A Clinical Study of the Oral Condition of Paediatric Liver Graft Recipients

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With grateful thanks to my husband Edward,

for his unfailing love,

patience and support.

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Author's Declaration

I, Marie-Therese Hosey, declare that the work contained within this thesis is my own work.

The contributions to this dissertation have already been acknowledged but can be listed as follows:

- the pilot study and protocol which I later developed into the cross-sectional study were initially devised by Dr. Linda Shaw
- the remaining four studies were entirely my own design but were presented to both Dr. Linda Shaw and to Dr. Deirdre Kelly, their recommendations were gratefully received
- the trough cyclosporin concentrations quoted in these studies are the mean of three blood tests, the wisdom of which I owe to Dr. Deirdre Kelly
- the CMV status of the subjects in the final study was supplied by Dr. Susanne Davison who personally matched the dates of my examinations to her CMV data base.

Synopsis

The liver transplantation procedure was first carried out in 1967 and, since that time, survival rates have been steadily improving. The introduction of the split liver graft procedure has now made liver transplantation available to babies under 1 year of age for the first time. This study is the largest in the world into the oral condition of paediatric liver graft recipients, it was carried out between 1992 and 1996 and contains five separate prospective clinical investigations.

It has already been reported that there is a high prevalence of intrinsic pigmentation, enamel hypoplasia, delayed eruption and cyclosporin-induced gingival overgrowth in children with liver grafts. However, the previous studies are few and the number of children in the samples were small. Therefore, the impact of both the increased availability of liver grafts to babies and the improved survival of liver graft recipients on the future paediatric dental need of these children is still to be fully ascertained.

Paediatric liver graft recipients also have a high nutritional demand both before and after liver grafting but, in spite of this, they have been shown to 'catch-up' on their peers one year after liver grafting. The effect of malnutrition on the eruption of the primary dentition and whether the same 'catch-up' growth occurs has never been investigated.

The bioavailability of cyclosporin is already highly variable, especially in liver graft recipients, and is likely to be further compromised in babies and infants who receive a split liver graft due to poorer absorption. The effect of age, age at the time of transplantation and duration of cyclosporin therapy not only on the prevalence and severity of gingival overgrowth but also on the erupting primary dentition also merits investigation.

Organ transplant recipients also have a higher risk of cytomegalovirus infection than the general population. It has recently been suggested that there is a link between cytomegalovirus infection and cyclosporin-induced gingival overgrowth. However, this has never been the subject of a clinical investigation.

In the first study, fifty-five paediatric liver graft recipients, who represented a cross-section of the children who attended the Liver unit at Birmingham Children's Hospital, were examined. Thirty-seven of the children were below 5 years of age. Forty-seven percent of the study group had intrinsic pigmentation but only 11% were found to have enamel hypoplasia. The prevalence of delayed eruption was found to be in excess of 40%. Fifty-five percent of the children had gingival overgrowth. There was a significant inverse relationship between the duration of cyclosporin therapy and the trough cyclosporin concentration but analysis of variance failed to show any association between the trough cyclosporin concentration and the severity of the gingival overgrowth.

In the second study, thirty-seven children with liver grafts who had intrinsic pigmentation of the dental hard tissues were examined and the severity of the intrinsic green pigmentation measured using a specially developed colour scale. The primary molar teeth were the most severely discoloured but the results of this investigation also suggest that the permanent incisors and first permanent molars are also likely to be similarly, if less severely, affected. The clinical evidence suggested that the deposition of the green pigment was incremental in nature and occurred in the immediate post-natal period. The study also found that the severity of the pigmentation did not to improve with time.

