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Fusion of teeth – Heredity, Trauma, or still a Dilemma?

*Halasagundi V.S., Tegginamani A.S., Hesarghatta P.R.,
Fernandes B.A., Rath A.*

Abstract

Fusion is a dental anomaly that arises through the union of two adjacent teeth. The etiology may be attributed to evolution, trauma, heredity, and environmental factors. Tooth germs in the same developmental stage and located close to each other are also found to have a high chance of undergoing fusion. Early diagnosis of the anomaly has a considerable importance. It should be followed by careful clinical and radiographic observations to prevent complications such as pulpal, periodontal, aesthetic concerns, space problems, oral hygiene maintenance, occlusal disturbances and delayed eruption of the permanent successors. The present case report describes one such case of fusion in lower anterior region in a nine year old girl.

Keywords: Fusion, Dental anomaly, Gemination, Tooth germ

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Introduction

Fusion is a dental anomaly caused due to union of two normally separated tooth germs (Jain et al., 2014). This anomaly generally leads to alterations in number of the teeth, as the union results in one tooth less than normal (Faria, 2011). Dental fusion may be total or partial based on the stage in which it happens. Fusion occurring during the calcification stage will be partial, in which case the tooth will have the size of two crowns fused together, a bifid crown, or both. Whereas a total fusion occurs, if it is at the early stages of development before calcification begins, resulting in a normal sized or slightly bigger than normal tooth (De la Hoz Calvo, Beltri & Chung-Leng, 2014). Although the exact etiology of fusion is unclear, pressure or physical force producing close contact between two developing tooth buds has been reported as a possible cause. Trauma, genetic and environmental factors have been implicated as contributing factors (Tuna, 2009; Schuurs, 2000). Fused teeth may also be formed as part of syndromes such as achondrodysplasia, chondroectodermal dysplasia, focal dermal hypoplasia, and osteopetrosis (Crawford, 2006). It is very important for health professionals to be familiar with dental developmental anomalies as these abnormal morphology demands prophylactic and early interceptive treatment in order to avoid the complications like pulpal problems, aesthetic concerns, space problems, oral hygiene maintenance, occlusal disturbances, and delayed eruption of the permanent successors. The present case reports fusion of lower deciduous anterior in a 9 year old girl.

Case Presentation

A 9-year-old girl reported to the paediatric dental clinic with the chief complaint of the unpleasant appearance due to presence of a large anterior tooth in lower jaw. Her medical history was not significant. There was no family history of dental anomalies, and there was no history of previous trauma to the teeth or jaws.

No abnormality was detected on extra oral examination. Intraoral examination revealed a large mandibular right lateral incisor, with a small groove observed on the labial and palatal aspects of the crown (Figure 1). Response to pulp testing was normal, no caries was detected in that tooth, and no pockets were present. The patient was in mixed dentition stage and had crowding in the anterior region due to a lack of space. Dental caries was observed on 85 and 84 was grossly decayed. Oral hygiene was fair with moderate amount of plaque and extrinsic stains. Also the mandibular arch showed one tooth lesser than the maxillary arch. The case was provisionally diagnosed as Fusion of 82 and 83.



Figure 1: showing fused lower right deciduous lateral incisor and canine

Intra Oral Peri Apical Radiograph (IOPAR) of that region revealed that the root of 82 had two pulp chambers, which confirmed the diagnosis for fusion of teeth. IOPAR also revealed root resorption of the fused teeth, and permanent tooth buds were also seen (Figure 2). The other oral structures were normal. No other anomalies were found.



Figure2: IOPAR showing two pulp chambers and two root canals irt 82.

The diagnosis was confirmed as Fusion irt 823.No further investigations were carried out. Patient was explained that no treatment was required as the teeth would be exfoliated shortly and would be replaced by permanent teeth.

Discussion

This report demonstrates a case of fusion of deciduous lateral incisor and canine. The clinical examination (crown morphology and number of teeth), as well as the radiographic examination, suggests it is a case of fusion and not germination, as there was one tooth less than normal in the mandibular arch. Tooth buds may fuse or geminate during their

development. The etiology of double teeth may be attributed to evolution, trauma, heredity, and environmental factors (Tuna et al., 2009). Tooth germs in the same developmental stage and located close to each other are found to have higher chances of fusion, resulting in double teeth (Veerakumar, Pari & Prabhu, 2011). Some researchers have proposed that this alteration occurs as a result of physical forces that bring the developing teeth in contact, by causing necrosis of the epithelial tissue which usually separates them and finally leading to fusion. Other school of thought says that fusion results from embryological persistence of the inter-dental lamina between two germs (Rakesh et al., 2012). Clinically, the crowns appear as joined together, with a small groove between the mesial and distal parts. Fused teeth may be characterized by one pulp chamber divided into two root canals, two independent endodontic systems, or one common pulp canal (Reeh & ElDeeb 1989; Hülsmann, Bahr & Grohmann, 1997).

While Fusion results from two separate tooth germs joining together during formative periods, Gemination results from cleavage of a single tooth germ. If there is union by enamel and dentin it is called true fusion, and union by dentin and/or cementum is called a concrescence (Jain et al., 2014).

The literature suggests fusion has a higher incidence in deciduous dentition (0.5%–2.5%) than in permanent dentition (0.1%–1.0%) (Ahmet et al., 2011; Schulze, 1970; Veerakumar, Pari & Prabhu, 2011). Fused teeth are found predominantly in the anterior region, with incisors and canines the most frequently affected (Penumatsa et al.,

2011; Saxena, Pandey &, Kamboj, 2008), as in this present case. Fusion of teeth appearing in anterior region can cause aesthetic problems including diastema, crowding and protrusion. In the present case, as the fusion was between the lateral incisor and canine, the major problem was esthetics. However, no specific treatment for the fused tooth was carried out as it was deciduous dentition and close to the time of exfoliation. However, the patient was recalled after 3 months for a follow up as the adjacent molar was carious and the underlying permanent tooth was about to erupt.

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Non-surgical management of Amlodipine induced Gingival overgrowth: A short review and a case report

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Abstract

Drug-induced gingival overgrowth (DIGO) remains a potential area of concern for the dental practitioners certain group of drugs may be implicated in this unwanted side effect, which may interfere with esthetics, mastication or speech. Among the calcium channel blockers (CCBs), Nifedipine administration has been most frequently associated with medication-related gingival overgrowth. Amlodipine, a second-generation dihydropyridine CCB, can also cause gingival hypertrophy. Here, we present a case of Amlodipine-induced gingival overgrowth in a 60-year-old male patient. A comprehensive oral health care regimen with non-surgical periodontal therapy and substitution of the drug along with oral hygiene reinforcement resulted in improvement of this condition without surgical intervention.

Keywords: Amlodipine, Calcium channel blockers, Drug induced gingival overgrowth, Non-surgical periodontal therapy, Pathogenesis of gingival overgrowth

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Introduction

Drug-induced gingival overgrowth is a histomorphological alteration due to the side effect of medication on the extracellular matrix (Trackman & Kantarci, 2015). It manifests as an abnormality caused by adverse drug reaction in patients under anticonvulsants, immunosuppressants, and calcium channel blockers (Murat et al., 2011). Increase in gingival size is a common feature of inflammatory gingival condition as well. Gingival overgrowth, being the most accepted terminology, as it is strictly a clinical descriptive term to avoid the erroneous pathologic connotations of terms used in the past, such as hypertrophic gingivitis or gingival hyperplasia (Sinha et al., 2014).

Drug-induced gingival overgrowth as a possible side effect has been reported with systemic use of anticonvulsants, immunosuppressants and calcium channel blocking agents (Trackman & Kantarci, 2015). There are also rare case reports on gingival overgrowth associated with erythromycin, trimethoprim and sulphamethoxazole (Valsecchi & Cainelli, 1992). Among the calcium channel blockers, gingival overgrowth is most commonly associated with Nifedipine and with other drugs of the same family like Amlodipine, Verapamil, Nicardipine, Nitrendipine, Oxodipine, Felodipine and Diltiazem (Jose et al., 2011). Amlodipine, a newer agent of dihydropyridine, used for treatment of hypertension and angina, was first reported for causing gingival overgrowth as a side effect by Seymour et al in 1994.

Pathogenesis of Drug induced gingival overgrowth

Drug-induced overgrowth has been associated with a patient's genetic pre-disposition (Bharti, 2012). Drugs causing this differ at the primary tissue target, but these drugs act similarly on the secondary target tissue cell, which is the connective tissue and causes gingival hyperplastic response. These drugs have the mechanism of action of inhibiting influx of calcium ion at cellular level. There are investigations done to show that drug induced gingival overgrowth are multifactorial (Figure 1) (Seymour et al., 1996). There are suggestions of increase in plaque eventually leading to gingival inflammation, which will exacerbate the overgrowth. Therefore, it is important to recognize dental plaque as a cofactor that causes gingival overgrowth.

