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Contents

Knowledge and Perception Assessment of Childhood Autistic Disorder among University Students

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Abstract

Background and objective: Autism has increased incidence and prevalence rates nowadays. The sufficient and adequate amount of knowledge and perception is important to enable early detection and provide appropriate care towards autistic children. **Objective**: This study was to determine the knowledge and perception of childhood autistic disorder among university students. **Materials and Methods**: A cross-sectional study was conducted on a sample size of 200 participants in SEGi University Kota Damansara, Malaysia. A set of hybrid validated questionnaire was used to assess their knowledge and perception of childhood autistic disorder by calculating the scores according to their answers on the questionnaire. Data analysis was done using ANOVA test with the help of SPSS software version 22. **Results**: Based on the overall scores of 195 respondents, the overall score of knowledge and perception on autistic spectrum disorder (ASD) was 45.7%. There was a significant difference between medical and non-medical science students in overall knowledge and perception about autism at *P- value* 0.012. Also, there were significant differences between males and females at *P- value* 0.016, while there was no significant difference in the knowledge assessment between male and female and races towards childhood autistic disorder. **Conclusion**: The knowledge and perception among university students on childhood autism are inadequate, students who are undertaking medical courses were more knowledgeable than those taking non-medical science subjects.

Keywords: Knowledge, Perception, Childhood, Autistic Disorder, University Students

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Introduction

Autism spectrum disorder (ASD) is a brain-based disorder characterized by a developmental disability that affects social interaction, behaviors, activities, and interests. It is a spectrum condition and people that are in different parts of the spectrum might have different learning disabilities, mental health issues and ability to interact with others. People with autism often show impairment in verbal and non-verbal communication (Sungji et al 2015). The concept of autism has evolved steadily since 1943. National Survey of Children's Health included the estimation of developing ASD in children aged 6-17 years. However, the estimated ASD prevalent by sex, race and geographic area (Christensen et al 2016).

Per the Diagnostic and Statistical Manual of Mental Disorders (published by the American Psychiatric Association), autism is classified into four sub-types, First called Autistic Disorder, which is also known as childhood autism and Kanner's Syndrome. The second is an Asperger's Syndrome that children with Asperger's Syndrome usually have Intelligence Quotient (IQ)

that is normal or above average and can handle his/her daily life but they face difficulties in communication and also have unusual behaviors. Third is called a Childhood Disintegrative Disorder, it is also known as dementia infantalis and Heller's syndrome. This is the most severe and rare form of autism where children who develop normally until the 2 years old, then lose all their abilities that they had before such as walking and talking. They also might develop seizure disorders. The fourth is called Pervasive Developmental Disorder: It is also known as atypical autism. It usually manifests after 3 years of age and can go without being diagnosed for years (Ousley and Cermak 2014).

There is no known exact cause of autism, one of the main risk factors for autism are genetic factors. As some of the studies show that the critical period for developing ASD is before birth, there is evidence that harmful drug intake during pregnancy has been linked with a higher risk of ASD such as thalidomide and valproic acid (Frances de et al 2009). Autistic signs differ from other developmental delays as the children face problems in early learning, they have challenging behaviors and interacting with others (Kasari et al 2013). During infancy, some autistic children do not use specific vocal sounds and older children have difficulties in non-verbal behaviors during interaction with others. Delays in language skills, less ability to adapt to the new routine as well as trouble in expressing their needs using typical words or motions are found. They may also have attention deficit hyperactivity disorder (ADHD) and anxiety or depression (Tager-Flusberg and Kasari 2013).

Furthermore, a study that includes awareness in addition to knowledge and perception was reported displayed that autism is largely known among the public due to the great efforts made by campaigns and organizations, however, knowledge about how to provide intervention and service provider responsibilities was still lacking and inaccurate (Dillenburger et al 2013).

Assessment of the knowledge about childhood autism among health care workers is more than non-health workers (Monday 2010). However, in Southern Asian countries such as Malaysia and Indonesia, the prevalence of ASD is still not fully known and this could be because there are still not enough efforts made into building infrastructure and other awareness centres for autism research (Shaukat et al 2014). The ASD has been increased globally and there are only a few publications that are reported on this topic among university students in Malaysia. Therefore, the objective of this study was to determine the knowledge and perception of childhood autistic disorder among university students.

Materials and Methods

The research study was set up to assess the knowledge and perception of autism spectrum disorder (ASD) among the young generation according to their field of education, gender and races. The research project was a cross-sectional study. This research was carried out among University students which primarily targeted the students in SEGi University, Kota Damansara. A sample size of 200 students from medical and non-medical faculties with approximately 200 students from each faculty were included. These faculties and the students were selected randomly from the first and second years. The

participants who were included in this study were aged between 18 to 26 years.

A set of validated questionnaire with 27 questions was prepared for the participants attached with an information sheet and consent form. The validated questionnaire consisted of three parts. The first part was about their demographic information, the second and the third parts were testing their knowledge and perception of the childhood autism spectrum disorder, respectively. The Statistical Package for the Social Science, SPSS software (version 22.0) was used to analyze the collected data. By using SPSS version 22, ANOVA test was implemented to compare the variables, p-value of <0.05 was considered as significant. This research study has been ethically approved by Ethics committee, SEGi University.

Results

Demographic Characteristics

A total of 200 responses were collected during the data collection but there were five incomplete questionnaires and were rejected. Therefore, data from 195 respondents were taken for analysing and interpreting. The response rate for this study was 97.5%. The majority (53.3%) of the participants were aged between 18-20 years. The majority of the respondents were female (65.6%), Chinese (53.4%). In this study, the total number of autistic child was 4.1% in participants' family (Table 1).

Table 1: Demographic characteristics of study population and presence of autistic child among participants' family

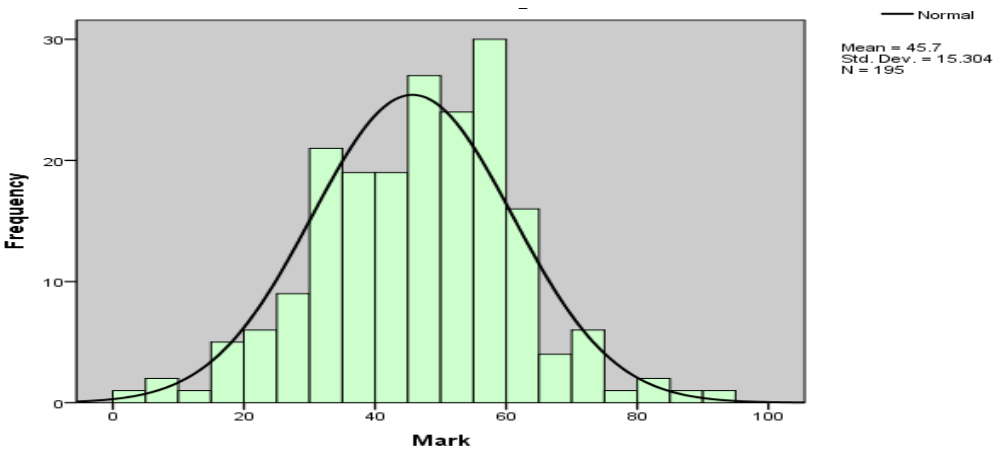
	N=195 (%)	Medical	Non-medical

Demographic Characteristics		N=100(%)	N=95(%)
Gender			
Male	67(34.0)	23(34.0)	44(66.)
Female	128(65.6)	77(60.0)	51(40.0)
Race			
Malay	27(13.8)	20(10.0)	7(3.0)
Chinese	104(53.4)	42(21.6)	62(31.8)
Indian	23(11.8)	13(6.7)	10(5.1)
Others	41(21.0)	25(12.8)	16(8.2)
Presence of autistic child in family			
Yes	8(4.1)	6(6.0)	2(2.1)
No	187(95.9)	94(94.0)	93(97.9)

Knowledge Assessment

Based on the overall value of 195 respondents, it showed that participants regardless of their fields of education, gender, and races, had no adequate knowledge and perception on autistic spectrum disorder (ASD) where the overall score was 45.7% (Fig. 1).

Figure 1: Knowledge and Perception on Childhood Autism of 195 Respondents



Meanwhile, the knowledge overall score was 51.9%. Majority of participants (31.8%) knew that autism is more common in males than female, also 73.3% agreed that children can outgrow autism with proper treatment. Almost, half of the participants (47.7%) thought that there is a medical test that can diagnose autism. A great number of respondents (83.1%) agreed that early identification can improve therapeutic outcomes for autistic children. A total of 104 respondents (53.3%) believed that people with autism can live independently. Also, 87 participants (44.6%) understood that delay in early communication is an early sign of autism. 41.5% of respondents believed that autism is a lifelong disorder. For the causes of childhood autism, majority of the respondents did not think that autism is caused by vaccination and poor parenting (67.7% and 64.1% respectively) but most of them believed that environmental factors (49.2%) and family history (71.8%) could be the causes of autism (Table 2).

