuberculosis* whereas the other proteins express the virulence of TB bacilli. GLA-SE is a synthetic adjuvant formulated in squalene oil. Four *M. tuberculosis* fusion proteins and GLA-SE adjuvant are combined. GLA-SE induced antigen-specific Th1 immune responses in mice (Penn-Nicholson et al., 2018). ID93/GLA-SE increased the production of polyfunctional T cell responses in BCG-vaccinated or non-BCG-vaccinated mice and guinea pigs (Baldwin et al., 2012). To evaluate the safety and immunogenicity of ID93/GLASE candidate vaccine, Phase-I and phase-II clinical trials have been completed in healthy adults in both the United States and South Africa. In these studies, ID93/GLASE induces a broad polyfunctional T-cell response, and there is an enhancement in multifunction antibodies (Penn-Nicholson et al., 2018). In phase-II trial, ID93/GLA-SE provided long-lived immunity due to an increase of CD4+ T-cell responses in humans (Panga et al., 2016). Several new phase-II studies related to the safety, immunogenicity, and efficacy of ID93/GLA-SE for prevention of TB infection is planned with high-risk health care workers in Korea.

TB/FLU-04L

Recombinant vaccine TB/FLU-04L, expressing Ag85A/ESAT6 proteins of TB bacilli has developed by using replication-deficient influenza virus strain A/Puerto Rico/8/34 H1N1 (Safrit et al., 2016). In a phase-I trial study performed with TB/FLU-04L vaccine, increasing antigen-specific IFN-γ response has been demonstrated in experimental animals (mice and cynomolgus monkeys). The vaccine was well-tolerated without serious adverse events. When the vaccine tested as a nasal spray, nasal cytokines (IL-1b, TNF α, and IL2) were detected after vaccination (Barry et al., 2016). After phase-I trials with TB/FLU-04L designed as a mucosal boost vaccine completed, a phase-II clinical trial is currently planned for this vaccine candidate (Soundarya et al., 2019).

4.3. *Phase-IIb TB Vaccine*

DAR-901

DAR-901 is a heat-inactivated, whole-cell preparation derived from *Mycobacterium obuense. M. obuense,* non-tuberculous mycobacterium (NTM), has identical multiple antigens with *M. tuberculosis*. Thus, this vaccine candidate may provide cross-protection against TB. DAR-901 is developed to be a booster vaccine for the prevention of TB infection in adolescents and adults. In a phase-I study, the safety and tolerability of the DAR-901 were displayed among adults with BCG (Panga et al., 2016). Also, assessment of tolerability and immunogenicity of DAR 901 at different doses has been accomplished in HIV-infected and HIV-uninfected adults who received the BCG vaccine (Khoshnood et al., 2018). The results indicated that DAR-901 induced a Th1 immune response and boosted protection against TB. Phase-IIb trial to prevent TB among adolescents is underway in Tanzania (Soundarya et al., 2019).

M72/AS01E

M72/AS01E is another subunit vaccine consisting of Mtb39A and Mtb32A antigens of *M. tuberculosis.* Two immunogenic proteins are incorporated with the liposome-based AS01 adjuvant system (Van Der Meeren et al., 2018). Mtb39a and Mtb32a proteins can stimulate peripheral blood mononuclear cells (PBMCs) again in healthy individuals who are positive for Tuberculin Skin Test. A membrane-associated protein, Mtb39a, and a fundamentally expressed protein and a serine protease, Mtb32a were selected by cell antigen screening. Both of them induce Th1 responses.

They are only expressed in *M. tuberculosis* and BCG but not in other mycobacteria (Schrager et al., 2019). Phase-I and phase-II trials have been completed to test the safety and immunogenicity of this vaccine. Overall, M72/AS01E was well-tolerated and induced a cell-mediated and humoral immune response in the recruited populations (Leroux-Roels et al., 2013). A phase-IIb study of the M72/AS01E candidate vaccine has been carried out in HIV uninfected adults in clinics in South Africa, Kenya, and Zambia (Van Der Meeren et al., 2018).