Earlier studies had reported rapid development of gingival overgrowth in patients who received 10 mg per day of Amlodipine within two months of onset (Madi et al., 2015). Clinical manifestation of gingival overgrowth frequently appears within one to three months after initiation of treatment with the associated medication (Bharti & Bansal, 2013).

There is less data on reports of overgrowth with amlodipine at a dose of 5 mg, even after taking for more than six months (Madi et al., 2015). This report outlines a case of gingival over growth, which was induced by Amlodipine, rather than the more common CCB drug, Nifedipine, in the presence of plaque and its non-surgical periodontal management.

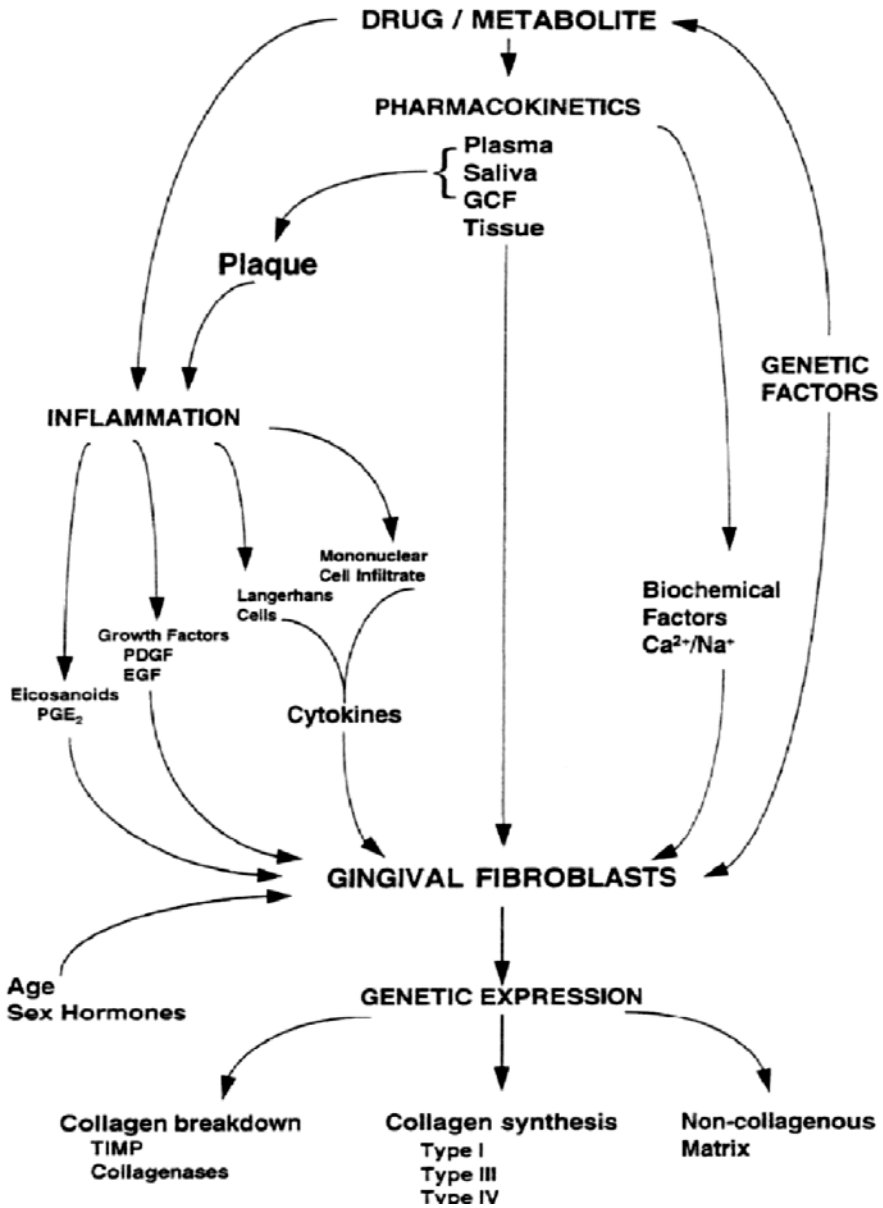


Figure 1. Schematic diagram to illustrate the potential multifactorial features and interactions involved in the pathogenesis of drug-induced gingival overgrowth (Seymour et al. 1996)

Case Presentation

A 60-year-old male patient came to SEGi University Oral healthcare center with the chief complaint of having bad breath and swollen gums which was present in upper and lower front region. Patient also complained of having bleeding gums upon brushing and pain associated with it. (Figure 2). Periodontal condition of the patient was poor with two teeth having Grade II mobility. Generalized gingival recession with clinical attachment loss (CAL) of 4-5mm was also recorded. Medical history revealed that the patient was known hypertensive since 2005 and was under Amlodipine 5mg, once daily, since then. Patient didn't suffer from any other systemic disease. Intraoral examination revealed that overgrowth originating from marginal, interdental and attached gingival area mostly around the maxillary and mandibular anterior teeth. The overgrowth was soft and edematous in consistency.

Slight pain and bleeding upon probing were presented at the hypertrophied areas. Inflammatory component of the gingival overgrowth was contributed mainly by the presence of plaque and calculus. Patient's physician was then consulted regarding the change of drug. The physician substituted amlodipine 5mg to hydrochlorothiazide 25mg. Treatment for the patient was complete debridement of local factors (scaling , polishing and root planing) along with oral hygiene instructions and was prescribed 0.12% Chlorhexidine mouthrinse twice daily for 15 days. Patient was then recalled for a reevaluation after 1 week and follow up visits were

scheduled at 1 month after the change of drug (Figure 3), 2 months (Figure 4), 3 months (Figure 5) and 12 months (Figure 6).



Figure 2. Preoperative front and side view showing gingival overgrowth



Figure 3. Re-evaluation post 1 month after drug replacement, front and side view



Figure 4. Re-evaluation post 2 months after drug replacement, front and side view



Figure 5. Re-evaluation post 3 months after drug replacement, front and side view



Figure 6. Re-evaluation post 12 months after drug replacement, front and side view

On review and follow up of 12 months post the non-surgical therapy, there was significant decrease in gingival overgrowth clinically along with improvement of oral hygiene.

Discussion

Drug induced gingival overgrowth remains the most widespread side effect of systemic medication on the periodontal tissues. Currently, more than 20 prescription medications are associated with gingival enlargement (Triveni et al., 2009). Despite their popularity and wide acceptance by the medical community, the oral impact of CCB therapy is rarely recognized or discussed. CCBs, as a group, have been

frequently implicated as an etiologic factor for a common oral condition seen among patients seeking dental care: drug-induced gingival overgrowth (Livada & Shiloah, 2014). Several factors namely; age, genetic predisposition, presence of preexisting plaque, and gingival inflammation influence the relationship between the drugs and gingival tissue. There is a variable gingival response in patients taking drugs based on which they are categorized as “responders” and “non-responders” (Seymour et al., 1996, 2000). Furthermore, within the group of patients that develop this unwanted effect, there appears to be variability in the extent and severity of the gingival changes.

The most common calcium channel blocker that causes gingival overgrowth are the dihydropyridones such as Nifedipine than any other subgroups of calcium channel blockers such as verapamil, diltiazem, felodipine or amlodipine. However, amlodipine has a distinctive physiochemical profile characterized by having near complete absorption, late peak plasma concentrations, high bioavailability and slow hepatic biodegradation. There is also slow elimination of this drug which cause a long duration of its action, which means that only a single dose is required. This will eventually lead to a similar or reduced severity of side effects compared with Nifedipine (Triveni et al., 2009).

The prevalence of gingival overgrowth on patient who are taking Nifedipine and Amlodipine were reported ranging between 20-83% and 3.3% respectively (Gopal et al., 2015). As compared to that the occurrence rate for amlodipine gingival overgrowth is very rare. There

is increased risk of gingival overgrowth on patient who are on Nifedipine as compared to patient who are taking Amlodipine, and the difference between these 2 drugs are of interest. As Amlodipine is more polar with the pKa values of 8.7 than Nifedipine which is lipophilic and will dissolve within the cell membrane and pass into the cytoplasm. While the etiology of DIGO is said to be multifactorial, the drug cellular interaction is also crucial in the pathogenesis of this effect (Trackman & Kantarci, 2015).