The third study was a controlled study that compared the effect of malnutrition, the underlying liver disease, and cyclosporin medication in the aetiology of delayed eruption of the primary dentition. This study confirmed that the prevalence of delayed eruption of the primary dentition in children with liver grafts was 43%, and 48% in those who also had malnutrition. Children with liver grafts had significantly fewer teeth than their age-matched controls with liver disease. There was a highly significant association between the trough cyclosporin concentration and the number of teeth in liver graft recipients who did not have malnutrition. The results showed that malnutrition alone was not a significant aetiological factor. This findings suggest that cyclosporin caused delayed emergence of the primary dentition when there had previously been delayed eruption due to severe liver disease.

In the fourth study, ninety-seven paediatric liver graft recipients were examined and the study population was divided into groups according to age, then by age at

transplantation and by duration of cyclosporin therapy. The results showed that the prevalence of cyclosporin-induced gingival overgrowth varied with the age of the study sample. It was found that there was lower prevalence of cyclosporin-induced gingival overgrowth in babies and infants, 33% and 55% respectively, compared to 100% in adolescents.

Although the prevalence of gingival overgrowth was found to increase with duration of cyclosporin therapy, no significant association was found between the duration of cyclosporin therapy and either the presence or the severity of gingival overgrowth in any of the age-banded groups.

There was a significant inverse correlation between the trough cyclosporin concentration and the duration of cyclosporin therapy in all of the age groups which confirms the finding in the cross-sectional study. This is probably related to the difficulty in maintaining optimal therapeutic levels of the drug in the growing child.

Recipients of liver transplants are becoming ever younger and more numerous. This investigation shows that paediatric liver graft recipients will require dental care to restore aesthetics, and manage delayed eruption of the primary teeth and gingival overgrowth.

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Publication: *Oral findings in children with liver grafts, 1995*

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Glossary of Abbreviations

OLT	orthotopic liver transplantation
EHBA	extrahepatic biliary atresia
FTT	failure to thrive
Cmax	maximum serum concentration
AUC	area under the curve
CMV	cytomegalovirus
ANUG	Acute Necrotising Ulcerative Gingivitis

Chapter One

Introduction

Since 1967 more than a thousand liver transplantations have been carried out in North America and Europe. This increase in numbers is due to earlier diagnosis and referral, improved patient selection and operative techniques, and to the successful development of immunosuppressive agents such as Cyclosporin A and FK506 (Tacrolimus) which was first clinically introduced in 1989 (Calne *et al* 1980: Starzl *et al* 1981a, 1989b: Hadley *et al* 1995). One and two year actuarial survival post OLT in world centres is now in excess of 85% and survival rates in paediatric liver transplantation approach 80% at one year and 70% at five years (Whittington & Ballisteri 1991).

Orthotopic liver transplantation (OLT) is a technique in which the recipient's liver is removed and a donor's liver is implanted in the original site. Liver grafts are matched by ABO blood group, size and, when possible, by cytomegalovirus status.

To overcome the shortage of paediatric donors reduction hepatectomies have recently been developed. This is a procedure in which part of an adult liver is cut down to fit a child. Reduction hepatectomies now make up 46-50% of the total grafts in children and have enabled babies under 1year of age and 10kg in weight to be offered liver transplantation for the first time (Beath *et al* 1993). The physiology and anatomy of babies under 1 year produces unique medical complications such as respiratory and ventilatory problems, and infection but in spite of this, 1 year survival rates have been reported in the region of 65% (Sokal *et al* 1990).

Some children with liver disease and liver transplantation also suffer from malnutrition. It has been shown that chronically malnourished liver transplant recipients have a higher prevalence of infection, surgical complications, and lower survival rates (Moukazel *et al* 1990).

1.1. Indications for Liver Transplantation

The diseases for which liver transplantation has been performed can be divided into the following groups: metabolic diseases, acute and chronic hepatitis, intrahepatic cholestasis, obstructive biliary tract disease, tumours, and other miscellaneous conditions such as cystic fibrosis. The critical factors that indicate a need for transplantation can be classified as; liver disease expected to progress to hepatic failure, liver-based metabolic disease, and primary hepatic malignancy. Transplantation is not usually considered if there is an alternative therapy or if the outcome will be poor (Whitington & Balisteri 1991) but the only absolute contraindications to liver transplantation are severe cardiopulmonary disease and irreversible organic brain disease (Kelly 1991).

