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EARLY CHILDHOOD CARIES (ECC) IS STILL A PROBLEM? AN EPIDEMIOLOGICAL AND AETIOLOGICAL APPROACH.

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Introduzione

La prevalenza della patologia cariosa nei paesi occidentali è diminuita in maniera decisa negli ultimi 20 anni, nonostante ciò, all'interno di tali società, esistono gruppi a rischio quali i bambini e gli anziani (Curzon e Preston, 2004). Per anni la patologia cariosa nell'età infantile è stata descritta con vari termini quali: carie da biberon, carie rampante o carie infantile (De Grauwe e Martens, 2004). Attualmente, si preferisce utilizzare il termine Early Childhood Caries (ECC), coniato dall'American Academy of Paediatric Dentistry (AAPD, 2008), per descrivere la presenza di almeno un elemento dentario cariato, otturato o estratto per carie nella dentatura decidua. Tale termine comunemente accettato nella letteratura più recente come definizione più generale include tra le cause della patologia sia l'allattamento al seno sia l'uso di sostanze edulcorate (De Grauwe e Martens, 2004).

Il quadro clinico dell'ECC è caratterizzato da lesioni cariose multifocali e gravi a livello delle superfici vestibolari e palatali degli incisivi superiori e di quelle palatali dei molari superiori. Il processo carioso ha inizio 3-6 mesi dopo l'eruzione dei denti interessando gli incisivi superiori e raggiunge l'apice prima dei 18 mesi (Thitasomakul *et al.*, 2006). Gli incisivi inferiori sono coinvolti solo tardivamente dal processo carioso e nei casi estremamente gravi grazie alla loro posizione vicino ai dotti escretori delle ghiandole sottomandibolari e sottolinguali che determina una continua detersione delle superfici. Inoltre, la posizione d'appoggio della lingua su tali elementi dentari durante la suzione ha una funzione protettiva permettendo a una minore quantità di cibo di aderire alle superfici dentali (De Grauwe e Martens, 2004).

Abitudini alimentari scorrette e l'allattamento al seno prolungato, sia in termini di ore giornaliere sia complessivamente oltre i 12 mesi, sono direttamente correlati all'insorgenza dell'ECC (Campus *et al.*, 2007; Al-Dashti *et al.*, 1995; Olak *et al.*, 2007).

Non si deve, infine, sottovalutare il potenziale cariogeno dei farmaci in uso in pediatria; ai piccoli pazienti sono somministrati regolarmente, in corso di malattie sistemiche, sciroppi zuccherati o

medicinali che riducono la salivazione (nei pazienti asmatici) (Horowits, 1998; Curzon e Preston, 2004).

Fattore rilevante nell'eziologia dell'ECC è lo stato socio-economico. Da vari studi effettuati nelle scuole dell'obbligo è risultato che la distribuzione della carie è concentrata maggiormente nei quartieri popolari dove vivono immigrati e famiglie con problemi socio-economici. Dai questionari compilati dai genitori è emerso che la maggior parte delle famiglie è composta da tre o più figli, vive con lo stipendio del padre, e sia il padre che la madre, spesso di giovane età, hanno conseguito un livello primario d'istruzione e in gran parte sono extracomunitari. Dal punto di vista odontoiatrico gran parte dei genitori hanno dichiarato di non conoscere le misure di prevenzione primaria della carie, di aver avuto esperienze di patologia cariosa e di ricorrere alle cure odontoiatriche solo in caso di urgenze. L'inconsapevolezza di avere problemi dentali, l'assenza di mal di denti, la spesa troppo alta per le cure odontoiatriche, la paura del dentista e la perdita dei rimborsi per le cure effettuate sono le ragioni più citate in merito alla scarsità delle visite odontoiatriche (Martens *et al*., 2006; Azogui-Lèvy *et al.*, 2003; Donaldson et Kinirons, 2001; Blair *et al.*, 2006).

Con tale tesi si è voluto analizzare il problema dell'ECC sotto diversi aspetti: eziologico, epidemiologico e preventivo.

Il lavoro è stato diviso in tre fasi:

1. revisione sistematica della letteratura sulla prevalenza dell'ECC e dei fattori di rischio;
2. studio epidemiologico cross-sectional sulla prevalenza di ECC e fattori di rischio nella popolazione prescolare della città di Sassari.
3. studio clinico randomizzato sull'efficacia della somministrazione nelle donne, durante l'allattamento, di un integratore contenente fluoro.

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De Grauwe A, Aps JK, Martens LC. Early Childhood Caries (ECC): what's in a name? Eur J Paediatr Dent 2004;5:62-70.

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Olak J, Mändar R, Karjalainen S, Söderling E, Saag M. Dental health and oral mutans streptococci in 2-4 years old Estonian children; International Journal of Paediatric Dentistry 2007;17:92-97.

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PAPER I

Early Childhood Caries (ECC) prevalence and background factors. A review.

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Summary

Aim: to describe the prevalence of ECC by reviewing published reports, and secondly to assess the rule of background factors on the ECC prevalence.

Design: Included studies were: reviews, meta analysis, RCT assessing the prevalence of ECC including more than 100 subjects; reporting subjects without any stated medical condition; in vivo studies; issued from January 1st, 2000 to May 31st, 2011. The age range for the inclusion was 0 to five years of age.

Results: Database searching showed 411 findings as potentially eligible. After abstract review 8 papers were retrieved as full-text and assessed for eligibility: three using ECC as mesh word, four using the term Early Childhood caries, and one using S-ECC as mesh word.

Conclusions: The main risk factor for ECC is the low socio-economical level of the parents. ECC is a public health problem and it requires involvement of all health professionals that provide care to children together with efforts from family members.

The dental caries is still an endemic disease affecting especially young subjects. Dental caries is a preventable disease, and it can be stopped and even potentially reversed during its early stages. People remain susceptible to the disease throughout their lives. Children in the age range of 12–30 months have a special caries pattern that differs from that in older children. The disease develops and affects the maxillary primary incisors and first primary molars in a way that reflects the pattern of eruption, longer the tooth has been present and exposed to the caries challenge, the more it is affected. So the teeth in the upper jaw are most vulnerable, while teeth in mandibular are protected by the tongue and by saliva from submandibular and sublingual glands. Caries lesion in young child is a worsening form of decay. Whereas certain feeding patterns, such as "at will" breast feeding and bedtime bottle feeding, have long been known to be risk factors for ECC, current research has demonstrated that other factors may also play a role.

This pattern of dental caries has been labeled variously as "bottle caries", "nursing caries", "baby bottle tooth decay", or "night bottle mouth". These terms suggest that the prime cause of dental caries in early childhood is inappropriate bottle-feeding. Current evidence suggests that use of a sugar-containing liquid in a bottle at night may be an important etiological factor, although it is not necessarily the only etiological factor. Therefore, it is recommended that the term Early Childhood Caries (ECC) be used when describing any form of caries in infants and preschool children. Various systems of classification have been used to define Early Childhood Caries (ECC) (Drury *et al.,* 1999; Psoter *et al.,* 2003). Two commonly accepted classifications for ECC include simple ECC and severe ECC, as defined by the American Academy of Paediatric Dentistry (AAPD, 2008). These two definitions were used for this study as follows:

- Simple ECC any deciduous presence of 1 or more decayed, missing, or filled tooth surfaces in any primary tooth in a child under 6 years of age;

- Severe ECC any sign of smooth-surface caries in children under 3 years of age; 1 or more cavitated, missing, or filled smooth surfaces in primary maxillary anterior teeth in children between the ages of 3 and 5; or decayed, missing, or filled scores of ≥ 4 (ages 3 to < 4), ≥ 5 (ages 4 to < 5), or ≥ 6 (ages 5 to < 6).

The main objective of this paper is aimed to describe the prevalence of ECC by reviewing published reports, and secondly to assess the rule of background factors on the ECC prevalence.

