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บทบรรณาธิการ | Editorial

ความสำเร็จระดับนานาชาติของเรา
Our international achievement

ในฐานะคนไทย และชาวกรุงเทพธนบุรี กระผมมีความภูมิใจเป็นอย่างยิ่งที่จะเรียนให้ทุกท่านทราบว่า เมื่อเดือนสิงหาคมที่ผ่านมา คุณเทนนิส พาณิภัค วงศ์พัฒนกิจ นักศึกษาปริญญาเอก คณะรัฐศาสตร์ มหาวิทยาลัยกรุงเทพธนบุรี และนักกีฬาตัวแทนทีมชาติไทยสามารถคว้าเหรียญทองในการแข่งขันกีฬาเทควันโดในมหกรรมการแข่งขันกีฬาโอลิมปิกเกมส์ 2024 ที่กรุงปารีส ประเทศฝรั่งเศส โดยคุณพาณิภัคนักกีฬาเทควันโดมือวางอันดับ 1 ของโลก สร้างผลงานการแข่งขันกีฬาเทควันโดได้อย่างยอดเยี่ยม สามารถป้องกันแชมป์คว้าเหรียญทองได้เป็นผลสำเร็จ และเป็นนักกีฬาทีมชาติไทยคนแรกที่คว้าเหรียญทองในกีฬาโอลิมปิก 2 สมัยซ้อน นอกจากนี้ ยังเป็นนักกีฬาเทควันโดทีมชาติไทยคนแรกที่สามารถครองเหรียญในกีฬาโอลิมปิก 3 สมัยซ้อนอีกด้วย ได้แก่ เหรียญทองแดงที่เมืองริโอ เด จาเนโร ประเทศบราซิล ในปี ค.ศ. 2016 เหรียญทองที่กรุงโตเกียว ประเทศญี่ปุ่น ในปี ค.ศ. 2020 และล่าสุดเหรียญทองที่กรุงปารีส ประเทศฝรั่งเศส ในปี ค.ศ. 2024

คณบดีและอาจารย์คณะทันตแพทยศาสตร์ร่วมแสดงความยินดีกับคุณเทนนิส

งานแสดงความยินดีกับทัพนักกีฬาไทยของมหาวิทยาลัยกรุงเทพธนบุรี วันที่ 14 สิงหาคม พ.ศ. 2567

นอกจากคุณพาณิภัค ที่ทำผลงานได้อย่างยอดเยี่ยมแล้ว นักกีฬาที่เป็นชาวกรุงเทพธนบุรีท่านอื่น ก็สามารถสร้างผลงานได้อย่างดีเยี่ยมเช่นเดียวกัน ซึ่งนี่น่าจะเป็นสิ่งที่สะท้อนคุณภาพคนของมหาวิทยาลัยของเรา ว่าไม่ได้สร้างผลงานแค่ในระดับประเทศ แต่สามารถสร้างผลงานที่ยอดเยี่ยมในระดับนานาชาติ เช่นเดียวกับนักศึกษาและคณาจารย์ในคณะทางสุขศาสตร์ที่มุ่งมั่นสร้างผลงานทางวิชาการให้เป็นที่ยอมรับทั้งในระดับประเทศและระดับนานาชาติเช่นกัน ดังที่ท่านผู้อ่านจะสามารถติดตามผลงานส่วนหนึ่งของเราได้ในวารสารการแพทย์โลกาภิวัตน์ที่ตีพิมพ์มาอย่างต่อเนื่อง ตั้งแต่ปี พ.ศ. 2565 ครับ

ยสนันท์ จันทรเวคิน

การนำเสนอผลงานทางวิชาการของนักศึกษาและคณาจารย์ของมหาวิทยาลัย

Original Article

เปรียบเทียบฤทธิ์ต้านไวรัสเฮอร์ปิสซิมซ์เพล็กซ์ของน้ำมันมะพร้าวสกัดเย็นและน้ำมันแร่ : การศึกษาในหลอดทดลอง

Comparison of anti-herpes simplex virus activities of virgin coconut oil and mineral oil : an *in vitro* study

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บทคัดย่อ น้ำมันมะพร้าวสกัดเย็นผลิตจากผลมะพร้าว (*Cocos nucifera* Linn) โดยการสกัดเย็นทำให้ยังคงมีฤทธิ์ทางชีววิทยา เช่น ต้านแบคทีเรียและรา ยังไม่เคยมีรายงานฤทธิ์ต้านไวรัสของน้ำมันชนิดนี้ ส่วนน้ำมันแร่เป็นน้ำมันทาผิวทั่วไป ไวรัสเฮอร์ปิสซิมเพล็กซ์มีสารพันธุกรรมเป็นดีเอ็นเอ และมีเปลือกหุ้ม อยู่ในวงศ์ *Herpesviridae* ตระกูลยา *Simplexvirus* ซึ่งประกอบด้วย HSV ชนิดที่ 1 (HSV-1) และชนิดที่ 2 (HSV-2) ทั้งสองชนิดสามารถก่อโรคติดเชื้อร้ายแรงและติดเชื้อซ้ำในคน acyclovir เป็นยาต้านไวรัสชนิดปฐมภูมิ ซึ่งมีรายงานพบ HSV สายพันธุ์ที่ดื้อยา ส่วนยาต้าน HSV อื่นๆ ยังมีราคาสูง การศึกษาเพื่อวัตถุประสงค์เพื่อตรวจฤทธิ์ต้าน HSV ของน้ำมันมะพร้าวสกัดเย็นในหลอดทดลอง โดยเปรียบเทียบกับน้ำมันแร่ วิธีการ plaque reduction assay ซึ่งถูกนำมาปรับใช้ 3 วิธี ได้แก่ inactivation, pretreatment และ posttreatment เพื่อตรวจฤทธิ์ต้าน HSV-1 (สายพันธุ์ KOS) และ HSV-2 (สายพันธุ์ Baylor 186) ผลการทดลองโดย inactivation พบว่า น้ำมันมะพร้าวสกัดเย็นความเข้มข้นร้อยละ 2 และ 2.5 (ปริมาตร/ปริมาตร) สามารถลดจำนวน plaque ของ HSV-1 และ HSV-2 ได้อย่างมีนัยสำคัญ ($p < 0.05$) เมื่อเทียบกับผลของน้ำมันแร่ ค่าความเข้มข้นที่ยับยั้งไวรัสได้ร้อยละ 50 (IC50) ของน้ำมันมะพร้าวสกัดเย็น ต่อ HSV-1 และ HSV-2 เท่ากับร้อยละ 0.51 และ 1.20 ตามลำดับ IC50 ของน้ำมันแร่ ต่อ HSV-1 และ HSV-2 เท่ากับ ร้อยละ 1.30 และ 1.80 ตามลำดับ โดย pretreatment และ posttreatment ไม่พบฤทธิ์ต้านไวรัส สรุปผลได้ว่าที่เป็นน้ำมันมะพร้าวสกัดเย็นความเข้มข้นร้อยละ 2 – 2.5 มีฤทธิ์ฆ่า HSV นอกเซลล์

คำสำคัญ: ฤทธิ์ต้านไวรัส ไวรัสเฮอร์ปิสซิมเพล็กซ์ น้ำมันแร่ น้ำมันมะพร้าวสกัดเย็น ฤทธิ์ฆ่าไวรัสนอกเซลล์

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ABSTRACT Virgin coconut oil (V) is produced from a fruit of coconut (*Cocos nucifera* Linn) by cold processing retaining biological properties, including antibacterial and antifungal activities. Antiviral property of V has not been reported. Mineral oil (M) is a common topical oil. Herpes simplex virus (HSV) is an enveloped DNA virus and a member of the family *Herpesviridae*, genus *Simplexvirus*. This genus consists of HSV type 1 (HSV-1) and HSV type 2 (HSV-2). Both HSV-1 and HSV-2 can cause serious and recurrent infections in humans. Acyclovir (ACV) is used as a primary treatment of the HSV infections. ACV-resistant HSV strains had been reported. Other anti-HSV drugs are still expensive. The objective of the study was To determine *in vitro* anti-HSV activities of V compared with those of M. For the method, plaque reduction assay (PRA) was modified into 3 methods, inactivation, pretreatment and posttreatment, for determining anti-viral effects of V and M against HSV-1 (KOS strain) and HSV-2 (Baylor 186 strain). For the results, by inactivation, 2 - 2.5% (V/V) V could reduce HSV-1 and HSV-2 plaque numbers significantly ($p < 0.05$) compared with those of M. The 50% inhibitory concentrations (ICs_{50}) of V against HSV-1 and HSV-2 were 0.51% and 1.20%, respectively. The ICs_{50} of M were 1.30% and 1.80%, respectively. By pretreatment and posttreatment**,** antiviral effect was not found. For Conclusion, 2 – 2.5% V possess virucidal effects on HSV-1 and HSV-2.

Keywords: Anti-viral activity; herpes simplex virus; mineral oil; virgin coconut oil; virucidal effect

INTRODUCTION

Virgin coconut oil (V) is widely used in Asian and Pacific regions as an edible and topical oil produced from fresh coconut milk or meat. It is a white solid fat below around 25°C (77°F), and a clear liquid oil in warmer climates.[1] Coconut (*Cocos nucifera* Linn) is in the family Arecaceae and the only viable species in the genus Cocos.[2] V is considered as the purest coconut oil and obtained by processing the mature coconut meat mechanically or naturally without heating, chemical treatment, bleaching, or deodorization, retaining many valuable components such as lauric acid, myristic acid, caprylic acid, and capric acid.[1] Lauric acid and myristic acid are resulted from V digestion whereas monolaurin is a monotriglyceride of lauric acid derived from V digestion, absorption and metabolism.[3] V has various biological properties, including antioxidant,[3,4] anti-inflammatory, analgesic and antipyretic,[5] antibacterial,[4,6-8] and antifungal activities.[4,9] Antiviral property of V has not been reported. Most antiviral activities came from lauric acid or monolaurin. Lauric acid inhibited vesicular stomatitis virus, an enveloped RNA virus.[10] Monolaurin showed *in vitro* inhibition effects on enveloped RNA and DNA viruses.[4,11]

Mineral oil (M) is a common topical oil and an ingredient in lotions, cold creams, ointments, and cosmetics. It is a lightweight inexpensive oil that is odorless and tasteless, composed mainly of alkanes and cycloalkanes from a mineral source, particularly a distillate of petroleum.[12]

Herpes simplex virus (HSV) is an enveloped spherical medium-size (155 - 240 nm) DNA virus and a member of the family *Herpesviridae*, genus *Simplexvirus*. This genus consists of HSV type 1 (HSV-1) and HSV type 2 (HSV-2). Both HSV-1 and HSV-2 can cause serious infections in humans. HSV-1 can lead to encephalitis, keratoconjunctivitis, gingivostomatitis, and skin infections whereas HSV-2 usually bring to genital infection.[13] For treatment of the infection caused by HSV, many antiviral drugs such as acyclovir (ACV), valacyclovir, famciclovir, penciclovir (PCV), and cidofovirare have been introduced. ACV is widely used as a primary treatment of this viral infection. Because of an increase in viral mutations and long term treatments, ACV-resistant HSV strains had been reported in both immunocompromised and immunocompetent patients. The prevalence of ACV-resistant HSV was higher in severely immunocompromised patients than in immunocompetent patients. PCV-resistant HSV was obtained from immunocompetent patients.[14-17] Many anti-HSV drugs are still expensive. Edible and topical oil with low cost of production such V attracted our attention to investigate ant-HSV effects of V. Therefore, this study aimed to determine *in vitro* antiviral activities of V against HSV-1 and HSV-2 compared with those of M by using plaque reduction assay.