Extrahepatic biliary atresia (EHBA) is the most common disease leading to liver transplantation. EHBA affects 0.8-1.0 per 10000 live births. It is the most important cause of death from hepatic failure in children in North America, Europe, Australia and Japan.

EHBA is a disorder of foetal and early postnatal life in which an inflammatory sclerosing process produces progressive obliteration of the extrahepatic biliary tree. The majority of infants with EBHA have an atretic or small gall bladder and complete obliteration of the bile ducts. For these children, hepatic portoenterostomy: the Kasai procedure is the treatment of choice but if it fails these children then proceed to liver transplantation, (Johnston 1991).

1.2 Morbidity and mortality of liver transplantation

There is a relatively low prevalence of chronic rejection in liver allografts compared to other transplant procedures. This could either be due to the lower immunogenicity of the liver or to its dual blood supply. When chronic rejection occurs in liver transplant recipients it is characterised by progressive loss of bile ducts, increasing cholestasis and obliterative vasculopathy (Lowes *et al* 1993). However, it is infection that is the major cause of morbidity and mortality in liver transplant recipients. Risk factors for infection include: preoperative morbidity, prolonged antibiotic use and operating time, the number of operations, and the magnitude of immunosuppressive therapy (Dummer *et al* 1983 : Stratta *et al* 1992). In the immediate post-operative stage bacterial infections such as Streptococcus faecalis, Streptococcus viridans, Pseudomonas and Staphylococcus aureus are likely. Systemic fungal infections are also common but can be prevented using oral anti-fungal drugs and cotrimoxazole is prescribed to prevent Pneumocystis carinii (Dummer *et al* 1983 : Kusne *et al* 1988 : Kelly 1991a : Kelly 1991b : Davison 1993).

1.3 The Liver Unit in Birmingham Children's Hospital

In 1989 the Liver Unit at Birmingham Children's Hospital began to provide a national service for the management of liver disease in children. It has a multidisciplinary team providing expert care for children of all ages ranging from small babies to the 16 year old child. This includes the management of a range of diseases from inherited liver disease to acute liver failure and liver transplantation. Approximately 30 liver transplants in children are performed each year. The one year survival following liver transplantation is greater than 90% and the five year survival is 75%. The unit has also developed a combined small bowel and liver transplant programme for children in Britain so that they do not have to go abroad for this procedure.

The pre-transplant assessment for children who have been referred to Birmingham for liver transplantation includes: anthropometry; blood tests; viral serological screening (CMV, Epstein-Barr, varicella, measles, hepatitis A, B & C, HIV); urinalysis; chest and bone-age X-rays; abdominal ultrasound and cardiology evaluation. In addition, since 1991, the children undergo a dental examination by a paediatric dentist (MTH). Following transplantation the following oral medications are prescribed:

- Acyclovir: under 5 years 200mg/dose qds; over 5years 400mg/dose qds
- Azathioprine 2mg/kg/dose daily
- Cyclosporin 1mg/kg dose, increasing to 1.5 mg/kg if renal function is good
- Ranitidine 3 mg/kg tds if under 10 years, 150mgs bd if over 10 years
- Prednisolone 1 mg/kg dose bd to a maximum dose of 30 mg daily. Stopped at 3 months

1.4 The anticipated dental needs of paediatric liver graft recipients

Children with liver disease require careful oral management due to their inadequate drug and protein metabolism and tendency towards prolonged bleeding. Intrinsic discoloration in children with biliary atresia has also been reported. It has been suggested that general malnutrition may have an effect on dental development, but there is no information in relation to liver disease.

It is clear that as liver transplantation becomes not only increasingly common but is also made available for babies and infants, the dental management will be more complex. So far published studies have not included preschool children and sample sizes have been small. Oral manifestations such as delayed eruption, intrinsic staining, and enamel hypoplasia have been subject to anecdotal reports but have not been fully investigated.