Table 2: Respondents' Knowledge about Childhood Autism

Knowledge Questions	Number (%)		
	Yes	No	Don't know
Do you think autism is more common in males than females?	62(31.8)	44(22.6)	89(45.6)
Do you think children can outgrow autism with proper treatment?	143(73.3)	29(14.9)	23(11.8)
Is there any medical test that can diagnose autism?	93(47.7)	28(14.4)	74(37.9)
Do you think early identification improves therapeutic outcomes for autistic children?	162(83.1)	8(4.1)	25(12.8)
Do you think people with autism can live independently?	104(53.3)	68(34.9)	23(11.8)
Do you think delay in early communication is an early sign of autism?	87(44.6)	69(35.4)	39(20.0)

Do you think autism is a lifelong disorder?	81(41.5)	80(41.0)	34(17.4)
Do you think autism is caused by vaccination?	19(9.7)	132(67.7)	44(22.6)
Do you think autism is caused by poor parenting?	43(22.1)	125(64.1)	27(13.8)
Do you think that the environmental factors could cause autism?	96(49.2)	63(32.3)	36(18.5)
Do you think family history increases chances of developing autism?	140(71.8)	25(12.8)	30(15.4)

Perception Assessment

The perceptions of the participants were assessed and the results revealed that majority of the respondents did not show enough perception towards ASD. An average score of 39.1% was obtained by 195 participants and it is the lowest if compared to the knowledge section and the overall value section. However, 112 (57.4) out of 195 participants responded strongly agree (27) and agree (85) that autism is a kind of neuro-developmental disorder that accompanied with a strong genetic basis. While 37.9% of them had the perception about that those autistic children not necessary show an intellectual disability. Less than half of them (40.5%) acquiesced in the statement that autistic children have mental disabilities. 51.7% of the participants were aware that autistic children could not play normally like others with their peers and have different eating habits with their peers. Majority of participants are not sure the children having autism can respond to directions recommendations easily, low perception that autistic children could perform a strong memorizing power, that less or no eye-contact and children having autism can have eating habits different from their peers were 44.6%, 37.9%, 44.1% and 51.3% respectively. Respondents' awareness towards autistic children could have talent in math, music, and drawing were high enough

whereby 66.1% of them agree and strongly agree with that statement. 46.7% of respondents were said to possess sufficient perceptions as they knew that autism cannot be cured by any drugs (Table 3).

Table 3: Respondents' Perception about Childhood Autism

Perception Questions	Number (%)				
	Strongly agree	Agree	Not sure	Disagree	Strongly disagree
Autism is a neuro-developmental disorder with a strong genetic basis.	27(13.8)	85(43.6)	69(35.5)	10(5.1)	4(2.0)
Autistic children are intellectually disabled.	8(4.1)	46(23.6)	67(34.4)	52(26.7)	22(11.2)
Autistic children have mental disabilities.	11(5.6)	68(34.9)	58(29.7)	41(21.0)	17(8.7)
Autism can play with their peers just like other children.	35(17.9)	66(33.8)	48(24.6)	39(20.0)	7(3.6)
Autism can respond to directions recommendations easily.	6(3.1)	44(22.6)	87(44.6)	48(24.6)	10(5.1)
Little or no eye-contact is a sign of autism.	19(9.7)	44(22.6)	74(37.9)	39(20.0)	19(9.8)
Autism can have stronger memories than other children.	15(7.7)	61(31.3)	86(44.1)	24(12.3)	9(4.6)
Autism can have eating habits different from their peers.	13(6.7)	48(24.6)	100(51.3)	26(13.3)	8(4.1)
Autism can be very talented in math, music and drawing.	50(25.6)	79(40.5)	52(26.7)	12(6.2)	2(1.0)
Autism is a disease that can be cured with drugs.	6(3.1)	12(6.2)	86(44.1)	62(31.8)	29(14.9)
Autism can immediately answer when they're called.	5(2.6)	34(17.4)	80(41.0)	54(27.7)	21(10.8)

The overall score of the knowledge and perception among the medical students was 50.1 with the minimum score of 9% and the maximum score of 91%. The overall score among non-medical students was 41.1 with minimum score of 0% and maximum score of 73%, there was a significant difference between medical and non-medical science students in overall knowledge and perception about autism.

For the comparison of knowledge, the value for medical students was 56.8. The minimum value was 9% and the maximum value was 91%. On another hand, non-medical science students scored was 46.7. The minimum and maximum values were 0% and 91% respectively. There was a significant difference between medical and non-medical science students on the knowledge of autism at P- value 0.012. For the comparison of perception, the value for medical students was 42.7% with a minimum of 0% and the maximum of 100%. Non-medical science students had a lower score compared to medical science students, which was 35. There was respondent that scored 0%, contributed to the minimum score whereas the maximum score for this part was 82%. Since the *P- value* was 0.012, therefore, there was a significant difference in perception about autism among health science and non-health science students (Table 4).

Table 4: Knowledge and perception assessment regarding medical and non-medical students

Variables	NO	Percentage	Minimum	Maximum	*p*-value
Overall Value					
Medical	100	50.1	9	91	0

Non-medical Science	95	41.1	0	73	
Total	195	45.7	0	91	
Knowledge Value					
Medical	100	56.8	9	91	
Non-medical Science	95	46.7	0	91	θ
Total	195	51.9	0	91	
Perception Value					
Medical	100	42.7	0	100	
Non-medical Science	95	35.4	0	82	0.012
Total	195	39.1	0	100	

Note: The mean difference is significant at the 0.05 level.

Results showed that there was significant differences between males and females for the overall mean score on perception of childhood autistic disorder. Females scored higher than males, which were 47.6(SD ±15.0) and 42.1(SD ±15.4) respectively. There was no significant difference between the scoring of male and female in the knowledge part but comparatively females scored a higher mean mark 53.2(SD ±15.7 than males 49.4(SD ±18.2). In assessing the perception of childhood autistic disorder, females also showed better than males by having 41.6(SD ±20.6) against 34.5(SD ±20.0)(Table

Table 5: Knowledge and perception assessment–among males and females

Overall Mark/ Variables	NO	Mean (±SD)	Minimum	Maximum	Sig.
Male	67	42.1(15.4)	0	91	
Female	128	47.6(15.0)	9	86	
Total	195	45.7(15.3)	0	91	
Knowledge Mark					
Male	67	49.4(18.2)	0	82	0.127

Female	128	53.2(15.7)	18	91	
Total	195	51.9(16.6)	0	91	
Perception Mark					
Male	67	34.5(20.0)	0	100	0.022
Female	128	41.6(20.6)	0	82	
Total	195	39.1(20.7)	0	100	

Note: The mean difference is significant at the 0.05 level.

The overall results of knowledge and perception levels among the races showed the mean mark for Malay students was 48.2(SD ±15.4). Chinese students got a mean mark of 43.6(SD ±15.4), Indian students was 45.8(SD±16.2). There was no significant difference between any of the races. In the assessment of knowledge alone, Malay students had the highest mean mark, which was 59.7(SD ±14.1), The Chinese students scored the lowest mean mark among all the races 48.4(SD ±17.0), the Indian students had a mean mark of 55.8(SD ±15.5). There was a significant difference between Malay and Chinese students with the *p*-value of 0.006 regarding knowledge value. Therefore, other races had the highest mean value of 43(SD ±18.6), followed by Chinese 39(SD ±20.9), Malay 36.6(SD ±22.8), and Indian 35.9(SD ±20.6). However, there were no significant differences between the races regarding overall value and perception value (Table 6).

Table 6: Knowledge and perception assessment among races

Overall Value/ Variables	N	**Mean (±SD)**	Minimum	Maximum	*p*-value
Malay	27	48.2(15.4)	27	82	
Chinese	104	43.6(15.4)	0	82	
Indian	23	45.8(16.2)	18	86	0.188
Other	41	49.2(14.0)	18	91	
Total	195	45.7(15.3)	0	91	
Knowledge Value					
Malay	27	59.7(14.1)	36	82	0.006

Chinese	104	48.4(17.0)	0	91	
Indian	23	55.8(15.5)	27	91	
Other	41	53.4(15.9)	27	82	
Total	195	51.9(16.6)	0	91	
Perception Value					
Malay	27	36.6(22.8)	0	82	
Chinese	104	39.0(20.9)	0	82	0.498
Indian	23	35.9(20.6)	0	82	
Other	41	43.0(18.6)	0	100	
Total	195	39.1(20.7)	0	100	

Note: The mean difference is significant at the 0.05 level.

Discussion

Knowledge and perception assessment of childhood autistic disorder was evaluated using a set of the questionnaire aimed to ascertain students' understanding of childhood autistic disorder (ASD) and their perception towards autism. Along the study, the majority of the respondents had heard about autism. However, the overall knowledge and perception towards ASD were considerably low with overall score of 45.7(SD ± 15.3). It was consistent with the low level of knowledge and awareness among middle school students in the previous report of the earlier study and it reflected a deficit in knowledge and perception of autism among in this study (Campbell et al 2011). In fact, education students should possess higher levels of knowledge and perception than the middle school children. This could be because of the local educational system as most of the participants (78.9%) were local students and they rarely learn–about autism in their primary and secondary schools. On the other hand, the result of low mean mark on knowledge and perception is in contrast with the study done in the United Arab Emirates which proved that university students were having good knowledge and perception on childhood autism. The contrariness can

be seen in some countries had more effective interventions or programs that successfully increased the knowledge and perception of the population on childhood autism (Ibrahim et al 2016).

The overall knowledge scores out of 195 participants in this study were 51.9% compared with the previous finding done in Nigeria in which the sample scored 56.2%. However, it should be noticed that this study reports a slightly lower knowledge score which can be attributed to the difference in the study setting (Monday et al 2010). This study presided over a population of interest who were from medical and non-medical sciences background who at least had some basic knowledge towards autism.

Based on the result, our respondents scored higher mean marks for the knowledge part than the perception part, which was 51.9% and 39.1% respectively. Comparing this result with the erstwhile study done in Northern Ireland, it revealed that the awareness was low enough about ASD. This might because of the less contact with the autistic children throughout their life. As shown by the results, there were only eight participants (4.1%) among the total of 195 participants had an autistic child in their family while the previous study showed that 60% of their respondents knew someone with ASD in their own family, circle of friends or work colleagues (Dillenburger et al 2013). This factor might cause their viewpoints on autism to be insufficient. The more exposure to the autistic children, the higher the understanding they would have towards autism. Autism awareness may also be low because it is categorized under learning disability and although autism may cause learning difficulties and disability affected. A local survey revealed that 1 in 625 Malaysian children had autism. As per the National

Autism Society of Malaysia ((NASOM) 2013), a 30% rise in an organization's intake of individuals with autism in the past three years has been found. Although there are programs in place that can potentially detect autism in children in Malaysia, such as the screening program known as Literacy and Numeracy Screening (LINUS) program for school entry in Year 1 which refers children with learning disabilities to medical professionals, it can be considered quite late time-wise (Xiang et al 2014).