H1/H56: IC31

Subunit adjuvant vaccine, H1/H56:IC31 developed as an adjuvanted fusion protein of three immunogenic *M. tuberculosis* antigens. Ag85B, 6-kDa early secretory antigenic target (ESAT-6) and Rv2660c formulated with the IC31 adjuvant (Luabeya et al., 2015). After TB bacilli are phagocytosed by macrophages at the beginning of infection, both Ag85B, and ESAT-6 antigens of *M. tuberculosis* are thought to be important for the survival of bacteria. When H1/H56:IC31 candidate vaccine administered either priming or booster, it was indicated highly immunogenic in preclinical vaccine studies that were done with mice and guinea pigs (Kamath et al., 2008). Phase-I and phase-II studies were completed for safety and immunogenicity in adults and adolescents that cured the active TB. In these studies, H1/H56:IC31 has been proved that it is a dependable vaccine. Now, a phase-IIb trial of H1/H56:IC31 is planned to test for the prevention of TB infection and prevention of the risk of relapse in previously treated TB patients (Schrager et al., 2019; Soundarya et al., 2019).

4.4. *Phase III TB Vaccine*

Mycobacterium vaccae

Mycobacterium vaccae is an environmental saprophyte mycobacterial species found in the soil. This mycobacterium is a rapidly growing species that is estimated to have immunogenic properties increasing the host's immune response. Initially, *M. vaccae* vaccine is improved as an immunotherapeutic vaccine rather than a preventive vaccine by using heat-killed preparations of *M. vaccae* strain. The safety and immunogenicity of *M. vaccae* vaccine were demonstrated in HIV-infected adults previously vaccinated with BCG in Phase-I and II clinical trials performed in Finland, and Zambia (Vuola et al., 2003). This vaccine was also found as safe and immunogenic and to ensure prominent protection against TB infection in phase-III clinical trial in Tanzania (Von Reyn et al., 2010). While the vaccine protects against the disease in some geographical settings, it may not ensure in the other places. This inconsistency is a major drawback of *M. vaccae* (Usman et al., 2017).

VPM1002

Another recombinant BCG vaccine VPM 1002 is being evaluated either replacement for BCG vaccination for newborn or prevention of TB in adolescents and adults (Nieuwenhuizen et al., 2017). In VPM1002, a listeriolysin (hly) encoding gene from the facultative anaerobic bacterium *Listeria monocytogenes* was added to the BCG genome and the gene encoding urease-C (ureC) was deleted. (Montagnani et al., 2014). Perforation of the phagosomal membrane by listeriolysin allows the release of recombinant antigens into the cytosol of the host. Also, the loss of ureC gene provides an acidic pH of 5.5 inside the phagosome for the optimal listeriolysin function (Orme et al., 2013). By gene modification, microbial antigens are released into cytosol with better CD8+ T-cell stimulation (Hesseling et al., 2007). In early clinical trials, VPM1002 tested in adults with BCG was found as safe and immunogenic in a phase-Ib trial in South Africa. In recent years, a phase-II trial has been concluded for assessment of the safety and tolerability of the vaccine in HIV-exposed and HIV-unexposed newborns in sub-Saharan Africa. To assess of the immunogenicity and safety of VPM1002 in 10,000 South African infants, a phase-III trial is programmed to begin in 2019 and it is planned to complete in 2021 (Schrager et al., 2019).

Mycobacterium indicus pranii

Mycobacterium indicus pranii (MIP) (also known as Mycobacterium w.) is a heat-inactivated non-tuberculosis mycobacterial vaccine for patients undergoing chemotherapy (Gupta et al., 2012). MIP contains antigens similar to *Mycobacterium leprae* (Gupta et al., 2012). In phase-II study of MIP has been shown to have immunotherapeutic influence on both leprosy and TB diseases (Kamal et al., 2016). Currently, two phase-III trials were conducted to test the efficiency and safety of MIP in pulmonary TB patients. In these studies, MIP was found that it does not have adverse effects and it plays a significant role in the elimination of the mycobacterium (Sharma et al., 2017).

Conclusion

A safe, efficient, and affordable vaccine is one of the most essential ways of protection against many infectious diseases. The improvement of new and effective TB vaccines is inevitable to control and end to TB. Especially, it is an urgent need to prevent the spread of drug-resistant strains of *M. tuberculosis*. To find a new, more effective vaccine as a booster vaccine for BCG or to replacement of BCG with a better alternative is a priority of the TB vaccine research. Recently, this research has been focused on the recombinant vaccine production using both BCG and *M. tuberculosis* strains. New TB vaccines are developed as preventive, post-exposure, and even therapeutic. In the past several years, tremendous progress has been made in clinical trials with several vaccine candidates. Although TB is considered as a poverty-related disease, it still occurs in developing countries, or among those of high socioeconomic status. Therefore, the expectation is the development of better, more protective TB vaccines in a short span of time. Without new TB vaccines, TB will neither be controlled nor eliminated.