Chapter Two

Literature Review

The effect of paediatric liver disease and transplantation on the oral tissues

The number of publications pertaining to the dental aspects and dental needs of children with liver disease or liver transplantation are relatively few in number. Moreover, they are mainly confined to individual case reports or to studies with relatively small numbers and disparate variables. Guidelines on the dental management of patients with liver disease and transplant recipients have also been published but have not been based upon clinical investigations (Svirsky 1989: Ziccardi *et al* 1991 : Glassman *et al* 1993).

Hyperbilirubinaemia and malabsorption of the fat soluble vitamins A and D cause defects in the formation of enamel and dentine such as hypoplasia and intrinsic staining. It has been reported that children with liver disease and liver transplantation have enamel hypoplasia, delayed eruption, and intrinsic green staining of both the primary teeth and soft tissues, cyclosporin-induced gingival overgrowth, a susceptibility to dental caries and enlarged pulp chambers. However, sample sizes in these studies have been small, ranging from one to nine (Shapiro *et al* 1975 : Seow *et al* 1991: Funakoshi *et al* 1992 : Zaia *et al* 1993) and the age range, underlying medical condition and other contributory factors were not investigated. The published dental literature in respect of paediatric liver transplantation is summarised in Table 1 overleaf.

Author	study information				dental findings			
	Date	No. child subjects	Mean Age / Age Range (months)	Diagnosis	% Enamel hypoplasia	% Delayed eruption	% Gingival overgrowth	% Intrinsic staining
Shapiro *et al*	1975	2	48 / (36-60)	biliary atresia	N/A	N/A	N/A	100
Rosenthal *et al*	1986	2	20 / (13-26)	liver disease	N/A	N/A	N/A	N/A
Svirsky	1989	1	37	OLT	N/A	N/A	100	N/A
VanCleynen & Demars-Femault	1990	2	78 / (72-84)	liver disease	N/A	N/A	N/A	100
Seow *et al*	1991	7	39 / 1-34	OLT	100	29	86	100
Funakoshi *et al*	1992	5	53	OLT	N/A	N/A	100	*'most cases'*
Zaia *et al*	1993	1	84	OLT	N/A	N/A	100	100

Table 1 The Dental Aspects Of Paediatric Liver Disease And Transplantation

To date, no large clinical study has been carried out in children with liver grafts to elucidate the oral complications of liver transplantation.

In the following sections the literature pertaining to intrinsic discolouration, enamel hypoplasia, delayed eruption of the primary dentition and cyclosporin-induced gingival overgrowth will be presented.

2.1 Intrinsic dental pigmentation

Intrinsically pigmented green teeth and pigmented alveolar bone and oral mucosa, have been widely reported in children with liver disease (Marsland & Gerrard 1953 : Shapiro *et al* 1975: Herbert & Delcambre 1987 Van Cleynen & Demars-Fremault 1990 : Seow *et al* 1991: Majewski *et al* 1993 : Zaia *et al* 1993). The pigment is thought to be biliverdin but it is not clear if both the enamel and dentine are affected, or if the pigmentation occurs pre- or post natally or is incremental in nature. Nevertheless, intrinsic green pigmentation appears to be common in paediatric liver graft recipients.

2.1.1 Intrinsic pigmentation in children with liver disease

In 1991 , Seow *et al* reported intrinsic green pigmentation in the primary dentition of infants who had either end-stage liver disease or liver grafts. Although the crowns of the primary teeth were too carious to accurately date the onset, it was stated that the pigmentation ceased when the subjects received liver grafts. Funakoshi *et al* (1992) also reported intrinsic green stain in all five of their subjects, four of whom were children in the primary dentition, who had received liver grafts.

Intrinsic green pigmentation has also been reported in the permanent dentition in a seven year old child with a liver graft who had previously suffered from biliary atresia. The maxillary permanent central incisors were reported to have normal white enamel in the cervical third, but the incisal regions were 'very green and slightly hypoplastic' (Zaia *et al* 1993).