Results of this study showed that medical students had higher average marks than non-medical science students regardless of the knowledge or perception of childhood autistic disorder. There was a significant difference in the perception among medical and non-medical students about childhood autistic disorder, it is might be the medical students had different educational contents and different level of exposure towards autism in their studies. This result is similar to the result of the previous study that was done by the University of Nigeria among final year undergraduate Medical, Nursing and Psychology students on the topic of "Factors Influencing Knowledge about Childhood Autism". The medical students got an overall mean mark higher than non-medical students (Monday et al 2010).

It should be noted that there was no significant association between knowledge about childhood autism and gender of the respondents. This is consistent with the study done in Karachi among medical students from private and public universities which reported that were almost the same[9]. This could be said that the information provided by the government were equally reached to both male and female students. There were strong relations between overall mean mark and perception on autism with the

gender of the participants, notwithstanding the knowledge section showed a vice versa. The results of overall mean marks of participants and their attitudes towards autism were slightly low irrespective of their curricular level. Females scored 47.6% in total, slightly higher than males which were 42.1%. It is demonstrated similarly in the perception section at where the females scored higher than the males which were 41.6% and 34.5% respectively. This could be due to the difference in their personality. Females usually tend to be more concern and attentive about the things around them. This is indicated that the need of awareness about the autism during their basic science curricular which is warranted. Interestingly, there were no significant differences between races, except for the marks for knowledge. This is a new finding since previously there is no local research done on the comparison between races about the knowledge and perception on childhood autistic disorder. This could be reasoned as the information about childhood autistic disorder was equally distributed among different races in Malaysia. In conclusion, ultimately the knowledge and perception among university students of childhood autism are turned out to be approximately inadequate. Based on the finding, students who are undertaking medical courses were more knowledgeable than those taking non-medical science subjects. In addition, females' knowledge and perception towards autism were significantly higher than male students. On the other hand, there is no significant difference in knowledge and perception between the races. More campaigns and educations regarding autism should be provided by universities or government accordingly. The topic of autism can be included in the syllabus of the primary and secondary education system.

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The Effect of Different Text Background Colour on Accommodation Error among Myopes

Jayakumaran D.M. & Uma Mageswari B.

Abstract

This study aimed at investigating the changes in the accommodation error of the eye to the changes of different text background colours among myopes. Thirty two myopes participated in this study and dynamic fused-crossed cylinder was used to measure the accommodation error binocularly. One-Way Repeated Measure ANOVA revealed that there was a statistically significant difference in the mean accommodation error between the four different background colours among myopes, $F(3, 93) = 0.92$ ($p<0.05$). Pairwise comparisons showed that the mean accommodation error of the blue (-0.27 D ± 0.52) background colour was significantly different ($p<0.05$) compared to that of the white (-0.03 D ± 0.70) and red (0.15 D ± 0.61) while the mean accommodation error of the green (-0.06 D ± 0.45) text-background colour did not significantly differ from any of the other colours ($p>0.05$). The red background colour tends to elicit a higher accommodation error and that of the darker blue tend to elicit a lower accommodation error compared to that of the standard white background.

Keywords: Accommodation error, fused-cross cylinder, text-background, colour, myopes.

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Introduction

These days, information are being displayed with a multitude of letters and text background colour combinations on printed materials and digital displays alike, no longer rigidly conforming to the standard of the black print on white backgrounds. However, it is also common that nowadays, that prolonged reading is associated with asthenopic symptoms such headache, eye discomfort, dry eyes and eyestrain, although found to be significantly higher with the display units compared to printed materials (Chu et al. 2011). In general, any type of near work would stimulate the accommodation system of the eye, and the accommodation response to it is greatly influenced by the chromatic elements and the luminance of the stimulus given (Nakatsuka et al. 2003). The ordinary activity of reading a printed material positioned at 40cm will induce an accommodative stimulus of 2.50 Diopters (D), but a normal accommodative response would be slightly less, at about 2.00D (Ahmad, Chen & Yahaya 2014; Elliot 2013). During reading, there is a difference in the position between the visual target and retinal conjugate point of the image in the eye and thus, an accommodation error will occur as lag of accommodation due to the tweaking of accuracy between the accommodation response and the accommodation demand (Nakatsuka et al. 2003; Charman 2008; Ahmad, Chen & Yahaya 2014). Myopic individuals are highly likely to under-accommodate when doing near work such as reading printed materials compared to emmetropes (Allen & O'Leary 2006).

Most of the studies previously conducted involved the colour of the characters or text itself, only very little investigation were focused on the

relationship between the accommodation error and the text background colour. Furthermore, there is a lack of research regarding the differences between text-background colours and its consequence on the accommodation system. Hence, it was a study of interest to investigate the effects of different text background colours among myopic individuals when reading, in terms of accommodation error.

Methods and Materials:

Ethical approval was obtained from SEGi Ethics committee (RIMC) in SEGi University to ensure that the study adhered to the Declaration of Helsinki. Normal subjects of age ranging between 18 to 29 years old with myopic refractive error of more than -0.50 DS spherical component and cylindrical component up to -1.00 DC were recruited. It was ensured that all the subjects had visual acuity (VA) of 6/6 or better for distance and N5 at near with habitual correction, no colour vision deficiencies from the Farnsworth-Munsell D-15 assessment, normal Amplitude of Accommodation (AA) with respect to each subject's age, phoria measurement within the normal range, no remarkable external or internal ocular disease, and no remarkable systemic diseases. The subjects who were contact lens wearers, have more than 2 errors in the Farnworth-Munsell D-15 assessment, difference in the cylinder component of more than -0.75 DC between the two eyes and subjects who are antimetropia were excluded from participating from the study. Informed consent was taken from each selected subject before participation in the study. All of the procedures of this phase took place in Room 3 of SEGi EyeCare in SEGi University.

In this study, a hardcopy of a black rectilinear cross target on a selected coloured background was used as a target and was called "Test Card" (Figure 1). The four different-coloured Test Cards were the same in size, shape and with a black printed rectilinear cross target on each. There was a stick zone marked on the upper part of each Test Card of same distances to ensure that the black cross targets were always on the same position to the subject's eye when each of the different colour Test Cards were used.

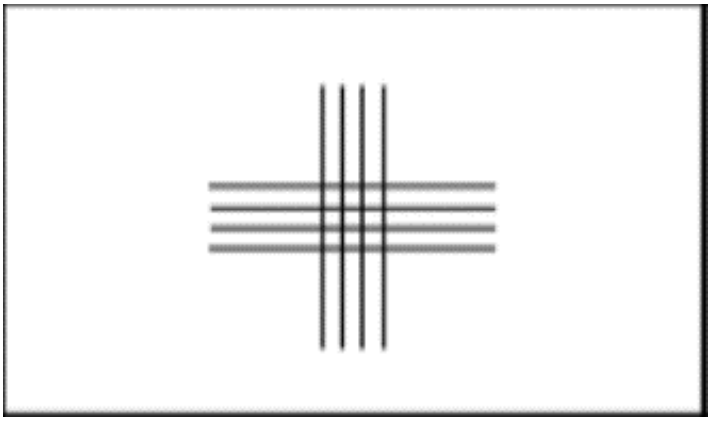

Figure 1 Rectilinear Cross Target used as "Test Card"

The selected background colour were red, green, and blue as according to Young-Helmholtz theory of trichromatic colour vision model (30). The approximate generic white, red, green and blue values of Test Card background were measured by using the Apple Digital Color Meter and Color Picker from Adobe Photoshop. The black cross target was scanned to the computer first before being printed on the colour code values in which red was 225-99-86, green was 110-205-85, blue appeared as 80-92-194 and the standard white background appeared as 255-255-255. The illumination of the room was ensured to be between 300-1000 lux (33) by using a lux

meter. The subjects were not exposed to the Test Cards prior to the participation of the study and was randomly selected from a shuffled pile, for each new subject, with all of the Test Cards facing down.

Each of the Test Cards were placed at a distance of 40 cm from the subjects and used together with the cross-cylinder lenses in the phoropter. The subjects were to note the difference in clarity between the horizontal and vertical lines of the rectilinear black target after placing the cross cylinder in the phoropter for each of the four different text-background colours. Astigmatism will be induced when the cross-cylinder were introduced binocularly upon viewing the rectilinear target, and the subject's subjective response indicates whether a lead or lag of accommodation exist (6). The accommodation error for each subject and for each text-background colour were measured binocularly when the subjects were asked to make the two meridional lines to be equally clear with the help of added neutralizing spherical lenses. The data obtained were analyzed by using the IBM SPSS Statistics version 24 (SPSS Inc., Chicago, IL, USA). A p-value for any data of less than 0.05 was considered to be statistically significant.

Results

Thirty-two subjects in total comprising of 8 males and 24 females participated in this study, all with the mean age being 21.78 years old (± 2.42). The number of participants with low myopia was 20, seven were medium myopes and 5 were high myopes, with the mean spherical component being -2.98 DS (±2.43) and the mean cylindrical component being -0.38 DC (± 0.33).

The mean changes of accommodation error for the text-background colour of white, blue, red and green were -0.031 D (± 0.70), -0.27 D (± 0.52), 0.15 D (± 0.61) and -0.06 D (± 0.45) respectively as shown in Figure 2. One-Way Repeated Measure ANOVA showed that there was a significant difference in the mean accommodation error for the text background of between white, blue, red and green colour among myopic individuals. $F(3, 93) = 0.92$ ($p<0.05$). Pairwise comparisons showed further showed that the mean accommodation error of the blue (-0.27 D ± 0.52) text-background colour was significantly different when compared to that of the white (-0.03 D ± 0.70) and red (0.15 D ± 0.61) text-background colours ($p<0.05$). In contrast, there was no significant difference when the mean accommodation error of the red background colour was compared to that of the white background colour. The mean accommodation error of the green (-0.06 D ± 0.45) text-background colour did not significantly differ from any of the other text-background colours ($p>0.05$).