MATERIALS AND METHODS

1.Preparation of tested samples: V and M used in this study were produced by Chemipan Laboratories Co., Ltd. (Thailand); and Johnson & Johnson (Thailand), respectively. Polyethylene glycol (PEG) 400 (Chemipan Laboratories, Thailand) was used as an emulsifying agent to make oil in water (O/W) emulsion in a ratio (v/v) 1:1 (PEG 400:oil). The certificate of Analysis (COA) (from the producer) of V was reported that it contained 0.047% lauric acid by AOAC method. Briefly, V or M was mixed thoroughly with PEG 400. The mixture were then added to minimum essential medium (MEM, Invitrogen®, USA) and mixed thoroughly to make an emulsion. The final concentrations of tested oil after mixing with the virus (tested sample) or MEM (cell control) in a ratio (v/v) 1:1 were 1 - 4% (v/v). MEM was mixed thoroughly with 1 - 4% (v/v) PEG 400 to make an emulsion and then

mixed with the virus in a ratio (v/v) 1:1 to be a negative control of each test sample.

2. Preparation of tested viruses and cell culture: HSV-1 (KOS strain), HSV-2 (Baylor 186 strain) and Vero cells (kidney cells of an African green monkey) obtained from the National Institute of Health, Thailand were used as tested viruses and cell culture. Vero cells are widely used for HSV-1 and HSV-2 infections. The cells were propagated confluently in a growth medium (GM) (MEM supplemented with 10% heated fetal bovine serum, Invitrogen®, South America) on the surface of a 25 cm^2-tissue culture flask in an incubator at 37°C and 5% CO_2 (Forma Scientific, USA) for 18 - 24 hr. After the GM was taken off, 2 mL of MEM containing the viruses were inoculated on confluent Vero cells with a multiplicity of infection (MOI) ≤ 0.01. After viral adsorption for 1 hr and then unadsorbed viruses were removed, 4 mL of MEM were added and the culture cells were reincubated for 2 - 3 days or until 90% cytopathic effect (CPE) were observed under an inverted microscope. The viruses were then harvested and collected as the stock viruses and kept at - 20°C until used. Quantification of the stock viruses were determined by a plaque assay. All the tested samples, cells, viruses were prepared and tested in a biosafety cabinet class II (Esco®, Singapore)

3. Plaque assay: A monolayer of Vero cells prepared on each well of a 24-well (for HSV-1) or 12-well (for HSV-2) tissue culture plate at 37°C and 5% CO_2 for 18 - 24 hr. After removing the GM, the stock viral solution was 5-fold diluted with MEM and then 0.25 (for HSV-1) or 0.5 (for HSV-2) mL of each dilution was added on Vero cells in each well (3 wells/dilution). The culture plate was reincubated for 1 hr. An overlayer medium (MEM containing 1.6% methylcellulose, 0.25 mL for HSV-1 or 0.5 mL for HSV-2) was added on the cells in each well and the plate was then reincubated for 2 - 3 days or until visible plaques were seen. Fixing solution (38% formalin:normal saline, 1:2) was added to and mixed with the overlayer medium in each well (0.5 mL/well for 24-well plate and 1 mL/well for 12-well plate). After fixing the infected cells at room temperature for 1 hr, the mixture solutions were removed and the cells were stained with 1% methylene blue with 10% methanol solution at room temperature for 1 hr. The extra methylene blue solution was washed with tapped water and plaques were counted in each well under an inverted microscope. The viral stock concentration was calculated as plaque forming unit (PFU)/mL.[18]

4. Plaque reduction assay (PRA): PRA was modified into 3 methods, inactivation, pretreatment and posttreatment, for determining anti-HSV activities of all samples as below.[19]

4.1 Inactivation: Appropriate amount of viruses (500 - 700 PFU/mL) was mixed with each concentration of the test sample or a control (MEM) in a ratio (v/v) 1:1 for 30 min at room temperature. The mixture were then added on each well (3 wells/concentration) containing Vero cell monolayer of a 24-well (for HSV-1, 250 µL/well) or 6-well (for HSV-2, 1.0 mL) tissue culture plate immediately after removing the GM. The culture plate was incubated at 37 °C and 5% CO_2 for 1 hr. The overlayed medium was added on the cells in each well (0.25 mL for HSV-1 or 1.0 mL for HSV-2) and the plate was then reincubated for 2 - 3 days or until visible plaques were seen. The infected cells were fixed and stained. The plaques in each test concentration were counted and calculated for viral titers. Dose-response curves were used to determined 50% inhibitory concentrations (ICs_{50}) of V and M.

4.2 Pretreatment: Monolayer Vero cells of each well of 24-well (for HSV-1) or 6-well (for HSV-2) tissue culture plate after removing the GM was pretreated with each concentration of tested samples or control, triplicately in the CO_2 incubator for 1 hr. Appropriate amount of viruses were adsorbed on each pretreated cells of the wells in the CO_2 incubator for 1 hr. The overlay medium was added to the wells. The plaques were enumerated and the ICs_{50} of the samples were calculated after 2 - 3 days.

4.3 Posttreatment: Appropriate amount of viruses were adsorbed on monolayer Vero cells of each well of 24-well (for HSV-1) or 6-well (for HSV-2) tissue culture plate immediately after removing the GM for in the CO_2 incubator 1 hr. The tested samples were added onto the infected cells and reincubated for 1 hr. The overlay medium was added to each well of the infected cultures. The ICs_{50} of the samples were calculated as previously mentioned.

5. Statistical analysis: All experiments were performed in triplicate, and three independent experiments were conducted. Data were analyzed using SPSS 29 (IBM, Armonk, New York, USA) (independent-samples Kruskal-Wallis test and Mann-Whitney test) and reported as means ± SD. p-value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

1. Cytotoxicity to Vero cells.

In this study, we found that Vero cells could not survive in the MEM containing 3 - 4% V or 3 - 4% M. Cytotoxic effects of the tested oil with more than or equal to 3% on the culture cells were found. Consequently, we continued to determine antiviral effect of 1.0 - 2.5% V and M. We also discovered that polysorbate 20 was not a suitable emulsifier for our study because it alone inhibited the viral titer and destroyed Vero cells. We found that polysorbate 20 less than or equal to 0.6% was safe for HSV and Vero cells but it was too low to emulsify V or M. An emulsifier helped an oil to dissolve in water and the

oil could react with the viruses. We chose PEG 400 as an emulsifying agent because it was nonionic and the used concentrations (1.5 - 2.5%) did not inhibit the viral titer or not destroy Vero cells.

2. Antiviral effects

By inactivation method in the PRA, the results showed that 2 - 2.5% V could reduce HSV-1 plaque numbers significantly ($p < 0.05$) compared with M (the same concentrations). In addition, 2 - 2.5% M could decrease HSV-1 plaque numbers significantly ($p < 0.05$) compared with the negative controls (MEM containing 2 - 2.5% PEG 400). The inhibition effects of V and M on HSV-1 were dose-dependent (Figure 1). The 50% inhibitory concentrations (ICs_{50}) of V and M against HSV-1 were 0.51% and 1.30%, respectively (Table 1).

Similarly, the results demonstrated that 2 - 2.5% V could lower HSV-2 titers significantly ($p < 0.05$) compared with M (the same concentrations). The inhibition effects on HSV-2 of V and M were also dose-dependent (Figure 2). The ICs_{50} of V and M were 1.20% and 1.80%, respectively (Table 1).

By pretreatment and posttreatment methods in the PRA, the results showed that V as well as M could not reduce HSV-1 and HSV-2 plaque numbers.