Conflict of Interests

Author declares no conflict of interests.

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Instruction for Authors

Aims and Scope

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The Aurum Journal of Health Sciences publishes *research articles, short reports, reviews, case studies* on all aspects of health sciences both in electronic and printed versions. Authors are encouraged to provide proofs of their research results and/or ethical committee approvals as a supporting material which will be published electronically separately with the article.

1. *Research articles:* The manuscripts that are describing findings of an original research in regard to all aspects of health sciences will be published as a "*Research article*". The research articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5.

Introduction; 6. Materials and Methods; 7. Results 8. Discussion; 9. Conclusion; 10. Acknowledgement; 11. Conflict of Interests; 12. References. Manuscripts submitted as a "*Research article*" do not have a wording limit. However, manuscripts that are submitted as "*Research article*" should be more than 5000 words, excluding the tables, figures and references.

2. *Short Reports:* The manuscripts that are describing preliminary findings obtained from an original research or/and results of pre-study performed on a topic in regard to all aspects of health sciences will be published as a "*Short report*". The short report articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Materials and Methods; 7. Results; 8. Discussion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Short report*" should not exceed 5000 words excluding the tables, figures and references.

3. *Reviews:* The manuscripts that are describing critical evaluation of the current situation in the literature and providing future prospects according to current knowledge on a topic in regard to all aspects of health sciences will be published as a "*Review*". The reviews should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Contents; 6. Introduction; 7. Sub-Topics Provided in Contents; 8. Conclusion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Review*" should not exceed 10,000 words excluding the tables, figures and references.

4. *Case studies:* The manuscripts that are describing a critical evaluation of an observed clinical cases in dentistry, medicine and clinical pharmacy will be published as a *"case study"*. The case studies should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Description of the Case; 7. Discussion; 8. Acknowledgement; 9. Conflict of Interests; 10. References. Manuscripts submitted as a "*Case report*" should not exceed 5000 words excluding the tables, figures and references. A written consent of the patient may be required if the case report contains images taken from the patients. All the case reports must contain ethical committee approvals.

Preparation of manuscript and general rules

The manuscripts should be written double spaced in Arial font type and 12 pts font size. Each page should be numbered, and consecutive line numbers should be provided. Title page, authors list and affiliations should be prepared as a separate file. Tables and Figures should also be prepared as a separate file.

Title Page: The title page should contain the full title of the work which should not exceed 200 characters. Abbreviations should be avoided in the title. Main title of the manuscript should be followed by the "*short title*" which should not be longer than 70 characters. Short title should be followed by the list of author names. Author names should be given as name and surname followed by superscript Arabic numbers indicating the affiliations. One author should be designated as the corresponding author and should be indicated in the authors list with the superscript asterix symbol after the affiliation indicator. Author list should be followed by the list of affiliations which indicate the department, institution, postal code, city, country and e-mail(s) of the author(s). Finally, corresponding author full mailing address, telephone, fax and e-mail should be provided. Acknowledgement and Conflict of Interests parts should be given in the title page.

Main text: Main text should be divided into sections and sub-sections using Arabic numerals, starting from the introduction part. Sections should be indicated with bold and non-italic characters. Sub-sections should be indicated with bold and italic characters (as given in example).

Section Example: 1. Introduction

Subsection Example: 2.1. *GC-MS Analysis*

First page of the main text should contain the title followed by a 300-word abstract. Abstract should not contain citations. Abbreviations could be used in the abstract however; full explanation of the abbreviations should be given at the first time that they have appeared in the abstract. Abstract should briefly summarize the study. Abstract should contain the following information: 1. Purpose/Aim of the study; 2. Materials and methodology used in the study; 3. Key results obtained in the study; 4. Conclusion remarks. Abstract part should be followed by 6 keywords that describe the work. Keywords should be separated from one another with a semicolon.

Depending on the type of article following parts should be given in the main text. Introduction part in the manuscript should contain a brief explanation of previous studies, aim of the current study and reasoning of the study. Materials and Method part should be given in full detail allowing replication of the performed experiments/clinical studies/technical studies by other scientists. In materials and methods section all the instruments, chemicals used in the study should be explained by their brand and model. Results, should be described without any comments. Discussion and conclusion parts should not contain any speculations. A clear and concise discussion and conclusion remarks should be given.

