Dental disease and caries related microflora in children with dystrophic epidermolysis bullosa

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Abstract

Purpose: The purpose of this study was to investigate dental caries, bacterial dental plaque, gingivitis and caries related oral microflora in children with predominantly autosomal recessive Dystrophic Epidermolysis Bullosa (DEB).

Methods: Thirty children with DEB from The Great Ormond Street Hospital for Children and 31 control children matched for age, gender and ethnicity were included in the study.

Results: The main findings were:

1. *A significantly greater mean dmft in the DEB children ($p < 0.05$).*
2. *A significantly greater mean plaque score for the DEB children for both the primary ($p < 0.001$) and permanent teeth ($p < 0.02$) compared with the control children.*
3. *A significantly greater mean gingivitis score for the DEB children for both the primary ($p < 0.002$) and permanent teeth ($p < 0.0001$) compared with the control children.*
4. *A significantly greater salivary total anaerobic count for the control children compared with the DEB children ($p < 0.001$).*

Conclusions: The results reflect the difficulties that children with DEB have with basic oral hygiene procedures combined with slow oral clearance.(Pediatr Dent 23: 438-443,2001)

Introduction

Epidermolysis Bullosa (EB) is the term used to describe a group of rare hereditary, chronic non-inflammatory skin diseases. The pattern of inheritance may be either autosomal dominant or autosomal recessive.

EB is characterised by the formation of bullae as a result of mild to moderate trauma which heal with atrophic scarring. Occasionally bullae can develop spontaneously. EB is classified into four main groups based on mode of inheritance, anatomic location and distribution of the lesions and associated morbidity. The main groups are Simplex, Recessive Dystrophic, Dominant Dystrophic and Junctional, in addition to at least 23 subtypes[1]. The incidence of recessive DEB is approximate1y 1 in 300,000 births and dominant DEB 1 in 50,000.[2]

Recessive DEB is characterised by blistering beneath the lamina densa of the basement membrane which causes separation of the sub lamina dura.[3] There are widespread bullae involving the skin and mucosa that heal with scarring (Fig. 1). The effects of this may be widespread including dysphagia[4] which can lead to oesophageal strictures[5] and ocular involvement[6,7] with corneal abrasion.[8] Scarring and contracture of the oral mucosa cause specific problems including obliteration of buccal sulci, ankyloglossia and microstomia.[9] Eating and swallowing is difficult and the children tend to eat small quantities of pureed food throughout the day. The diet is often supplemented with high carbohydrate drinks to increase the calorie intake, for example Enrich (Abbott Laboratories Ltd. UK). Toothbrushing is difficult because blistering of the hands and fingers causes contracture reducing the ability to grip and hold a toothbrush (Figs. 2 and 3). In addition, the oral mucosa readily blisters with the slightest trauma (Fig. 4). It is these factors which may account for the high caries experience reported in children with DEB.[10,11] The prevalence of dental caries was found to be significantly greater in individuals with junctional EB and recessive DEB.[11] The prevalence for simplex EB and dominant DEB were found to be similar to the controls. A number of other possible explanations for the increased

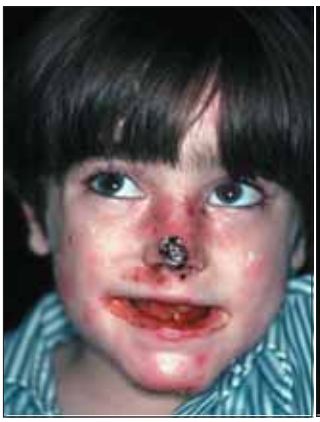

Fig 1. Scarring and healing lesions on the face and lips

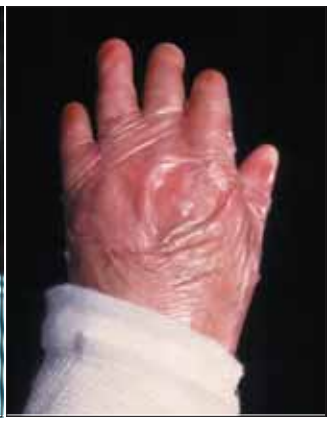

Fig 2. Contracture of the fingers due to blistering and scarring. Note dystrophic skin

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Fig 3. Primary dentition showing plaque, gingivitis and cervical caries. This child was on a high sucrose diet and unable to brush the teeth because of oral blistering.