The treatment should be individualized due to the medication used and clinical appearance of the individual case. Initial treatment plan should be change of the offending drug or eliminating it. Either way the decision should be taken after consulting the patient's physician. The primary aim of nonsurgical approaches is to reduce the inflammatory component in the gingival tissues and thereby avoid the need for surgery. Meticulous removal of plaque on a frequent basis helps in the maintenance of attachment levels. Patients at risk from, or who have developed drug-induced gingival overgrowth will benefit from effective oral hygiene measures, professional tooth cleaning, scaling, and root surface instrumentation. For some patients these measures alone could reduce the gingival overgrowth to acceptable levels, for others, it could make surgical correction easier (Mavrogiannis et al., 2006).

There is substantial evidence that states that good oral hygiene and frequent professional removal of plaque and calculus decreases the degree of gingival overgrowth and improves gingival health and prevent recurrence (Dhale & Phadnaik, 2009; Srivastava et al., 2010).

To date there has been no clear-cut literature which could consolidate the pathogenesis, clinical manifestations, and the management of affected patients (Bharti & Bansal, 2013).

Conclusion

The management of drug induced gingival overgrowth depends on the change to an alternate drug and patient cooperation. Additionally, dental surgeons should be able to identify the changes in the oral cavity related to the general health of their patients. Patients must be informed of the tendency of certain drugs to cause gingival overgrowth and the associated oral changes and the importance of effective oral hygiene.

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Accommodative lag with multifocal soft contact lenses

Barodawala F. S.¹, Thacker N.²

Abstract

Accommodative lag has been reported to be one of the factor responsible for myopic progression. This research was conducted to study the effects of centre near design of multifocal soft contact lenses (MFCLs) on accommodative lag in young myopic adults. Sixteen (16) subjects with a mean age of 20.62 ± 2.55 years were selected in this clinical cross-sectional study. The mean spherical equivalent refraction (SER) of the subjects was between the ranges of -1.00 to -5.50 D and cylinder less than 0.75 D with no ocular pathology. Subjects were fitted with a centre near design of multifocal contact lens with a lower addition. Accommodative lag was measured using the WAM5500 binocular auto refractometer with WCS-1 software. The mean SER of the subjects was $2.44 \text{ D} \pm 1.07 \text{ D}$. The mean accommodative lag was measured to be $0.55 \text{ D} \pm 0.19 \text{ D}$ which increased to $0.81 \pm 0.17 \text{ D}$ with MFCLs wear which showed statistical significant difference ($p < 0.05$, paired t-test). Centre near design of multifocal soft contact lenses increase the accommodative lag in myopic subjects. Longitudinal studies are indicated to study the effect of these lenses on myopic.

Keywords: Myopia, Accommodative lag, Multifocal soft contact lense

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Introduction

Myopia has been known for more than 200 years and was described by the ancient Greeks. Despite of recorded use of convex lenses for presbyopia correction in the 13th century, the correction of myopia refractive error had to await the development on the concave lenses in the mid-16th century. Myopia or near-sightedness has been a worldwide public health problem; its high prevalence and rate of progression with knowledge of its related complications has put a heavy burden on the health care system and deeply affected individual patients. Nowadays, uncorrected refractive error is increasingly recognized as a significant cause of avoidable visual impairment worldwide, suggested by the inclusion of uncorrected refractive error as one of the prioritized eye diseases of Vision 2020: The Right to Sight—a global initiative launched by a coalition of non-government organisations and the World Health Organization (Pizzarello & Abiose, 2004). Prevalence of myopia has been reported in previous studies. In an Indian population based studies, the prevalence of myopia in the Andhra Pradesh Eye Disease Study is reported to be 4.44% in subjects less than 15 years which increased to 19.39% in subjects above the age of 15 years, they also found a significant but a weak association of myopia with levels of education (Dandona et al., 1999). Whereas, the Chennai Glaucoma Study estimated the prevalence of myopia to be about 31% amongst adults aged 40 years or older (Raju et al., 2004).

Accommodation is the process whereby changes in the dioptric power of the crystalline lens occurs so that the object in- focus retinal image

of the object of regard is obtained and maintained at the high resolution fovea (Benjamin & Borish, 1998). Lag of accommodation is the amount by which the accommodative response of the eye is less than the dioptric stimulus to accommodation (Goss & Zhai, 1994; Rouse, Hutter & Shiftlett, 1984).

Clinical measurement of accommodative lag at near is typically done using dynamic retinoscopy. This is an objective method in which the patient views a near point target, while the examiner adjusts the lens power, uses a “skia ladder”, or his or her distance from the patient. Normally an individual can have an accommodative lag of +0.50 D to +0.75 D (Sternier, 2004). The Grand Seiko WR-5100K and WAM5500 are also available for the measurement other dynamic accommodation and pupil size and peripheral refractions. Both these instruments are comparable and facilitate the measurement of static refractive error and accommodative response to the real-world stimuli (Atchison, 2003; Davies, Mallen, Wolffsohn & Gilmartin, 2003; Fedtke, Ehrmann & Holden, 2009; Gwiazda & Weber, 2004; Kundart, Hayes, Gietzen & Sheedy, 2010). The present study used the WAM5500 for measurement of accommodative lag. Clinically, WAM5500 is proved to be reliable and comparable for refractive measurements (Sheppard & Davies, 2010). A research conducted observed that the measurement with WAM provides additional information on the accommodative response and pupillary constriction rather than response amplitude (Win-Hall, Houser & Glasser, 2010). Other techniques for measuring the lag of accommodation includes bells

retinoscopy (Tarczy-Hprnoch, 2013), photoretinoscopy using the PlusOptix PowerRefractor (PR) (Gabriel & Mutti, 2009).

The Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) study evaluated the accommodative lag in the subjects in children who had an annual myopic progression of about -0.45 D with a 4 D Badal stimulus; they stated that the accommodative lag in the subjects neither at the beginning nor at the end of the yearly progression interval was different. It was suggested that foveal hyperopic retinal blur during near viewing may not drive juvenile-onset myopia progression (Berntsen, Sinnott, Mutti & Zadnik, 2011). Gwiazda, Thorn and Held in (2005) studied evaluation of accommodation, accommodative convergence and AC/A ratios before and onset of myopia stated that children who became myopic later showed elevated AC/A ratios at one and two years before the onset of myopia, whereas accommodative convergence was greater at the onset of myopia. However, they found that the accommodative lag in subjects who turned myopic a year compared to emmetropic subjects showed no significant change in the lag post myopic development.

Accommodative demands in progressive myopia were studied in subjects where the refractive error and accommodative responses were monitored at 4 months interval for over a period of 1 year. The results indicated that almost all subjects exhibited accommodative stimulus-response gradients close to unity. Furthermore, no significant correlation was observed between the gradient of this function and

either the rate of myopic progression or the overall change in refractive error (Rosenfield & Desai, 2002).

Peripheral optical errors and their change with accommodation differ between emmetropic and myopic eyes were studied where the peripheral optical quality was measured for two accommodative states in emmetropic and myopic eyes. The results of the two groups were found to differ on three points where the myopes had a smaller relative peripheral myopia, they showed a larger asymmetry in defocus over the visual field, and their relative peripheral myopia, which increased with accommodation for emmetropes, did not change or decreased with accommodation (Lundström, Mira-agudelo & Artal, 2009).

Myopic progression studied earlier reported that accommodative lag is associated with progression of myopia in adults whereas other reports that lower accommodative lag is associated with myopia progression in adults (Allen & O'Leary, 2006; Rosenfield & Desai, 2002). Also it is observed that, myopic progression did not correlate positively with accommodation, but the shorter the average reading distance of the follow-up time the faster was the myopic progression. There are also controversies over whether accommodative lag in children is elevated prior to the onset of myopia (Gwiazda Thorn & Held, 2005; Mutti et al., 2006) and whether accommodative lag is associated with myopic progression (Berntsen, Mutti & Zadnik, 2011). Therefore, the research was conducted to study the effect of MFCLs (centre near design) on accommodative lag in young myopic adults.

Materials and Methods

A cross-sectional study was conducted where subjects aged between 8 to 25 years having myopia of - 1.00 D to -5.50 D with astigmatism less than 0.75 D, and anisometropia of less than 1.00 D were included. Other inclusion criteria was best corrected monocular visual acuity of 0.00 using logMAR for distance and 0.63M for near, stereo acuity of less than 40 seconds of arc at 40 cm. Whereas patients with any other ocular abnormalities, any history of rigid contact lenses wear or any other systemic diseases were excluded from the study.