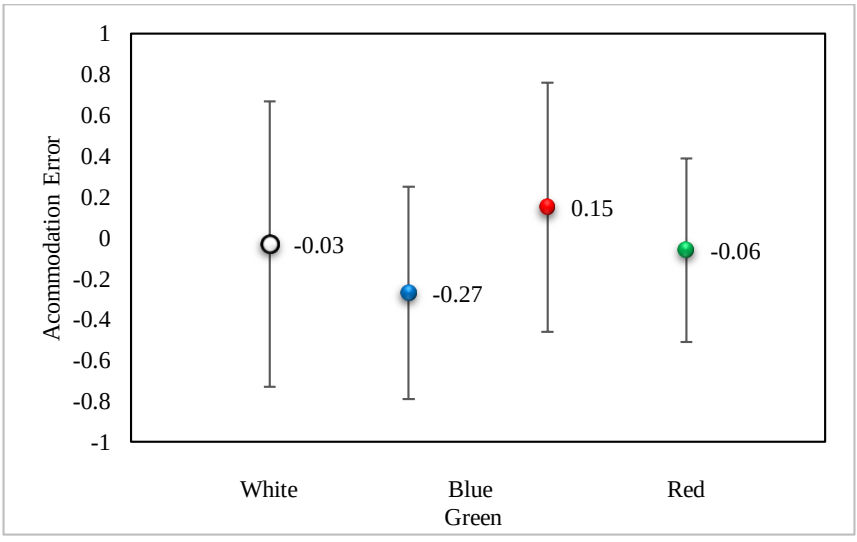

Figure 2 Mean Accommodation Error for all coloured Test Card

Discussion

A positive accommodation error, also known as accommodation lag, occurs when the accommodative response is lesser than the accommodative demand (Manny et al. 2009; Hinkley, Iverson-Hill& Haack 2014). The results showed that the red text-background colour has the highest mean accommodation error; while on the other hand, the blue text-background colour has the lowest mean accommodation error respectively among the rest. The findings implied that the accommodative response was the least when viewing a black target on a background colour with a longer wavelength like red, and the accommodative response was the highest when viewing a black target on a background colour with a shorter wavelength, i.e. blue. The accommodation error for the background colour of medium wavelength, represented by green was almost similar in value to that of the white background colour, suggesting that the accommodative error for viewing a black target on a white background were close to almost none and most similar in nature to that of the green background colour.

The mean accommodation error was the highest when red was used as the background colour because the target colour was presented as black, and the subjects were required to pay attention to the target with respect to the red background colour. A myopic eye will elicit a higher accommodation lag and low accommodative response when required to pay attention to a dark coloured target such as black compared to that with a bright colour, which is more attention-focusing and stimulating accommodation (Ahmad, Chen & Yahaya 2014).

Previous studies have shown that the negative lenses that were used to correct myopia will create a hyperopic retinal defocus (Jiménez et al. 2011; Lin et al. 2010; Mutti et al. 2006; Nakatsuka et al. 2005). This consequently will affect the longitudinal chromatic aberration (LCA) as well (Mutti et al. 2006; Nakatsuka et al. 2005). At near and with addition minus lenses, the longer-wavelength (red) of the LCA in a myopic eye will be focused further behind the retina than that for emmetropes (He et al. 2013; Rucker & kruger 2004). It may have caused a noticeable blurred retinal image at the margins of the target (Rucker & Wallman 2012). This may have resulted in a higher accommodation lag and hence, more plus lenses were required to re-adjust the image onto the retina.

The long-wavelength sensitive cones (R-cones) in the retina were claimed to have efficient detection of luminance contrast (Rucker 2013). A near task with a higher luminance contrast will cause the ocular system to detect it as high spatial frequency and consequently lower the accommodative error (Buehren & Collins 2006). The red colour that was used in another study (Ahmad, Chen & Yahaya 2014) was against a white background, which was known to demonstrate an almost no accommodative error (Rucker 2013). On the other hand, the black target on a red background in the present study was considered to have a reduced spectral bandwidth (Atchison, Strang & Stark 2004), and hence, a slightly lower luminance contrast was identified by the R-cones, and thus there would be a larger accommodation error (Lee et al. 1999).

It is found in previous studies that a blue stimulus will relax the accommodation response more than that of the red one because it is

positioned in front of the red wavelength in LCA (Ahmad, Chen & Yahaya 2014; Kröger & Binder 2000; Rucker & Kruger 2004). This would mean that an emmetropic eye will be more myopic and a larger and a more positive accommodation error will be induced (Graef & Schaeffel 2012; Kröger & Binder 2000). The current study, however, contradicts this. The blue colour used as the background colour in this study was found to have the most negative and lowest mean accommodation error instead. It is found that the accommodation effort surprisingly increased at wavelengths of colour below 430 nm or with colours of darker blue (Graef & Schaeffel 201; Seidemann & Schaeffel 2003), which was the colour similar to the RBG value used in this investigation to represent short wavelength, i.e. code 80-92-194. This led to a far lower accommodation error as well, and not exactly obeying the effect of LCA. This can be explained by the distribution of the short-wavelength sensitive cones (B-cones) in the retina. They are present less in the fovea but more in the periphery retina, and are more used to the myopic defocus since most of the use of daily technologies in the modern era emits blue light (Jiang et al. 2014). Furthermore, the processing of background information was mostly attributed by the peripheral retina (Alizaeh-Ebadi, Markowitz & Shima 2013). When a myopic defocus occurs, the darker blue image will be further in front of the retina, and additional minus lenses were needed to reduce the perception of the target being blurred on the retina (Gegenfurtner & Sharpe 2001). This consequently will decrease the accommodation error, supporting the finding of this experiment. The explanation was based on the assumption that B-cones were preferably stimulated by a darker blue colour of wavelength that lies below 430 nm (Seidemann & Schaeffel 2003; Gegenfurtner & Sharpe 2001). In the

meantime, a clear understanding of the mechanism of LCA in affecting the retinal processing of different wavelength of light is yet to be known (Jiang et al. 2014).

A black target on a blue background was also considered to have reduced spectral bandwidth (Atchison, Strang & Stark 2004) and thus, reducing the luminance contrast (Bhattacharyya et al. 2014; Buehren & Collins 2006). It was supported that this decreases the visual acuity of the target and resulting in a very negative accommodation error due to the inability of the myopic eye to detect high spatial frequency (Buehren & Collins 2006). In addition, it was recommended that black and blue combinations should be avoided as it offered poor intelligibility to the subjects and lowered cortical activation (Bhattacharyya et al. 2014). Hence, this study supports the notion that the optimum accommodation response will be elicited in higher luminance levels, such as a black target with white or green background colour.

A normal trichromatic eye is commonly focused at 550 nm to 570 nm in general, but follows the focal plane predicted by LCA with respect to the different wavelengths to accommodate, except at very short wavelengths like dark blue (Seidemann & Schaeffel 2003; Graef & Schaeffel 2012; Rucker 2013). This is because the R-cones and medium-wavelength sensitive cones (G-cones) are very closely packed in the fovea compared to the B-cones, providing higher spatial acuity and have “specialized channel pathways” to the visual cortex (Kruger et al. 1993; Graef & Schaeffel 2012). Consequently, in the present study conducted, there was no significant difference of the mean accommodation error of the green colour as background compared to the other colours because the yellow-green colour

range (550 - 570 nm) was effortlessly focused onto the retina when accommodation is active (Kruger et al. 1993). This also explains that the value for the mean accommodation error of green to be almost no accommodation error.

In contrast, in the investigation conducted in a previous study, the mean accommodation error for a black target on green background was the highest among the other background colours. However, a previous study conducted (Bakaraju, Yeotikar & Srinivas Rao 2007) involved emmetropic subjects instead of myopes and the accommodation error was measured for coloured backgrounds in the conditions of with and without additional minus lenses in front of the subject's eye.

The findings and explanation that a black target on a white background induced a slightly lower to almost no accommodation error claimed that the most precise compensation the lens-induced defocus was found in white light (Rucker 2013). The white colour involves the merging of the spectrum of wavelengths and follows the chromatic dispersion in the human eye (Kruger et al. 1993). Therefore, when there is a summation of different defocus via the combination of different wavelength of lights from a white coloured background, there was almost no accommodation error (Rucker 2013) as found in the present study.

It was found in this study that different wavelengths and colours cannot all be focused at the same time onto the retina. The present investigation support that the accommodation system involving accommodation error is influenced by colour vision perception (Seidemann & Schaeffel 2003;

Rucker 2013; Ahmad, Chen & Yahaya 2014). It was previously stated that children should read text from paper that reflects short or blue wavelength or with a background that is luminated by a blue light (Kröger & Binder 2000). The present study, however, contradicts by revealing that not all short wavelength colour as the background would relax the accommodation, since the accommodation effort was found to be increased again at wavelengths shorter than 430 nm or a colour of darker blue (Graef & Schaeffel 2012).

Furthermore, it was found that subjects have higher accommodation lags with reduced spectral bandwidth illumination such as when using black target on red background and black target on blue background, regardless if it was on visual display units or on paper (Kruger et al. 1993). In addition, a study emphasized that a combination of a black target on blue background was to be eluded (Bhattacharyya et al. 2014). Hence, a lighter colour of the spectrum was recommended for the background of a dark target which will result in a higher spectral bandwidth (Lee et al. 1999). The findings in the current study were on par with the findings of two different earlier investigations (Shieh & Ko 2005; Bakaraju, Yeotikar & Srinivas Rao 2007) which showed that the preferable background colours for a black target were white and green due to the more accurate response by the eye. Many previous studies were conducted for visual display units (VDUs) instead of printed materials and it was foreseen that the colour perception will be slightly more distinguished when using VDUs compared to that of a printed text material (Ahmad, Chen & Yahaya 2014). However, the accommodation response with respect to accommodation error was noted to be similar between the two mediums (Sorkin, Reich & Pizzimenti 2003). Hence, the

findings can be applied to the background colours of both VDUs and printed materials.

