Dental Decay and Oral Findings in Children and Adolescents Affected by Different Types of Cerebral Palsy: A Comparative Study

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Objective: *To compare dental caries and oral findings in patients affected by different types of Cerebral Palsy (CP).* ***Study design:*** *This cross-sectional study involved 120 children and adolescents with a diagnosis of CP. WHO diagnostic criteria were used to determine DMFT (caries diagnosis), the pocket depth and attachment level (periodontitis diagnosis). Additionally, the study evaluated dental erosion, traumatic dental injuries, treatment needs index (TNI), oral habits, malocclusions, gingival overgrowth, and dental fluorosis.* ***Results:*** *The most frequent CP type was spastic (62.5%), followed by mixed (18.3%), ataxic (10%), and athetoid (9.1). Patients affected by mixed CP showed a higher prevalence in decayed, DMFT index and TNI compared with the other types of CP ($p<0.05$). The frequency of malocclusion in the clinical evaluation was 87.5% and in plaster models was 49.2%.* ***Conclusions:*** *Dental caries was an important issue in mixed and athetoid CP groups. Oral habits and malocclusions were the most significant oral health problems in individuals with CP.*

Key Words. *Cerebral Palsy; Periodontal Disease; DMFT index; Dental Caries; Malocclusions.*

INTRODUCTION

Dental caries (DC) is one of the most prevalent diseases in the oral cavity with a multifactorial etiology. There is practically no ethnical group in the world that does not exhibit some evidence of DC[1]. Multiple factors (interaction of bacteria, diet, oral hygiene, plaque, immunity, time, and host response) influence DC initiation and progression, and dissemination of the oral bacteria[2,3]. On the other hand, particular segments of the population presents a high frequency of DC, such as people of lower socio-economic strata, lower education level or those affected by disabilities[4].

Developmental disabilities are a heterogeneous group of chronic conditions defined by cognitive, behavioral, or physical functioning problems. Cerebral palsy (CP) is one of the most frequent cause of motor disability in children; however, its prevalence (affects 2 to 3 per 1000 births[5]) has been stable for a long time. In Mexico[6], the precise prevalence of CP in children and adolescents is unknown, but it has been reported a CP prevalence of 85% in reported a life expectancy of 50 years old, for 20 years old individuals affected by CP[7]. Patients affected by CP have several medical and oral disorders, including gastroesophageal reflux disease (GERD), feeding intolerance, difficulty to handle oral secretions, poor motor control, dental caries, periodontal disease, traumatic dental injuries, bruxism, excessive tooth wear, damage of temporomandibular joint, and swallowing problems[8]. Furthermore, these patients have an increased risk of infections by Gram-negative and Gram-positive bacteria associated with oral infections[9]. The aim of the present study was to compare dental caries and oral findings in children and adolescents affected by different types of CP.

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PATIENTS AND METHOD

This cross-sectional study involved 305 children and adolescents with a clinical diagnosis of CP, but only 120 fulfilled the selection criteria. The inclusion criteria were: diagnosis of CP including spastic, ataxic, athetoid and mixed type (two o more types of CP affecting one patient). Sixty individuals with CP lived in San Luis Potosi City, and sixty were recruited in Morelia City (both cities located in the north-central area of Mexico). Completed health and dental questionnaires that included information about systemic health, dental and periodontal status were completed for then study population. Before the clinical examination, the parent or guardian signed a written informed consent[10]. This consent embodied the ethical principles of the Declaration of Helsinki (Version 2008). The Clinical Studies Committee of the Master´s Degree in Advanced Education General Dentistry Program at San Luis Potosi University, Mexico, approved the study (2014/02/01). Besides, this study followed the Strengthening the Reporting of Observational studies in Epidemiology **(**STROBE), guidelines for observational studies.