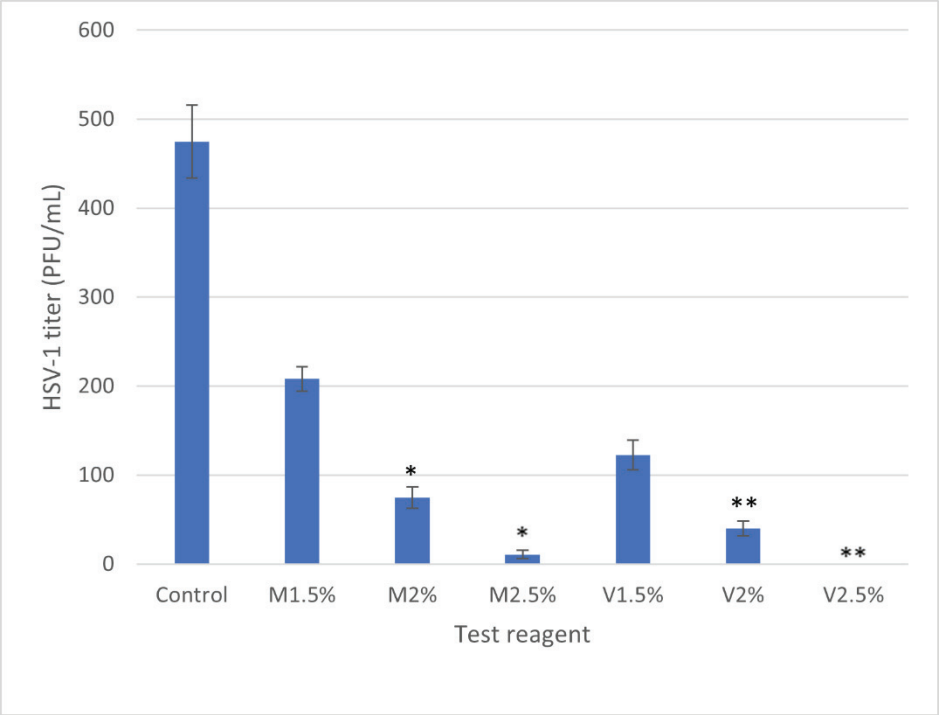

Figure 1 Effects of virgin coconut oil (V) and mineral oil (M) on herpes simplex virus type 1 (HSV-1)(KOS strain) determined by plaque reduction assay (inactivation). Results were represented as mean (n = 3) ± SD. * represented as different from the negative controls (minimum essential medium containing PEG 400) and ** represented as different from M significantly ($p < 0.05$)

Table 1 Comparison the 50% inhibitory concentration (IC_{50}) of virgin coconut oil and mineral oil against herpes simplex virus type 1 (HSV-1) (KOS strain) and herpes simplex virus and type 2 (HSV-2)(Baylor 186 strain) determined by plaque reduction assay (inactivation)(n = 3). Minimum essential medium containing PEG 400 as negative controls.

Test reagent	IC_{50} (mean ± SD)(%)	
	HSV-1	HSV-2
Mineral oil	1.30 ± 0.05	1.80 ± 0.04
Virgin coconut oil	0.51 ± 0.22	1.20 ± 0.09

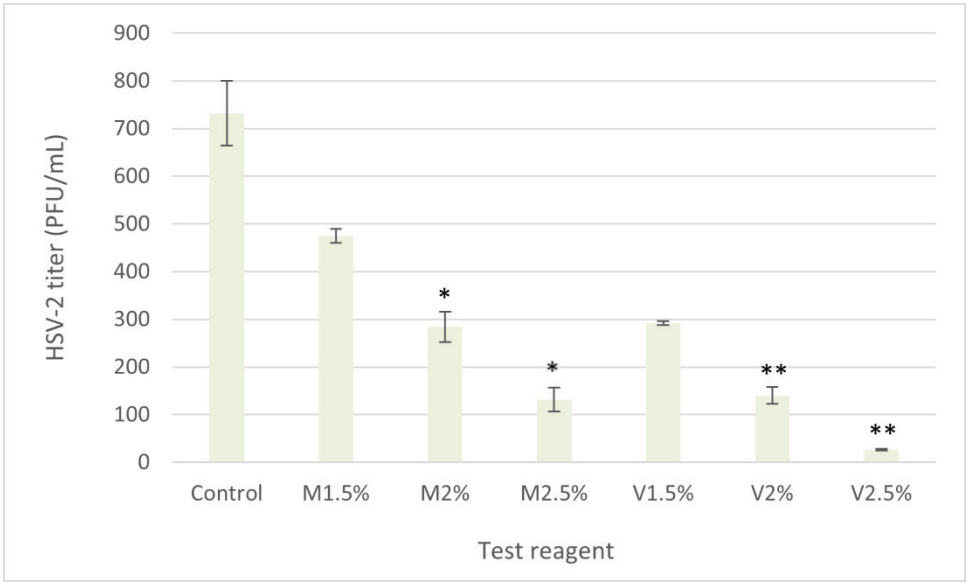

Figure 2 Effects of virgin coconut oil (V) and mineral oil (M) on herpes simplex virus type 2 (HSV-2)(Baylor 186 strain) determined by plaque reduction assay (inactivation). Results were represented as mean (n = 3) ± SD. * represented as different from the negative controls (minimum essential medium containing PEG 400) and ** represented as different from M significantly ($p < 0.05$).

DISCUSSION

In this study, we compared V with M because M is a common topical oil. Agero and Verallo-Rowell[20] determined the effectiveness and safety of V compared with M as a therapeutic moisturizer for mild to moderate xerosis by a randomized double-blind controlled clinical trial. Subjective grading of xerosis by the investigators showed a general trend toward better (though not statistically evident) improvement with V than with M. They concluded that V was as effective and safe as mineral oil when used as a moisturizer.

Antibacterial and barrier repair properties of V and M were reported by Ramos[21] in children with mild to moderate atopic dermatitis. By using SCORAD (SCORing for Atopic Dermatitis) and bacterial culture, a randomized controlled doubleblind trial was conducted in two tertiary hospitals. The SCORAD and level of erythema were significantly decreased throughout the 4-week duration for both treatment groups, but much lower in the V group. The antibacterial property of V group was better than that of M group since there was no growth of bacteria after the 4-week treatment.

V and M were compared with the control using Kruskal-Wallis test. The nonparametric Kruskal-Wallis test is preferable to a one-way analysis of variance with at least three groups of independent samples, the differences between these sets of data estimated.[22] V was compared with M using the Mann-Whitney test. The Mann-Whitney U test is the nonparametric equivalent to the two samples t-test when the dependent variable is a continuous variable measured for all observations in two groups.[23]

Plaque formations were significantly reduced, when HSVs were incubated with certain amount of V or M prior to adsorption indicating that V and M had virucidal effects or direct inactivation on HSV-1 and HSV-2. Interestingly, V exhibited virucidal effects greater than those of M significantly. The same results were found when star anise oil was examined for antiviral activity against HSV-1 using PRA. Anti-HSV-1 activity of star anise oil was direct inactivation of free virus particles.[24] The antiviral effects of peppermint oil against HSV-1 and HSV-2 were tested *in vitro* on RC-37 cells using PRA. This oil affected the viruses before adsorption, but not after penetration into the host cells.[25]

In order to determine the mode of antiviral action of V and M, either Vero cells were pretreated before viral infection or viruses were incubated with the tested oils before infection or tested oils were added after viral adsorption on the host cells. Pretreatment of Vero cells with V or M had no effect on the production of infectious HSV and plaque formation. So, V and M could not prevent penetration of the viruses to their susceptible cells . To observe the intracellular anti-HSV activities of the tested samples, the posttreatment experiment was carried out. V and M had no intracellular effect on HSV infection. Future study about a more emulsifier may approve the intracellular effect since this effect is important for treatment of an infectious disease.

V is composed of valuable components such as lauric acid (C12, a major component), myristic acid (C14), caprylic acid (C8), capric acid (C10). However, coconut oil contained only trace amounts of free fatty acids (about 0.03% by mass).[1] Caprylic acid and capric acid are short chain fatty acid (SCFA). Lauric acid and myristic acid are medium chain fatty acid (MCFA).[3] V, primarily a MCFA is not packaged into chylomicrons for circulation through lymph vessels like long-chain fatty acids (LCFA), and instead is broken down quickly upon consumption instead of being stored in adipose tissues. MCFA is also easily soluble and digestible by salivary and pancreatic lipases when compared to LCFA.[4] Many studies suggested lauric acid had better antimicrobial properties amongst all fatty acids, including monolaurin, a secondary metabolite. Vesicular stomatitis virus (VSV) is an enveloped RNA virus that infects cattle, horses, and pigs. In the presence of lauric acid (C12) (purchased from Sigma)(60 - 100 µg/ml), the production of infectious VSV was inhibited reversibly in a dose-dependent manner. After removal of C12 the antiviral effect disappeared. In addition, the chain length of the monocarboxylic acids proved to be crucial, as those with shorter or longer chains were less effective or had no antiviral activity. C12 prevented the binding of the viral M protein to the host cell membrane, resulting in inhibition of virus release. Thus, lauric acid had an intracellular effect on VSV.[10] In our study, 2 – 2.5% V containing few lauric acid (about 2.35 - 2.94 µg/ml) showed anti-HSV effects. Physical property of the oil may help virucidal effect. However, the comparison was difficult because of different methods and evaluations. We used PRA and evaluated V as IC50 values. Hornung et al.[10] used viral titer assay and evaluated lauric acid as 10-fold viral reduction.

Monolaurin (obtained from Med Chem Laboratories, Monroe, MI, USA) with tert-butyl hydroxyanisole (BHA) (used for prevent oil from oxidative deterioration) showed *in vitro* virucidal effects on human enveloped RNA (influenza virus, pneumovirus, paramyxovirus, rubeola virus) and DNA viruses (HSV-1, HSV-2, cytomegalovirus). When the 1% concentration was added to the reaction mixture, after 1 hr (but not 30 min) at 23°C, all viruses were reduced in infectivity by more than 99.9%. Electron micrograph of the treated viruses showed that loss of viral infectivity was associated with disintegration of the virus envelope. Destruction of lipids and phospholipids in the viral envelope is the key factor in the virucidal activity of monolaurin.[11] Our results showed that V had virucidal effect after inactivation at least for 30 min (but not 15 min). The virucidal effects of V and M on enveloped DNA viruses like HSV-1 and HSV-2 may come from a physical effect.

Antiviral property of V has not been reported. Moreover, antiviral effects of lauric acid[10] or monolaurin[11] reported previously were produced from commercial companies not extracted from V.

CONCLUSION

The results of this study showed that 2 - 2.5% V possess in vitro virucidal effects against HSV-1 and HSV-2 better than those of M.

ACKNOWLEDGE

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CONFLICT OF INTEREST

The authors declare that we have no financial interests or personal relationships that could be influent the results in this study.