The research was approved by the Research and Ethics Committee, Lotus College of Optometry, India and complied with the tenets of the Declaration of Helsinki. Written consent from all subjects were taken before enrolment. Accommodative lag measurements were carried out using WAM5500 binocular open field refractometer (Figure 1) with the WCS-1 software in the high speed mode attached to a desktop. Distance refraction was obtained using the WAM5500 under natural viewing conditions one eye at a time where the other eye fixated at a 3 m fixation target. After the distance readings were obtained, it was fully corrected by the full aperture trial lenses to obtain a zero reading. With the correction on, the near target was shown at 50 cm distance and then the near refraction was carried out where the pupil size started to change which confirmed that the patient was accommodating. All the readings were captured on a high speed mode for 30 seconds unioocularly. Readings were captured by the software and were produced into an excel sheet file. Missing data on pupil size

and refractive values were deleted for obtaining 20 consecutive readings, mean of which was taken for the analysis.

A thorough soft contact lens evaluation was performed. Soft lens multifocal design from Bausch + Lomb was fitted. Parameters of the contact lens used are shown in Table 1. An adaptation of 20 minutes was given to the patient till the watering subside and the patient felt comfortable. After adaption, visual acuity and accommodative lag were measured with the lenses on. Accommodative lag using the same method was then measured with the contact lenses on Data was collected and analysed using the statistical package SPSS version 20.0 for windows (IBM SPSS Statistics, Armonk, NY, USA). Shapiro-Wilk test for normality was conducted. Paired t-test was used to compare the measurement obtained from the right and left eye, and also to compare the mean accommodative lag obtained pre and post contact lens wear. A p-value of less than 0.05 was considered statistical significance.



Figure 1:WAM5500 binocular open field auto refractometer

Parameter	Value
Material	Polymacon
Water Content	38%
Dk	8.4
FDA Group	Group I (Low water content, Non-ionic)
Production Method	Cast moulding
Base Curve	8.5 mm and 8.8 mm
Modality	2-week replacement
Visibility tint	Light Blue
Diameter	14.5 mm
Power Range	-10.00 D to +6.00 D in 0.25 D steps Addition: Low Add: up to +1.50 D, High Add: +1.75 D to 3.00 D
Centre thickness	0.10 mm at -3.00 D
Design	Aspheric, Centre-near design (CN)

Table 1: Parameters of Bausch +Lomb multifocal lens

Results

Sixteen (16) young adult myopes with a mean age of 20.62 ± 2.55 years (age range 18 to 25 years) including 7 males and 9 females were studied. The mean SER obtained from right and left eye were $-2.44 \text{ D} \pm 1.07 \text{ D}$ and $-2.23 \text{ D} \pm 1.17 \text{ D}$ respectively. The mean accommodative lag in the right eye and left eye were $0.55 \text{ D} \pm 0.19 \text{ D}$ and $0.58 \text{ D} \pm 0.20 \text{ D}$ respectively. No statistical significant difference was found in the SER and accommodative lag measurements obtained between both the eyes ($p > 0.05$). Therefore, data obtained from the right eye was used for statistical analysis. Table 2 shows the mean \pm SD of SER and accommodative lag without contact lenses.

Parameter	Right Eye	Left Eye	p-value
Spherical Equivalent Refraction (SER)	2.44 D ± 1.07 D	2.23 D ± 1.17 D	p > 0.05
Accommodative lag without contact lenses	0.55 D ± 0.19 D	0.58 D ± 0.20 D	p > 0.05

Table 2: Mean ± SD of SER and accommodative lag without contact lenses

After the subjects were fitted with the multifocal contact lenses, the mean accommodative lag increased to 0.81 ± 0.17 D. Paired t-test showed a statistical significant difference between the mean accommodative lag before and after wearing MFCLs, ($p < 0.05$).

Discussion

The results of the present study are in agreement in previous studies which states that accommodative lag increases with the use additional plus power at near. These studies have used bifocal spectacles, progressives spectacles and near glasses (Aller & Wildsoet, 2008; Berntsen, Mutti & Zadnik, 2011; Cheng, Schmid, Woo & Drobe, 2010; Weizhong, Zhikuan, Wen, Xiang, & Jian 2008). The long term use of MFCLs with centre near design is suggested to study the effect of the change in accommodative status on myopic progression. Also the effect of accommodative status on the axial elongation and the effect on the cycloplegic refraction would help to comment on the usefulness of the multifocal centre near design contact lens to arrest myopic progression. A research was carried out to evaluate whether

myopes show poor accommodative response and have a larger accommodative lag under natural seeing conditions. The research subjects included early onset myopes with spectacle and contact lenses and were compared to age matched emmetropes. It was observed that in adults with an early onset of myopia, the habitual accommodative lag was small. It was probably due to reduced accommodative demand. The results of the present study also demonstrated that the accommodative lag s under a habitual seeing condition can be different from that under an experimental condition, i.e., monocular viewing after full correction with contact lens. Also, the previously reported data obtained under the experimental conditions are not sufficient for discussing the causative relationship between accommodative lag and myopia progression (Nakatsuka, Hasebe, Nonaka & Ohtsuki, 2003).

It is reported that late onset myopes also showed a significantly extended accommodation response times after a sustained near vision task which were consistent with previous reports of refractive shifts in late-onset myopes and early-onset myopes and provide a corollary between reflex and adaptive components of the accommodation response (Culhane & Winn, 1999). The present study population were young adults and the measurements done were taken after adaptation of MFCLs, it still showed change in lag of accommodation. Hence, it is suggested to study the long term effect of MFCLs on accommodative lag and also monitor the changes in myopia in early as well as late onset of myopia.

Clinical trials designed to slow the progression of myopia using bifocal spectacles have found either modest or non-significant treatment effects. The role of accommodative lag in myopia progression, is still unknown. There has also been differences in age, measurement methods, and accommodative demand levels among these three studies make comparisons difficult. The effects of the differences in population or study design are unknown. Also many studies have reported that myopes tend to have larger accommodative lags from at the time of onset (Berntsen. Mutti & Zadnik, 2011; Fredrick, 2002). Increased accommodative lag occurred in children only at the onset myopia, elevated accommodative lag is unlikely to be a useful predictive factor for the onset of myopia. Increased hyperopic defocus from accommodative lag may be a consequence rather than a cause of myopia (Mutti et al., 2006). The present study recruited myopes visiting the hospital for eye examination, no consideration was made if they were newly diagnosed myopes or were known myopes.

The important part of the measurement of accommodative lag with multifocal lens was whether the patient was accommodating and using the near portion for viewing the near accommodative target. This was taken care by the observation of the pupil diameter changes which was able to measure with the help of the WAM5500 while taking the near measurements (Kundart, Hayes, Gietzen & Sheedy, 2010; Sheppard & Davies, 2010). A novel and reliable method to investigate the relationship between the degrees of accommodative lag in myopes on a clinically standard instrument to measure accommodation is the use

of the open field binocular auto refractometer WAM-5500 which is used in the present study (Win-Hall, Houser & Glasser, 2010).

Bifocal and multifocal glasses have also been used so as to attempt to arrest myopia progression. The use of a +1.50 D addition is used to reduce but not prevent accommodation and is a compromise between peripheral distortion and wearability on one hand and accommodation reduction on the other. This is the reason why low add MFCLs were used in the present study as the low add according to manufacturer's guidelines is less than +1.50 D. It is suggested that bifocal lenses may reduce the accommodative demand and thus decrease the rate of myopia progression but bifocals may not control accommodation at all distance because of they having only two focal zones and this can lead to slightly out of focus retinal images at other distances (Cheng, Schmid, Woo & Drobe, 2010; Fulk, Cyert & Parker, 2000). Progressive addition lenses (PALs) is more cosmetically acceptable and allow children to have clear vision at all distances without adjustment of the focal mechanism of the eye. In young myopes, however, PALs must be fitted very high to facilitate the use of the segment for reading. In a clinical trial it was proven that PALs reduce the myopic progression and it also communicated that PALs with accommodation system is the cause of myopic reduction (Shih, Hsiao & Lin, 2000). The result of the present study is in conjunction with these studies where it was also found that accommodative lag increased with the use of additional plus at near. However, bifocal and progressive spectacle lenses may be less cosmetically acceptable, are difficult to adapt to, and compliance with spectacle wear may be a problem. In addition, children may not always use the lower segment

of the lens for reading (Fulk, Cyert & Parker et al., 2000; Grosvenor, Perrigin & Perrigin, 1987).