The WHO caries diagnostic criteria were used to determine the permanent tooth DMFT index (decayed, missing and filled tooth surfaces)[11]. Additionally, a calibrated examiner determined the diagnosis of periodontitis. The pocket depth and clinical attachment loss (periodontitis diagnosis) were measured by inserting a probe parallel to the teeth long axis and crossing each tooth's surface circumferentially[12]. The diagnosis of periodontitis was determined when the pocket depth was ≥ 3 mm, and the attachment loss was ≥ 2 mm in at least 30% of the measured sites[13]. Epithelial attachment to the cement-enamel junction was used to estimate the clinical attachment loss. Dental erosion evaluation was carried out according to the clinical assessment of erosive enamel lesions[14]. Traumatic dental injury classification[15] was used to classify traumatic dental injuries (TDI) to the maxillary and mandibular anterior permanent teeth. For each patient, the treatment needs index was used to calculate the necessities of dental treatment and the modified Dean's index to determine dental fluorosis[16,17]. In all individuals in the study WHO criteria were applied to analyze the clinical evaluation. Angle's classification was used to record the occlusion of each subject by using orthodontic plaster models. The overgrowth index in plaster models[18-20] was used to examine gingival overgrowth. Furthermore, the study included information about the pharmacological treatment of epilepsy and seizures. Each patient received a professional tooth cleaning, long/term preventive measures, and dental treatment strategy.

Statistical analysis

Two examiners were calibrated in all variables with an expert through Kappa and intraclass correlation coefficient tests prior the study. For descriptive statistics were used means and standard deviation. The present study used parametric tests like ANOVA adjusted by Dunnet test and non-parametric analysis. The Spearman correlation test was used to correlate age and dental caries. For the statistical analysis was used JMP program version 9.0 (SAS Institute, Cary, NC, USA) and Stata Intercooled version 11.0 (Stata Corp LP, College Station, TX, USA).

RESULTS

Table 1 shows the distribution of clinical types of CP according to age and sex. People with the spastic type were the most frequent, followed by the mixed, ataxic, and athetoid type. Youngest people were more affected by the athetoid type of CP while the ataxic type affected oldest people. Males were more affected by all types of CP than females.

Table 2 shows caries experience evaluated with the DMFT and its different components related to the various types of CP. The patients affected by the mixed CP showed a higher prevalence in decayed, DMFT index and TNI compared with the other types of CP. The DMFT index and the decayed components showed a statistically significant difference ($p<0.05$) among groups. There were statistically significant differences in the missing and filled components.

Table 3 shows DMFT comparisons between different types of CP according to ANOVA test. The difference between DMFT (permanent teeth) in the four types of CP showed a statistically significant difference ($p<0.05$). DMFT was significantly higher for mixed individuals with CP when comparing with spastic and ataxic type. The individuals with CP showed low frequency (15.8%) of periodontal disease (2.2±0.8mm pocket depth profile and 0.1±0.3 mm for clinical attachment loss).

There was a presence (37.5%) of dental erosion that was classified as follows; 23.3% for code 1 (loss of surface features); 4.1% for code 2 (loss of enamel with an exposure of dentin <1/3 surface) and 9.1% for code 3 (enamel loss with exposure of the dentin >1/3 surface). Regarding to traumatic dental injuries, 20.9% had traumatisms on anterior teeth, which were divided into the following categories: 1.6% with enamel crack, 15% with enamel-dentin fracture without pulpal exposure, and 4.1% enamel-dentin fracture with pulpal exposure. Besides, 53.3% of the CP population was affected by dental fluorosis, most of the participants showed a mild fluorosis and San Luis Potosi City residents.

The clinical evaluation of dental occlusion indicated that 87.5% of the participants had malocclusions. According to the Angle´s classification, the evaluation in plaster models showed more frequency of participants with class I (50.8%), and less frequency of individuals with subdivision 2 (6.6%).

There was a high prevalence of different single oral habits or its combinations. A total of 103 (85.3%) individuals affected by CP showed oral habits. The most frequent single habit was bruxism, followed by mouth breathing, and lingual interposition. Drooling is defined as the presence of saliva beyond the margins of the lip, which was reported in 1.6%. In Table 4 is shown a wide range of oral habits combinations, the most prevalent was drooling-mouth-breathing-bruxism (23%) and the drooling-mouth-breathing (15%). There was a low frequency of gingival overgrowth that affected only nine individuals (7.5%).