Methods

Protocol for this review was the PRISMA 2009 checklist (available at www.prisma-statement.org)

Eligibility criteria

Included studies were: reviews, meta analysis, RCT assessing the prevalence of ECC including more than 100 subjects; reporting subjects without any stated medical condition; in vivo studies; issued from January 1st, 2000 to May 31st, 2011. The age range for the inclusion was 0 to five years of age.

Only studies published in English language were considered due to the virtual absence of research published in other languages as resulted from preliminary electronic database searches.

Included studies:

- Assessed prevalence and morbidity outcomes for ECC and background variables;
- Measured dental caries as DMFT and/or DMFS Index according to WHO standards;
- Reported dietary, oral hygiene habits and Socio-economic level of the family;
- Clearly described objective, methods and results, with no significant discrepancies;
- Case reports, case series, outbreak investigations and abstracts were excluded.

Possible outcomes for included studies were:

- Increased risk of developing ECC in lower socio-economical families;
- Increased risk of developing ECC associated with dietary habits;

- Increased risk of developing ECC associated with behavioral and/or oral hygiene habits.

Information sources and search

From March 2011 the following electronic databases were searched: Medline, Embase®, The Cochrane Library and Google Scholar®. Two preliminary searches were conducted in June 2011 to obtain an overall idea of findings and to polish searching terms (MeSH words) and limits. The MeSH words was accessed to identify entry terms and compose the final Boolean search: Caries, ECC, S-ECC, Severe Early Childhood Caries, Baby Bottle Caries associated to epidemiology an background factors.

Time coverage was from January 1st, 2000 to May 31st, 2011. No topic related nor relevant finding resulted from both The Cochrane Library and Google Scholar®; these electronic databases were therefore excluded from final Boolean search.

Final search was conducted on September 29th, 2011. Reference lists of included and relevant papers were reviewed. Abstract was collected for all findings.

Study selection and data collection process

One reviewer screened all collected findings and registered title, author and whole reference in two Excel files (one for included and one for excluded findings, according to eligibility criteria) using a screening guide created on eligibility criteria. Kind of source was registered as reason for exclusion. The same reviewer conducted a new and independent screening after 15 days. Duplicates from different electronic databases were excluded. The full text of all studies judged potentially eligible in at least one screening were retrieved. Then, one reviewer screened the full text for inclusion using a screening guide and a second reviewer screened all findings. When disagreement occurred a third reviewer was consulted.

Data items

One reviewer used a standardized and pilot-tested form to abstract data. Then, a second reviewer screened data abstraction. When disagreement occurred, a third independent reviewer was consulted. Abstracted date were about study design, PICOs, population, exposure, outcomes, methodological features, results and funding and were defined through STROBE Statement—checklist of items that should be included in reports of observational studies.

The overall quality of evidence for each outcome was rated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Results

Figure 1 displayed the flow chart of the study. Database searching showed 411 findings as potentially eligible (appendix 1). After abstract review 8 papers were retrieved as full-text and assessed for eligibility: three using ECC as mesh word, four using the term Early Childhood Caries, and one using S-ECC as mesh word. Apart for this single finding, reference list checking highlighted a large number of duplicates with database searching results.

Table 1 describes characteristic of included studies assessing the prevalence of ECC. One study (Bernabè and Hobdell, 2010) was a review paper on the prevalence of ECC in 22 different countries; results reported confirmed that ECC is strictly related to SES level of the family. The authors observed that dental caries prevalence and levels decrease as the Gini per capita increase. The countries were divided in rich countries (according to World Bank criteria) and poor countries.

In rich countries, income inequality is the strongest factor associated to Early Childhood Caries. Further epidemiological data must be gathered through surveys in other regions of the country to support oral health programmes. It also needs to raise the awareness on the diagnosis prevention and treatment of ECC among health care workers including paediatricians, physicians, nurses and midwives. Especially facilities for preventive dental work (Fluoride varnish/gel application, fissure sealants) should be enhanced. Two papers were from North America (Tiberia *et al*., 2007; Nunn *et*

al., 2009) and reported a quite different prevalence rate 3.0% in US and 74.0% in Canada. The two groups were not comparable. Tiberia *et al.* reported about a sample selected from surveys of parents seeking dental care for children in 5 Canadian dental practices so an important bias in selection is present. On the other hand Nunn *et al.* compared data of ECC prevalence among 1- to 3-year-old children sampled from paediatric clinics in Boston area and compare ECC prevalence among these children with similarly aged children who were surveyed as part of the Third National Health and Nutritional Examination Survey (NHANES III). Overall ECC prevalence in Boston children was 3.0 % compared to a double value of prevalence in children derived from NHANES III (6.3%). Two papers were conducted in BRICS (Brazil, Russia, India, China, South-Africa) countries (Tyagi 2008; Feldens *et al.*, 2010). The two papers depicted a prevalence of 19.2% in India, and 63.0% in Brasil. Tyagi described a cross-sectional study on 813 children aged (2-6 years) living in Davangore (India) and no statistical significant difference was detected among gender. No figures were provided how the sample was selected or if it was representative of the area. Feldens *et al.* performed a birth cohort study on southern Brazilian 4 years olds. 500 infants were recruited at birth and the paper was focus only on S-ECC (Severe- Early Childhood Caries) at 4 years of age (final sample 340 subjects). Twenty-six per cent presented simple-ECC and 37.0 % S-ECC.

Two papers were from Japan (Niji *et al.*, 2010) and Greece (Maragakis *et al.*, 2007). Niji *et al.* performed a cross-sectional investigation on the relation between mother age and behavioral and caries prevalence in children. Overall 646 mother-child pairs were examined. ECC prevalence was 41.0%. In Greece, a cross-sectional study on ECC, location and treatment needs was completed. ECC prevalence recorded was quite high (60.1%). A longitudinal observational community-based study was conducted in Thailand (Thitasomakul *et al.*, 2009) using negative binomial regression procedure to analyze data. Overall mean dmfs ranged from 0.1±0.4 to 2.8±2.7.

Screening for dental caries should start as soon as the first primary tooth erupts or not later than one year of age. Oral health programmes should be established focusing on mothers, caregivers, community health workers, preschool teachers and children.

Table 2 describes the background factors associated with ECC. The paper of Maragakis *et al.* was excluded, because no association with background factors was made. The socio economical level of the family (recorded using several proxy variables like the Gini coefficient, the Annual household income, low income of the family) was reported associated with ECC in four papers (Thitasomakul *et al.*, 2009; Nunn *et al.*, 2009; Barnabé *et al.*, 2010; Feldens *et al.*, 2010). Dietary and behavioral habits like breast-feeding habits, sweetened food, bottle-feeding at night, holding liquids in the mouth for prolonged time, frequency of between meal snacks more than 4 time a day, mother's age at childbirth were positively associated to ECC in six papers (Tiberia *et al.*, 2007; Tyagi, 2008; Nij *et al.*, 2010; Thitasomakul *et al.*, 2009; Feldens *et al.*, 2010).

Discussion

The prevention and management of Early Childhood Caries (ECC) should be an important objective of public health. The onset of the disease is in children with less 2 years of age. The prevention should begin during the prenatal period, continuing with the mother and the child during preschool in collaboration with paediatrician, paediatric dentist and teacher. The vertical transmission (from mother to the child) of cariogenic bacteria involves women in all kind of prevention programmes. This review was aimed to describe and analyze the prevalence of ECC and relative risk factors.

A lot of risk factors are described associated with ECC. However, the main risk factor for ECC is the low socio-economical level of the parents.

Children with ECC have a high frequency of sugar consumption, not only of fluids given in the nursing bottle, but also of sweetened solid foods characteristic is likely to be one of the most

significant caries risk factors. World Health Organization (WHO) recommended that non-milk products and cellular extrinsic sugar should not represent more than 10% of the total daily caloric intake, and that sugars should comprise no more than 10-20% of the human diet. While the scientific basis of the messages to promote breastfeeding for general health are well accepted, prolonged and nocturnal breastfeeding is associated with an increased risk of ECC. However, the evidence for such an association of is limited and inconsistent, and is based primarily on cross-sectional studies relying on retrospective recall of infant feeding practices.