Similar effect of bifocal contact lenses that it helped to reduce myopic progress in identical twins in a cross over study compared to single vision soft contact lenses was seen (Aller & Wildsoet, 2008). Proclear multifocal dominant design was used to study the peripheral myopisation with additions from +1.00 D to +4.00 D. It was observed that with a plano dominant design, up to -6 D of peripheral spherical equivalent could be achieved; a +1.00 D and +2.00 D addition did not show practical effects on peripheral myopisation but there was an increased in the central and peripheral refractive values with a +3.00 D and a +4.00 addition which could be a negative effect (Lopes-Ferreira et al., 2011).

Conclusion

Centre near multifocal soft contact lenses on 20 minutes adaptation increases the accommodative lag in young adult myopic subjects. Longitudinal studies need to be carried out to monitor the effect of MFCLs on accommodative lag in myopic progression.

Disclosure

This paper is a part of the thesis submitted by the author for the partial fulfillment of the requirements of the Masters in Optometry (M. Optom) at Lotus College of Optometry, Mumbai, India. There is no financial or personal interest for any of the brand of product(s) mentioned in the paper by the author and co-author. No financial grant

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Effect of hues on accommodation in high myopes

Ramlugun Y., Batumalai U.M.

Abstract

This study aimed to investigate the effects of hues on accommodation in high myopic subjects by measuring their mean accommodative error after colouring. Thirty eight subjects aged between 18 to 28 years old were recruited. The experiment was carried out on the subjects for three consecutive days using randomization technique. A colouring application named “Recolor” which is accessible on the hand phone was used. The phone used was an iPhone 6s with phone dimensions of 138.3 x 67.1 x 7.1 mm and screen size 4.7 inches. Prior to the test, accommodation error was measured using the Fused Cross-Cylinder technique. Subjects were asked to perform colouring using red, green and black hues for a period of 45 minutes. After the colouring session, accommodation lag was measured again. Paired t-test result showed a significant difference between the mean of accommodation error before and after colouring for red and green hues with ($p < 0.05$). However, black hue had no difference. Pairwise comparison further showed that the mean accommodation error of the black hue ($0.07D \pm 0.23$) was significantly lower when compared to the mean accommodation error of red hue ($0.20D \pm 0.29$) and green hue ($0.19D \pm 0.29$) respectively. The study demonstrated that red and green hues tend to initiate a high accommodation error and that black hues tend to elicit almost no accommodation

Keywords: Accommodation error, Hues, Colouring, Adults

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Introduction

As art is making its way back to the human visual creativity and skills, colouring books have once again become an international trend, but this time not for children but for adults, and most likely as an art therapy (Belchamber, 1997). This art therapy is a mental health profession which uses the creative artwork to explore feelings, reconcile emotional conflicts, manage behaviour and addiction, develop social skills, improve reality orientation, reduce anxiety, and increase self-esteem and foster self-awareness (Curry & Kasser, 2005). Colouring definitely has therapeutic potential in reducing anxiety, create focus. The soothing and calming effects of colouring also act as a mean for the brain to switch off from other thoughts and focus only on the moment, helping to eliminate any kind of free-floating anxiety.

Color is one of the stimulant likely to influence accommodation (Kergoat & Lovasik, 1990). The accommodative level varies as a function of the color of a target. Red targets tend to induce better accommodation response than green ones. Furthermore, color vision may play an important a role in the control of accommodation (Seidemann & Schaeffel, 2002). As the role of chromatic aberration effects in controlling the accommodation response, a monochromatic individual may not be able to distinguish the defocus effect due to the changes in target colors (Rucker & Kruger, 2004).

The colouring books emphasize on geometrical and floral patterns, as well as figures for colouring, which alleges that these forms of art

therapy provides emotional release and calming of the mind (Grossman, 1981; Christenfeld & Creager, 1996; Cronin, 1994). However, effect on vision and visual discomfort was not investigated. The purpose of this study was to investigate the effect of the hues on accommodation errors on young myopic adults after colouring for 45 minutes.

Materials and Methods

A. Study Population

The chosen subjects were aged between 18 to 28 years of age. Subjects with mean spherical equivalent refraction (SER) of $\leq 6.00D$ with best corrected visual acuity (BCVA) of 6/6 and N6 for near were included. Subjects with binocular vision defects and any form of ocular disorders were excluded.

B. Screening Assessment

A screening assessment was carried out on the subjects entitled for this study. Each qualified candidate was given a consent form.

A complete history taking was carried out. The habitual VA status were taken monocularly and binocularly using Snellen chart at 6 meter and near reading chart at 40 cm. The distance VA was further assessed using the +1.00 test as an indication of over corrected lens power. Colour vision test was performed using OCULUS HMC-Anomaloscope. The test was performed monocularly at near during which the subject was asked to match the colours which resemble closest to the colour displayed on the apparatus. For stereo acuity,

Randot test stereograms was used. The near point of accommodation (NPA) and the near point of convergence (NPC) were measured using the modified Royal Air Force (RAF) rule. Subjects with normal range of NPA and NPC were qualified to proceed with near task.

C. Research Test

The Fused Cross-cylinder (FCC) test was performed using the phoropter to measure the initial lag. This step was done in room illumination of 758 lux. The illumination was measured using a lux meter. Cross cylinder $\pm 0.50D$ was introduced binocularly in front of the subject's eyes while viewing the rectilinear target. If the subject reported that the horizontal lines were darker and clearer when looking at the target, $+0.25D$ were added binocularly to the phoropter until the vertical lines or both meridional lines were equally clear. When the initial horizontal lines appeared to be clearer, the subject was reported to experience lag of accommodation. In contrary, if the vertical lines were initially seen to be clearer, $-0.25D$ were added binocularly until the horizontal or both meridional lines were equally clear. Vertical lines were darker when the subject experienced lead of accommodation. If both meridional lines could not be made equally clear, the accommodation was measured based on the lenses that made the previous blurred lines to be clearer. The Patient was then informed to perform the colouring test.

A colouring application named "Recolor" which is accessible on the hand phone was used for the study. Recolor- colouring book 1.3 is available in Apple App Store or Google Play Store and offers a variety

of hues in red, green and black. The hand phone used was an iPhone 6s with measurements 138.3 x 67.1 x 7.1 mm and screen size at 4.7 inches. The hand phone was held at the subject's working distance (35-45cm). The screen was set at medium (50%) brightness. The span of the test was set for 45 minutes. The subject performed the same tests for 3 consecutive days using different colour which was chosen by the randomization technique. During this experiment, a pre coloured sample was projected on an iPad as a guideline and the subject had to look at the sample and follow the hue. After each colouring session, accommodation lag was measured again using the FCC method. The data collected was analysed using SPSS Version 23 (Chicago, IL, USA). This study was conducted according to the tenets of the Declaration of Helsinki.

Results

Thirty six subjects from 18 to 28 years of age with mean age of 22.64 years \pm 1.69 were recruited for this study. 24 of them were male subjects while 12 were female subjects.

The mean accommodation error for the red, green and black hues for both pre and post assessment are as shown in Figure 1.

Paired t-test was conducted to compare the mean of pre and post red, green as well as black respectively. Paired t-test result showed that there was a significant increase in accommodation error when compared before and after colouring for red and green hues ($p < 0.05$). However, black hue showed no significant difference ($p > 0.05$).

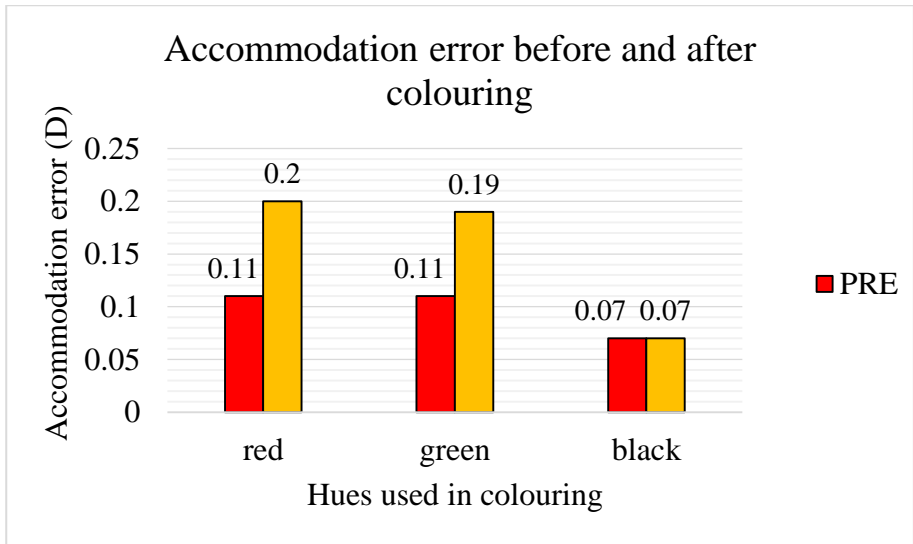


Figure 1: Descriptive data of mean, standard deviation of pre and post accommodation error.