Table 1. Distribution of CP patients according to age and sex

CP type	Frequency (%)			*Age
		Male	Female	Mean ± SD (Range)
Spastic	75 (62.5)	39 (52.0)	36 (48.0)	16.8 ± 6.8 (9-44)
Mixed	22 (18.3)	12 (54.5)	10 (45.5)	18.6 ± 6.4 (9-31)
Ataxic	12 (10.0)	6 (50.0)	6 (50.0)	21.5 ± 9.4 (11-40)
Athetoid	11 (9.1)	7 (63.6)	4 (36.4)	14.2 ± 5.7 (9-25)
Total	120 (100)	64 (53.3)	56 (46.6)	17.3 ± 7.1 (9-44)

CP: Cerebral palsy; SD: Standard deviation; * Expresed in years.

Table 2. Decayed, missing, filled, DMFT index and TNI in study groups

Variables	Spastic	Mixed	Ataxic	Athetoid	P
Decayed	3.3 ± 3.4	8.4 ± 3.3	3.3 ± 5.0	3.5 ± 5.4	<0.0001**
Missing	0.3 ± 1.2	0.5 ± 1.4	1.4 ± 2.9	0 ± 0	0.14
Filled	1.2 ± 2.7	0.8 ± 1.4	1.5 ± 3.5	0.8 ± 1.7	0.9
DMFT	5.3 ± 4.4	9.5 ± 3.6	6.7 ± 5.2	8.1 ± 5.6	0.0045**
TNI	61.7%	90.2%	43.9%	40.8%	

DMFT: decayed, missing, and filled tooth index; TNI: treatment needs index; **statistically significant difference between the means (p<0.05)

Table 3. DMFT comparisons among individuals with different types of CP.

CP types	Mean±SD	P
Spastic vs Mixed	5.3 ± 4.4 vs 9.5 ± 3.6	0.023*
Spastic vs Ataxic	5.3 ± 4.4 vs 6.7 ± 5.2	0.2
Spastic vs Athetoid	5.3 ± 4.4 vs 8.1 ± 5.6	0.9
Mixed vs Ataxic	9.5 ± 3.6 vs 6.7 ± 5.2	0.001*
Mixed vs Athetoid	9.5 ± 3.6 vs 8.1 ± 5.6	0.5
Ataxic vs Athetoid	6.7 ± 5.2 vs 8.1 ± 5.6	0.7
Global DMFT 6.5 ± 4.7		

DMFT (decayed, missing, filled tooth) index; CP: cerebral palsy; SD: standard deviation; ANOVA adjusted by Dunnett test were used to identify mean differences across rows: Statistically significant difference between the means (p<0.05)*

Table 4. Frequency of malocclusions and orla habits in CP

Malocclusions		Oral Habits
Clinical evaluation	Angle Classification	Single
Frequency (%)		
Normal 15 (12.5)	Class I 61 (50.8)	Bruxism 12 (10.0)
Mild 39 (32.5)	Class II 27 (22.5)	Mouth Breathing 9 (7.5)
Moderate 66 (55.0)	Division 1 19 (15.8)	Lingual Interposition 8 (6.6)
Severe 0 (0)	Division 2 8 (6.6)	
OAH 0 (0)	Clase III 12 (10.0)	Combinations
		DL,MB,Bx 28 (23.0)
		DL, MB 18 (15.0)
		MB,Bx 6 (5.0)

CP: cerebral palsy; OAH: Orthodontic Appliance Holder; DL: drooling; MB: mouth breathing; Bx: bruxismo subjetcs

DISCUSSION

There is a wide variety of research about the oral health of individuals with CP. However, only a small percentage had studied patients during childhood. Most of the studies had been focused on analyzing single oral traits and did not include the complete oral health status of individuals with CP.