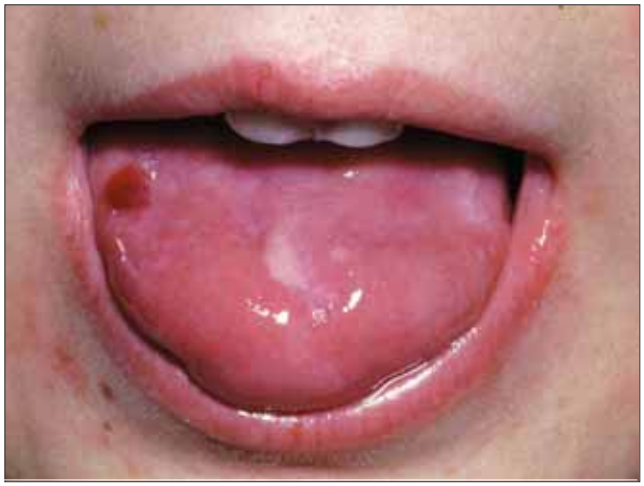

Fig 4. Spontaneous bullous with haemorrhage on the right side of the tongue. Note scarring from previous bullae.

caries experience have been suggested. The salivary flow has been investigated and no difference was found between the EB individuals and controls. The IgA secretion was greater in the EB group which was probably due to blistering[12] and the investigators concluded that increased caries prevalence was due to non-salivary factors such as diet. A decreased secretory IgA antibody response has been demonstrated in the children in the foregoing investigation which suggests that there may be a defect in salivary secretory immunity in DEB.[13] The extent to which this might influence caries susceptibility is not known. A change in the oral microflora has been identified as a possible cofactor.[11] There is no direct relationship between the extent of oral blistering and caries experience[11] and there is no explanation for the marked variation in caries experience between individuals with the same EB sub-type.

Generalized enamel hypoplasia is well documented in junctional EB.[10,14,15] Dental anomalies ranging from mild hypoplasia to missing teeth have been reported in individuals with Simplex EB and DEB.[16] Other work has demonstrated that the mineral and chemical composition of dental enamel in DEB is no different from normal and does not predispose the teeth to caries[17]. More recently serum albumen, which is a known inhibitor of enamel crystal growth, was detected in junctional EB enamel but not in either control or DEB enamel.[18] It was concluded that junctional EB enamel was developmentally compromised.

Since there is little oral health data for children with DEB, this study investigated the prevalence of dental caries, plaque, gingivitis and caries related microflora in children with that condition.

Ethical approval was obtained from The Great Ormond Street Hospital for Children NHS Trust, and the Lewisham and North Southwark Committee on Ethical Practice. Written consent was obtained from the parent(s) and verbal consent from each child.

Children with established DEB that were inpatients, or those attending as outpatients to the EB clinic at The Great Ormond Street Hospital for Children, aged between 2 years and 17 years, were included in the study.

Children attending either the Orthopaedic Outpatient Department or Dental Trauma Clinic at Guy's Hospital UMDS were matched for age, gender, ethnicity and social class with the subjects. Ethnicity was assessed using a standard but comprehensive scheme at The Great Ormond Street Hospital. Social Class was assessed on the basis of the main parental occupation using the Office of Population Censuses and Surveys Classification of Occupations.[19]

Six toothblocks were examined by one investigator (RB), on 2 separate occasions, 1 week apart, to assess intra-examiner reproducibility.[20] A toothblock is a block of plaster set with several natural teeth that have been extracted because of caries.

Clinical Procedures

Debris was not removed from the teeth prior to examination because of the risk of trauma to the oral tissues. All the teeth were visually examined for caries with a mirror using the World Health Organisation criteria.[21] The indices were recorded as the dmfs/dmft (decayed, missing and filled surfaces of the primary teeth/decayed, missing and filled primary teeth) and the DMFS/DMFT (decayed, missing and filled surfaces of the permanent teeth/decayed missing and filled permanent teeth).