Comparison of hues and accommodation

One Way Repeated Measures ANOVA test was conducted to compare the mean accommodation error of the three colour hues among high myopes. The result showed that there is a significant difference in the mean accommodation error for the red, green and black hues among high myopic subjects $F(2, 70) = 4.478$ ($p < 0.05$).

Pairwise comparison further showed that the mean accommodation error of the black hue ($0.07D \pm 0.23$) was significantly lower when compared to the mean accommodation error of red ($0.20D \pm 0.29$) and green hue ($0.19D \pm 0.29$). The mean accommodation error of red and green showed no significant difference.

Discussion

The study investigated the mean changes of accommodation error on red, green and black hues among high myope individuals when using a colouring application for 45 minutes. The results showed that red and green hues affected the mean accommodation error while black on the contrary did not induce any accommodation error.

The findings implied that the accommodative response was least when viewing a target with longer and medium wavelength. The accommodation error for the black hues presented no changes before and after the experiment, suggesting that viewing the black target did not affect the accommodative response. Intensive near tasks such as reading and use of digital gadgets on a regular basis greatly influence myopic eyes (Goss et al., 1997). It has been speculated that larger accommodative lags might be tolerated by myopes, as compared to emmetropes, because of their reduced sensitivity to defocus blur, thus the human myopic eyes did not respond to any hyperopic defocus.

According to a study done by Ahmad, Chen and Yahaya (2014), myopic subjects demonstrated greater accommodation error for near visual targets than emmetropes. They claimed that this increase in accommodation lag with red target could be due to how attentive the myopic subjects were to the coloured targets. Their findings confirmed there was a significant increase in accommodation lag in red target compared to black target. The black target produced the highest accommodation error among all visual targets used in this study.

In the present study, red, green and black hues were used. The results showed that the mean accommodation errors were significant between black and red and green and black respectively. However there was no significant change in accommodation error between red and green hues since it is the long (red) and middle (green) wavelength components of the broadband retinal image that provides a chromatic signal for accommodation errors (Tavassoli & Ringach, 2010). Subsequently, in the present review, it was inferred that there was no significant difference in the mean accommodative lag among red and green shades. Long and medium wavelength similarly incite a greater of accommodation while doing delayed near works.

Previously, study have shown that minus lenses that are used to correct myopic eyes and the negative lenses will create peripheral hyperopic defocus (Nakatsuka et al., 2005). Upon the addition of negative lenses in myopic eyes, the longitudinal chromatic aberration could be affected as a long wavelength hue will be focused further behind the retina compared to that of emmetropic eyes (He et al., 2013). Therefore, a higher accommodation error was observed while colouring in red and green as more plus lenses were required to re-focus the image onto the retina.

Furthermore, other studies attempted to evaluate the effect of the Longitudinal Chromatic Aberration (LCA) and Transverse Chromatic Aberration (TCA) on the visual quality. Marcos et al. (1999) theoretically computed the effect of LCA and transverse chromatic aberration on the visual quality and evaluated which one was more detrimental to the visual performance of human eye. They found that

TCA could degrade the visual quality as much as LCA. Correcting TCA and leaving LCA uncorrected could improve the visual quality more than correcting the LCA and leaving the TCA uncorrected.

It was found in the present study conducted that black hue induced no accommodation lag. This result was supported by a study done by Rucker (2013) whose findings showed that a black target or background will induce slightly lower to almost zero accommodation error. The human eye is affected by spectrum of wavelength and its chromatic dispersion (Kruger et al., 1993). Therefore, in the presence of more distinct colours with short, medium and long wavelength, the eye is more sensitive to defocus caused thus giving a more accurate response to changes in accommodation.

Consequently, it was found that subjects had a greater lag of accommodation when subjected to a shorter spectral bandwidth illumination, regardless of whether it was on paper or visual display units (Kruger et al., 1993 cited in Lee et al., 1999). A study was conducted on VDU and it was observed that colours were perceived slightly better than printed materials (Ahmad et al., 2014). Nevertheless, according to Sorkin, Reich and Pizzimenti (2003), it was found that the accommodation response with respect to accommodation error was the same irrespective of the medium used. Therefore, the findings found during this current study can be compared to an experiment done on a printed material.

Another factor that can be linked to the changes in accommodation error after the near task can be due to visual discomforts which

includes illusion of colours and shapes, eyestrain and eye fatigues. In the study done by Tosha et al. (2009), results demonstrated that subjects with low visual discomfort had normal accommodative response while those with high visual discomfort experienced accommodative fatigue. In the present study a majority of the subjects did complain of eye strain and eye fatigues after colouring within the first 20 minutes of the procedure. Tosha et al. (2009) suggested that the high visual discomfort group was characterized by accommodative fatigue, developing a higher accommodation lag over time when viewing at a near distance.

Further studies on different range of hues affecting accommodation error would be useful to conclude whether or not colouring habit may elicit some accommodation error. Effect on convergence and accommodation on this colouring habit should also be investigated to sustain a clearer effect on the eye.

Conclusion

The study concludes that red and green hues tend to initiate a high accommodation error among myopic individuals. Whereas, the black hue did not elicit any accommodation error. This shows that colours does affect accommodation in the eye while performing a colouring task.

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Microbial Activity of Ophthalmic Multipurpose Solution Marketed in Klang Valley, Malaysia

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Abstract

There are approximately 140 million contact lens wearer worldwide who are using it to improve their vision or solely for cosmetic purpose. The development in contact lens care system especially in enhancing multipurpose solution (MPS) is one of the attempts to improvise the safety, efficacy as well as promoting comfort to contact lens users. This study investigated the pattern of microbial activity in commercially marketed MPS in Malaysia. Six different brands of MPS that are available locally were selected as the sample for this research. The observed aspects included the growth of microorganisms in MPS at different temperatures after opening the sealed packaging, and its relationship to disinfectant content in the MPS. Measured by colony forming unit per milliliter (CFU/mL), the microbial growth increased with the increased of storage temperature from 4⁰C to room temperature and it was at the peak at 37⁰C. The result showed the growth was at a minimal rate at the early days and started to increase and fluctuate over time. Newer generation of MPS that have dual disinfecting agents showed better outcome in inhibiting the microbial growth. As a conclusion, the growths of microbial contaminants in MPS were divergent at different temperatures. Observations on the growth were also made prior to time and it showed a fluctuating growth pattern. In this experiment, the potency of MPS was compared between single disinfecting agent MPS and dual.

Keywords: Ophthalmic Multipurpose Solution, Contamination, Bioburden

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Introduction

There are approximately 140 million contact lens wearers worldwide (Jaya Dantam et al., 2016). The development in contact lens care systems especially in enhancing multipurpose solutions (MPS) is one of the attempts to improve the safety, efficacy as well as promoting comfort to contact lens users (García-Porta et al., 2015). MPS is an essential gear used in cleaning, disinfecting, rinsing, storing the contact lens and as a lubricant in rewetting the eyes. Besides, MPS has become the choice in lens care product option over hydrogen peroxide (H_2O_2) –containing solution due to the tolerability, simplicity and cost efficiency which encourage compliance among contact lens users (García-Porta et al., 2015).

Since the 1980s, contact lens care products, such as lens cases and MPS, must undergo numerous tests before these products received approval to be marketed (Rosenthal et al., 2002). These standards are all controlled by national bodies in every country that followed the guidelines of the international regulatory requirements. The outbreak of microbial contamination in contact lens users, lens cases and MPS has alarmed the society on the importance of having such requirements and standards in manufacturing contact lens related products. The main international body that is responsible in governing the approval of these products is the Food and Drug Administration (FDA).

One of the important requirements in the production of MPS is to calculate the D-Value of MPS (Rosenthal et al., 2002). The D-value

explains about the period that is needed to impoverish the number of microorganisms in a group by 90 percent in one log unit. Researchers have found several ways in estimating the antimicrobial effectiveness of lens care solutions and one of it is the analysis of the D-value. In daily users practice situation, the presence of microorganisms that adhered to or penetrated the contact lenses are normal but not desirable. Hence, investigators emphasise more on the time required for various MPS to penetrate the lens and eliminate the contamination in order to come out with a good lens care system (Gopinathan et al., 1994).