Conclusion

The study found that the text background colours of printed materials affect the accommodation error among myopes. This study suggest that green background colour and the chromatic or the standard white background colour were better used for comfort of accommodation for myopes as they minimize the accommodation error elicited.

Acknowledgement

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Influence of Storage Temperature on Probiotic Survivability in Cultured Milks

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Abstract

Cultured milks are a common source of probiotics that impose many health benefits when administered viable in sufficient amounts to the host. This research studies the impact of varying storage temperatures on probiotic survivability in cultured milks. Six commercially-available fermented milk brands (labelled A-F) claiming to contain different species of Lactobacillus were stored at 4°C and room temperature (RTP) for 24 hours prior to the first sampling, all throughout the 5-week period of the study. Colony-forming units (CFU) were counted after a 2 days of incubation at 37°C. Products A, B and F – all containing L. casei ssp. – showed better probiotic survivability at 4°C than RTP; however the opposite results were seen for products D and E which consisted primarily of L. paracasei and L. rhamnosus HN001 respectively, whereby higher numbers of probiotics were obtained at RTP than at 4°C. Product C failed to observe any probiotic growth throughout the study. Apart from C, all products managed to sustain at least the minimum recommended number of probiotics (6 log CFU/mL) throughout their shelf lifes at 4°C.The results show that storage temperature plays an important role in maintaining survivability of probiotics in cultured milks.

Keywords: Lactobacillus, probiotic storage, survivability, temperature

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Introduction

Cultured milks are products of lactic acid fermentation of milk via incorporation of lactic acid bacteria (LAB). Being economically inexpensive, easily available and tremendously palatable, fermented milks have become a common and popular source of probiotics amongst members of the community. Probiotics comprise of microorganisms like yeasts and bacteria; but are more famously known as "good bacteria" that strive in the gastrointestinal tract. Lactobacilli spp. and Bifidobacteria spp. are examples of probiotic bacteria commonly found in cultured milk products (Ferdousi et al 2013; Varga-Visi and Pápai 2015) which confer health benefits like preventing infections and cancer, aiding digestive problems, and strengthening the immune system when taken in sufficient viable quantities into the body (Kailasapathy and Chin 2000; Saad et al 2013). The minimum number of probiotics recommended to be present viable in fermented dairy products is at least 106 colony-forming units per mL (CFU/mL) at the time of consumption or at the end of their shelf life to accommodate and compensate for any losses during processing, transferring and storage (Calinoiu et al 2016; Damin et al 2008; Dave and Shah 1997; Hsiao et al 2004; Ferdousi et al 2013; Freitas et al 2015; Gueimonde et al 2004;

Kailasapathy and Chin 2000; Ranadheera et al 2012; Rozada et al 2009; Wang et al 2012).

To achieve this requirement, it is of utmost importance to determine optimal production procedures and various conditions that can maximise the survivability of probiotics in media. These include during manufacture, distribution, sale, storage and also in the host. Probiotics need to survive throughout the entire transit in hosts from the start of ingestion, to surviving the harsh acidic gastrointestinal conditions, hydrolytic enzymes and bile salts in the intestines, before finally being able to exert their effects at their site of action. As a result, numerous research has been done, be it in the field of microbiology, such as studying the effects of different combinations of probiotic LAB and starter LAB cultures (Dave and Shah 1997; Donkor et al 2006; Gilliland et al 2002; Kristo et al 2003; Mani-López et al 2014; Ng et al 2011; Shah 2000; Vinderola et al 2002;), or biotechnology and food processing, such as studying different techniques and materials for probiotic microencapsulation (Abbaszadeh et al 2014; Anal and Singh 2007; Capela et al 2006; Ding and Shah 2009; Homayouni et al 2008; Hsiao et al 2004;Kanmani et al 2011; Krasaekoopt et al 2006; Wang et al 2012).

Storage conditions, either in warehouses after production, during distribution, sale, or consumer handling and home storage of the product, is another factor that greatly influences the survivability of probiotics. These conditions include presence of oxygen, hydrogen peroxide and other metabolites as well as changes in pH and also temperature fluctuations (Damin et al 2008; Donkor et al 2006; Hsiao et al 2004; Ferdousi et al 2013;Freitas et al 2015; Mortazavian et al 2007; Ng et al 2011; Rozada et al

2009;Sadaghdar et al 2012; Scharl et al 2011; Talwalkar and Kailasapathy 2004; Vinderola et al 2011).

Post-manufacture and beyond manufacturing plant storage warehouses, storage temperature, in particular, is the only variable affecting probiotic survivability that can be directly controlled by distributors and consumers. Dairy products are generally known to require refrigeration during storage to maximise their shelf lives. Numerous studies on storage temperatures in fermented dairy products, whether exclusively at 4°C (Damin et al 2008; Gueimonde et al 2004; Khalf et al 2010;Ranadheera et al 2012; Shah et al 1995; Wang et al 2012), varied within the refrigerated ranges (Freitas et al 2015; Gilliland et al 2002; Mortazavian et al 2007; Nighswonger et al 1996), or comparing refrigerated and room temperatures (Ferdousi et al 2013; Hsiao et al 2004; Rozada et al 2009; Scharl et al 2011) have shown that probiotics attain better survivability in refrigerated conditions. However, different species and strains of probiotics have slightly different storage temperatures preferred for survival (Damin et al 2008; Ferdousi et al 2013; Hsiao et al 2004; Kailasapathy and Chin 2000; Mortazavian et al 2007; Scharl et al 2011;Shah et al 1995; Wang et al 2012; Varga-Visi and Pápai 2015).

From the findings of these studies, probiotic survivability in cultured milk is also expected to be significantly affected by storage temperature, in which refrigerated temperatures are preferred for maximising probiotic viability. Hence, this research looks to investigate and compare the effects refrigerated and room temperatures on probiotic survivability in several brands of commercially-available cultured milks in Malaysia. This study was also

slightly extended further into estimating and testing the cultured milk products for compliance with the minimum recommended number of probiotics at recommended refrigerated temperatures.

Material and Methods

Procurement of samples

A fresh batch of the 6 fermented milk brands (labelled A-F, all unflavoured) was commercially obtained just a day before commencement of the study to secure maximum prolongation of expiry dates for the entire 5-week duration of laboratory work. Besides approximately standardising the manufacturing dates across all 6 samples, this also helps to achieve at least a good 3 to 4 week-period for testing the adherence of cultured milk manufacturers to the minimum recommended number of probiotics at refrigerated temperatures, at the end of their shelf lifes.

Determination of dilution factors

Six different brands of cultured milks labelled A-F were spread undiluted up to 6 times dilution (dilution factor of 1 to 10-6) using sterile 1× PBS and incubated for 24-48 hours. Plates were then observed for presence or density of probiotic growth and the dilution factor that facilitated best visualisation for the most convenient counting was chosen for each sample.

Preparation of samples

Upon procurement, each cultured milk brand was separated into two samples from the same bottle: one to be kept in 4°C and the other in room temperature

(RTP) for 24 hours. Assays were conducted in triplicates, which were then drawn out from each of the samples kept at both temperatures for serial dilution and spreading.

5 mL of each product stored in 4°C was also drawn out separately, sealed with parafilm, labelled, and kept aside at 4°C to act as controls for each of the samples. These controls were then spread at the end of the sampling period as a comparison to ensure that colonies cultured from the test samples throughout the research period were not contaminated or originated from foreign sources.

Aseptic techniques were consistently practiced throughout the sampling procedures to minimise microbial contamination.

Data collection

The petri dishes were removed from the incubator after 24-48 hours of incubation and observed for presence of probiotic colonies. Pictures of each plate were also taken as reference before counting of colonies using a colony counter pen. Colony-counting for each plate was done at respective dilution factors that were predetermined before spreading to facilitate easy visualisation of the colonies for manual counting.

Data analyses

The average numbers of colonies for each sample at each temperature were converted from CFU/100 µL into CFU/mL as the standardised unit for

analyses. From these results, log CFU/mL was calculated to ease data analysis and comparison.

Results

Dilution factors

The dilution factors for products A-F were determined (Table 1) to facilitate best visualisation for the most convenient counting for each sample.

Table 1. Determined dilution factors for products A-F at the start of study

Product	Dilution factor
A	10^{-6}
B	10^{-6}
C	1 (undiluted)
D	10^{-4}
E	10^{-4}
F	10^{-6}

Probiotic survivability

Products A, B and F had shown similar results, in which probiotic survivability was higher in 4°C than in RTP. Under both storage temperatures, a consistent declining pattern was seen in product A (Figure 1); however, the death of probiotics occurred much more rapidly in RTP than in 4°C. When stored in 4°C, probiotic survivability remained above 7 log CFU/mL even after its expiry date. Storage in RTP resulted in a plummet to

less than the minimum recommended probiotic quantity of 6 log CFU/mL at the 5th harvest, one week before the end of its shelf life.

Products B and F had similar results (Figure 1). The survivability curves for both products generally showed that at RTP, probiotic survivability declined more rapidly than at 4°C. Despite that, both temperatures for both products still managed to sustain more than 7 log CFU/mL of probiotics throughout their shelf lives.