Four gingivally related quadrisections of each tooth (mesiobuccal, distobuccal, mesiolingual, distolingual) were visually examined for bacterial dental plaque deposits to give the plaque score, using a modification of the index of O'Leary.[22] This has been shown to be reproducible in small children.[23]

The gingivae were visually examined for inflammation using a simplified gingival index based on the number of tooth quadrisections associated with gingival inflammation to give the gingivitis score.[23] Spontaneous gingival bleeding was also recorded. Developmental dental anomalies were recorded using the FDI notation.[24]

Each parent was asked if the child was taking fluoride supplements. The parents and child were also asked to complete a prospective diet diary of all food and drink consumed over a 3 day period and to return it in the stamped addressed envelope provided. To highlight differences in composition of the diet, highly cariogenic foods were given additional weighted scores — hence the term "weighted daily sugar intake."

Supragingival plaque was collected from the upper left permanent molar tooth, using a sterile excavator, or from the most

distal tooth present in any quadrant if this tooth was either missing or unerupted. The plaque was stored in a bijou bottle containing 1ml of reduced transport medium[25] with glass beads. Three ml of unstimulated saliva was collected from each child in a sterile universal container over a 10-minute period. The samples were transported on ice to the laboratory for processing.

Microbiological Procedures

Saliva samples were collected from 7 children. Each sample was divided into halves, inoculated onto, and processed on both selective and non-selective media.

Tenfold serial dilutions of the plaque samples were prepared in reduced transport fluid and 100 ml aliquots of the appropriate dilutions were inoculated onto both selective and non-selective media. Mitis Salivarius agar (Oxoid Unipath Ltd., Basingstoke, Hampshire) 0.2 units/ml bacitracin (Sigma-Aldrich Co. Ltd., Poole, Dorset) and sucrose 15% w/v (BMSA) [26] was prepared for the growth of Mutans Streptococcci. The plates were incubated anaerobically at 37˚C for 4 days. Rogosa agar was prepared for the growth of Lactobacilli (Oxoid) and the plates were incubated anaerobically for 4 days. Sabouraud Dextrose agar was prepared for the growth of Candida species (Oxoid). The plates were incubated aerobically at 37°C for 4 days. Columbia agar (Oxoid) supplemented with 5% (v/v) defibrinated horse blood (CBA) was prepared to determine the total anaerobic count. The plates were incubated anaerobically in a gas jar supplemented with 5% carbon dioxide and 10% hydrogen, at 37˚C for 7 days.

Streptococcal colonies from the BMSA plates were characterized initially by gram-staining. Two colonies of presumptive *S. mutans* were subcultured onto separate plates of CBA and incubated anaerobically for 24 hours. Using a sterile cotton swab, colonies were removed from the CBA plate and inoculated into 2ml of sterile normal saline at a density of at least McFarland standard 2. Each bacterial suspension was subjected to a small range of carbohydrate fermentation tests comprising glucose, mannitol and sorbitol in addition to arginine and aesculin hydrolysis.[27,28] Any mixed or contaminated cultures were subcultured on blood agar until pure. Quality testing was carried out by culturing the Guy's C *S. mutans* type strains in the same manner. *S. mutans* and *S. sobrinus* were not differentiated.

Lactobacilli were identified by colonial morphology, gram-staining and positive catalase reaction. Candida species were identified by colonial morphology and gram staining.

Statistical Analysis

All data were tested for normality using the Shapiro-Wilks test[29] and the distribution was found to be non normal. The Mann Whitney test was used for continuous variables and the Chi Square Test for categorical data.

Results

Thirty children with DEB and 31 controls matched for age, for gender as far as possible, ethnicity and social class were included in the study. Of these 24 had been diagnosed with recessive DEB and 6 with dominant DEB. One other child with Junctional EB was excluded from the final analysis. The mean age of the DEB children was 9 years, range 3 to 18 years. The mean age of the controls was 10 years, range 4 to 17 years. There were 20 girls and 10 boys in the DEB group and 12 girls and 19 boys in the control group. Plaque and saliva samples were collected from 23 children; 18 with recessive DEB and 5 with dominant DEB, and from 23 controls.