The present study involved 120 individuals affected by different types of CP. The mean age was 17.3 years and males were the most affected by CP than females[21]. This could be explained due to the recessive X-linked chromosome variants that may contribute to this difference. As a consequence, men were more vulnerable to a genetic mutation than females[22].

The mean values of the DMFT index were high compared to studies carried out in other countries. The differences could be explained due to age, educational level of parents, family income, and the different methods used to analyze the variables involved[23-26]. When the various types of CP were examined, patients affected by mixed CP had the highest DMFT index. This could be attributed to the presence of involuntary movements, pathological oral reflection, spasticity of mastication muscles that may contribute to poor oral hygiene, and consequently to a higher dental caries[27]. On the other hand, there was a low prevalence of periodontal disease with low values of both pocket depths (2.2 mm) and attachment loss level (0.1 mm). These findings agree with a report in European population that showed a low prevalence of periodontal disease[28].

Additionally, the main problem for CP patients is the presence of malocclusion. Nevertheless, there were questionable results in different reports. The difference could be explained by the method used for diagnosis. Clinical evaluation showed that there is a high frequency of malocclusion (87.5%), but a more detailed analysis of Angle´s classification based on plaster models demonstrated a low rate (49.2%). The clinical evaluation takes into account other factors such as the presence of dental crowding, overjet, and deviation of medium line. One possible explanation for the difference between the reported studies could be that there is and overestimation of the malocclusions frequency due to individuals with CP having involuntary movements [29-31].

More than a third (36.6%) of the studied sample used anticonvulsive drugs (magnesium valproate and topiramate), but there was a very low prevalence of gingival overgrowth (7.5%). There is a study reporting 42% of gingival overgrowth in patients with epilepsy diagnosis under treatment with magnesium valproate[32]. A high prevalence of GERD has been observed in individuals affected by CP; the presence of GERD leads to a high frequency of dental erosion[33]. However, in this study dental erosion was found in only 37.5%; some reports showed a high prevalence (>75%) of dental erosion[34]. The discrepancies of both gingival overgrowth and dental erosion could be explained by other factors such as hygiene, genetic predisposition, drug administration, diet, and salivary flow.

Dental traumatic injuries had a prevalence of 20.8%; maxillary central incisors were the most affected teeth and most patients had overjet associated as well. It has been reported a high prevalence of dental injuries in individuals with CP , and the presence of overjet increase the risk of traumatic injuries[35]. Enamel crack was the most frequent DTI, followed by fracture of enamel and dentin with or without pulp exposition. These findings agree with several reports

that suggested CP patients have restricted mobility that requires a wheelchair, walker or crutches as an aid in walking; this produces a high risk for DTI[36]. Fluorosis was found in 53.3% in the studied population. This could be explained due to half of clinical sample was collected in San Luis Potosi City that is an endemic area of dental fluorosis[37]. The primary strength of the present study is that all traits were evaluated and compared to the same population rather than the fragmented information from various communities. The other strength is the comparisons of several features, among the individuals affected by different types of CP.

CONCLUSIONS

Dental caries, oral habits, and malocclusions were the most significant clinical oral findings in children and adolescents affected by Cerebral Palsy. Dental caries is a major issue for individuals with CP. The CP groups most affected by DMFT were athetoid and the mixed. DMFT, the decayed component of DMFT, and TNI showed a statistically significant difference ($p<0.05$) among groups.

In the CP population, oral habits and malocclusions were significant findings that require long-term preventive measures and dental treatment strategy. However, gingival overgrowth, dental erosion, and traumatic dental injuries had a low prevalence. Furthermore, dental fluorosis showed a high prevalence mostly due to a portion of the sample was collected in an endemic area of fluorosis.