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Original Article

บทบาทของโคนบีมคอมพิวเตดโทโมกราฟฟีในการวินิจฉัยใบหน้าแหว่งข้างเดียวประเภทเทสเซียร์ 5

The role of CBCT on the diagnosis of Unilateral Tessier Facial cleft 5

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บทคัดย่อ ใบหน้าแหว่งข้างเดียวประเภทเทสเซียร์ 5 เป็นลักษณะใบหน้าแหว่งที่พบได้ยากที่สุด และมีผลกระทบต่อผู้ป่วยค่อนข้างมาก การวินิจฉัยโรคใบหน้าแหว่งหากเป็นกรณีที่แสดงความผิดปกติที่เนื้อเยื่ออ่อนภายนอกสามารถวินิจฉัยได้ง่ายโดยการตรวจทางคลินิก แต่การตรวจทางภาพถ่ายรังสีจะช่วยในการประเมินตำแหน่งของรอยแหว่งในกระดูกได้ อย่างไรก็ตามเมื่อถ่ายภาพรังสีที่เป็นภาพสองมิติ อาจพบการซ้อนทับกันของกระดูกขากรรไกรและใบหน้า นำไปสู่การวินิจฉัยโรคที่ผิดพลาดได้ ในปัจจุบันการถ่ายภาพโคนบีมคอมพิวเตดโทโมกราฟฟี ซึ่งแสดงภาพแบบสามมิติ สามารถให้ข้อมูลที่ถูกต้องแม่นยำมากและมีการนำมาใช้อย่างกว้างขวาง แต่ยังไม่ค่อยได้นำมาใช้ในงานวินิจฉัยและวางแผนการรักษา

บทความนี้จึงขอนำเสนอกรณีศึกษาการใช้การถ่ายภาพโคนบีมคอมพิวเตดโทโมกราฟฟีในการวินิจฉัยและวางแผนการรักษาลักษณะใบหน้าแหว่งข้างเดียวประเภทเทสเซียร์ 5 และอภิปรายลักษณะทางภาพรังสีของโรคดังกล่าว

คำสำคัญ: โคนบีมคอมพิวเตดโทโมกราฟฟี, โรคใบหน้าแหว่ง, ใบหน้าแหว่งข้างเดียวประเภทเทสเซียร์ 5

Abstract Tessier cleft 5 is the rarest of oblique clefts and has a big impact on those who were born with it. Cranio-facial clefts (CFCs) can be easily diagnosed by clinical examination in cases with external soft tissue manifestations. Radiographic investigations were also used to evaluate the position of the cleft. However, two-dimensional radiographs may lead to

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misdiagnosis due to the superimposition of the craniofacial skeleton. Recently, cone-beam computed tomography (CBCT) provided accurate three-dimensional imaging that is widely used but not routinely in diagnosis and treatment planning.

This paper presented a rare case of Tessier cleft 5 with no extraoral soft tissue manifestations using a CBCT scan for the definite diagnosis and also the treatment planning and discussed a radiographic approach to the diagnosis of the CFCs.

Keywords: Cone-beam computed tomography; Cranio-facial cleft; Tessier cleft 5

INTRODUCTION

Cranio-facial clefts (CFCs) are malformations that affect both the face and cranium in various forms. The incidence of CFCs is approximately 1.4-6.0 per 100,000 live births[1, 2] CFCs do not only involve the lip, palate, nose, and alveolar ridge but also eyes, maxilla, and forehead. Most disfigurations related to facial anomalies are attributed to CFCs.[3] The most known cleft that occurs in the craniofacial region is cleft lip and palate.[4] There are several systems of classification and phenotype characterization of the CFCs, such as the classification systems of Morian, American Association of Cleft Palate Rehabilitation (AACPR), Boo-Chai, Karfik, and Tessier.[3-7] However, the most common and widely used is Tessier classification.

Tessier classification involves not only soft tissues but also skeletal anomalies. The numbers 0 to 14 were used to define the location of the clefts as shown in Fig. 1. The clefts are also classified about the facial midline and eye socket: 0 to 3 as middle clefts, 4 to 6 as oblique clefts, 7 and 8 as lateral or transverse clefts, and 9 to 14 as an extension of 0 to 5 respectively. When using the eye socket as a reference, those above the upper eyelids are called cerebro-cranial clefts (numbers 0 to 7) while those that are below the lower eyelids are craniofacial clefts (numbers 8 to 14). Soft tissues are often involved with the occurrence of cleft resulting in external or surface manifestations.[4, 8] Craniofacial clefts may occur as unilateral or bilateral and complete or incomplete. In case of bilateral occurrence, one side may be more prominent than the other side and usually different type of cleft occurs on each side.[4]

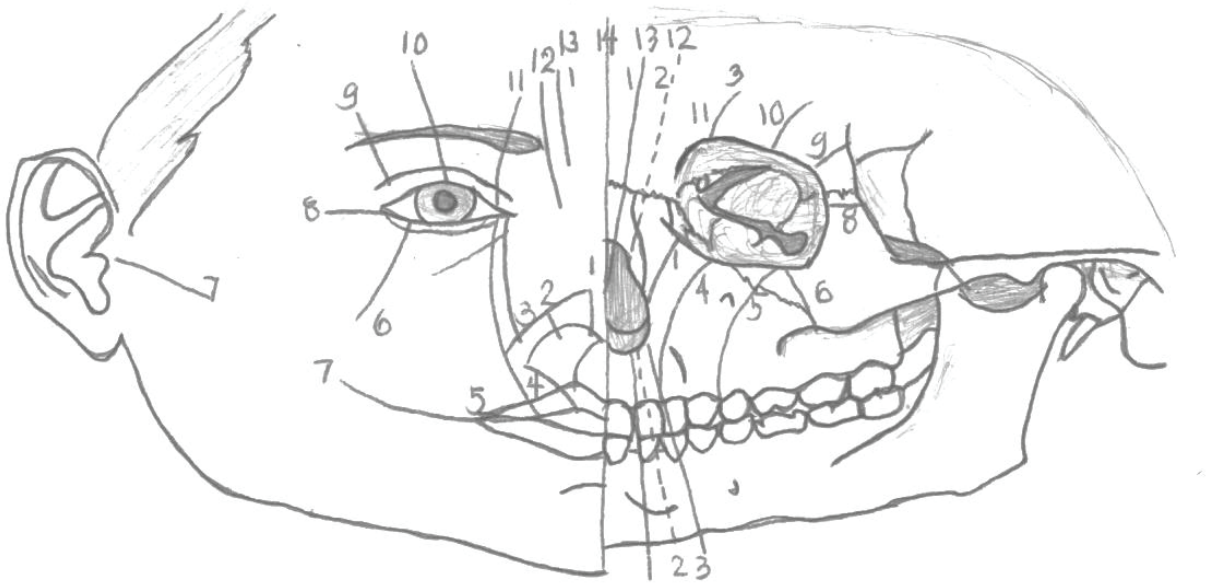

Fig. 1 Tessier classification of the craniofacial clefts; on the right (R) are the lines of the bone tissue cleft and shown on the left (L) are the lines of the soft tissue cleft.

Oblique clefts are uncommon facial clefts and may involve the palate and temporal bone. Their incidence is estimated to be 0.075-0.54% [9] which is only 0.22-0.25% of all CFCs.[3] Tessier cleft 5 is the rarest of the oblique clefts.[3, 10] In literature, there are other names for Tessier cleft 5 according to different authors and institutions, such as *"orofacial canthal cleft"* (AAPCR), *"type II oro-ocular cleft"* (Boo-Chai), *"true cleft"* (Karfik), and *"lateral maxillary dysplasia"* (van der Meulen).[4, 11] Moreover, Pereira et al.[12], Whitaker[5], and David et al.[13] described the features of Tessier cleft 5 in different characteristics.

Infants who are born with CFCs, like Tessier cleft 5, have a big impact on their lives. They are prone to nutritional deficiency due to feeding difficulties, have poor oro-motor development, and may have hearing loss.[14] In severe cases of CFCs, abnormalities relating to the eyes, such as eyesight impairment, can occur.[15] Older patients with untreated CFCs have remaining problems with speech, hearing, and eating. These problems may give an individual with CFCs low self-esteem and psychosocial effects, such as social adjustments.

Diagnosis of CFCs can be easily made by clinical examination of different anatomical areas, such as orbit, cheek, lip, alveolar bone, and soft palate. Radiographic examinations, such as panoramic and intraoral radiographs, were also performed to evaluate the position of the cleft. However, these two radiographic techniques could not cover the whole morphology of the facial skeleton and its relation to the infraorbital foramen which is a necessary anatomy for the diagnosis. Meanwhile, a cone-beam computed tomography (CBCT) scan allows an accurate 3D imaging of the complex hard tissues that aids in diagnosis and planning for the extent of surgical reconstruction of CFC malformations. In addition to the standard 3D image, the presence of serial, coronal, axial, and sagittal formats gives finer details on the skeletal distortions. Even though medical CT produces superior images, CBCT is more advantageous for maxillofacial applications especially for dental use because of its lower dose of radiation and comparable images of osseous structures at lower cost.[16]

This paper aimed to present a rare case, Tessier cleft 5, including an analysis of its characteristics which differ from the other reported cases and to introduce a radiographic approach to diagnosis. Moreover, the radiological differential diagnosis of this abnormality is also discussed.

CASE REPORT

A 17-year-old male patient came to the Dental Hospital, Faculty of Dentistry, Khon Kaen University, Thailand in 2015 with a chief complaint of having facial asymmetry since birth and an enlarged tissue at the right posterior area of the maxilla. He was born of a non-consanguineous marriage and had no remarkable antenatal history. He had good physical and mental development except for his facial deformity. Extraoral examination showed facial asymmetry with a deviation of the mandible towards the right. There was no prominent extraoral manifestation of a cleft except for the right deviation of the patient's mouth and the tissue tags anterior to the left ear (Fig. 2). Intraoral examination showed an anterior open bite with unilateral crossbite at the right posterior teeth. The alveolar bone at the area of the right posterior maxilla expanded bucco-palatally with bony hard consistency on palpation. Severe crowding and supernumerary teeth can also be observed on the upper arch especially on the right side (Fig. 3).

From the panoramic radiograph (Fig. 4), a double maxilla was found on the right side including supernumerary teeth, of which the shape like the second premolar and molars and the deciduous retained root remained between the premolar and molar. The distorted shape and enlarged size of the right zygomatic process of the maxilla were seen. Moreover, the right maxillary sinus was recessed and the right posterior hard palate was lifted. However, it is difficult to interpret accurately by the general dentist due to the superimposition of bony structures. The first interpretation led to a mixed radiolucent-radiopaque lesion or cystic lesion which could be a differential diagnosis as fibrous dysplasia, ameloblastoma, and odontoma. Therefore, the biopsy was performed to rule out the impression of the previous differential diagnosis. The histopathological report indicated that the specimen was consistent with normal compact and cancellous bone or a mature stage of benign fibro-osseous lesion. The pathologist suggested that clinical-radiologic-pathologic correlation was required.