Six toothblocks were examined on 2 separate occasions 1 week apart and the Kappa value was 0.7913. This shows substantial agreement.[29]

The proportion of DEB children who were caries free was 20%, which was not significantly different from the 26% of the matched control. The mean combined DMFS and dmfs was significantly greater for the DEB children, 18.1 (± 25.4), compared with the controls, 4.4 (± 5.5) ($p < 0.05$). The mean combined DMFT and dmft was also significantly greater for the DEB group, 6.2 (± 6.5), compared with the control group, 2.3 (± 1.9) ($p < 0.03$) (Table 1). The mean dmft was significantly greater for the DEB children, 6.5 (± 5.7), compared with the controls, 2.2 (± 2.0) ($p < 0.05$). The number of missing primary tooth surfaces was also significantly greater for the DEB children, 14.9 (± 23.4), compared with the control children,

Table 1: Decayed, Missing, Filled Surfaces and Teeth: DEB and Matched Control Groups

	DEB Group (n = 30)				Matched Control Group (n = 31)				
	Mean	Median	Min	Max	Mean	Median	Min	Max	Sig
DMFS + dmfs	18.1 ±25.4	4	0	88	4.4 ±5.5	1	0	20	p< 0.05
DMFT + dmft	6.2 ±6.5	4	0	25	2.3 ±1.9	2	0	6	p < 0.03
dmfs	21.4 ±25.4	8.5	0	88	2.6 ±2.9	1	0	7	p< 0.06
dmft	6.5 ±5.7	6	0	20	2.2 ±2.0	2	0	5	p < 0.05
msd	14.9 ±23.4	0.5	0	88	0.5 ±1.5	0	0	5	p < 0.03
DMFS	1.8 ±4.1	0	0	18	0 0	0	0	ns	
DMFT	1.4 ±2.8	0	0	11	0 0	0	0	ns	

Sig = statistically significant difference .
ns = no statistically significant difference

0.5 (± 1.5) ($p < 0.03$) (Table 1). There was no significant difference in the mean dmfs.

The mean plaque score was significantly greater for the primary teeth for the DEB children, 33.7 (± 31.1), compared with the controls, 1.8 (± 3.3) ($p < 0.001$) (Table 2). Similarly, the mean plaque score for the permanent teeth was also significantly greater for the DEB children, 28.6 (± 31.6), compared with the controls, 4.6 (± 5.6) ($p < 0.02$) (Table 2).

The mean gingivitis score was significantly greater for both the primary teeth 21.5 (± 29) compared with the controls, 0.00, ($p < 0.002$) and the permanent teeth in the DEB children, 27.5 (± 34.9) compared with the controls, 2 (± 4.6) ($p < 0.0001$).

Table 2: Plaque and Gingival Inflammation Scores: DEB and Matched Control Groups

	DEB Group (n = 30)				Matched Control Group (n = 31)				
	Mean	Median	Min	Max	Mean	Median	Min	Max	Sig
Primary Dentition									
Plaque	33.7 ±31.1	24	0	80	1.8 ±3.3	0	0	8	p<0.001
Gingival inflammation	21.5 ±29	4	0	25	0	0	0	0	p<0.002
Permanent Dentition									
Plaque	28.6 ±31.6	13.5	0	100	4.6 ±5.6	3	0	16	p<0.02
Gingival inflammation	27.5 ±34.9	6.0	0	20	2.0 ±4.6	0	0	14	p<0.0001

Sig = statistically significant difference

Table 3: Total Bacterial Counts As Colony Forming Units per ml Saliva and Plaque (log_{10}): DEB and Control Groups