ECC is a public health problem and it requires involvement of all health professionals that provide care to children. Oral health cannot be seen as separate from general health. The objective of dentistry surpasses the preservation of the teeth; it aims at maintaining oral and systemic health.

Children receive notions of oral care and values from their families. Therefore, we should change the risk factors for caries development by establishing a partnership with the family, having prevention as the main focus. Prevention is inexpensive, but it demands efforts from family members, who sometimes are not aware of the consequences that caries can bring, or when they realize the problem, the disease is already installed resulting in consequences to the child and family's life.

Essential References

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Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administration. J Public Health Dent 1999;59:192-7.

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Niji R, Arita K, Abe Y, Lucas ME, Nishino M, Mitome M. Maternal age at birth and other risk factors in early childhood caries. Pediatr Dent 2010;32:493-98.

Nunn ME, Dietrich T, Singh HK, Henshaw MM, Kressin NR. Prevalence of early childhood caries among very young urban Boston children compared with US children. J Public Health Dent 2009;69:156-62.

Psoter WJ, Zhang H, Pendrys DG, Morse DE, Mayne ST. Classification of dental caries patterns in the primary dentition: a multidimensional scaling analysis. Community Dent Oral Epidemiol 2003;31:231-8.

Thitasomakul S, Piwat S, Thearmontree A, Chankanka O, Pithpornchaiyakul W, Madyusoh S. Risks for early childhood caries analyzed by negative binomial models. J Dent Res 2009;88:137-41.

Tiberia MJ, Milnes AR, Feigal RJ, Morley KR, Richardson DS, Croft WG, Cheung WS. Risk factors for early childhood caries in Canadian preschool children seeking care. Pediatr Dent 2007;29:201-8.

Tyagi R. The prevalence of nursing caries in Davangere preschool children and its relationship with feeding practices and socioeconomic status of the family. J Indian Soc Pedod Prev Dent 2008;26:153-7.

Figure 1. Flow chart of the study

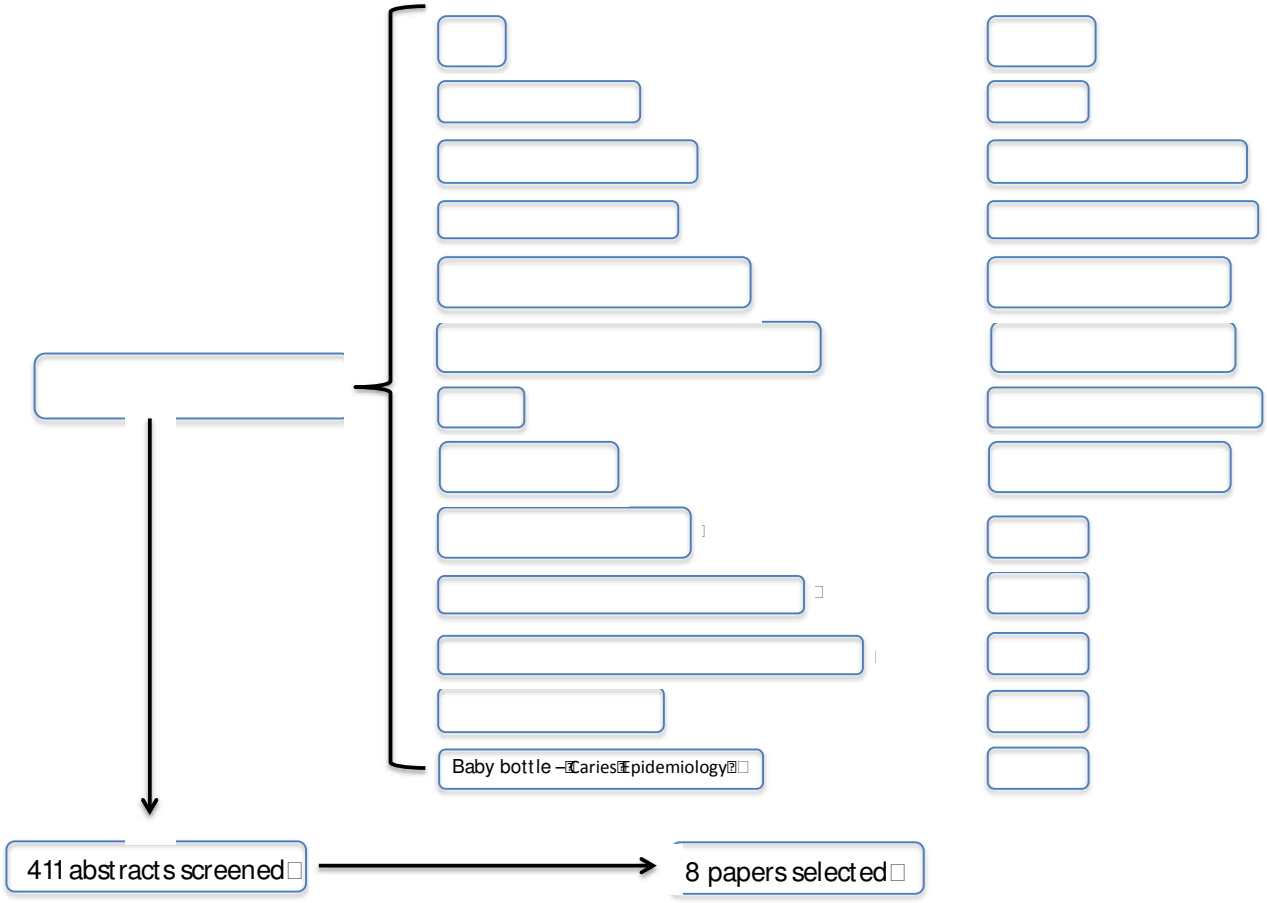

Table 1. Papers selected for the review.

Authors	*Age gropus*	*Size*	*Country*	*Prevalence/dmft mean*
Thitasomakul *et al.* (2009)	9-18 months	495	Thailand	Mean range 0.1±0.4 2.8±2.7
Nunn *et al.* (2009)	1-3 years	787	United States	3.0 %
Barnabé *et al.* (2010)	5-6 years	--	22 countries	1.68±0.59 mean (0.70-3.31 range)
Niji *et al.* (2010)	1.5-3 years	646	Japan	41 %
Maragakis *et al.* (2007)	2-6 years	684	Greece	60.1 %
Tyagi (2008)	2-6 years	813	India	19.2 %
Tiberia *et al.* (2007)	1-5 years	137	Canada	74.0 %
Feldens *et al.* (2010)	4 years	340	Brazil	26.0% simple-ECC 37.0 S-ECC

Table 2. Association between background factors (socio-economical levels, dietary behaviours, oral hygiene) and ECC.

Authors	*Background factors associated*
Thitasomakul *et al.* (2009)	• Children aged 12-18 months • Low income • Mothers did not have a daily intake of milk • No calcium during pregnancy • Children who were breast-fed • Mother's poor oral health status • Mothers had only primary school education • Mothers with 10 or more decayed teeth • Children who were not fed cooked rice or commercial cereal by the age of 3 months • Children who had soft drinks at 9 months • Children who had local traditional desserts at 9 months • Children who had started eating vegetables later than 6 months • Sweetened food • Sugary food by the age of 5 months • Soft drink • Sugary snacks • Children who did not have their teeth brushed at 9 months
Nunn *et al.* (2009)	• Race • History of a child's visit to the dentist • Parent's education • Annual household income
Barnabé *et al.* (2010)	• Gini coefficient
Niji *et al.* (2010)	• Mother's age at childbirth (< 22 years) • Frequency of between meal snacks more than 4 time a day • Child's CAT score of 1.5 year old equal to greater than +1.5
Tyagi (2008)	• Children who were bottle-fed • Use of dummy
Tiberia *et al.* (2007)	• Leaving the bottle with the child • Having problems brushing • Holding liquids in the mouth for prolonged time • Being Caucasian
Feldens *et al.* (2010)	• Breastfeeding duration • Frequency of breastfeeding • Night-time bottle use for liquids other than milk • High density of sugar

- High density of lipids
- Maternal schooling
- Per capita income
- Teeth at 12 months

Appendix 1. List of 411 paper retrieved by MESH search. Papers are listed in date of publishing order.