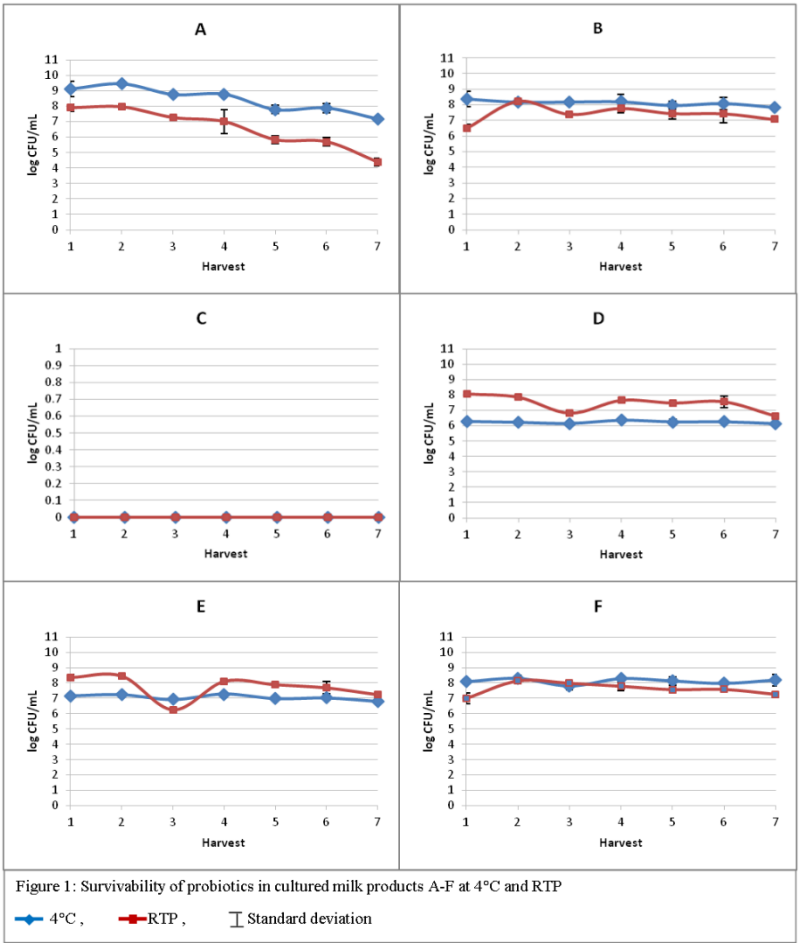

Figure 1. Survivability of probiotics in cultured milk products A-F at 4°C and RTP.

Contrary to products A, B and F, products D and E demonstrated a completely opposite trend: RTP consistently had a higher survivability than 4°C (except for harvest number 3 for product E which may be associated with human error). Survivability in RTP was approximately 2 log CFU/mL and 1 log CFU/mL higher than 4°C for product D and E respectively throughout the research duration. Despite that, both temperatures for both products still managed to maintain a probiotic survivability of above 6 log CFU/mL throughout their shelf lives.

According to our observation, product C was the only sample which had unchangingly failed to accommodate even a single colony of probiotic growth in both storage temperatures for the whole 5 weeks of experimental work.

Discussion

On the labels, product A claims to contain L. casei Shirota; product B, L. acidophilus (LA) and L. casei (LC); product C, Lactobacillus; product D, L. paracasei (LP), Streptococcus thermophilus (ST) and L. delbruekii ssp. bulgaricus (LB); product E, L. rhamnosus HN001 (LR), Bifidobacterium lactis (BL), LA and ST; and product F, LC.

The three fermented milks containing LC, A, B and F, had demonstrated better probiotic survivability in 4°C than in RTP. These results are in agreement with studies by Ferdousi et al. (2013) and Mortazavian et al. (2007), and can be associated to higher probiotic metabolism rates at higher temperatures, hence, incurring a higher death rate.

Contradictory results were observed for products D and E which employed yoghurt starter LAB (LB and/or ST) co-cultured with probiotic LAB. Both these brands resulted in higher probiotic survivability in RTP storage as compared to in 4°C.

Product D contained LP as the probiotic and LB and ST as the starter cultures. One limitation to this study is that selective enumeration of the probiotic bacteria was not done; hence, the colonies cultured may possibly include those of the starter cultures as well. A possible reason for enhanced probiotic survivability in product D at RTP may be caused by starter culture LB growth being suppressed under refrigerated temperatures (Kneifel et al. 1993), thereby causing more growth to be seen in the RTP plates as compared to at 4°C. However, this finding contradicts to the Teixeira et al. (1995) study which found higher LB survivability at 4°C compared to 20°C. Post-acidification may also contribute to better survivability of probiotics in RTP than in 4°C. LB is responsible for this action. Production of lactic acid and hydrogen peroxide by LB under refrigerated temperatures has been observed to be detrimental to some strains of LA (Lourens-Hattingh and Viljoen 2001, Ng et al. 2011, Shah 2000). Further studies need to be done to confirm if LP, and the strain employed in product D is affected. Again, a contradicting finding by Kneifel et al. (1993) in which growth of LB was suppressed under refrigerated temperatures would also suggest that lactic acid and hydrogen peroxide production is also suppressed. There may also be a potential of LP co-cultured with ST and LB to not require refrigerated storage. A study by Ferdousi et al. (2013) demonstrated that LP survived second best under RTP storage, albeit less than at 4°C. It is important to note that probiotic behaviour is strain-specific (Vinderola et al. 2002); hence the

possibility of synergistic effects in co-cultures of certain strains of LP with ST and LB under RTP holds. Further studies need to be done to identify the LP strain used in product D and its interaction with the starter cultures is needed to clarify this result.

The higher CFU/mL achieved in RTP as compared to in 4°C for product E containing LR co-cultured with LA, BL and ST may suggest the potential of LR with these co-cultures to not require refrigerated storage. Ferdousi et al. (2013) demonstrated that LR had the best survivability in 20°C (despite having a better survivability in 4°C) and recommended LR to be used in production of yogurts in warmer countries. It is important to note that in the study, LR was co-cultured with yoghurt cultures, ST and LB. Different species and strains of co-cultures bring about different interactions, synergistic, or antagonistic effects (Vinderola et al 2002). Synergism between BL with LA and ST is already known, however, interaction of LR with LA and BL in the presence of ST has not been explored as of yet.

Another important issue to consider is the incorrect identification and labelling of probiotics, which has been proven to be fairly common in previous researches (Coeuret et al 2004; Gueimonde et al 2004; Schillinger 1999). This discrepancy not only causes misleading information to be conveyed, but also affects the viability and quality of cultured milk products due to the very diverse types of interactions between different LAB spp. (Vinderola et al 2002). The worst-case scenario is reflected in product C, whereby the manufacturer's claims of incorporating Lactobacillus in its cultured milk was tested to be inaccurate. Consequently, tighter quality

controls and research and development efforts should be employed to ensure that probiotics identified are accurate.

One shortcoming of this study was that it was only conducted under aerobic conditions. As a result, the presence of obligate anaerobes like Bifidobacteria, which may play a part in influencing survivabilities of the probiotics, was unable to be detected.

Conclusion

Despite the mixed results pertaining to the comparison of probiotic survivability in 4°C and RTP achieved from this research, all products, except for C, demonstrated a survivability of more than the minimum recommended number of probiotics (106 CFU/mL) at recommended storage temperature (4°C) throughout their shelf lives. This research, hence, agrees fully with the manufacturers' suggestions of storing cultured milks in refrigerated temperatures.

Some recommendations for improvement in future research include usage of proper enumeration media for each species of LAB to enable proper isolation, avoid overlapping growth and hence, clearer results to be attained. Proper identification of the LAB species and strains in each product should also be done to accurately study their interactions with co-cultures, especially in combinations that have yet to be scientifically explored. Also, one of the products included in this research contained BL, which is an obligate anaerobe. As this study only considered aerobic conditions, it is suggested that future studies also include anaerobic conditions to further enhance the accuracy of the findings.

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Comparison between aerobic and anaerobic methods of semi quantitative culture for estimation of salivary lactobacilli count

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Abstract

Aim: To compare the aerobic and anaerobic methods of semi quantitative culture for estimation of salivary lactobacilli count.

Methodology: This cross sectional study was conducted on twenty five children aged 3-5 years with at least one cavitated carious lesion. Whole unstimulated salivary samples were collected, diluted and cultured on two sets of Petri dishes containing Rogosa agar medium. One set of Petri dishes (25 plates) were cultured aerobically for 72 hours. Another set of petri dishes (25 plates) were cultured anaerobically with Anaero gas pack system (Hi Media) for 72 hours. The colonies were counted and compared with inferential statistics using SPSS version 20.$p<0.05$ was considered significant.

Results: Out of 25 plates cultured aerobically, the lactobacillus growth was seen only in 8 plates (32%), as compared to 22 plates (88%) cultured using anaerobic method. While plates cultured aerobically showed an average of 0.28 colonies per plate, plates cultured anaerobically showed about 8.4 colonies per plate($p<0.001$)

Conclusion: The present study found that anaerobic method of culture was better than aerobic method in semi quantitative estimation of salivary Lactobacilli on Rogosa agar medium.

Keywords: Aerobic, Anaerobic, Culture, Salivary Lactobacilli

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Introduction

Dental caries is one of the most common preventable childhood diseases (Am Acad Paed Dent 2007) associated with excessive exposure to carbohydrates and early infection with cariogenic microorganisms like Mutans Streptococci (MS) and Lactobacilli (LB) (Colak et al 2013; Botha 2001; Palmer 2010).Lactobacilli were the first microorganisms implicated in the development of dental caries (Van Houte et al 1982). LB are prolific lactic acid producers (acidogenic) as well as acid-tolerant (aciduric), by the virtue of which they can create a low pH environment. Hence, they can be considered as key determinants in severity and progression of carious lesions (Ambade and Bhadbhade 2015)

Many studies have shown that children with Severe-Early Childhood Caries exhibit a significantly different microbiota from that of their caries-free counterparts and that lactobacilli comprise a significant portion of the cariogenic biota (Li et al 2015; Piwat et al 2012).Studies have also shown a strong correlation between the salivary Lactobacillus count and dental caries (Shi et al 1992; Gabris et al 1999). Quantitative estimation of the lactobacilli count in saliva of children may also serve as one of the useful indicators in prediction or evaluation of caries risk especially while screening large

number of children at risk for caries development (Syed et al 2015; Zhang et al 2014; Yoshizawa et al 2013).Though, a large number of epidemiological studies have shown a wide range of Lactobacillus colonization in newborn, preschool children, adolescent, and adult populations, valid and reliable measures for assessment of lactobacilli colonization and prediction of caries outcome are still lacking (Li et al 2015).