The patient was then sent for CBCT (WhiteFox Cone Beam Computer Tomography Scanner®, Acteon Group, France) imaging. The CBCT scan demonstrated a cleft on the right premolar region of the patient's maxilla extended to the area slightly below the lateral third of the orbit and the right zygomatic arch lower than the other side. The cleft separated maxillary structures posterior of the premolar from the rest of the upper arch resulting in a double maxilla. (Fig. 5a, 5b, and 6). The cleft continued inward medially ending in the posterior area of the soft palate. The right eye socket also appeared to be smaller and the floor of the right orbit was slightly pushed superiorly. The right maxillary sinus and nasal cavity were intact but were smaller relative to the other side and were displaced superiorly showing asymmetry on both sides. There was also skeletal disturbance of the sphenoid and pterygoid plates. Right pterygoid plates seemed to have asymmetry in size and were closer to the midline compared to the pterygoid plates

on the other side. The lateral wall margin of the sphenoidal sinus was thicker on the right, which might have caused the sphenoidal sinus to be slightly compressed towards the left (Fig. 7). Bucco-palatal expansion of the alveolar bone on the right side of the posterior maxilla could also be observed. Supernumerary teeth and malocclusion of teeth could also be seen on the radiographs (Fig. 6). Both condyles were similar in shape and size. Their positions were relative to the glenoid fossa (Fig. 7C). There was no asymmetry of the cranial base and calvarium. The patient was currently undergoing pre-orthodontic surgical treatment in preparation for his orthodontic therapy. This case report was approved by the Khon Kaen University Ethics Committee for Human Research (HE612338).

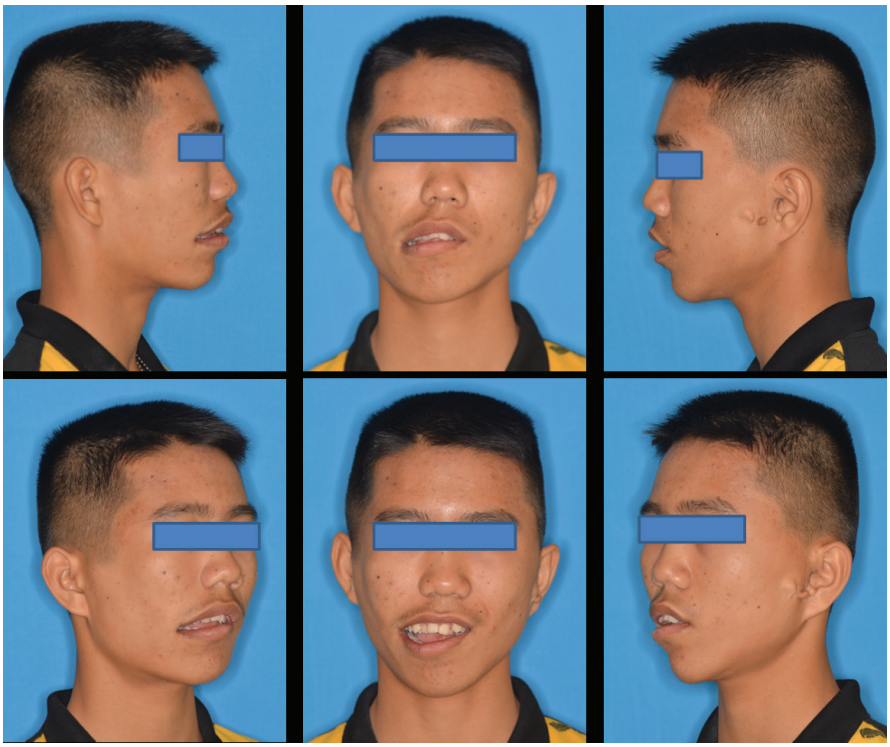

Fig. 2 Extraoral photographs of the patient showing facial asymmetry with incompetent lip and mandible deviated to the right side.

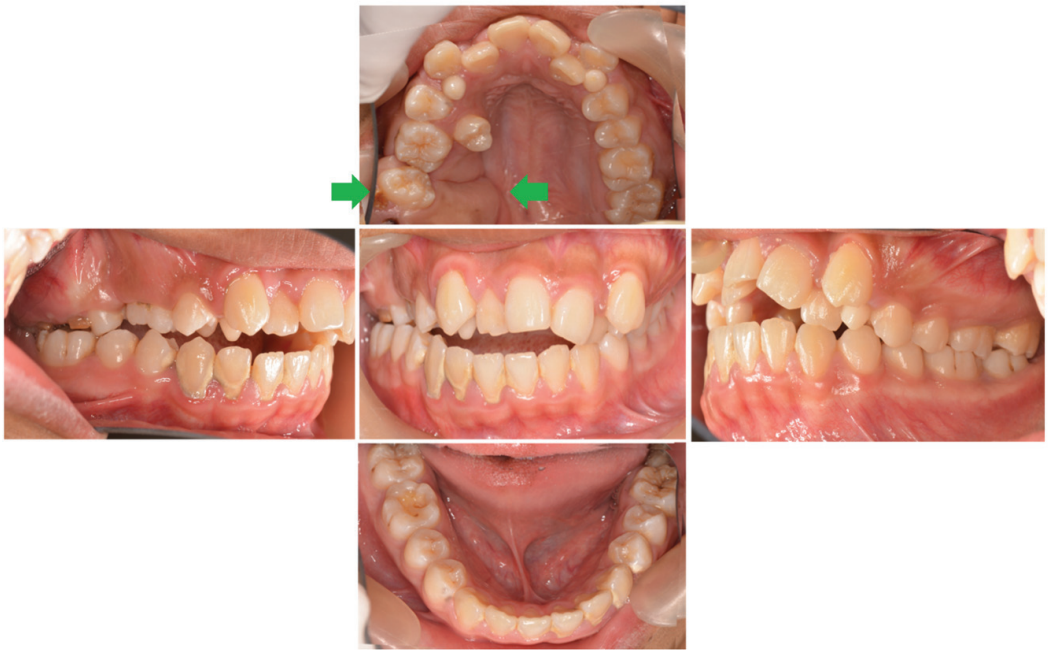

Fig. 3 Intraoral photographs of the patient showing anterior open bite and severe crowding with unilateral crossbite (right side). The alveolar bone at the area of the right posterior maxilla expanded bucco-palatally with bony hard consistency on palpation (arrow).

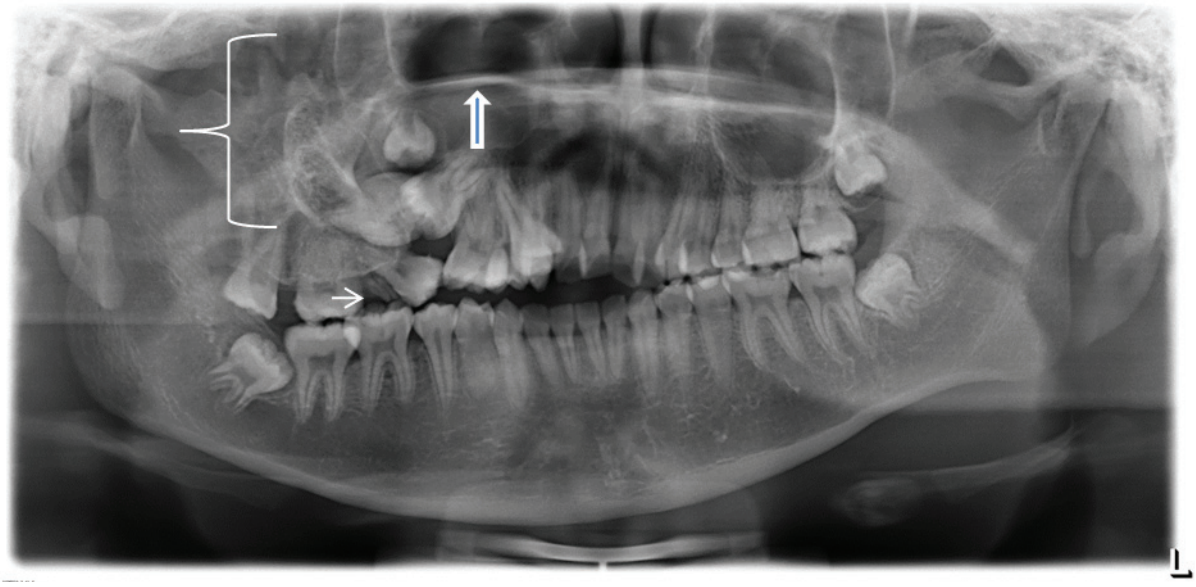

Fig. 4 Panoramic radiograph showing double maxilla on the right side including supernumerary teeth and the deciduous retained root (arrow). The zygomatic process of the right maxilla showed a distorted shape and enlarged size (parenthesis). The right maxillary sinus was recessed. The right posterior hard palate was lifted (thick arrow). The lower border of the mandible on the right side was lower than that of the left side.

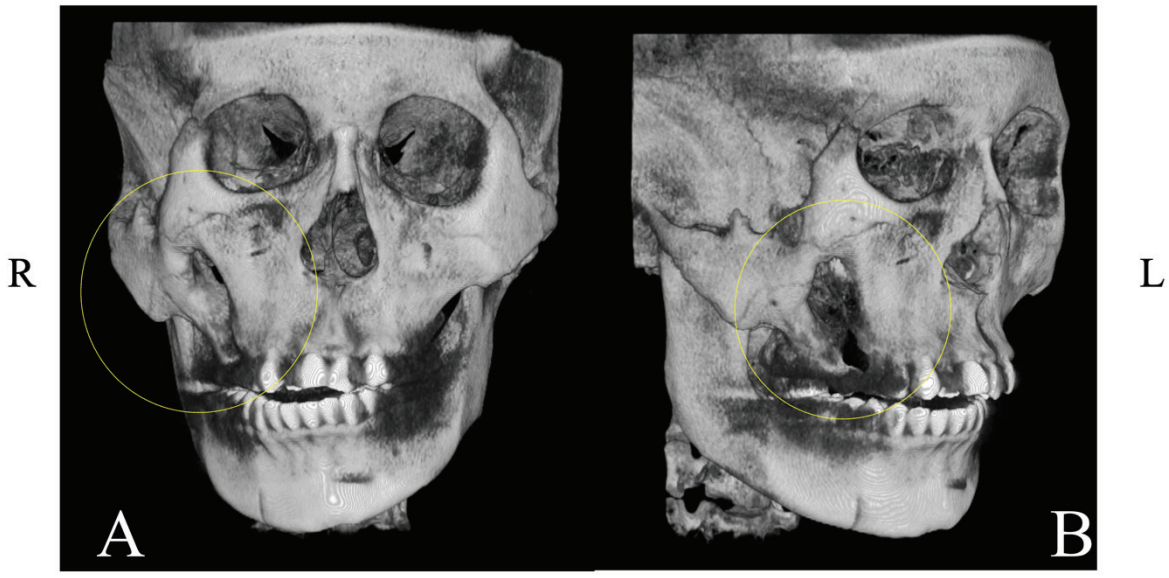

Fig. 5 Three-dimensional CBCT reconstruction in frontal (A) and oblique (B) views show the extent of Tessier cleft 5.