Acknowledgement

Authors should indicate any acknowledgement related to the study in this part.

Conflict of Interests

Authors should clearly indicate any kind of conflict of interests for the study in this part. If the authors do not have any conflict of interests, they should indicate "Authors declare no conflict of interests".

Tables & Figures

The authors should indicate the position of Tables and Figures in the text by indicating the Title of the table (as given in the example). All figures should be provided as a tiff file with at least 300 dpi resolution. The images given as figures should be authentic, no manipulations should be done. Color figures are welcome in the journal and does not require a publication fee.

Example: Figure 1. ^{1}H-NMR spectrum of *Ulubelenolide*.

The figures and tables should be given as a separate file. Each table and figure given should contain a title and if required footnotes should be given. Each figure and table given should be self-explanatory.

Reference Format

Citation of references in the text

Authors must check their manuscript that every reference cited in their text should also be in the given reference list and every reference listed should be in cited in the text. Citations of unpublished results and personal communications should be avoided. Citations of literatures that were accepted by a journal and which have doi number, issue and page numbers could be cited in the text however, authors should indicate that this work is "*in press*". The citations of the web pages should be avoided. The citations in the text should adhere to the following style.

Cited reference which have a single author: (author's last name, year of publication)

Example: (Biyikoglu, 2017)

Cited reference which have two authors: (last name of the first author and second author, year of publication)

Example: (Biyikoglu and Polatoglu, 2017)

Cited reference which have three authors or more: (last name of the first author et al., year of publication)

Example: (Polatoglu et al., 2017)

Cited references which have the same first author(s) that were published in the same year: (last name of the author, year of publication and uncapitalized letters for separation)

Example: (Biyikoglu, 2017a; Biyikoglu 2017b)

Cited references as lists: The references that are going to be given as a list in a single parentheses should be first arranged alphabetically than chronologically.

Example: (Biyikoglu 2017a; Biyikoglu 2017b; Polatoglu et al., 2013)

Cited references given in text: If author names are going to be mentioned in the text for the citation than it should be given as: "….Polatoglu et al. (2013)……"

Examples: "……..Polatoglu et al. (2013) have indicated….

"……Biyikoglu (2017) demonstrated that……"

Reference formatting

The reference formatting should be given according to the following style (APA). DOI numbers should be given after the reference if available.

Reference style

Reference to a journal publication:

Polatoglu, K., Demirci, F., Demirci, B., Gören, N., Baser, K. H. C. (2010). Antibacterial activity and the variation of Tanacetum parthenium (L.) Schultz Bip. essential oils from Turkey. Journal of Oleo Science, 59(4), 177-184. https://doi.org/10.5650/jos.59.177

Reference to a book:

Preedy, V. R. (Ed.). (2015). Essential oils in food preservation, flavor and safety. 1st Ed., Academic Press, Elsevier, Oxford, UK.

Reference to a chapter in an edited book:

Polatoğlu, K., Karakoç, Ö. C. (2015). Biologically Active Essential Oils against Stored Product Pests. 1st Ed., In Preedy, V.R. (Eds.), Essential Oils in Food Preservation, Flavor and Safety. Academic Press., Elsevier, Oxford, UK, pp. 39-59.

Reference to a website:

National Cancer Institute, A success storyTaxol® (NSC 125973) https://dtp.cancer.gov/timeline/flash/success_stories/s2_taxol.htm (accessed 14 December 2017)

Reference to a Thesis:

Knight, K.A. (2011). Media epidemics: Viral structures in literature and new media (Doctoral dissertation).

Abbreviations

Full explanation of the abbreviations should be given at the first time that they have appeared in the text. Title should not contain any abbreviations. After the explanation of the abbreviations are given in the text authors could use abbreviations throughout the text.

Example: "…..Acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes were ……."

Chemical and Biological Nomenclature

The names of the biological organisms should be given in full of the author name at the first time they appear in the text. The genus and species names should always be written in italics. Authors could use the short name of the organism after the full name was indicated. Local names of the organisms could be mentioned however, throughout the manuscript these organisms should be referred to with their binominal names.

Chemical compounds should be preferably named according to the IUPAC nomenclature.