A large number of studies in the past have correlated high Lactobacillus count in saliva with a high DMFS index, most of them through semi quantitative analysis (Badet and Thebaud 2014).However, it has not been systematic and cannot be compared, as some have used aerobic method for culture of Lactobacilli, and others have used anaerobic method. No study has been carried out to compare the results obtained by different methods of culture using the same medium. Hence, the present study was undertaken to compare the salivary lactobacilli count on the same culture medium using two different semi quantitative culture techniques-aerobic and anaerobic, so that predicting caries risk in children using lactobacilli count as indicator could be done more precisely.

Materials and methods:

This study was part of a study conducted to find out the association between severe childhood caries, streptococcus mutans and lactobacillus in young children and has been published elsewhere. The study was approved by Institutional Ethical committee. Informed consent was obtained from mothers of all children. The study was conducted on 25 children aged 3-5 years with at least one cavitated carious lesion, randomly selected. Clinical

examination was carried out by a single examiner using mouth mirror and CPI probe under natural light. Caries experience was recorded using dmft index. Children with systemic diseases, on long term medication or who had taken antibiotics within 3 months were excluded.

Sample collection and processing:

All samples were collected in mid-morning session. Children were made to rinse their mouths with water to remove any debris. Children were refrained from swallowing for a minute and made to spit in disposable plastic containers to collect whole unstimulated saliva.1 ml of saliva was transferred to vials containing ringer's lactate transport media, labelled and transported to lab on ice and processed within one hour.

All salivary samples were vortex mixed and diluted by 100 fold using saline. 0.1ml of this diluted sample was cultured on each of the two Petri dishes, one for aerobic and other for anaerobic incubation. All samples were labelled accordingly.

One set of Petri dishes (25 plates) were placed in incubator for aerobic culture for 72 hours. Another set of petri dishes (25 plates) were placed in jar with Anaero gas pack system (Hi Media) for 72 hours. The colonies were identified by morphology and gram staining. Confirmatory tests were also done for colonies which included sorbitol and mannitol fermentation. Colonies were counted with the help of colony counter and expressed as actual number of CFUs x 10^4

Statistical analysis:

Data was analysed using statistical software SPSS 20. Chi-square test and student's t-test were used to find out differences in percentage and mean number of colonies between two types respectively. $P<0.05$ was considered as significant.

Results.

The mean age of children was 3.9+0.4 years. The mean caries experience of children was 5.8+2.8 and mean carious surfaces was 7.9+1.8.

Out of 25 plates cultured aerobically, the lactobacillus growth was seen only in 8 plates (32%). Whereas out of 25 plates cultured anaerobically, 22 plates (88%) had lactobacillus colonies. This difference was significant with $p<0.001$(Table.1).

Table 1: comparison of percentage of samples positive for LB growth in Aerobic VS Anaerobic method

Culture method	Sample size (n)	Positive	Percentage	P value
Aerobic	25	08	32%	<0.001
Anaerobic	25	22	88%	

A significant difference was also seen in mean number of colonies. While plates cultured aerobically showed an average of 0.28 colonies per plate, plates cultured anaerobically showed about 8.4 colonies per plate(Table 2).

Table 2: comparison of mean number of LB colonies in Aerobic VS Anaerobic method

		n	Mean (x 104)	standard deviation(+)	p-value
Children with s-ECC	Aerobic	25	0.28	0.09	<0.001
	Anaerobic	25	8.4	0.8	

Discussion

Early childhood caries (ECC) is considered to be a public health problem especially in developing countries, with the prevalence and severity being highly variable (Vadiakas et al 2008). The prevalence of dental caries in a study has been reported to be 54.1%, 42.6%, 50.7% among three, four and five years old children respectively. About 60.9% of children are found to have one or more carious lesions (Jose and King 2003).Thus, an in-depth understanding and awareness about the natural history of ECC is required to apply preventive strategies to inhibit dental caries in very young children (Piwat et al 2012).

The most common organisms that have been associated with caries in children are Mutans Streptococci and Lactobacilli (Kanasi et al 2010).Lactobacilli levels and dentinal caries have been correlated since decades, much before S. mutans-caries link. Infact some studies have suggested that a number of Lactobacillus species can be niche-specific

colonizers that vary according to the environment and that certain Lactobacillus species found in caries lesions show more cariogenic characteristics than others (Gross et al 2010). Hence they can be better predictors of caries than Mutans Streptococci (Granath et al 1994). The Lactobacillus count which means number of lactobacilli present in 1 ml of saliva (CFU/mL), can serve as an indirect indicator of the content of fermentable carbohydrate. Hence it can be used to determine the efficiency of dietetic measures as well. Also, new clinical treatment interventions of S-ECC require the regular evaluation of cariogenic bacterial colonization in the oral cavity. In this regard, semi quantitative culture to estimate the lactobacilli colonies in saliva may prove to be a useful measure which is both simple and economical. However there is a variation in the semi quantitative culture technique used for estimation of lactobacilli count in saliva by different studies in the past.

In the present study, children with cavitated caries were selected as most of the studies in the past have shown a positive correlation between salivary lactobacillus counts and dentinal caries (Bonecker et al 2003). At the same time, many studies have been able to detect lactobacilli in only less than half of the samples of saliva of young caries free children (Carlsson et al 1975; Köhler et al 1984).

In the present study about 88% of samples cultured with anaerobic technique were positive with lactobacilli colonies as compared to only 32% of samples with growth in aerobic method though samples from same children and same media were used.

There was a significant difference in the mean number of colonies as well with anaerobic method demonstrating higher number of colonies as compared to aerobic methods.

Never the less, few chair-side commercial test kits are available which can quantitatively assess the microbial risk indicators like Mutans Streptococci and Lactobacilli.[7] But these are expensive and thus may have limited application in predicting caries risk in a large number of children as in screening programmes, as compared to traditional quantitative analysis.

In conclusion, the present study found that anaerobic method of culture was better than aerobic method in relation to semi quantitative estimation of salivary Lactobacilli on Rogosa. Agar medium. However this was a pilot study done on a small sample and thus the results cannot be generalized. A large scale study would be useful in the same direction.

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A comparative evaluation of sealing ability of four different retrograde filling materials – an in vitro study.

Sylvia western J

Abstract

Aim: To evaluate the sealing ability of four different retrograde filling materials : Zinc free Silver Amalgam, IRM (Intermediate Restorative Material), MTA (Mineral Trioxide Aggregate) and Biodentine.

Objective: To evaluate the apical microleakage in retrograde cavities filled with Zinc free Silver Amalgam, IRM, MTA and Biodentine by determining linear dye penetration with a stereomicroscope.

Materials and Methods: 60 single rooted teeth were collected and decoronated. After standard endodontic treatment, 3mm of the root apices was resected at 90 degrees to the long axis. Root end cavities of depth 3 mm were prepared and were divided into four experimental groups: Group 1 - Zinc free Silver Amalgam, Group 2 – IRM, Group 3 – MTA and Group 4 - Biodentine. After retrofilling, the teeth were immersed in 2% methylene blue. The extent of dye penetration was measured using stereomicroscope.

Results: statistical analysis done using one way ANOVA and Post HOC Tukey's test revealed overall statistically significant difference between all the groups at $p<0.05$ ($p=0.00001$). The extent of apical microleakage can be summarized as: IRM > Zinc Free Silver Amalgam > MTA > Biodentine.

Conclusion: In terms of sealing ability, Biodentine is a better retrofilling material than the commonly used retrograde filling materials.

Keywords: Apical microleakage, Biodentine, Dye penetration, Retrofilling materials, Stereomicroscope.

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Introduction

The most important factor for a successful endodontic treatment is the complete three dimensional obturation of the root canal system and development of a fluid tight seal (Ozata, Erdilek and Tezel 1993), which restricts the passage of microorganisms and byproducts from the pulp space into the periapical tissues and vice versa. When this is not possible due to complexity of root canal systems or presence of physical barriers, surgical endodontic therapy becomes the next alternative (Erkut et al 2006). This procedure includes reflection of a mucoperiosteal flap, exposure of involved root apex resection of the root apex, preparation of root end cavity and insertion of a root end filling material. Various root end filling materials have been tried and tested so far in the past (Kokate and Pawar 2012). However, no material has been found, which has all or most of the properties of an ideal root end filling material (Ahmetoglu Topçu and Oruçoglu 2014), especially the property of preventing microleakage completely. Biodentine with its improved physical and chemical properties, seems to be a promising material to be used for retrograde filling. Comparison of apical microleakage of biodentine with the commonly used retrograde filling materials is needed to comparatively evaluate its sealing ability.

The purpose of this study was to evaluate the apical dye microleakage of four different retrograde filling materials (Zinc free Silver Amalgam, IRM , MTA and Biodentine) by dye penetration using 2 % methylene blue and the extent of microleakage measured in millimeters under a stereomicroscope of magnification (× 20x). The tested null hypothesis is that there is no difference in sealing ability between the materials; Zinc free Silver Amalgam, IRM, MTA and Biodentine.