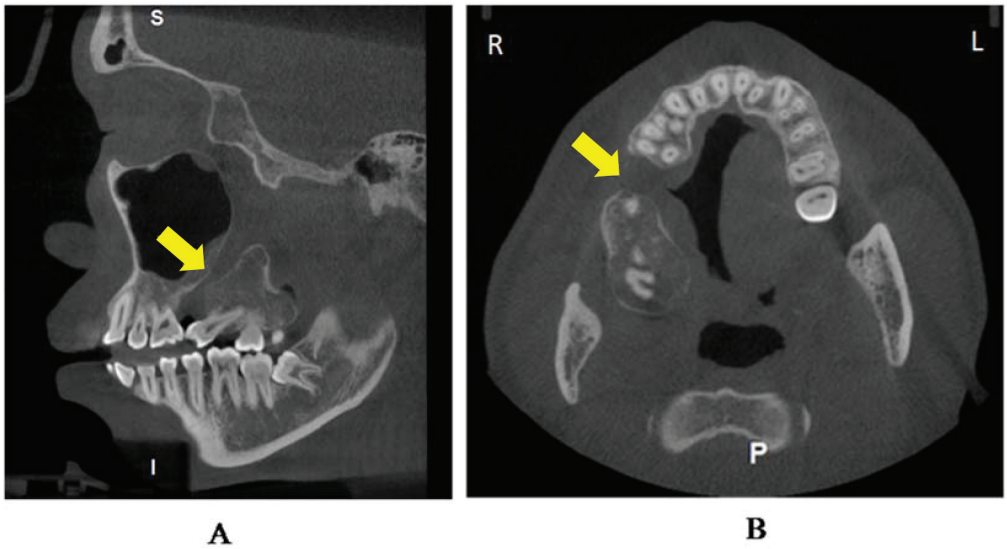

Fig. 6 Sagittal (A) and Axial (B) views of CBCT scan showing separation of the right maxilla (S = superior, I = inferior, P =posterior, R= right, L = left) .

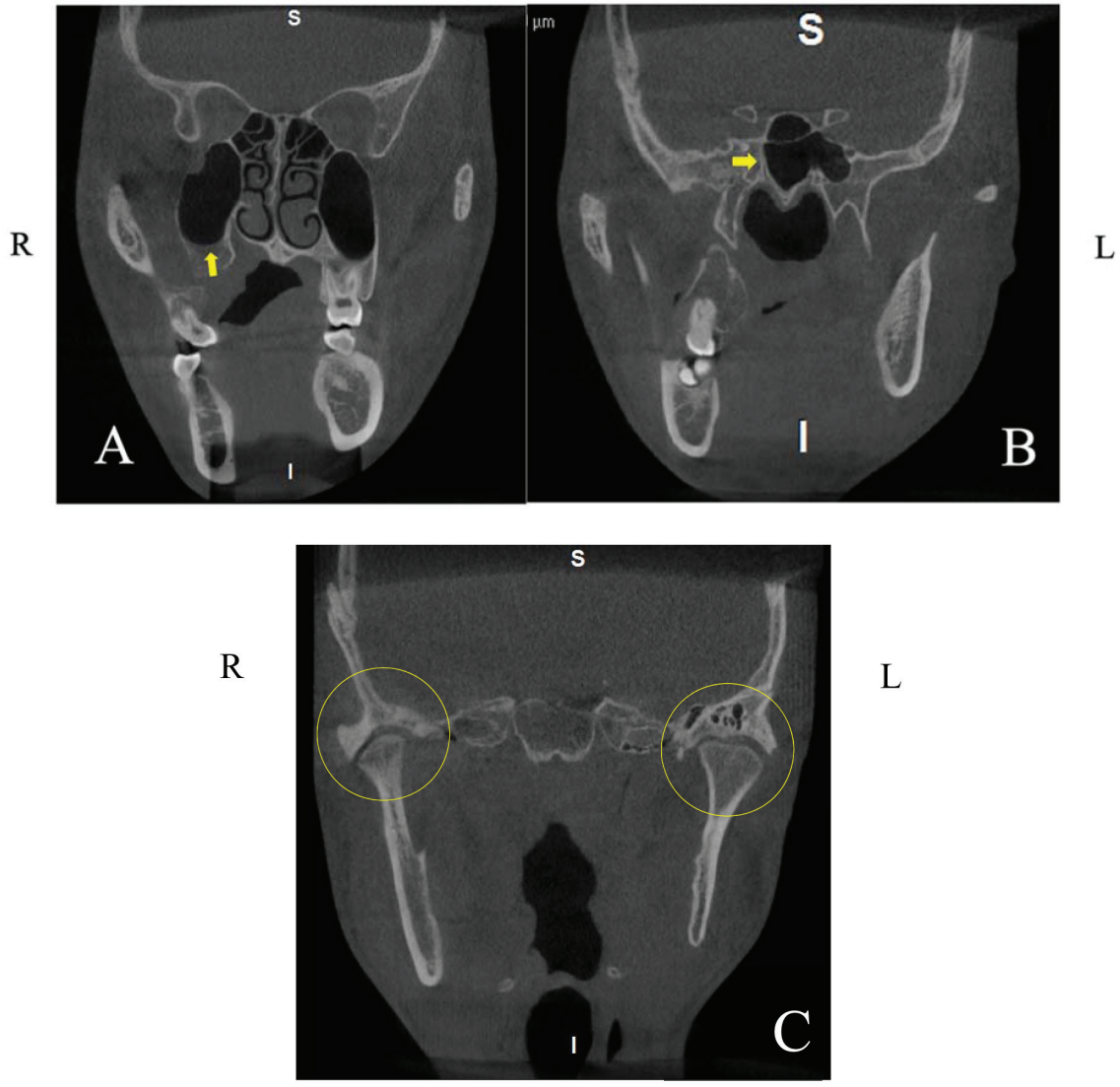

Fig. 7 Coronal view of the patient's CBCT scan showing – A. displacement of the right maxillary sinus and nasal cavity; B. compression of the sphenoidal sinus to the left; C. position of condyles in relation to the glenoid fossa.

DISCUSSION

In this report, we analyzed a Tessier cleft 5 using CBCT. Unlike most of the reported cases of Tessier cleft 5, the patient showed no significant external soft tissue manifestation of the cleft except with the presence of tissue tags anterior to the left ear and facial asymmetry with a downward oblique lip line deviating towards the right. Lack of external soft tissue manifestation combined with the rare occurrence of Tessier cleft 5 made it somehow difficult to readily diagnose this case.

Since no known embryologic grooves correspond to Tessier cleft 5, it was theorized that oblique facial clefts were due to stop in development, tissue disruption secondary to maxillary terminal branches lesions, or disturbances in the development of the maxilla. [3, 4, 17] Recent findings suggest that Tessier cleft 5 is a combination of tethered tissue migration, e.g. amniotic band, and cellular ischemia due to pressure [4], and are strongly associated with other congenital malformations such as limb constrictions. Amnion rupture syndrome exemplifies the relationship of tethered tissue migration with CFCs, which includes craniofacial clefts, amniotic bands, and visceral and extremities malformations.[3] However, Tessier cleft 5 was not linked to limb malformation and/or constrictions based on Coady.[18] Aside from genetic causes, risk factors including the use of drugs, smoking, exposure to radiation, vitamin deficiency and toxicity, and infection during pregnancy are also related to the development of CFCs.[19] The patient presented here was born with no remarkable antenatal history and malformations of extremities. The concept of tethered tissue migration cannot explain the presence of skeletal Tessier cleft 5 in this case. Furthermore, no external soft tissue manifestation in the present case can support that tethered tissue migration occurred in the patient during development in the uterus. But, we also cannot exclude the possibility that the former theories may be the reason for this Tessier cleft 5 case.

The external manifestation of Tessier cleft 5 shows on the lateral third of the eyelid and oral commissure of the lip.[8] Facial asymmetry is one of the main characteristics of Tessier cleft 5. Facial asymmetry can also be present in other conditions, such as hemifacial microsomia (HFM), which is a first branchial arch syndrome involving underdeveloped temporomandibular joint (TMJ), mandibular ramus, mastication muscles, and the ear.[20] Clinical appearance of HFM ranges from a slight facial asymmetry to severe stunted facial half, which can include orbital problems, a partially formed or total absence of ear, deviation of the facial midline to the affected side, and an upward oblique lip line towards the affected side.[20] The case presented here, although it showed a facial asymmetry that includes an oblique lip line, could not be considered as HFM because our patient had a downward oblique lip line deviating to the right contrary to the clinical appearance of the patients with HFM. Furthermore, our patient had a complete ear with normal hearing. Radiographic images also showed that our patient's affected side showed no sign of under-development of the TMJ, ramus, mastication muscles, and ear.

CFCs usually involve both bone and soft tissue deformities. Duplication of the jaw occurs more in the maxilla than the mandible which is commonly associated with cleft lip and palate. In some cases, the presence of a supernumerary alveolar process which may contain supernumerary teeth and commonly seen in the molar areas was observed.[21] Bone cleft of Tessier cleft 5 is located distal to the canine on the premolar area traversing the maxilla lateral to the infraorbital foramen through the middle third of the orbital rim to enter the orbit.[21] The case presented here had no extraoral soft tissue manifestations such as cleft lip but enlarged maxillary alveolar process and alveolar cleft resulting in a double maxilla were observed. This case different from the previously reported cases of Tessier cleft 5 that manifested external soft tissue deformities. In reported cases of Tessier cleft 5, supernumerary teeth were also observed to be included with either duplication of the jaw or supernumerary alveolar process.[21] This patient present a full set of vital permanent dentition and supernumerary teeth with normal forms on the maxilla, hence, overcrowding was still observed in addition to the size of the osseous structure of the maxilla.