1. Erdem AP, Guven Y, Balli B, Ilhan B, Sepet E, Ulukapi I, Aktoren O. Success rates of mineral trioxide aggregate, ferric sulfate, and formocresol pulpotomies: a 24-month study. Pediatr Dent 2011;33:165-70.
2. Buzalaf MA, Levy SM. Fluoride intake of children: considerations for dental caries and dental fluorosis. Monogr Oral Sci 2011;22:1-19.
3. Foster GR, Downer MC, Tickle M. Modelling the impact of process variables in community fluoridated milk schemes on a population of UK schoolchildren. Br Dent J 2011 May 28;210:E17.
4. Rayen R, Hariharan VS, Elavazhagan N, Kamalendran N, Varadarajan R. Dental management of hemophiliac child under general anesthesia. J Indian Soc Pedod Prev Dent 2011;29:74-9.
5. Lukacs JR. Gender differences in oral health in South Asia: metadata imply multifactorial biological and cultural causes. Am J Hum Biol 2011;23:398-411.
6. Yengopal V, Bhayat A, Coogan M. Pediatric oral HIV research in the developing world. Adv Dent Res 2011;23:61-6.
7. Frazão P. Effectiveness of the bucco-lingual technique within a school-based supervised toothbrushing program on preventing caries: a randomized controlled trial. BMC Oral Health 2011;22;11:11.
8. Curtis B, Warren E, Pollicino C, Evans RW, Schwarz E, Sbaraini A. The Monitor Practice Programme: is non-invasive management of dental caries in private practice cost-effective? Aust Dent J 2011;;56:48-55.
9. Maupomé G, Karanja N, Ritenbaugh C, Lutz T, Aickin M, Becker T. Dental caries in American Indian toddlers after a community-based beverage intervention. Ethn Dis 2010;20:444-50.
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13. Taji S, Seow WK. A literature review of dental erosion in children. Aust Dent J 2010;55:358-67.
14. Ferracane J, Hilton T, Korpak A, Gillette J, McIntyre PS, Berg J; Northwest PRECEDENT. Use of caries prevention services in the Northwest PRECEDENT dental network. Community Dent Oral Epidemiol 2011;39:69-78.
15. Peters MC, Tallman JA, Braun TM, Jacobson JJ. Clinical reduction of S. mutans in pre-school children using a novel liquorice root extract lollipop: a pilot study. Eur Arch Paediatr Dent 2010;11:274-8.
16. Martignon S, Tellez M, Santamaría RM, Gomez J, Ekstrand KR. Sealing distal proximal caries lesions in first primary molars: efficacy after 2.5 years. Caries Res 2010;44:562-70.
17. van der Zee V, van Amerongen WE. Short communication: Influence of preformed metal crowns (Hall technique) on the occlusal vertical dimension in the primary dentition. Eur Arch Paediatr Dent 2010;11:225-7.
18. Armfield JM. Community effectiveness of public water fluoridation in reducing children's dental disease. Public Health Rep 2010;125:655-64.
19. Vilhena FV, Olympio KP, Lauris JR, Delbem AC, Buzalaf MA. Low-fluoride acidic dentifrice: a randomized clinical trial in a fluoridated area. Caries Res 2010;44:478-84.
20. Novaes TF, Matos R, Raggio DP, Imparato JC, Braga MM, Mendes FM. Influence of the discomfort reported by children on the performance of approximal caries detection methods. Caries Res 2010;44:465-71.

21. Parker EJ, Jamieson LM, Broughton J, Albino J, Lawrence HP, Roberts-Thomson K. The oral health of Indigenous children: a review of four nations. J Paediatr Child Health 2010;46:483-6.
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25. Wang SJ, Briskie D, Hu JC, Majewski R, Inglehart MR. Illustrated information for parent education: parent and patient responses. Pediatr Dent 2010;32:295-303.
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PAPER II

Early Childhood Caries (ECC) and background factors. A case control cross-sectional study.

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Summary

Aim: to evaluate the influence of several determinants, like as socio-economical level (SES), behavioural factors (dietary and oral hygiene) on the presence of ECC in pre-school children in the town of Sassari.

Design: a cross-sectional case control study was designed. The protocol consisted in a dental examination and a standardized questionnaire. The study group was of 544 subjects: 260 girls (47.8%) and 284 boys (52.2%). The sample was then categorized into two age groups: 185 (34.0%) children aged 48-60 months and 359 (66.0%) children aged 18-47 months**.**

Results: caries experience was 16.0% (28.6% in the older group and 9.5% in the younger one). Caries presence was linked to: a) parents' educational level in both parents in the old groups ($p<0.01$); b) mother's educational level ($p<0.01$) in the young group; c) use of bottle feeding ($p=0.02$) in the older group; d) use of sweetened dummy at night ($p=0.01$) in the young group; e) brushing habits after each meal ($p=0.04$) in the younger group; f) dental check-ups in the last six months ($p=0.01$) in the young group; g) eruption age of the first teeth ($p=0.04$) in the young group; h) number of siblings in both age groups ($p<0.01$). In the conditional logistic regression procedure a dummy variable of the parents' educational level was created. An educational level of parents shows a protective effect towards ECC prevalence (OR=0.44 $_{95\%}$CI 0.34 – 0.57). Also the presence in the family of one or more siblings was statically associated to ECC (OR=1.91 $_{95\%}$CI 1.30 – 2.81).

Conclusion: our results highlight the role played by socio-economic status and behavioural habits in the development of ECC.

During the past decades a severe decrease in caries experience in children in countries of western world (Szoke and Petersen, 2000; Campus *et al*., 2001; Vanobbergen, 2004; Campus *et al.*, 2007; Campus *et al.*, 2009) was observed. In industrialised countries, the prevalence, severity and patterns of caries are thought to be related to determinants which include social and educational background, dietary and oral hygiene practices (Roeters *et al.*, 1995; Stecksen-Blicks and Borssen, 1999). However, the pattern of caries disease reflects distinct risk profiles related to living conditions, lifestyles and environmental factors and the implementation of preventive oral health schemes (Petersen, 2005). Findings of relationships between socioeconomic status (SES) and health outcomes are quite ubiquitous across in the health literature. People with lower educational attainment and lower income usually have lower life expectancy; consequently, children from families with a low income are more likely to have a low birth weight and to suffer from childhood illnesses (Armfield, 2007). Breastfeeding is recommended by paediatricians to be continued for at least the first year of life and beyond for as long as mutually desired by mother and child (Gartner *et al.*, 2005). Prolonged and unrestricted breastfeeding, however, has been reported to be a potential risk factor for caries (Hallett *et al.*, 2006; Iida *et al.*, 2007). Various systems of classification have been used to define Early Childhood Caries (ECC) (Drury *et al.*, 1999; Psoter *et al.*, 2003). Two commonly accepted classifications for ECC include simple ECC and severe ECC, as defined by the American Academy of Paediatric Dentistry (AAPD, 2008) the first one Simple ECC any deciduous presence of 1 or more decayed, missing, or filled tooth surfaces in any primary tooth in a child under 6 years of age is the most used one.

Caries disease in preschool children has a complex multifactorial aetiology: it has also been associated with improper feeding practice (Ismail, 2003; Hallett *et al.*, 2006;

Campus *et al.*, 2007; Kramer *et al.*, 2007) and low socio-economic background (Ismail, 2003; Muirhead and Marcenes, 2004; Hallett *et al.*, 2006). Nowadays, increasing attention is being paid to differences in population subgroups in characterizing the distribution and correlation of dental disease (Seppä *et al.*, 2000; Muirhead and Marcenes, 2004; Bankel *et al.*, 2006). A link between preterm birth or low-birth weight and caries was proposed (Seow, 1997; Burt and Pai, 2001; Eastman, 2003; Shulman, 2005; Saraiva *et al.*, 2007), but no conclusive evidence has been found.