	DEB Group (n = 23)					Matched Control Group (n = 23)					
	% IF	Mean	Median	Min	Max	% IF	Mean	Median	Min	Max	Sig
Saliva											
Mutans Streptococci	74	3.42 ±2.37	4.48	0.00	6.58	78	3.56 ±2.14	4.51	0.00	6.18	ns
Lactobacilli	61	1.93 ±1.82	2.04	0.00	5.43	65	2.25 ±1.85	2.90	0.00	5.10	ns
Candida species	55	1.20 ±1.39	0.70	0.00	3.81	43	0.96 ±1.21	0.00	0.00	3.18	ns
Total Anaerobic Count	100	7.41 ±0.54	7.54	6.20	8.30	100	8.17 ±0.40	8.10	7.27	8.91	p<0.001
Plaque											
S. mutans	57	2.72 ±2.77	2.48	0.00	6.99	74	3.0 ±2.11	3.34	0.00	6.13	ns
Lactobacilli	39	0.79 ±1.11	0.00	0.00	3.05	30	0.54 ±0.88	0.00	0.00	2.45	ns
Total Anaerobic Count	100	7.63 ±0.46	7.60	6.64	8.57	100	7.53 ±0.32	7.61	6.92	8.24	ns

Sig = statistically significant difference
ns = no statistically significant difference

Table 4: Percentage of Each Bacterial Species As a Proportion of the Total Anaerobic Count (log_{10}): DEB and Control Groups

	DEB Group (n = 23)					Matched Control Group (n = 23)					
	Mean		Median	Min	Max	Mean		Median	Min	Max	Sig
Saliva											
Mutans Streptococci	1.13	3.16	0.09	0	13.19	0.11	0.21	0.02	0	0.74	ns
Lactobacilli	0.04	0.09	<0.001	0	0.35	0.01	0.03	<0.001	0	0.11	ns
Candida species	0.001	0.004	<0.001	0	0.02	<0.001	<0.001	<0.001	0	0.001	ns
Plaque											
Mutans Streptococci	1.05	2.44	0.001	0	9.80	0.38	0.87	0.004	0	2.7	ns
Lactobacilli	<0.002	<0.001	0.00	0	0.002	<0.001	<0.001	0.00	0	0.001	ns

Sig = statistically significant difference
ns = no statistically significant difference

The only developmental defect detected was chronological hypoplasia on the permanent central incisors and first permanent molars of one child with recessive DEB.

Fluoride mouthrinses or tablets were used by 9 (29%) of the DEB children and by 2 (6.5%) control children. Diet sheets were completed and returned by 12 (39%) of the DEB children and 11 (35%) of the controls. Both the DEB and control children reported an average of 5 sugar intakes a day. The weighted average daily intake was 8 for the DEB group and 6 for the controls, which were not significantly different.

Plaque and saliva were obtained from 18 children with recessive DEB and 5 with dominant DEB and from 23 controls. There was no significant difference in the isolation frequency in both plaque and saliva of Mutans Streptococci, Lactobacilli or Candida species between the DEB and control children (Table 3). The total salivary anaerobic count was significantly greater for the control group compared with the DEB group ($p < 0.001$) (Table 3). There was no significant difference in the mean salivary or plaque counts of Mutans Streptococci, Lactobacilli or Candida species between the DEB and control groups (Table 3). There was no significant difference in the proportion of plaque and salivary Mutans Streptococci, Lactobacilli or Candida species as a percentage of the total anaerobic counts between the DEB and control children (Table 4).

Discussion

Autosomal recessive DEB was the predominant type affecting most of the children, with a few cases of dominant DEB. The statistical analysis was completed on the whole subject group, with the exception of the one child with Junctional EB, because the numbers were relatively small. The child with Junctional EB had only minimal manifestations of EB and was thus excluded from the final analysis.

Both the combined DMFS and dmfs and combined DMFT and dmft were significantly greater in the DEB children compared with the controls, which is in agreement with other workers[11]. A significantly greater number of teeth were extracted in the DEB group, which is a reflection of the difficulty in carrying out restorative treatment[30]. This is mainly because any manipulation of the soft tissues causes blistering and scarring. The dmft, 6.5, is greater than some other groups of chronically sick children of comparable ages at the same tertiary referral centre. The mean dmft for children with severe congenital cardiac disease in one recent study was 3.9 [23] and for children undergoing bone marrow transplantation 2.5 [31].