Shapiro *et al* (1975) described two cases of children with biliary atresia and severely pigmented primary teeth. They reported that all of the teeth, including the roots were stained dark green, as were the exposed alveolar bone and buccal plate.

Funakoshi *et al* (1992) also carried out a radiographic and histologic examination but they found no evidence of abnormality. However, Majewski *et al* (1993) examined ground sectioned primary molar and incisor teeth from a 3 year old child with biliary atresia and reported that there was prominent interglobular dentine present and focal cemental dysplasia.

2.1.2 Historical reports of intrinsic green stained teeth

Reports of intrinsic green pigmented teeth in the primary dentition are associated with hyperbilirubinaemia caused by rhesus incompatibility (Miller 1951: Farquahar 1951: Tank 1951: Barta *et al* 1989). In 1951, Miller reported finding green pigmented teeth due to rhesus factor in a child. Pigment was found in those areas of the tooth formed at birth but not in tooth tissue formed thereafter. He concluded that the pigment was laid down in those areas of dentine and enamel which were undergoing calcification during the period that the subject was jaundiced.

2.1.3 Hyperbilirubinaemia and its affect on the enamel organ

Hyperbilirubinaemia causes the deposition of pigment in all parts of the body. This pigment is the result of the catabolism of haem-containing proteins such as haemoglobin and myoglobin into bilirubin, carbon monoxide and iron. It is thought that this pigment is deposited in the enamel organ as biliverdin, which is the oxidation product of the bilirubin molecule. It has also been reported that concentrations of bilirubin have to be in excess of 30mg/100ml for the green pigmentation of the dental tissues to occur (Zaia *et al* 1993).

2.1.4 Tooth colour and discoloration

The colour of healthy teeth is affected by the colour, translucency and the thickness of the enamel together with the colour of the underlying dentine. Discoloration can arise from both extrinsic and intrinsic factors. The former includes chromogenic bacteria, food pigment, dye and tobacco. The latter includes dentinogenesis imperfecta, amelogenesis imperfecta, fluorosis, tetracycline, lepromatous leprosy, and haemolytic diseases of the newborn such as erythroblastosis foetalis, congenital erythropoietic porphyria and liver diseases (Dayan 1983).

2.1.5 Chronology of formation of the dental tissues

Hard tissue formation in the crowns of primary incisor teeth starts between the 13th to the 16th week of intrauterine life and continues until enamel calcification is completed three months after birth (Lunt & Law 1974). If the deposition of the green pigment is incremental in nature, there is a potential to date the onset of the hyperbilirubinaemia using the pattern of the pigmentation in these primary teeth. The foetal liver is not known to have a role before birth but if the intrinsic green stain is indeed biliverdin, and is deposited incrementally in tooth tissue formed prenatally, this could not only provide a clue about the true function of the foetal liver but also unlock the possibility of earlier medical detection and treatment of paediatric liver disease.

The Chronology of the Formation of the Dentition is shown in Table 2 overleaf.