The present study evaluates the influence of several determinants, like as socio-economical level (SES), behavioural factors (dietary and oral hygiene) on the presence of ECC in pre-school children in the town of Sassari. The null hypothesis was that no associations are present between ECC presence and socio-behavioural factors. To validate this hypothesis a cross-sectional case control study was designed and performed.

Material and methods

Design of study and sample size selection

The study was conducted from January 2010 to November 2010. Data on the total number of residents in Sassari from pre-school groups were derived from the National Statistical Institute (Italian National office of Statistics. ISTAT http://www.demo.istat). The total number of children aged 18-60 months was 5354 (2714 boys and 2640 girls). Sassari is the largest district of northern Sardinia with 127.893 inhabitants. The fluoride concentration in local tap water is low (0.03 mg/L) (Campus *et al.*, 2009). The study designed was approved by the ethical committee of the University of Sassari (n°184/2009).

Caries prevalence data on Sardinian preschool children was used (Campus *et al.*, 2009) to calculate sample size. The theoretical sample size with a Confidence Interval set at 5 was 529 children, the number was increased by 5% against the possible number of non-responders. Children were recruited at the kindergartens using systematic cluster sampling, where each class was identified as a cluster and compiled into a list. The first cluster was randomly chosen, while the others were selected at the systematic interval of three classes. Altogether 722 children were recruited in the study (Figure 1).

The protocol consisted in a dental examination and a standardized questionnaire. Parents or guardians were issued with an information leaflet, explaining the aim of the study and requesting their child's participation, after which they were asked for signed consent. Only children with questionnaire filled out and parents signed consent were enrolled in the study (621 subjects). The clinical examination was performed during the schooldays with the teacher/s present. Sixty-one (9.8%) children were absent on the school day of the clinical examination and another 16 (2.6%) refused to participate. The study group was of 544 subjects: 260 girls (47.8%) and 284 boys (52.2%). The sample was then categorized into two age groups: 185 (34.0%) children aged 48-60 months and 359 (66.0%) children aged 18-47 months.

Clinical examination

Clinical assessments were carried out by one examiner (GC) under standardized conditions using a drying tooth device, a plain mirror and a WHO-CPI probe under optimal artificial lighting. Dental caries experience (dmfs) was recorded. A carious lesion was diagnosed when a cavity at dentinal level was noted (Pitts *et al.*, 1995).

The examiner received training and intra- and inter-examiner reliability was assessed before the beginning of the survey. Fifty subjects were examined and re-examined after

72 hours. Inter-examiner reliability was evaluated through analysis of variance for fixed effect compared to a benchmark (Castiglia *et al.*, 2007), while intra-examiner reproducibility was assessed as percent agreement and Cohen's Kappa statistics. A good examiner reliability compared to benchmark was found without significant differences ($p = 0.15$) and with a low value of mean squares for error (0.51). As regard, the percent agreement, intra-examiner reproducibility, was high (Cohen's Kappa 0.86).
The standardized questionnaire consisted of closed questions. It was filled out by parents or guardians at home and brought to school by the child. It contained questions related to medical status, consumption of sweets and soft drinks (mean intake frequency/day), frequency of toothbrushing and use of fluoride supplements apart from fluoride toothpaste.

Data analysis

Data were coded and imputed into a database (Microsoft® Excel 2010) and checked to verify accuracy of data. Statistical analysis was performed using Stata® 10.0 for Mac (http://www.stata.com). Initially, clinical condition parameters and potential risk indicators were analyzed univariately to describe the variables and distributions. Descriptive statistical analysis was performed. To avoid the attenuating effect of unequal variability among groups on the value of t, a square root transformation was performed when the response variable was a count (Fleiss, 1986). The association between ECC and background factors was tested using χ^2 test. Multivariate analysis was performed at two levels, first a logistic regression forward stepwise procedure using as dependent variable caries experience positively dmfs>0 was run in the two age groups separately. The conditional logistic regression procedure was used to the

analysis of a sample of matched 1:2 case-control. Unless stated otherwise, the criterion for statistical significance was set at $\alpha=0.05$.

Results

Overall caries experience was 16.0% (28.6% in the older group and 9.5% in the younger one). Mean dmfs was 0.9±0.2 $_{95\%}$CI (0.6 – 1.3) in all subjects while in the older group was 1.9±0.5 $_{95\%}$CI (1.0 – 2.8) and was 0.4±0.1 $_{95\%}$CI (0.2 –0.6). The need of dental care (d>0) was predominant in all age groups. The distribution of demographic and social variables by caries prevalence is displayed in Table 1. The parents' educational level was significantly associated to caries prevalence in both parents in the old groups ($p<0.01$), in the young group caries prevalence was associated to the mother's educational level ($p<0.01$). Regarding dietary habits the use of bottle feeding was associated to caries prevalence ($p=0.02$) in the older group; in the young group the use of sweetened dummy at night was statically associated to caries ($p=0.01$). In the younger group, brushing habits after each meal ($p=0.04$), dental check-ups in the last six months ($p=0.01$), the eruption age of the first teeth ($p=0.04$) were statistically linked to caries prevalence. The number of siblings was statistical associated in both age groups ($p<0.01$). Two logistic regression models were constructed and run for each age group separately. A high educational level of the father was protective to the presence of caries lesion in older sample, while the educational level of the mother was statistically significant in the younger group (Table 2). The presence in the family of more than one sibling was positively associated to caries (OR=1.63 $_{95\%}$CI 1.06 – 2.49). In the conditional logistic regression procedure a dummy variable of the parents' educational level was created (Table 3). An educational level of parents shows a protective effect towards ECC prevalence (OR=0.44 $_{95\%}$CI 0.34 – 0.57). Also the

presence in the family of one or more siblings was statically associated to ECC (OR=1.91 $_{95\%}$CI 1.30 – 2.81).

Discussion

Caries experience in Sardinian kindergarten children was similar than that reported in other surveys (Sundby and Petersen, 2003), moreover an impressive decrease was observed respect to those previously reported (Campus *et al.*, 2007). As reported in literature, the socio-economical status of the family and behavioural habits has a substantial impact on the development of ECC (Armfield, 2007). Low socio-economic status compromises the ability of individuals to care for their health leading to a reduced resistance to oral and other diseases.

Uncorrected nursing habits (bottle-feeding, use of pacifier at night, etc.) are the most frequently reported causes of ECC (Ismail, 2003), but the disease may also occur in children who are breast-fed. In the United States, for example, the majority of babies are fed using a nursing bottle, yet the majority of them do not develop ECC (Iida *et al.*, 2007).

In Italy, primary dental health service is based on private health care providers; thus, oral care is mainly financed by direct payment or, to a lesser extent, through public or private insurance schemes. The low level of caries experience described in this paper supports the hypothesis that public dental services play a minor role in the caries experience decrease observed in all developed countries.

Several numbers of limitations should be also considered when interpreting these findings. For example the study was cross-sectional and, consequently, no information is available regarding to lesion progression; the methods used for caries diagnosis

(visual inspection with probe and mirror) may be considered a limitation, but these methods are considered to have excellent reliability (Castiglia *et al.*, 2007).

In conclusion, our results highlight the role played by socio-economic status and behavioural habits in the development of ECC. We hope that the results of our study help in the promotion of improved oral health amongst infants and small children in Sassari.