REFERENCES

1. Moses J, Rangeeth BN, Gurunathan D. Prevalence of dental caries, socieconomic status and treatment needs among 5-15 year old school going children of Chidambaram. J Clin Diagn Res 5(1): 146–5, 2011.
2. Kaur N, Sahni P, Singhvi A, Hans MK, Ahluwalia AS. Screening the Drug Resistance Property Among Aerobic Pathogenic Microorganisms of Dental Caries in North-Western Indian Population: A Preliminary Study. J Clin Diagn Res 9(7): ZC05–8, 2015. doi: 10.7860/JCDR/2015/11989.6143.
3. Nomura R, Otsugu M, Naka S, Teramoto N, Kojima A, Muranaka Y, Matsumoto-Nakano M, Ooshima T, Nakano K. Contribution of the interaction of Streptoccoccus mutans serotype K stains with fibrinogen to the pathogenicity of infective endocatditis. Infect Immun 82(12): 5223–5234, 2014.
4. Macek MD, Heller KE, Selwitz RH, Manz MC. Is 75 percent of dental caries really found in 25 percent of the population? J Public Health Dent 64(1): 20–25, 2004.
5. Duruflé-Tapin A, Colin A, Nicolas B, Lebreton C, Dauvergne F, Gallien P. Analysis of the medical causes of death in cerebral palsy. Ann Phys Rehabil Med 57(1): 24–37, 2014.
6. Poblano A, Arteaga C, Garcia-Sanchez G. Prevalence of early neurodevelopmental disabilities in Mexico. Arq Neuropsiquiatr 67(3A): 136–740, 2009.
7. Haak P, Lenski M, Hidecker MJ, Li M, Paneth N. Cerebral Palsy and aging. Dev Med Child Neurol 51 suppl 4: 16–23, 2009.
8. Erasmus CE, van Hulst K, Rotteveel JJ, Willemsen MA, Jorgerius PH. Clinical practice: swallowing problems in cerebral palsy. Eur J Pediatr 171(3): 409–14, 2012.
9. Young NL, McCormick AM, Gilbert T, Ayling-Campos A, Burke T, Fehlings D, Wedge J. Reasons for hospital admissions among youth and young adults with cerebral palsy. Arch Phys Med Rehabil 92(1): 46–50, 2011.
10. Von Elm E, Altam DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting od Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Gac Sanit 22: 144–50, 2008.
11. Loyola-Rodriguez JP, Villa-Chavez C, Patiño-Marin N, Aradillas-Garcia C, Gonzalez C, de la Cruz Mendoza E. Association between caries, obesity and insulin resistance in Mexican adolescents. J Clin Pediatr Dent 36: 49–53, 2011.
12. Rodríguez- Martínez M, Patiño Marin N, Loyola-Rodríguez JP, Brito-Orta MD. Gingivitis and periodontitis as antagonistic modulators of gingival perfusion. *J Periodontol*. 2006;77(10);1643–1650.
13. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 34: 9–21, 2000.
14. Lussi A. Dental erosion clinical diagnosis and case history taking. Eur J Oral Sci 104(2(Pt 2)): 191–198, 1996.
15. Garcia-Godoy F. A classification for traumatic injuries to primary and permanent teeth. J Pedod 5(4): 295–297, 1981.
16. Mann J, Sgan-Cohen HD, Asher RS, Amir E, Cohen S, Sarnat H. A treatment need index: a pilot study. Int J Paediatr Dent 3(3):129–134, 1993.
17. Pontigo-Loyola AP, Islas-Marquez A, Loyola-Rodriguez JP, Maupome G, Marquez-Corona ML, Medina-Solis CE. Dental fluorosis in 12-and 15-year-olds at high altitudes in above-optimal fluoridated communities in Mexico. J Public Health Dent 68(3): 163–166, 2008.
18. Bendgude V, Akkareddy B, Panse A, Singh R, Metha D, Jawale B, Garcha V, Jathar P. Correlation between dental traumatic injuries and overjet among 11 to 17 years Indian girls with Angle´s class I molar relation. J Contemp Dent Pract 13(2): 142–146, 2012.