	Tooth	Commencement of Hard Tissue Formation	Amount of Enamel at Birth	Enamel Completed	Eruption	Root Completed
					months	years
MAXILLA	A	14 weeks I.U.	5/6	1 1/2 months	10 (8-12)	1 1/2
	B	16 weeks "	2/3	2 1/2 months	11 (9-13)	2
	C	17 weeks "	1/3	9 months	19 (16-22)	3 1/4
	D	15 1/2 weeks "	1/2 - 3/4 crown	6 months	16 (13-19)	2 1/2
	E	19 weeks "	1/5 - 1/4 crown	11 months	29 (25-33)	3
MANDIBLE	A	14 weeks I.U.	3/5	2 1/2 months	8 (6-10)	1 1/2
	B	16 weeks "	3/5	3 months	13 (10-16)	1 1/2
	C	17 weeks "	1/3	9 months	20 (17-23)	3 3/4
	D	15 1/2 weeks "	cusps united	5 1/2 months	16 (14-18)	2 1/4
	E	18 weeks "	cusps united	10 months	27 (24-30)	3
					Years	
MAXILLA	1	3 - 4 months	-	4-5 years	7 - 8	10
	2	10-12 "	-	4-5 years	8 - 9	11
	3	4 - 5 "	-	6-7 years	11-12	13 - 15
	4	1 1/2 - 1 3/4 years	-	5-6 years	10-11	12 - 13
	5	2 - 2 1/4 "	-	6-7 years	10-12	12 - 14
	6	At Birth	trace	2 1/2 years	6 - 7	9 - 10
	7	2 1/2 - 3 years	-	7-8 years	12-13	14 - 15
	8	7 - 9 "	-	12-16 years	17-21	18 - 25
MANDIBLE	1	3 - 4 months	-	4-5 years	6 - 7	9
	2	3 - 4 months	-	4-5 years	7 - 8	10
	3	4 - 5 months	-	6-7 years	9 -10	12 - 14
	4	1 3/4 - 2 years	-	5-6 years	10 -12	12 - 13
	5	2 1/4 - 2 1/2 "	-	6-7 years	11 -12	13 - 14
	6	At Birth	trace	2 1/2 -3 years	6 - 7	9 - 10
	7	2 1/2 - 3 years	-	7-8 years	11-13	14 - 15
	8	8 - 10 "	-	12-16 years	17-21	18 - 25

Table 2 Chronological Table of Development of the Human Dentition (Lunt and Law 1974)

2.1.6 The incremental nature of intrinsic green pigmentation

It is generally believed that the green pigment is laid down in tissues that are undergoing calcification rather than by diffusion through the dentine following hard tissue formation (Brearley 1968: Zaia *et al* 1993). The cessation of deposition of the green pigment following liver grafting also lends further weight to this hypothesis (VanCleynen & Demars-Fremault 1990: Seow *et al* 1991: Funakoshi *et al* 1992).

Chronological deposition has also been reported in relation to green pigmentation in a premature child who had suffered from hyperbilirubinaemia. All of the clinical crowns of the maxillary incisors were green in colour but the mandibular central incisors were only green in the incisal one-third. This suggested that the intrinsic pigmentation occurred in the immediate post natal period (Herbert & Delcambre, 1987).

2.1.7 Acquired pigmentation of the dental hard tissues occurring in utero

Van Cleynen and Demars-Fremault (1990) reported that incremental intrinsic green pigmentation in the primary dentition of children with biliary atresia appeared to have started during hard tissue formation in utero. This report not only suggests an incremental distribution of the pigment but also that the intrinsic green stain might have been incorporated into the tooth substance before birth.

Rosenthal *et al* (1986) also reported two cases where the deposition of intrinsic stain may have occurred in utero. One of the subjects was a 2kg infant boy of 32 weeks gestation who had green intrinsic staining of the incisal and middle thirds of the maxillary and mandibular central incisors. The second subject was a 26 month old boy with biliary atresia who had intrinsic green pigmentation of all of his primary teeth.

There is already evidence, based on reports of tetracycline pigmentation of the dentition, that substances can cross the placental barrier and affect the primary teeth of the neonate, (Genot *et al* 1970 : Anthony 1970 : Dayan 1983). However, Marsland and Gerrard (1953), who previously examined 170 children with neonatal jaundice in Birmingham, reported that dentine laid down pre-natally was not affected.

2.1.8 Distribution of the intrinsic green pigmentation

It is not clear if the intrinsic green pigmentation is present in both the dentine and enamel. Marsland and Gerrard (1953) reported that they found green pigment to be present only in dentine. Green pigmentation which was confined to the dentine was also reported by Tank (1951) who further concluded that unstained enamel merely reflected the underlying pigmented dentine. However, Funakoshi *et al* (1992) stated that the enamel was also stained but to a lesser extent than the dentine.