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Table 1. Distribution of Demographic and Social Variables by caries prevalence (simple-ECC).

	dmfs=0 *(n %)*	dmfs>0 *(n %)*	Person chi-sqaure p-value
Sample n=544	457 *(84.0)*	87 *(16.0)*	
males n=284 *(52.2)*	237 *(83.4)*	47 *(16.6)*	
females n=260 *(47.8)*	220 *(84.6)*	40 *(15.4)*	
48-60 months			
males n=100	76 *(76.0)*	24 *(24.0)*	
females n=85	56 *(65.8)*	29 *(34.2)*	χ^2=1.66 p=0.54
18-47 months			
males n=184	161 *(87.5)*	23 *(12.5)*	
females n=175	164 *(93.7)*	11 *(6.3)*	χ^2=4.04 p=0.04
Mother's educational level			
48-60 months *(no responders =5)*			
• Primary schools	36 *(20.0)*	28 *(15.6)*	
• Secondary schools	62 *(34.4)*	20 *(11.1)*	
• University degree	30 *(16.7)*	4 *(2.2)*	χ^2=12.54 p<0.01
18-47 months *(no responders =6)*			
• Primary schools	64 *(17.8)*	14 *(3.9)*	
• Secondary schools	138 *(38.4)*	14 *(3.9)*	
• University degree	123 *(34.3)*	6 *(1.7)*	χ^2=10.04 p<0.01
Father's educational level			
48-60 months			
• Primary schools	52 *(29.0)*	36 *(20.1)*	
• Secondary schools	44 *(24.6)*	13 *(7.3)*	
• University degree	31 *(17.3)*	3 *(1.7)*	χ^2=13.83 p<0.01
18-47 months			
• Primary schools	95 (27.0)	15 *(4.3)*	
• Secondary schools	129 (36.8)	11 *(3.1)*	
• University degree	94 (26.8)	7 *(2.0)*	χ^2=3.43 p=0.180
Mother's occupational status			
48-60 months *(no responders =3)*			
• Unemployed	42 *(23.0)*	26 *(14.3)*	
• Employed	88 *(48.4)*	26 *(14.3)*	χ^2=4.96 p=0.03
18-47 months *(no responders =0)*			
• Unemployed	62 *(17.4)*	14 *(4.0)*	
• Employed	261 *(73.3)*	19 *(5.3)*	χ^2=9.62 p<0.01
Father's occupational status			
48-60 months *(no responders =5)*			
• Unemployed	6 *(3.3)*	7 *(3.9)*	
• Employed	78 *(43.4)*	29 *(16.1)*	
• Self working	45 *(25.0)*	15 *(8.3)*	χ^2=4.57 p=0.10
18-47 months *(no responders =7)*			
• Unemployed	18 *(5.1)*	7 *(2.0)*	
• Employed	191 *(54.3)*	18 *(5.1)*	
• Self working	109 *(31.0)*	9 *(2.5)*	χ^2=10.45 p<0.01
Sweetened dummy at night			
48-60 months *(no responders =4)*			
• Yes	26 *(14.4)*	14 *(7.7)*	
• No	104 *(57.5)*	37 *(20.4)*	χ^2=1.18 p=0.27
18-47 months *(no responders =1)*			
• Yes	56 *(15.7)*	12 *(3.3)*	
• No	268 *(74.9)*	22 *(6.1)*	χ^2=6.48 p=0.01

Bottle feeding			
48-60 months *(no responders =8)*			
• Yes	111 *(62.7)*	34 *(19.2)*	
• No	18 *(10.2)*	14 *(7.9)*	χ^2=5.46 p=0.02
18-47 months *(no responders =10)*			
• Yes	260 *(74.5)*	26 *(7.5)*	
• No	57 *(16.3)*	6 *(1.7)*	χ^2=0.01 p=0.91
Meals a day			
48-60 months *(no responders =4)*			
• 3	27 *(14.9)*	10 *(5.5)*	
• 4	69 *(38.1)*	19 *(10.5)*	
• >4	34 *(18.8)*	22 *(12.2)*	χ^2=5.32 p=0.07
18-47 months *(no responders =5)*			
• 3	35 *(9.9)*	5 *(1.4)*	
• 4	199 *(56.2)*	15 *(4.2)*	
• >4	87 *(24.6)*	13 *(3.7)*	χ^2=3.43 p=0.18
Dental check-ups			
48-60 months *(no responders =4)*			
• Yes	32 *(17.7)*	15 *(8.3)*	
• No	97 *(53.6)*	37 *(20.4)*	χ^2=0.31 p=0.57
18-47 months *(no responders =2)*			
• Yes	20 *(5.6)*	6 *(1.7)*	
• No	303 *(84.9)*	28 *(7.8)*	χ^2=5.97 p=0.01
Eruption of the first teeth			
48-60 months *(no responders =13)*			
• 6 months	47 *(27.3)*	16 *(9.3)*	
• 9 months	46 *(26.7)*	17 *(9.9)*	χ^2=0.11 p=0.94
• >9 months	33 *(19.2)*	13 *(7.6)*	
18-47 months *(no responders =12)*			
• 6 months	105 *(30.3)*	18 *(5.2)*	
• 9 months	123 *(35.4)*	10 *(2.9)*	
• >9 months	86 *(24.8)*	5 *(1.4)*	χ^2=6.07 p=0.04
Brush teeth after meal			
48-60 months *(no responders =2)*			
• Yes	48 *(26.3)*	20 *(10.9)*	
• No	82 *(44.8)*	33 *(18.0)*	χ^2=0.01 p=0.91
18-47 months *(no responders =4)*			
• Yes	51 *(14.4)*	10 *(2.8)*	
• No	270 *(76.0)*	24 *(6.8)*	χ^2=3.95 p=0.04
Number of siblings			
48-60 months *(no responders =6)*			
• 0	48 *(26.2)*	13 *(7.1)*	
• 1	41 *(22.4)*	6 *(3.3)*	
• >1	41 *(22.4)*	34 *(18.6)*	χ^2=17.49 p<0.01
18-47 months *(no responders =2)*			
• 0	108 *(30.2)*	5 *(1.4)*	
• 1	67 *(18.8)*	4 *(1.1)*	
• >1	148 *(41.5)*	25 *(7.0)*	χ^2=9.53 p<0.01

Table 2. Logistic estimates of the model (forward stepwise logistic regression) for caries experience, by age groups.

48-60 months

Number of observations = 178 Log Likelihood = 89.43, χ^2_4 =30.54 $P < 0.01$

Variable	*P*	OR	$CI_{95\%}$
Mother's educational level	0.06	0.55	0.30-1.03
Father's educational level	0.02	0.48	0.26-0.90
Meals a day	0.05	1.66	1.00-2.77
Number of siblings	0.02	1.63	1.06-2.49