Similar to other ophthalmic products, MPS container and content must be sterile until it is opened for the first usage. Unwanted microorganism can harvest and contaminate the MPS during the contact with fingers, lids, air, contaminated contact lens and even contact lens cases (Dermatologic and Ophthalmic Drugs Advisory Committee, 2012). With the increase in the number of contact lens users nowadays, it is important to emphasise good compliance in using the MPS especially during the process of cleaning, storing and rinsing (Thakur & Gaikwad, 2014). According to research done on contact lens care compliance, there are certain personal limitation that have to be considered as the factors that allow contamination on the MPS, such as poor vision and forgetfulness among the contact lens users (Raghad et al., 2011).

Several types of contact lens disinfectant and care solutions are commercially available in the market, including hydrogen peroxide-based solutions as well as MPS. This study attempted to investigate

the pattern of microbial growth in commercially locally marketed MPS in Malaysia. The sample were stored at different temperatures and observed for microbial growth in different aspects (Aminou & Alam-Eldin, 2014). Also, bacterial challenge tests were performed to compare the effectiveness of MPS products with single- or combination disinfection agents.

Materials and Methods

Procurement of ophthalmic multipurpose solutions

Six different brands of ophthalmic multipurpose solution available in Malaysia market were purchased and anonymously labelled (Table 1). Three of the MPS brands (MPS A, MPS B and MPS C) were made up of combination disinfecting agents while the other three MPS brands (MPS D, MPS E and MPS F) consist of single disinfecting agent.

Multipurpose Solution (MPS)	Disinfecting Agent
MPS A	Polyquaternium-1 0.001% Myristamidopropyl dimethylamine 0.0005%
MPS B	Alexidine dihydrochloride 0.00016% Polyquaterium- 1 0.0003%
MPS C	Polyaminopropyl Biguanide 0.00013% Polyquaternium 0.0001%
MPS D	Polyhexanide
MPS E	Polyhexamethylene Biguanide 0.0001%
MPS F	Polyhexamethylene Biguanide 0.0001%

Table 1: Anonymously labelled multipurpose solutions (MPS) and disinfecting agents used.

Preparation of bacterial culture media

Nutrient agar (OXOID®) were used as the medium to test for the growth of microorganism (Margot McKerrell, 2004). Agar was prepared according to manufacturer's recommendations. All procedures were conducted under aseptic conditions.

Sampling and storing

Each brand of MPS was divided into three portions and transferred into sterile falcon tube with approximately 5mL of the solution in each tube. Each tube was labelled accordingly and stored under three different temperatures: 4°C, room temperature (25°C) and 37°C, respectively. Two negative controls were prepared. The first was conducted on the first day as the sample was taken and inoculated on nutrient agars directly after the product seal was opened under aseptic conditions. The second test was performed on the last day of the experiment. The sample was sealed and incubated at 37°C before used on the last day.

Microbial analysis

Samples were prepared in triplicates for each MPS at each temperature. The plates were labelled accordingly before the spreading. 100µl of the sample dispensed onto the nutrient agar plate and spread evenly. These plates were incubated at 37°C for 16 hours (Szczołka-Flynn et al., 2009; Tim Sandle, 2015). The samples were exposed to the air before and after the spreading process to mimic the usage of the end users in open air , 2013).

Serial Dilution and E. coli challenge

MPS A and F were selected for this assay based on their contrastive performance and relatively strong ability in suppressing microbial growth as shown in Figure 2. Serial dilution was conducted to observe the effectiveness of disinfecting agent in MPS in preventing the growth of microorganism. The dilution factors used in this experiment were 1, 0.5, 0.25 and 0.125. The diluted samples were challenged with 5 μL of *E. coli* at 4×10^8 CFU/mL (Ezekiel, 2013), followed by incubation at 37°C for 18 hours. Then, 100 μl of the sample was evenly spread onto the nutrient agar plates. These plates were incubated at 37°C for 16 hours before the bacterial counts were determined (Szczotka-Flynn et al., 2009; Tim Sandle, 2015).

Observation and colony counting

The number of colony appeared on the medium were quantified. Viable colony counting method was used and recorded in colony forming unit per mL (CFU/mL) (Scandinavian Pulp, Paper and Board Testing Committee, 2002). For colonies that have too dense growth in a particular medium, which falls under ‘too numerous to count’ (TNTC) category, pictures were taken and documented accordingly.

Results

Microbial contamination at different temperatures

No significant difference in microbial contamination was observed in MPS with combination disinfecting agents (Figure 1). This was

anticipated due to the presence of 2 disinfecting agents, which may carry different mechanism of actions against microorganisms. On the other hand, the mean CFU/mL of MPS D, E and F, which consisted of single disinfecting agents, were having significant increase in their bioburden level from 4°C, room temperature (25⁰C), to 37°C (Figure 1).

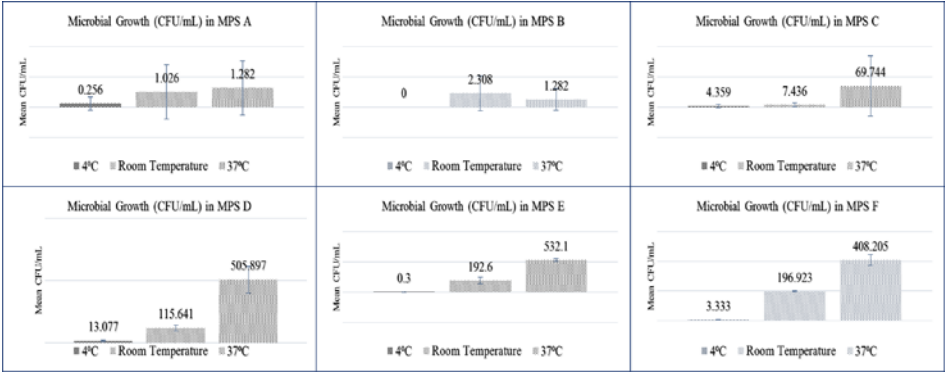


Figure 1: Mean colony forming unit per mL (CFU/mL) of MPS A-F under various storage temperatures.

Microbial contamination over time

The microbial contamination in MPS A, B, C, D, E and F show fluctuation from day 2 until day 32. Figure 2 shows the growth (mean CFU/mL) of microorganism in MPS A to F respectively. As illustrated, bioburden was observed in these MPS products from day-22 onwards (Figure 2). MPS solution with combination disinfecting agents (MPS A, B, and C) had significantly lesser bioburden than those with single disinfecting agents (MPS D, E, and F) throughout the 5-week study period (Figure 2).

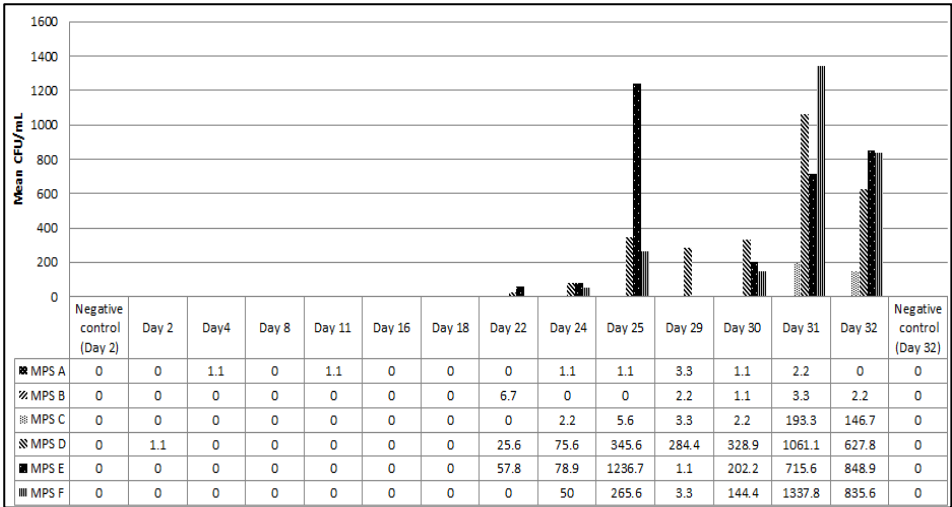


Figure 2: Mean microbial growth (CFU/mL) detected in MPS A to F over 32 days (5 weeks).

***E. coli* bacterial challenge**

E. coli challenge was performed on serially diluted MPS A and MPS F. This assay was conducted to compare the microbial growth pattern in single disinfecting agent against combination disinfecting agent in MPS. The result shows that the pattern of microbial growth increased as the MPS was diluted. By comparing the mean CFU/mL of these two products, MPS F has higher microbial growth compared to MPS A (Figure 3). At dilution factor of 0.125, the mean CFU/mL cannot be quantified as the growth of the colony has exceeded the ‘too numerous to count’ (TNTC) limit and forming bacterial lawn.