Malformations due to CFCs are being managed by surgical corrections, except for loss of sight and hearing. Speech problems are being addressed through speech therapy. Achieving a high success rate depends on the time of surgical intervention. The later the intervention, the more effect it has on craniofacial development. Management of CFCs needs a multispecialty medical team to perform the multistage treatment.[8] Depending on severity, CFCs are managed in a few months after birth or after a year. Severe or extreme CFCs are recommended to be treated in the first few months. In not severe case, it can be waited for a little longer. Treatment also includes reconstruction and/or repositioning of displaced structures because of the cleft such as the skeletal malformations.[5]

The success of surgical intervention is also dependent on diagnostic aids such as radiograph investigations. In addition to the commonly used intraoral and panoramic radiographs, more sophisticated radiographic imaging techniques are already available such as CBCT which is helpful in diagnosis and treatment planning for

maxillofacial applications. CBCT image of Tessier cleft 5 can just be a narrow furrow traversing the anterior maxilla or a broad maxillary cleft lateral to the maxillary sinus and infraorbital foramen. The latter may continue to the inferolateral orbital rim and floor with no inferior orbital fissure communication at the posterior. The lateral maxillary part is collapsed with a reduction in the maxillary arch transverse dimension. It also manifests in the sphenoid as shortening and thickening of the lateral orbital walls on the area of the greater wing. Pterygoid plates are also mildly displaced relative to the midline. The cranial base and calvarium are minimally displaced.[13] These abnormalities could not be easily detected clinically, thus radiographic investigations are needed not only for diagnosis but also for treatment planning. The CBCT images of this present case conformed to the skeletal descriptions of David *et. al.*[13] on Tessier cleft 5. In comparison to the radiographic images of HFM, our case showed that the structures on our patient's affected side presented no underdevelopment.

Adequate and appropriate imaging techniques, with emphasis on 3D imaging, are very important for proper diagnosis and treatment planning of CFCs especially when there is no external soft tissue manifestation. Lack of proper radiographic imaging may result in inadequate diagnosis and treatment planning. Facial reconstruction was not done in this case because there was no significant extraoral manifestation of the cleft, moreover, the patient's cleft was found after CBCT imaging and it did not affect the patient's functions. However, pre-orthodontic procedures, such as bone grafting, were done for his current orthodontic treatment to correct the patient's malocclusion.

CONCLUSION

Tessier cleft 5 is a rare oblique cleft, which is also uncommon among craniofacial clefts, and affects patients' health. The findings of the case presented here suggest that CBCT is a good modality of choice for diagnosis and successful management of Tessier cleft 5 with no extraoral soft tissue manifestations related to the affected side. CBCT helps the medical team in planning reconstruction procedures, both soft and hard tissues. Therefore, the CBCT scan is recommended for the diagnosis and treatment planning of CFCs. Further studies are still needed to be done about CBCT analysis on the characteristics of the phenotypes of Tessier cleft 5.

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Original Article

ประสิทธิผลของไมดาโซแลมผสมไฮดร็อกซีซีนเปรียบเทียบกับคลอรอลไฮเดรตผสมไฮดร็อกซีซีนในการทำให้ผู้ป่วยเด็กสงบ

The sedative efficacy of two combination of midazolam and hydroxyzine compares to chloral hydrate and hydroxyzine in pediatric dental patients

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บทคัดย่อ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบความปลอดภัยและประสิทธิภาพการระงับความกังวล ระหว่างการใช้ไมดาโซแลมกับคลอรัลไฮเดรตในผู้ป่วยเด็กที่เข้ามารับการรักษาทางทันตกรรม ออกแบบการศึกษาเป็นแบบสุ่ม สลับกลุ่ม และปกปิดสองทางในผู้ป่วยเด็กอายุ 20-60 เดือน ที่มีสุขภาพแข็งแรงจำนวน 50 คน ผู้ป่วยแต่ละรายอาจได้รับยาไมดาโซแลม (0.5 มก./กก.) ผสมกับไฮดร็อกซีซีน (25 มก.) หรือยาคลอรัลไฮเดรต (50 มก./กก.) ผสมกับไฮดร็อกซีซีน (25 มก.) ในการรักษาครั้งแรก และมีการสลับกลุ่มให้ยาอีกชนิดในการรักษาครั้งที่สอง วัดอัตราการเต้นชีพจร การหายใจ ความอิ่มตัวของออกซิเจน ประเมินความทรงจำและความวิตกกังวลในแต่ละช่วงเวลา ผลการศึกษาพบว่าไม่มีความแตกต่างระหว่างกลุ่ม ทั้งในแง่สัญญาณชีพ ความทรงจำ และความวิตกกังวล แต่กลุ่มไมดาโซแลมมีข้อดีในแง่ความง่ายของการบริหารยาและผลข้างเคียง เช่น อาการคลื่นไส้ อาเจียน การร้องไห้ ที่น้อยกว่าคลอรัลไฮเดรต ซึ่งถือเป็นข้อได้เปรียบของไมดาโซแลม

คำสำคัญ: ไมดาโซแลม, คลอรอลไฮเดรต, ภาวะการเสียความจำไปข้างหน้า, การคลายความกังวล

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Abstract The objective of the study was to compare the safety and sedating efficacy between midazolam and chloral hydrate in pediatric patients receiving dental treatments. The randomized, crossover, and double-blinded study was performed in 50 healthy patients, age between 20 to 60 months. The subjects received equal volume of either midazolam (0.5 mg/kg) with hydroxyzine (25 mg) or chloral hydrate (50 mg/kg) with hydroxyzine (25 mg) in first visit and alternative drug in second visit. The pulse rate, respiratory rate and oxygen saturation were monitored. Anterograde memory and anxiety were evaluated at specific time points. For the results, there were no differences of physiological signs, amnesia as well as anxiety between two groups. However, midazolam had better compliance, and less side effects such as nausea, vomiting and crying which was the advantages over chloral hydrate.

Key words: Midazolam, chloral hydrate, anterograde amnesia, anxiety.

INTRODUCTION

Although chloral hydrate is frequently used because of its safety and efficacy [1-5], the long-standing action and constant sedative effect are unpredictable[3]. Moreover, its anxiolytic effectiveness in pediatric dental patients is still unclear [6,7] It can induce bradycardia, apnea, and can decrease oxygen saturation. The cardiovascular effect of chloral hydrate is significant comparing to other agents, such as oral midazolam[8,9], whereas the duration of action is variable[10]. With less cardiovascular effect[8], midazolam properties includes the anxiolytic, hypnotic, anticonvulsive, muscle relaxant and amnestic[9]. Midazolam has been used for conscious sedation in dentistry with limited support documentation about the efficacy[11-13].

Both chloral hydrate and midazolam have been used in conjunction with hydroxyzine. This antihistamine has sedative and anti-emetic properties. In the recommended dose (25-50 mg), there is no respiratory depression with no-known side effect. Although few studies have compared chloral hydrate and midazolam in terms of patient cooperation[14,15] and anxiety[16-18], there was no investigation of effects on memory function, especially in pediatric patients receiving dental treatment. The objective of the study was to compare the combination of oral midazolam and hydroxyzine with the combination of chloral hydrate and hydroxyzine in the anterograde amnesia, anti-anxiety, onset of action, complication, and compliance aspects.

METHODS

This study was approved by the ethics committee of the Faculty of Dentistry, Prince of Songkla University (PSU), Thailand. The pediatric dental patients who came to the Pediatric Dental Clinic, PSU Dental Hospital were included in this study. The inclusion criteria were as follows, 1) age between 20-60 months, 2) Class I anesthetic risk (the American Society of Anesthesiologists), 3) unable to cooperative ("definitely negative" in Frankl's Behavior Rating Scale[19], 4) require a minimum of restorative treatment and 5) have informed consent from the guardian. The patients were randomly allocated using the coin flipping to get either oral chloral hydrate (50 mg/kg, not exceeding 1 gm) combined with 25 mg hydroxyzine (Group A) or oral midazolam (0.5 mg/kg) combined with 25 mg hydroxyzine (Group B) at first visit. Then they got the alternative drug regimen as crossover design. The schedule timing, examiner, operator, and dental assistant were similar at both visits. The anesthesiologist allocated the patient to drug regimen and dispensation. The patient's guardians got information of the study and signed the informed consent before sedation and operation. The NPO time was at least six-hour.

At the beginning of the visit, the anesthesiologist recorded initial vital signs as well as the oxygen saturation, then continued recording until the patient met the discharge criteria (capability to maintain airway, accomplishing the baseline cardiorespiratory function, normal hydration, and capability to sit up unaided, at least 10 seconds). Critical complications, including respiratory decline, less than 90% oxygen saturation, and more than 25% decrease in mean arterial pressure, were observed.

For the anxiety assessment, an Anxiety Score System modified from Wilton[20] (Table 1) was used to assess the patient. The score was recoded at the following steps, oximeter probe placement, before transferring to treatment room, after papoose board placement and after treatment. Then the freshly prepared cherry distinguished liquid containing either chloral hydrate + hydroxyzine or midazolam + hydroxyzine was given, in body weight corresponding volume according to the assigned group. Each child stayed with guardian in the quiet room until the sign of drowsy was detected, then was transported to the treatment room. The examiner and operator were blind to the sedation regimen.

Table 1 Anxiety Scoring System

Criterion	Score
Agitated: clinging to the parent and/or crying	1
Alert: awake but not clinging to the parent; may whimper but not cry, anxious	2
Calm: sitting or lying with eyes open; relaxed	3
Drowsy: Eyes open, dull reaction. Responds to minor stimulus	3.5
Very drowsy: Eyes closed, dull reaction. Responds to minor stimulus	4
Asleep: Sleeping, no response to minor stimulus	5

For memory assessment, each child was asked to select a picture from the Stanford-Binet Intelligence Scale-Memory for Objects Subjects Subtest [21]. One hour after finishing treatment, the test was repeated.