It has been suggested that the key to the difference between the deposition in enamel compared to dentine lay in the different patterns of development of these two hard tissues. Marsland and Gerrard (1953) believed that the green stain, which was

thought to be contained in the organic matter, was removed from the enamel during the phase of enamel maturation. Interestingly, they also reported that they found no correlation between the colour of the teeth and either the duration or the severity of jaundice.

2.1.9 Variation in the intrinsic pigmentation with time

The nature and intensity of the intrinsic pigmentation has been reported as being variable with time. Marsland and Gerrard (1953) reported that the green pigmentation appeared to fade over the follow-up period and suggested that this was due to changes in the translucency of the enamel of the primary dentition with age. The pigmentation has also been reported to undergo a colour change from an initial yellow, changing to green, before fading completely (Thursfield 1912: Langmead 1912: Barta *et al* 1989).

It has also been reported that the primary molar teeth are more deeply stained than the incisors (Marsland & Gerrard 1953) and that the pigmentation becomes darker towards the pulp (Rosenthal *et al* 1986). This latter finding might simply be attributable to the increased area of dentine in primary molars. However, the primary molar teeth are predominantly formed post natally whilst the crowns of the primary incisors predominately form in utero (Lunt & Law 1974). Therefore, if the hyperbilirubinaemia occurs only after birth, it is the primary molar teeth that are calcifying at the time. Thus if the pigmentation does indeed occur in an incremental fashion and affect only those teeth that are calcifying at the time, these primary molar teeth are likely to be more stained than the primary incisors.

The colour change and loss of intensity is more difficult to explain. It might be attributable to the authors (Marsland & Gerrard 1953: Barta *et al* 1989) relying on the hear-say of the parents reports of the affected children rather than a clinical evaluation. Nevertheless, Thursfield (1912) and Langmead (1912) carried out sequential examinations and both did report that the primary teeth were initially a *'vivid'* yellow in colour which later turned to green.

2.1.10 Summary

The most extensive studies into intrinsic green staining in the primary dentition were published in the 1950's (Tank 1951: Farquhar 1951: Marsland & Gerrard 1953). Neonatal hepatitis due to Rhesus incompatibility was eradicated over thirty years ago and so investigations into the nature and onset of intrinsic green staining in infants were never fully pursued. Today, children with liver disease have a much greater life expectancy, particularly now that liver transplantation is increasingly available. Since caries-free, green stained primary teeth could be used to elucidate the role of the foetal liver the nature, the chronology of the pigmentation requires further examination.

2.2 Enamel Hypoplasia in children with liver disease and liver grafts

The mineralised enamel contains a history of the child's early life. Enamel does not remodel and so disturbances during development remain in the tooth as a permanent record (Fearne *et al* 1994). Enamel hypoplasia of the primary dentition is a valuable clue to the child's well-being before and after birth. The primary central incisors start to mineralise during the fourteenth gestational week and the crowns of the second primary molars are completed by 11 months of age (Lunt & Law 1974).

2.2.1 Definition

Enamel hypoplasia is defined as a quantitative defect of enamel, visually and morphologically identified as involving the surface of the enamel and associated with a reduced thickness. The defective enamel may occur as shallow or deep pits either arranged horizontally in a linear fashion or generally distributed around all or part of the enamel surface. The defective enamel may also occur as small or large, wide or narrow grooves (Ainamo & Cutress 1982).

2.2.2 Aetiology of enamel hypoplasia

The aetiology of developmental enamel defects can be hereditary or acquired and can be associated with numerous systemic disorders including, Trisomy 21, epidermolysis bullosa, pseudohypoparathyroidism, oculodentodigital dysplasia and mucopolysaccharidoses. Enamel defects are also strongly associated with inborn errors

of metabolism such as phenylketonuria, erythropoietic porphyria, and primary oxaluria. Enamel hypoplasia is also related to neonatal disturbance such as premature birth, haemolytic anaemia and viral exanthematous diseases of childhood (Pindborg 1982).