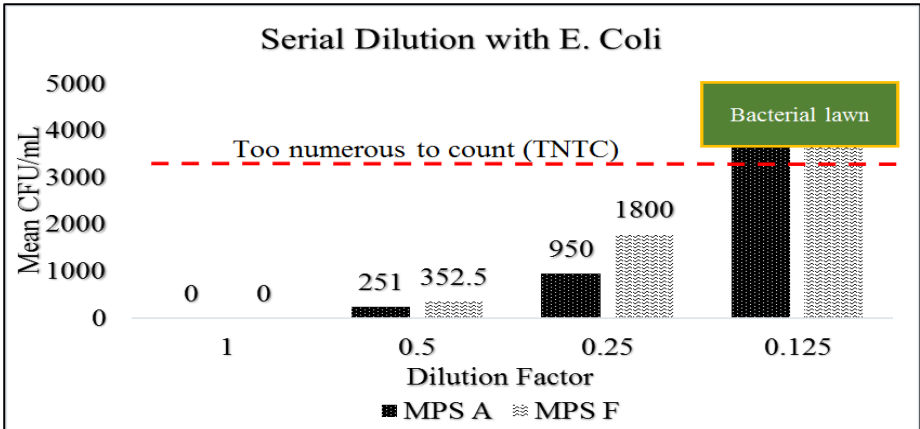


Figure 3: *Escherichia coli* challenge test on MPS with single disinfecting agent (MPS A) and dual disinfecting agents (MPS F).

Discussion

Polyquaternium based disinfectants have predominantly antibacterial activity, in addition to activity against fungal strains such as *Candida albicans*. These biocides show activity at the cell membrane of microorganisms, inducing potassium leakage from cells. Biguanide based disinfectants have a number of synonyms such as PHMB, polyhexanide, polyaminoprophyl biguanide, (DYMED) (Bausch and Lomb's trade name for PHMB) and Trischem (Abbott Medical Optics, AMO's tradename for PHMB). This group of disinfectants has established antibacterial, antifungal and antiprotozoal activity. They are cationic disinfectants with unique chemical structure that allows the molecules to selectively engage cell walls of microorganisms (Debarun Dutta, 2016). Currently, several preservative based multipurpose lens care solutions employ dual disinfectants to provide high antimicrobial activity against a wide range of microorganisms including bacteria, fungus and Acanthamoeba (Debarun Dutta, 2016).

In addition, determining the influence of temperature on microbial growth is significant in predictive microbiology as well as in prophesising the period of shelf life of MPS (Huang et al., 2011). Based on the result obtained in this experiment, the antimicrobial capacity of disinfecting agents was shown to be influenced by storage conditions such as temperature and humidity (Boost et al., 2006). As shown in Figure 1, contamination was observed at low temperature (4°C) as well as room temperature. As the temperature increased to 37°C, microbial growth was observed at a much higher rate. This is because most microorganism have their optimum growth temperature that fall within 30°C to 40°C (Kenneth Todar, 2012.). The storage temperature and conditions of MPS can vary among contact lens wearers. It is important to emphasise and to educate the lens users of these effects as changes in temperature can enhance or reduce the rate of biological burden of the MPS. Higher risk of contamination incident may be seen in tropical and sub-tropical areas where the temperature and humidity are much higher, which are conditions preferred by microorganisms to manifest and grow (Boost et al., 2006).

In this experiment, the samples were categorised into two distinct groups which are MPS that consist of single disinfecting agent (MPS D, E, and F) and MPS that were made up of combination disinfecting agents (MPS A, B, and C). The results showed that the reading of mean CFU/mL for single disinfecting agent MPS were much higher compared to MPS-containing combination disinfecting agents (Figure 2). This indicates that MPS with combination of disinfecting agent

were more effective compared to those with single disinfecting agent. In addition, from our observation, the general survivability rate of microorganism in all six MPS products remained minimal at the early stage of the experiment (first 3 weeks after opening of product seals), followed by detectable contamination from day-22 onwards (Figure 2). According to some previous studies, this may be due to the alteration of the concentration of MPS's active ingredient over time (Kal et al., 2016). Besides, greater contamination was observed in solution that were used for a prolong duration compared to those used for shorter period (Gopinathan et al., 1994).

Although human error might be the cause of inconsistent growth pattern of some MPS throughout the 5-week period in this experiment, a published report demonstrated the viability and invasiveness between microorganism of different species and morphological groups (Hiti *et al.*, 2002). Microorganisms have unique defense mechanism to sustain their growth in an adverse environment, which may also be a contributing factor in obtaining an inconsistent growth curve in this study.

MPS E and MPS F are made up of the same disinfecting agent (Table 1). However, their mean CFU/mL differed from each other (Figure 2). A literature demonstrated different efficacies of the same disinfecting system when tested. This is attributed to the different formulations of each commercial disinfecting solution, including buffering agents (such as sodium chloride), cleaning agents (such as sodium citrate), conditioning agents (such as tetriconic), and surfactants (such as propyleneglycol) (Aminou and Alam-Eldin, 2014). Some of these

inactive substances may participate in the reaction either by reducing or enhancing the effect of the disinfecting agents. Other publications had stated that certain formulation of MPS has been found to significantly influence the efficacy of the solution as a disinfectant. For example, some MPS contain several types of non-ionic surfactants (poloxamer, poloxamine, propylene glycol) which were claimed to support the growth of *Acanthamoeba* spp. as these inactive ingredients were able to form biofilm layer which may protect the contaminants from being killed by the disinfecting agents (Aminou and Alam-Eldin, 2014). However, further studies are still needed to clarify the individual and collective effects of these compounds on the general disinfectant efficacy.

Since there were no specific standard in managing MPS after seals are removed and content is exposed to open air, contact lens users are generally advised to discard the content of MPS a few months after breaking the seal of the bottle. This period is known as discard duration and the MPS contents are assumed to be safe from contamination and effective in disinfecting the lenses before the discard date. Despite of this recommendation, according to this study, it was found that several MPS products do not comply with the criteria of maintaining the products' sterility for more than one month (Figure 2).

The current available dual-disinfection MPS in the market are the latest evolution from the older generation of MPS. The major improvement of the latest preserved lens care systems is that the biocides and disinfecting agent molecules are much larger than the

first generation preservatives that made up of only single disinfecting agent such as thimerosal and chlorhexidine (Debarun Dutta, 2016). This has led to a lower uptake of the preservative into the lens matrix, which has been shown to reduce allergic reactions (Debarun Dutta, 2016).

MPS A and F were selected to conduct *E. coli* challenge tests. Both of these MPSs were from different distinguished categories, where MPS A contained combination disinfecting agents while MPS F consisted of single disinfecting agent. The rationale behind choosing these two MPSs were due to the lowest mean CFU/mL that have been observed in both categories. Both MPS A and MPS F have the lowest mean CFU readings which indicate that they were the most effective MPS from each category (Figure 2). From the results obtained, when the MPSs were diluted by 0.5 and 0.25, the mean CFU/mL reading for MPS A was lower than MPS F. This shows that the MPS with dual disinfecting agents over performed MPS with single disinfecting agent. Also, our result supports the claims made by MPS manufacturers where the minimal disinfecting agents are used to minimise the chance of allergy reactions to the end users.

Nevertheless, the balance between effectiveness of disinfecting agents used and their safety profile towards the ocular system must be taken into consideration before formulating a MPS. Although it is crucial for a MPS to achieve specific disinfecting capacity which is stable over the recommended period of use, it is also important to note that since MPS are intended to be introduced into the eye, they should not adversely affect the cornea (Begley et al., 1994; Dutot, 2013;

Mowrey-McKee et al., 2002). Hence, although dual disinfectants containing MPS can provide superior performance compared to single disinfectant containing MPS (Mark Wilcox, 2011), a good MPS shall not be rated as a greater choice solely based on the fact that their disinfection ability is stronger. This study has provided evidence that recent improvements in formulations of MPS, such as the use of combined disinfecting agents, have improved their efficacy compared to single disinfecting agent-containing MPS, based on the results obtained.

Conclusion

In conclusion, the growths of microbial contaminants in MPS were divergent at different temperatures. The growth increased with the increase of storage temperature from 4°C to room temperature (25°C) and at the peak at 37°C. It also shows that MPS can be stored at 4°C to minimise the tendency of microbial invasion towards the content of the solution. However, future studies must be done to test on the suitability and stability of other ingredients in the MPS, as it is crucial to confirm the safety and effectiveness of the other content prioritising the ocular health is the outmost important. In summary, newer generation of MPS products with dual disinfecting effect show better performance in inhibiting the growth of microbial contaminants.

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