Data Analysis

Demographic data were presented with descriptive statistics. The differences of physiological effects (vital signs and oxygen saturation) in group A and B were assessed using paired t-test at 95% level of significance. The difference in group A and B patient's anxiety were determined using Wilcoxon matched-pairs signed-ranks test. The non-parametric McNemar matched pairs analysis test was done for nominal-scale memory test.

RESULTS

Fifty patients (24 girls and 26 boys) were recruited in this research. Mean age was 36 months as showed in Table 2. Both regimens were well tolerated as showed in Fig. 1. Midazolam group showed better drug compliance and less nausea and vomiting. The respiratory rates, heart rate, blood pressure and oxygen saturation of both groups were not different. The Fig. 2 and 3 revealed heart rates from the beginning until the termination of treatment with no difference between two groups. For the assessment of anxiety, the scores at different time including anxiety on arrival, applying pulse oximeter, anxiety before treatment, applying papoose board, and anxiety after treatment were showed (Table 3). The Wilcoxon matched-pairs signed-ranks test demonstrated no difference between two groups. The assessment of memory could not be demonstrated in every patient, Fifteen out of 50 failed to recall the picture (33.3% in midazolam group and 66.7% in chloral hydrate group), whereas 18 out of 50 succeed to recall (44.4% in midazolam group and 55.6% in chloral hydrate group). The non-parametric McNemar matched pairs test revealed no difference between two groups (Table 3).

Table 2 Characteristics of the groups

	Total	Drugs	
		Chloralhydrate	Midazolam
Mean Age (months)	50	36.2 ± 8.02	36.4 ± 7.83
Sex	50	25 (50.0%)	25 (50.0%)
Female	24	12 (50.0%)	12 (50.0%)
Male	26	13 (50.0%)	13 (50.0%)
Visit	50		
1st Visit	25	13 (52.0%)	12 (48.0%)
2nd Visit	25	12 (48.0%)	13 (52.0%)

Table 3 Mean anxiety scores in each step and memory test result by drug used

Result	Midazolam	Chloralhydrate	*p*-Value
Anxiety			
- On arrival	2.96 ± 0.046	2.88 ± 0.09	0.414
- Pulse oximeter	2.36 ± 0.20	2.46 ± 0.18	0.258
- Before Treatment	3.46 ± 0.06	3.70 ± 0.20	0.225
- Papoose board application	2.59 ± 0.25	3.31 ± 0.27	0.107
- After treatment	2.94 ± 0.06	2.98 ± 0.05	0.581
Memory test			
- Failed to recall picture	5 (33.3%)	10 (66.7%)	0.515
- Succeeds to recall picture	8 (44.4%)	10 (55.6%)	0.515
- Refused to recall pictures	11 (68.8%)	5 (31.3%)	-
- Questionable	1 (100%)	0 (0%)	-

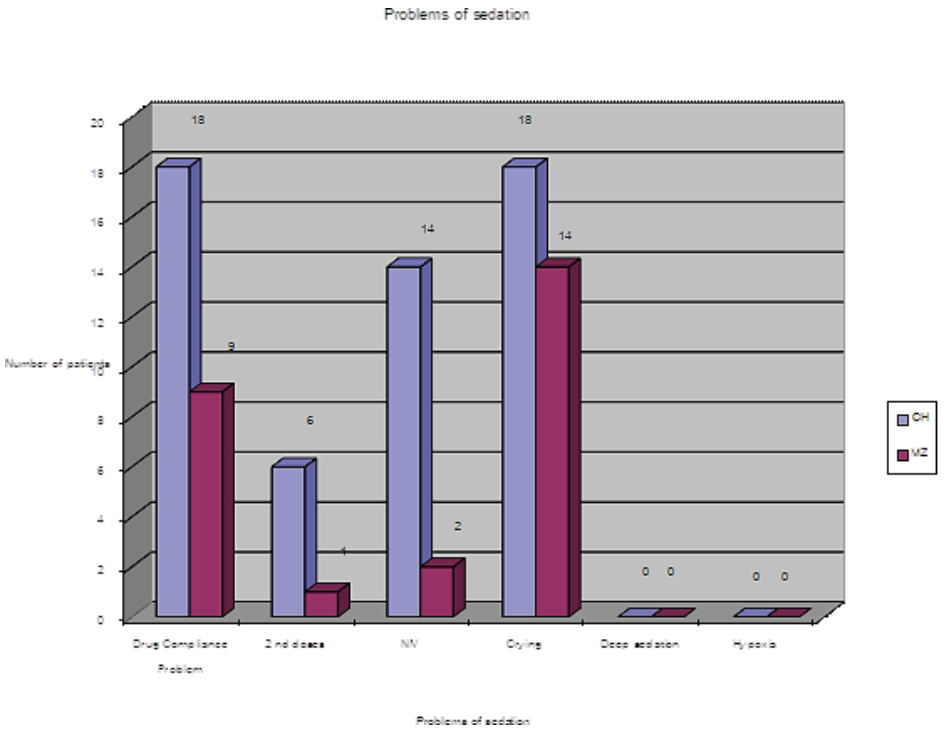

Figure 1 Number of children in each category of problems of sedation

(Drug compliance problem, 2nd dose, N/V (= nausea/vomiting), Crying, Deep sedation, Hypoxia).

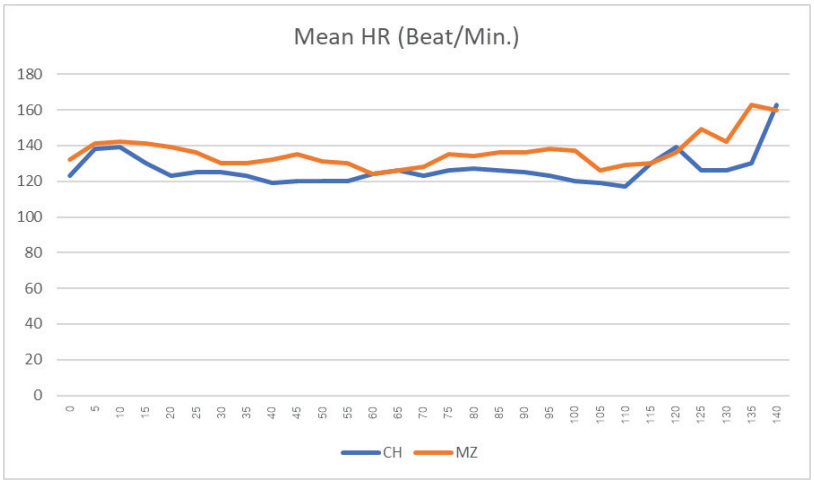

Figure 2 Mean heart rate of subjects compare between chloralhydrate and midazolam.

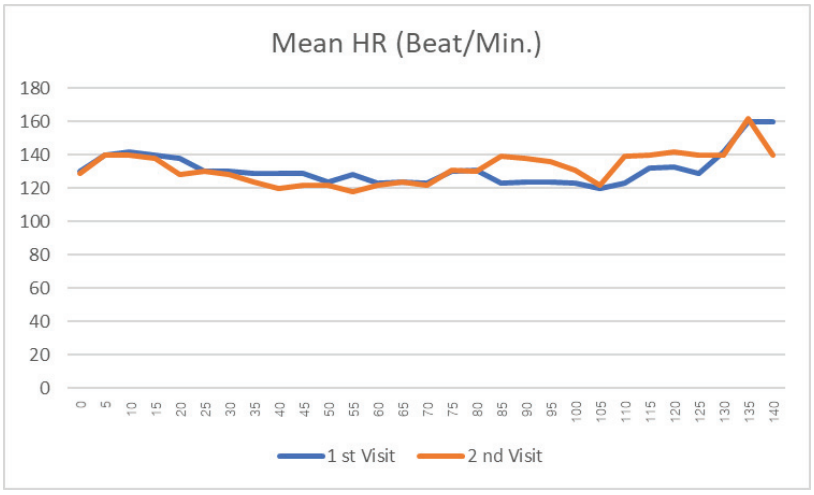

Figure 3 Mean heart rate of subjects during the first and the second visits.

DISCUSSION

Conscious sedation is a preferred choice in pediatric patient. It can reduce the pain distress and worry about treatment, as well as promote co-operation in the next visit. The sedation can be done with various sedative agents and routes, but the oral route is the most preferred route. Midazolam is a safe and effective sedative agent[22,23] with rapid onset[24,25]. In this study, the 0.5 mg/kg dose was selected. This dose was as effective as larger dose for children [26-29]. Field et al reported that the 0.5 mg/kg dose of midazolam was as effective as the 0.75 mg/kg dose in child sedation[30], whereas McMillan et al reported equal effects of 0.5, 0.75 and 1.0 mg/kg doses of oral midazolam in children[31]. Chloral hydrate is sedative-hypnotic agent in non-opioid, non-benzodiazepine group. Because the commercial liquid formulation is unavailable in the market, each hospital prepares its own formulation. The low-dose chloral hydrate (10-25 mg/kg), combined with other sedatives, is used in pediatric dentistry [32]. In this study, chloral hydrate with an anti-emetic, hydroxyzine, was used. A total dose did not exceed 1 gm to avoid the toxicity. The proper level of sedation for management behavior has to be considered in performing extensive procedures in children under 6 years old[33]. In this study, observation was continued throughout post drug-administration time, and the changes in vital signs was within limit. No complication, such as hypoxia or sedation, was detected in both groups. However, in previous articles, unfavorable complications were reported[34,35]. Assessment of memory function is complicated in young children because of variation of child development and imprecise reactions which is not related to recall function[36]. The realizing and recall ability may be affected by increased

apprehension or exhaustion from dental treatment. Recognition tests are less difficult to perform and less sensitive to different developments. Boyd recommended the clinical assessment of memory in children[37], using memory task of recognition, which was used in this study.

CONCLUSION

We performed a clinical trial to compare the efficacy of oral midazolam (0.5 mg/kg) to those of chloral hydrate (50 mg/kg) in less-than five-year pediatric patient receiving dental procedures. The effectiveness in term of anterograde amnesia (effects on memory function) and anti-anxiety was comparable in both medications. However, midazolam had at least 3 advantages; less complication, better drug compliance and shorter onset of action which could reduce a dental visit time.

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