19. Szoke J, Petersen PE. Evidence for dental caries decline among children in an East European Country (Hungary). Community Dent Oral Epidemiol 28(2): 155–160, 2000.
20. Seymour RA, Smith DG, Turnbull DN. The effects of phenytoin and sodium valproate on the periodontal health of adult epileptic patients. J Clin Periodontol 12(6): 413–419, 1985.
21. Murphy DJ, Sellers S, Mackenzie IZ, Yudkin PL, Johnson AM. Case-control study of antenatal and intrapartum risk factors for cerebral palsy in very preterm singleton babies. Lancet 346(8988): 1449–1454, 1995.
22. Jacquemont S, Coe BP, Hersch M, Duyzend MH, Krumm N, Bergmann S, Bergmann JS, Rosenfeld JA, Eichler EE. A higher mutational burden in females supports a "female protective model" in neurodevelop- mental disorders. Am J Hum Genet 94(3): 415–425, 2014.
23. Du R.Y, Mcgrath C, Yiu C.K, King N.M. Oral health behaviors of preschool children with cerebral palsy: a case-control community-based study. Spec Care Dentist 34(6): 298–302, 2014.
24. Cardoso AM, Gomes LN, Silva CR, Soares Rde S, Abreu MH, Padilha WW, Cavalcanti AL. Dental Caries and Periodontal Disease in Brazilian Children and Adolescents with Cerebral Palsy. Int J Environ Res Public Health 12(1): 335–353, 2014.
25. Jain M, Mathur A, Sawla L, Ghoudhary G, Kabra K, Duraiswamy P, Kulkarni S. Oral health status of individuals with mental disabilities in India. J Oral Sci 51(3): 333–340, 2009.
26. Altun C, Guven G, Akgun OM, Akkurt MD, Basak F, Akbulut E. Oral health status of disabled individuals attending special schools. Eur J Dent 4(4): 361–366, 2010.
27. de Carvalho RB, Mendes RF, Prado RR Jr, Moita Neto JM. Oral health and oral motor function in children with cerebral palsy. Spec Care Dentist 31(2): 58–62, 2011.
28. Altun C, Guven G, Akgun OM, Akkurt MD, Basak F, Akbulut E. Oral Health status of disabled individuals attending special schools. Eur J Dent 4(4): 361–366, 2010.
29. Strodel BJ. The effects of spastic cerebral Palsy on occlusion. ASDC J Dent Child 54(4): 255–260, 1987.
30. Carmagnani FG, Goncalves GK, Correa MS, dos Santos MT. Occlusal characteritics in Cerebral Palsy patients. J Dent Child 74(1): 41–45, 2007.
31. Miamoto CB, Ramos-Jorge ML, Pereira LJ, Paiva SM, Pordeus IA, Marques LS. Severity of malocclusion in patients with cerebral palsy: determinant factors. Am J Orthod Dentofacial Orthop 138(4): 394, 2010.
32. Gurbuz T, Tan H. Oral Health status in epileptic children. Pediatr Int 52(2): 279–283, 2010.
33. Guare RO, Ferreira MC, Leite MF, Rodrigues JA, Lussi A, Santos MT. Dental erosion and salivary flow rate in cerebral palsy individuals with gastroesophageal reflux. J Oral Pathol Med 41(5): 367–371, 2011.
34. Su JM, Tsamtsouris A, Laskou M. Gastroesophageal reflux in children with cerebral palsy and its relationship to erosion of primary and permanent teeth. J Mass Dent Soc 52(2): 20–24, 2003.
35. Holan G, Peretz B, Efrat J, Shapira Y. Traumatic injuries to the teeth in young inidividuals with cerebral palsy. Dent Traumatol 21(2): 65–69, 2005.
36. Jalihal S, Nagarajappa R, Sharda A, Asawa K, Tak M. Assessment of dental trauma among cerebral palsy individuals in Udaipur city. Dent Traumatol 28(6): 448–451, 2012.
37. Loyola-Rodriguez JP, Pozos-Guillen AJ, Hernández-Guerrero JC, Hernández-Sierra JF. Flurosis in primary dentition in a region with endemic water fluoride. Salud Publica Mex 42(3): 194–200, 2000.