Materials and methods

Freshly extracted 60, intact, non-carious single rooted human maxillary upper central incisors and canines that were extracted for periodontal reasons within six months period of start of the study were collected from the Department of Oral and Maxillofacial Surgery where?. After removal of all soft tissues and debris, the selected teeth were stored in normal saline at room temperature. The specimens were decoronted at CEJ using a diamond disc mounted on a micromotor contra angle handpiece. After decoronation, standard endodontic access cavities were prepared using a No.2 Endo access bur (Dentsply Maillefer, Ballaigues, Switzerland). ISO 15 K file (Mani, Japan) was used to confirm the root canal patency. Working length was determined by passing an ISO 15 K file into the root canal until the tip of the file was visible at the apical foramen, and then 1mm was subtracted from that length. The apical third of the roots of the teeth were instrumented to a size ISO 50 K file which was the master apical file. The step back technique was used to flare the root canal. 10 ml of 2.5% Sodium hypochlorite was used as an irrigant in between successive files. Recapitulation with smaller size files was done during biomechanical preparation. The smear layer was

removed by irrigation with 10 ml of 17% EDTA (please mention the full form) solution and 10 ml sodium hypochlorite, each for 3 min. Final rinse was done with 10 ml of normal saline. All canals were dried with paper points and obturated by lateral condensation technique using AH plus sealer and guttapercha. The access cavities were sealed with Glass Ionomer cement (Fuji IX GP, GC Corporation, Japan). The teeth were resected apically at 90 degrees to the long axis of the tooth using cross cut fissure bur (Mani, Japan) removing 3mm of the root apices. Root end cavities were prepared using a No. 2 round bur (Mani, Japan) with a contra angle micromotor handpiece to a depth of 3mm under continuous irrigation with water. The coronal portion of the root was covered with sticky wax. The external surfaces of all specimens were coated with two coats of nail polish except at the apical 2mm. The specimens were then divided into four experimental groups: Group 1- Zinc free Silver Amalgam (DPI Alloy and Vensons, India), Group 2 – IRM (Caulk, Dentsply Maillefer, Ballaigues, Switzerland), Group 3 – MTA (Angelus, Londrina, PR, Brazil) and Group 4 – Biodentine (Septodont, Saint-Maur-des-Fossés, France). The respective retrofilling material for each group was manipulated according to the manufacturer's instructions and root end filling was completed accordingly. The teeth were then immersed in 2% methylene blue dye for 48 hours. The roots were washed and split longitudinally with a diamond disc using a water coolant. The depth of dye penetration was examined under a stereomicroscope (*20x) to evaluate the roots for the extent of apical microleakage and the stereomicroscopic images are presented in Figure 1, 2, 3, 4. The greatest depth of dye penetration along any one of the cavity walls was taken and measured in millimeters using Motic Images Plus 2 software. The results

were then analyzed statistically using One Way Analysis of Variance (ANOVA) and Post Hoc Tukey's test using Statistical Package for Social Sciences (SPSS) version 13.0 software.

Results

The Mean, Standard deviation, Standard error and Coefficient of variance values of apical dye microleakage in the four groups are presented in Table 1. The mean apical dye microleakage value was found to be highest in group 2 (Intermediate restorative material) and lowest in group 4 (Biodentine). Apical dye microleakage values can be summarized as: IRM > Zinc Free Silver Amalgam > MTA > Biodentine. From various computations obtained from One way ANOVA and Post Hoc Tukey's test as presented in Table 2 and 3, it can be inferred that the difference in apical microleakage values between the groups is statistically significant $p<0.05$.

Table 1: Mean, Standard deviation, Standard error and coefficient of variance of apical dye microleakage in four groups.

Groups	N	Mean	SD	SE	CV
Group I	15	1.475	0.025	0.006	1.698
Group II	15	2.607	0.054	0.014	2.078
Group III	15	0.830	0.038	0.010	4.622
Group IV	15	0.149	0.038	0.010	25.115

Table 2: Comparison of four groups (I, II, III, IV) with respect to apical dye microleakage (in mm) by one way ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	P-value
Between groups	3	49.20	16.3988	10185.5763	0.00001*
Within groups	56	0.09	0.0016		
Total	59	49.29			

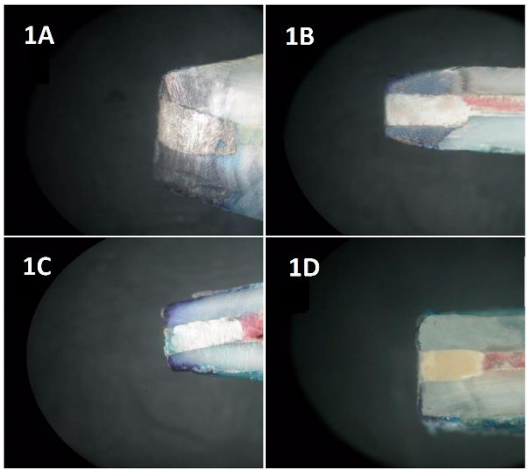
*p<0.05 (denotes statistical significance)

Table 3: Intergroup comparison of four groups (I, II, III, IV) with respect to apical dye microleakage (in mm) by Tukeys multiple post hoc procedures

Groups	Group I	Group II	Group III	Group IV
Mean	1.4747	2.6073	0.8300	0.1493
SD	0.0250	0.0542	0.0384	0.0375
Group I	-			
Group II	P=0.0002*	-		
Group III	P=0.0002*	P=0.0002*	-	
Group IV	P=0.0002*	P=0.0002*	P=0.0002*	-

*p<0.05 (denotes statistical significance)

Figure 1:



Discussion

It is certain that the prevention of microleakage of bacteria and their byproducts from the pulp space into the periapical tissues and vice versa contributes to the resolution of periapical lesions, justifying the importance of studies related to a material's sealing ability (Bernabé et al 2013). Several in vitro studies (Ozata, Erdilek and Tezel 1993; Erkut et al 2006; Kokate and Pawar 2012; Bernabé et al 2013; Ahmetoglu, Topçu and Oruçoglu 2014; Radeva, Uzunov and Kosturkov 2014; Ravichandra, PV, Harikumar, Vemisetty et al 2014; Soundappan, Sundaramurthy and Raghu 2014; Malhotra and Hegde 2015; Mandava et al 2015; Bolhari, Sharifi and Pirmoazen 2015; Torabinejad, Watson and Pitt Ford 1993; Fischer, Arens and Miller 1998; Chang 2012; Khandelwal 2015; Nanjappa 2015; Pradhan et al 2015; Saraswathi et al 2015; Seedat and Vyver 2016) have comparatively evaluated apical microleakage around various root end filling materials like Silver Amalgam, Zinc phosphate, Glass Ionomer Cement and

its continuum, Ethoxy Benzoic Acid, Intermediate Restorative Material, Mineral Trioxide Aggregate, bioaggregate, CEM (Calcium Enriched Mixture) , Chitra-CPC, and Biodentine. In the present study, commonly used root end filling materials of the past and present decade; Zinc free Silver Amalgam, Intermediate Restorative Material (IRM), Mineral trioxide aggregate (MTA) and Biodentine are all comparatively evaluated, in an effort to determine a retrofilling material showing least apical microleakage and better sealing ability using dye penetration method. Dye penetration method was chosen because of its ease of performance and the fact that if a material is able to prevent the penetration of smaller dye particles, it can obviously prevent the penetration of smaller particles like bacteria and their byproducts (Torabinejad, Watson and Pitt Ford 1993).

Results of the present study has shown that MTA seals better than IRM and Silver Amalgam, which is consistent with the previous studies (Torabinejad, Watson and Pitt Ford 1993; Fischer, Arens and Miller 1998; Erkut et al 2006; Chang 2012; Seedat and Vyver 2016). The reason for better sealing ability of MTA when compared to Silver Amalgam and IRM may be attributed to the formation of MTA - dentin interfacial layer which prevent microleakage (Chung et al 2011). However, MTA has its own set of drawbacks which includes difficulty in handling, longer setting time and initial looseness of the mixed cement. Use of MTA for periradicular surgery has become still challenging because, contamination of blood before the complete set of MTA could adversely affect the hydration reaction of MTA.

In this study, Biodentine group showed significantly lower microleakage compared to MTA which is consistent with the previous studies (Kokate and

Pawar 2012; Ravichandra, Harikumar and Deepthi 2014; Khandelwal et al 2015; Malhotra and Hegde 2015; Nanjappa et al 2015). The reason for better sealing ability of biodentine may be attributed to the formation of tag like structures due to Ca and Si ion uptake by dentin, faster setting time and better handling properties (Khandelwal et al 2015).

Five studies (Radeva, Uzunov and Kosturkov 2014; Bolhari, Sharifi and Pirmoazen 2015; Pradhan et al 2015; Saraswathi et al 2015; Seedat and Vyver 2016) have concluded that there is no significant difference in microleakage between MTA and biodentine. However two other studies (Soundappan, Sundaramurthy and Raghu 2014; Mandava et al 2015), have achieved results contrary to the present study, stating that MTA has better sealing ability compared to biodentine probably due to varied methodology and outcome assessment method.

Limitations of this study are 1) In the present study, conventional technique of using No.2 round bur in contra angle handpiece was used for root end cavity preparation instead of ultrasonic retrotips, which could have been a better option and more pertaining to the era of modern dentistry. However, in vitro studies have its own set of shortcomings compared to that of randomized controlled clinical trials

Conclusion

Within the limitations of this present study, it can be concluded that none of the materials tested were able to avoid apical dye microleakage completely. Of the four root end filling materials tested in this in vitro study, Biodentine has better sealing ability compared to Zinc free silver Amalgam,

Intermediate Restorative Material (IRM) and Mineral Trioxide Aggregate (MTA), when used as a root-end filling material. However, further in vivo studies are to be conducted to evaluate Biodentine as an ideal root end filling material.

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General

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All papers submitted should be of font type times new roman, font size 10, single-spaced and in duplicate on A4 sized paper. Submissions are to be made to the editor before every 30 June and 30 November. There should be a margin of 25mm on all sides of the papers. A soft copy of the papers can be submitted via http://segi.edu.my/onlinereview/papersubmission.php

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