18-47 months

Number of observations = 349 Log Likelihood = -167.32, χ^2_4 =33.70 $P < 0.01$

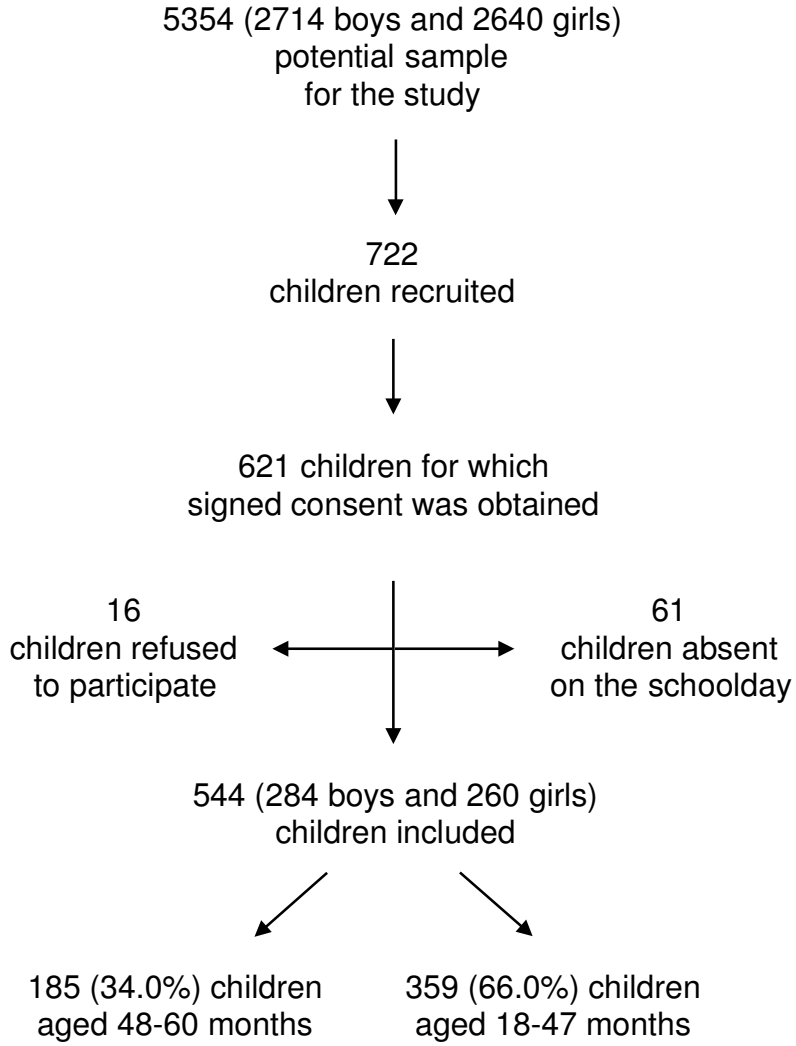
Variable	*P*	OR	$CI_{95\%}$
Mother's educational level	<0.01	0.52	0.36-0.77
Father's occupational status	0.08	0.65	0.40-1.05
Sweetened dummy at night	0.07	0.56	0.30-1.06
Number of siblings	0.10	1.23	0.96-1.59

Table 3. Odds ratio (OR) and 95% confidence intervals for ECC.

Number of observations = 239 Log Likelihood = -111.69, χ^2_4 =69.12 $P < 0.01$

	Odds Ratio	95% Confidence Interval
Parents'educational level	0.44	0.34 – 0.57
Meals a day	1.62	0.99 – 2.64
Number of siblings	1.91	1.30 – 2.81

Figure 1. Flow chart of the study sample.



PAPER III

Fluoride concentration in breast milk after the use of a fluoridated food supplement. A randomized placebo-controlled study.

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Summary

Background A way to administer fluoride to infants is to provide fluoride to the breastfeeding mother, but no sufficient scientific evidence is available.

Aim: The hypothesis behind this report was that the use of a fluoride food supplement in mothers during breastfeeding might increase the fluoride concentration in breast milk.

Design: A double-blind randomized placebo-controlled study was designed. 124 women were divided in two groups: a fluoride group, using a non-sucrose food supplement containing fluoride and a control group, using a non-sucrose food supplement without fluoride. The investigation had a randomized placebo-controlled study design with an experimental period of six weeks.

Results: at t_0, fluoride concentration values in both groups were quite comparable and no statistically significant differences were observed (p=0.52 respectively). The dropout rate was quite high (40.3%) mainly caused by the early interruption of breastfeeding. The comparison of the fluoride concentrations at the three different endpoints (t_0-t_1-t_2) between the two groups did not show statistical differences (p=0.12).

Conclusions No statistically significant differences were found between fluoride group and control group. Overall, the fluoride concentrations in the two groups did not significantly changed during the experimental period; only a statistically significant increase in F concentration was noted in the fluoride group.

Despite great efforts and achievements in oral health promotion were obtained, dental caries still remains a major health problem in childhood (Petersen, 2009). A striking reduction in dental caries in industrialized countries was registered, although the proportion with severe disease has remained at 10-15%, and the prevalence is increasing in developing countries. In Italy as in the majority of industrialized countries, recent data display an uneven distribution, with the highest burden of the disease among the underprivileged groups (Campus *et al.*, 2007; 2009; Wennhall *et al.*, 2008); this distribution calls for new strategies in caries prevention.

Milk and milk products contain nutrients that have potential anticaries properties: calcium, phosphate, casein, and lipids (Levine, 2001; Aimutis, 2004). A low prevalence of caries among schoolchildren who drink milk daily was recorded; the sample did not use fluoride regularly and had poor oral hygiene (Petti *et al.*, 1997). However, results of epidemiological studies on the association between dairy products intake and dental caries among young children are scant and inconsistent. A large debate was carried on the efficacy of fluoride during pregnancy. Some studies demonstrated fluoride ions capacity to pass the placental barrier (Toyama *et al.*, 2001; Gupta *et al.*, 1993); however still now scientific evidence on the possibility of fluoride ion to be incorporated into the child's teeth during pregnancy period is not available. A way to administer fluoride to infants is to provide fluoride to the lactating mother. The amount of fluoride in breast milk has been studied for many years (Sener *et al.*, 2007; Chuckpaiwong *et al.*, 2000; Latifah *et al.*, 1989). The fluoride concentration in breast milk was evaluated in women living in areas with low and high fluoride concentration in water (Spak *et al.*, 1983), but no statistically significant difference was found. The hypothesis behind this report was that the use of a fluoride food supplement in lactating mothers increases the fluoride concentration in breast milk.

Materials and methods

Study design and sample selection

A double-blind randomized placebo-controlled study was designed and approved by the ethical committee of the University of Sassari (n°184/2009). The study was conducted in Sassari, Sardinia, Italy, between October and December 2009. All the participants were resident in Sassari area, where a low natural fluoride content (≈0.3 ppm) in tap water is present. In 2008, the number of delivery was 1041. Women were recruited at the Gynaecologic Clinic of the University of Sassari before the delivery. Using systematic cluster sampling, women were compiled into a list; the first cluster was randomly chosen, while the others were selected at the systematic interval of five women. Subjects with a history of systemic antibiotic, topical fluoride or chlorhexidine treatment within 30 days before the baseline visit were excluded. Inclusion criterium was the possibility to use a breast pump (electric or manual).

Altogether 324 women were recruited. An information leaflet, explaining the aim of the study was delivered and a signed consent was requested. Only women with signed consent were called for examination (286 subjects). The flow chart of the study design is shown in Figure 1. In order to obtain interpretable results, the number of subjects to be included in each group was calculated. Two groups of women were formed: a fluoride group, using a non-sucrose food supplement containing fluoride and a control group, using a non-sucrose food supplement without fluoride. Considering significant a 25% difference between groups in fluoride concentration in breast milk, with values of 40% and 15% for the fluoride and the control groups respectively, and a 95% probability to obtain a significant difference between groups at 5% level, the number of subjects needed per group was 26.5. The acceptance rate was 55.8% (n = 160, mean

age 32.5±4.6). Using a computer program (Excel® 2003 for Mac OsX), the randomization was carried out on an individual basis by GC. 36 women refused to participate, thus the final study sample was formed by 124 subjects (63 women in the fluoride group and 61 in the control one). At t_1 interim evaluation, 27 women were excluded due to the early interruption of breastfeeding, (9 in the fluoride group and 18 in the control group), at t_2, 21 more women were excluded (10 received a systemic antibiotics therapy, 3 did not return the empty blisters and 8 due to the early interruption of breastfeeding). Thus, only 74 women concluded the experimental period: 40 in the fluoride group and 34 in the control group.

Treatment and sample collection

The two supplementations, powder form, were identical in weight (2.17g), form, colour and packing, but only one contained fluoride (1.5 ppm for each dose). They were produced and supplied by Milte® Italia spa (Italy), and coded as "green" or "red". The code was sealed by an independent monitor and not broken until the statistical analysis was finalized. The women were instructed to mix the powder into 100/150 ml of tap water and to drink the solution once a day in the morning after breakfast. Subjects were asked to make no changes in dietary and oral hygiene habits. All subjects received a fluoridated toothpaste containing 1450 µg/g NaF (Mentadent P, Unilever Italia, Milano) to be used during the experimental period. They were asked to avoid any other oral hygiene adjuvant and any commercial fluoride products during the study. Moreover women were instructed to drink bottled water with a low fluoride content (<0.04 ppm). In order to evaluate the success of administration of the food supplement, women were given food supplement necessary for a single week at a time and they were asked to return the empty blisters. Samples of milk were collected at baseline (t_0), after three weeks of food supplement use (t_1) and finally after six weeks of use (t_2).