Although only a small proportion of the DEB and control group completed the diet sheets, a relationship between the greater prevalence of caries in the DEB children and the number of daily sugar intakes was anticipated for the following reasons. Children with severe EB eat very small quantities of food at a time throughout the day and most supplement their diet with sugar and high carbohydrate drinks to increase the calorie intake. The sugar intake recorded may be similar to the controls but the slow oral clearance in the DEB children means that food and drinks are in contact with the teeth for most of the day.

The plaque and gingivitis scores were significantly greater in the DEB children compared with the controls and was not unexpected because of the difficulties with toothbrushing. A different group of children with craniosynostosis, who also experience difficulties with toothbrushing because of hand deformities, were also found to have a higher gingivitis scores than the control group[32]. An important part of the oral care was rinsing with 0.2% Chlorhexidine, which if used effectively, reduces plaque accumulation[33,34]. Rinsing is difficult for these children because of the microstomia and ankyloglossia and more recently compliance has been increased with the use of an 0.2% chlorhexidine spray.

The use of chlorhexidine may have been a contributory factor for the significantly lower total anaerobic count for the DEB children compared with the controls. If this were the case, a similar effect would have been expected with the absolute numbers of Mutans streptococci. However, decreased secretory IgA antibody responses to *Candida albicans, Lactobacillus casei* and strepto*coccus mutans* were demonstrated in these children suggesting a mucosal immune defect[13]. If there was a mucosal immune defect, higher absolute counts of Mutans streptococci, Lactobacilli and Candida species might have been expected. It is difficult to draw conclusions from these findings.

Conclusions

The prevalence of dental caries was significantly greater for a group of children with DEB compared with the matched controls. Because of the difficulties with oral health care all the DEB children should be actively encouraged to use fluoride supplements and 0.2% chlorhexidine either as a mouthrinse or a spray.

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References

1. Eady RAJ. The classification of epidermolysis bullosa. In: Epidermolysis Bullosa: A comprehensive review of classification, management and laboratory studies. Eds. Priestly GC, Tideman MJ, Weiss JB and Eady RAJ. DEBRA 1-9,1990.
2. Davidson BCC. Epidermolysis Bullosa. *J Med Genet* 2:233,1965.
3. Fine JD, Bauer EA, Briggerman RA, Carter DM, Eady RAJ, Esterly NB et al. Clinical and laboratory criteria for subtypes of inherited epidermolysis bullosa: a consensus report by the Subcommittee on Diagnosis and Classification of the National Epidermolysis Bullosa Registry. *J Am Acad Dermatol* 24:119-135,1991.
4. Travis SPL, McGrath JA, Turnbull AJ et al. Oral and gastrointestinal manifestations of epidermolysis bullosa. *Lancet* 340:1505-1506,1992.
5. Ergun GA, Lin AN, Danneberg AJ, Carter DM. Gastrointestinal manifestations of epidermolysis bullosa. A study of 101 patients. *Medicine* 71:121-127,1992.
6. McDonnell PJ, Spalton DJ. The ocular signs and complications of epidermolysis bullosa. *J R Soc Med* 316:576-578,1988.
7. Iwamoto M, Haik BG, Iwamoto T et al. The ultrastructual defect in conjunctiva from a case of recessive epidermolysis