Milk samples were gathered during 3 minutes by women into a polyethylene tube, after cleaning the breast with cotton wool and distilled water. An aliquot of this milk (5.0µl) was collected in a plastic tip and stored in a sterile plastic microbeaker.

Fluoride analysis

Prior to analysis, an appropriate volume of Total Ionic Strength Adjustment Buffer (TISAB, usually 0.5 ml) was added to all samples, in order to adjust the pH 5.0 and their ionic strength. The fluoride concentrations of the samples were measured after adding 5 ml of distilled water. The chemical analyses were done in a blind manner; fluoride was analyzed using a ion-specific electrode (model 9609, Orion Research) coupled to a potentiometer (model 710A, Orion Research). All measurements were made in triplicate and expressed as mean ± standard error (SE). A complete speciation protocol was carried out to determine the different fluoride fractions: a- Inorganic Free Fluoride (IFF), b- Inorganic Bonded Fluoride (IBF), c- Casein Organic fluoride (OCF), d- Globulinic Organic Fluoride (OGF) and finally e- Total fluoride concentration (TF).

Statistical Analysis

To normalize the data, fluoride concentrations were logarithmically transformed prior to analysis. Mean, standard deviation and standard error were calculated for each product and phase. The data were multiplied for 1100 (the dilution factor) to obtain the concentration of fluoride. Data were analyzed for statistically significant differences using repeated one-way measures analysis of variance (ANOVA). Anova was calculated after each milk collection and at the end of the research as the sum of fluoride concentrations at all the time intervals, as a single measure of substantivity.

Results

No adverse effect was referred by from women in both groups. A total of 74 women completed the experimental period (Figure 1) and data on fluoride concentration in breast milk are referred to 40 subjects from fluoride group and 34 from control group. The dropout rate was quite high (40.3%), mainly caused by the early interruption of breastfeeding.

The different fluoride fractions recorded at t_2 in the two groups are reported in Table 1. The fluoride concentration in the different fraction were quite similar in the two groups; only in Casein Organic Fluoride (OCF) fraction a slight difference was observed (141 μg/l in the fluoride group and 132 μg/l in the control group) (p=0.06).

At t_0, fluoride concentrations in both groups were quite comparable and no statistically significant differences were observed (p = 0.52 respectively).

At t_2, women in the fluoride group showed a statistically significant increase in fluoride concentration compared to baseline (from 472±118 μg/l to 510±123 μg/l) (p=0.03). In the control group, a slight not statistically significant increase in fluoride content was observed (from 480±148 μg/l to 486±150 μg/l). The comparison of the fluoride concentrations at the three different endpoints (t_0-t_1-t_2) between the two groups did not show statistical differences (p=0.12) (Table 2).

Discussion

The aim of the present study was to evaluate whether a fluoride food supplement administered to lactating women can increase the fluoride content in breast milk. A randomized clinical trial was designed and carried out. No statistically significant differences were found between fluoride group and control group. Overall, the fluoride concentrations in the two groups did not significantly changed during the experimental

period. This result can be explained with the existence of a plasma-milk barrier against fluoride, in order to protect the infant from excessive intake of fluoride (Ekstrand *et al.*, 1981). Nevertheless, in the present paper, a statistically significant increase in the halogen concentration was noted in the fluoride group, after six weeks of fluoridated food supplement use.

Breast milk possesses unique nutritional, biochemical, anti-infective and anti-allergic properties. From theoretical point of view, breast milk might represent an excellent way to administer fluoride to infants. A wide range of fluoride concentration in breast milk is reported, *i.e.* WHO reports a range from < 0.002 to 0.1 ppm (World Health Organization). In the present paper the fluoride concentrations measured were higher than those reported in literature (Sener *et al.*, 2007). A strength point of the paper is the inclusion criteria like the recent stipulated absence of recent use of fluoride supplements as a preventive measure. However it is necessary to underline that the large number of drop-out might have affected the study validity and it is possible to speculate that a larger sample could lead to different results.

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Figure 1. Flow chart of the study sample.

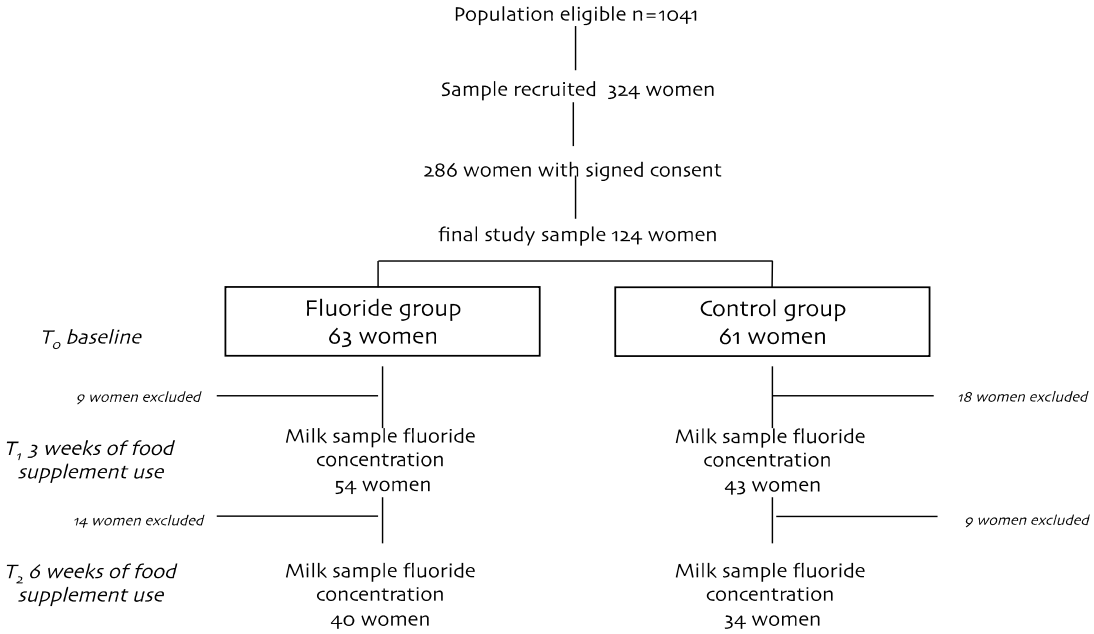

Table 1. Different Fluoride fractions concentrations in breast milk at t_2.

	Inorganic Free Fluoride (IFF) *mean range (µg/l)*	Inorganic Bonded Fluoride (IBF) *mean range (µg/l)*	Casein Organic fluoride (OCF) *mean range (µg/l)*	Globulinic Organic Fluoride (OGF) *mean range (µg/l)*	Total fluoride concentration (TF) *mean range (µg/l)*
Fluoride group	*73 (14 ÷ 77)*	*125 (53 ÷ 185)*	*141 (75 ÷ 208)*	*171 (70 ÷ 235)*	510 (*390 ÷ 620)*
Control group	*68 (40 ÷ 85)*	*118 (68 ÷ 140)*	*132 (88 ÷ 163)*	*168 (142 ÷ 256)*	*486 (413 ÷ 604)*
Anova one way	***NS***	***NS***	***0.06***	***NS***	***NS***

Table 2. Fluoride concentrations in breast milk measured in the two groups at the three different endpoints.

	Fluoride group *mean± Standard Deviation (µg/l)*	*Control group* *mean± Standard Deviation (µg/l)*	*Anova one way*
t_0 baseline	472±118	480±148	NS
t_1 three weeks of use	480±126	472±142	NS
t_2 six weeks of use	510±123	486±150	
Anova one way	0.03	NS	