bullosa. *Arch Ophthalmol* 109:1382-1386,1991.
8. Gans LA. Eye lesions of epidermolysis bullosa. Clinical features, management and prognosis. *Arch Dermatol* 124:763-4,1988.
9. Wright JT, Fine JD, Johnson LB. Oral soft tissues in hereditary epidermolysis bullosa. *Oral Surg Oral Med Oral Path* 71:440-446,1991.
10. Wright JT, Fine JD, Johnson L. Hereditary epidermolysis bullosa: oral manifestations and dental management. *Pediatr Dent* 15:242-248,1993.
11. Wright JT, Fine JD and Johnson L. Dental caries in hereditary epidermolysis bullosa. *Pediatr Dent* 16:427-432,1994.
12. Wright JT, Childers NK, Evans KL, Johnson LB, Fine JD. Salivary function in persons with hereditary epidermolysis bullosa. *Oral Surg Oral Med Oral Path* 71:553-559,1991.
13. Sweet SP, Ballsdon AE, Harris JC, Roberts GJ, Challacombe SJ. Impaired secretory immunity in dystrophic epidermolysis bullosa. *Oral Microbiol Immunol* 14:316-320,1999.
14. Gedde Dahl. Epidermolysis bullosa: A clinical, genetic and epidemiological study. Baltimore. John Hopkins Press. 1971.
15. Buckner Tuderman L, Rantal I, Renuala T. Evidence for a structural abnormality of collagen VII in a patient with dystrophic epidermolysis inverse. *J Invest Dermatol* 98:141-146,1992.
16. Pearson RW. Clinicopathologic types of epidermolysis bullosa and theirnon-dermatological complications. *Arch Dermatol* 124:718-725,1988.
17. Kirkham J, Robinson C, Strafford SM, Shore RC, Bonass WA, Brookes SJ et al. The chemical composition of tooth enamel in recessive dystrophic epidermolysis bullosa: significance with respect to dental caries. *J Dent Res* 75:1672-1678,1996.
18. Kirkham J, Robinson C, Strafford SM, Shore RC, Bonass WA, Brookes SJ et al. The chemical composition of tooth enamel in junctional epidermolysis bullosa. *Arch Oral Bio*l 45:377-386,2000.
19. Office of Population Censuses and Surveys Classification of Occupation. Her Majesty's Stationary Office. London. 1980.
20. Cleaton-Jones P, Hargreaves JA, Fatti LP, Chandler HD, Grossman ES. Dental caries diagnosis calibration for clinical field surveys. *Caries Res* 23:195-199,1989.
21. World Health Organisation. Oral Health Surveys. Basic Methods. 3rd Ed. Geneva 1987.
22. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 43:38,1972.
23. Franco E, Saunders CP, Roberts GJ, Suwanprasit A. Dental disease, caries related microflora and salivary IgA of children with severe congenital cardiac disease. *Pediatr Dent* 18:228-235,1996.
24. Ainamo J. An epidemiological index of developmental defects of dental enamel. (DDE Index). FDI technical report number 15. *Int Dent J* 32:159-166,1982.
25. Hoover CI, Newbrun E. Survival of bacteria from human dental plaque under various transport conditions. *J Clin Microbiol* 6:212-218,1977.
26. Gold OG, Jordan HV, van Houte J. A selective medium for *Streptococcus mutans. Arch Oral Biol* 18:1357-1364,1973.
27. Casals JB, Pringler N. Diagnostic tablets for bacterial identification. Rosco Diagnostica. Taastrup Denmark. 1987.
28. Casals JB, Pringler N. The Zym – Kits and identification tablets for bacteria and yeast. Rosco Diagnostica 2630. 1st Ed. Taastrup Denmark. 1993.
29. Altman DG. Practical Statistics for Medical Research. 2nd ed. London: Chapman and Hall. 1992.
30. Wright JT. Comprehensive dental care and general anaesthetic management of hereditary epidermolysis bullosa. *Oral Surg Oral Med Oral Path* 70:573-578,1990.
31. Lucas VS, Roberts GJ, Beighton D. Oral health of children undergoing allogeneic bone marrow transplantation. *Bone Marrow Transplant* 22:801-808,1998.
32. Mustafa D, Lucas VS, Junod P, Evans R, Mason C, Roberts GJ. The dental health and caries related microflora in children with craniosynostosis. Cleft Palate Craniof J 2001. In press.
33. Schiott CR, Loe H, Jensen SB, Kilian M, Davies RM, Glavind K. The effect of chlorhexidine mouthrinses on the human oral flora. *J Periodontol Res* 5:84-9,1970.
34. Joyston-Bechal S, Hernaman N. The effect of a mouthrinse containing chlorhexidine and fluoride on plaque and gingival bleeding. *J Clin Periodontol* 20:49-53,1993.