Enamel hypoplasia is probably most extensively reported in relation to fluorosis. Small and Murray (1978) reported that the prevalence of at least one tooth with an enamel opacity in children was 14.6% to 83.5% in low fluoride areas 8% to 36.4% in optimally fluoridated areas.

Enamel hypoplasia in the primary dentition has been attributed to various factors such as fever, and local infection (Pindborg 1982), prolonged hypocalcaemia (Ranggard *et al* 1995), and trauma to the mineralising primary teeth caused by pressure of the laryngoscope on the alveolar ridge during intubation has also been implicated (Noren *et al* 1993). Enamel hypoplasia of the primary canines has also been found amongst children with nutritional deficiency (Skinner 1994).

2.2.3 Incidence of enamel hypoplasia

The presence of enamel defects in permanent teeth has been reported to be between 30% (Suckling *et al* 1976) and 49% (Dummer *et al* 1986) in caucasians. Warnakulasuriya (1989) reported a prevalence of 20% in a study of Sri Lankan children but attributed this low finding compared with other studies to poor lighting. Goodman *et al* (1987) reported a prevalence of 47% in Mexican children but found only 6% of primary teeth to be affected. However, some of these studies have counted

tetracycline stained teeth together with those with enamel defects and no record was made as to the past medical history of the study sample (Suckling *et al* 1976).

2.2.4 Enamel hypoplasia and liver disease

Hyperbilirubinaemia and malabsorption of the fat soluble vitamins A and D can result in defects in the formation of enamel and dentine (Shapiro *et al* 1975). Therefore, children with liver disease are more likely to have enamel hypoplasia in the primary and permanent dentitions. Seow *et al* (1991) found enamel hypoplasia in all nine of the children in their study with liver disease. They reported that these enamel defects ranged from minor breaks in the enamel to large areas of missing enamel that appeared to be located in those areas of the primary teeth that were formed postnatally.

2.2.5 Summary

It is likely that paediatric liver transplant recipients have been malnourished and must have undergone numerous investigations including endoscopy. The prevalence of enamel hypoplasia has not yet been investigated in this population.

2.3 Delayed eruption in paediatric liver transplantation

2.3.1 Prevalence and aetiology

Seow *et al* (1991) reported delayed eruption of the primary dentition in 3 of the 9 patients in their study. Proffit (1993) diagnosed delayed eruption only when a tooth had failed to erupt six months after the expected time. Under these criteria only one of the patients in the study of Seow *et al* (1991) would be defined as having delayed eruption. Moreover, three of the patients in the study had body weights below the third percentile and all but one had heights on or below the third percentile but the effect of malnutrition was not taken into account.

2.3.2 Mechanisms of primary tooth emergence

Tooth *emergence* is the descriptive term applied to a tooth's entry into the oral cavity. The teeth develop either within a bony crypt or within a bony trough and, as such, an intimate relationship exists between tooth eruption and bone growth. Following calcification of the crown, bone resorption occurs in two regions; firstly in the base of the crypt and later around the gubernacular canal, expanding the crypt in the long axis of the tooth. The tooth itself appears to play no active role in the eruption process but it has been shown that removal of either the coronal or apical part of the dental follicle stops the eruptive process.

The density of bone and the rate of bone resorption directly influence the rate of eruption. Eruption provides the stimulus for growth of the alveolar process. As such the process of alveolar bone growth and tooth eruption are co-ordinated and the two processes are indistinguishable. Calcification of the crown of the tooth is a critical step in the eruptive process since it provides a rigid mass against which the forces within the follicle can act. Blood pressure in the periodontal ligament, osmotic tissue fluid pressure and repetitive impulses have all been proposed as the likely factors to provide the eruptive force. However, ultimately, tooth eruption depends upon the imbalance of forces acting upon the tooth and the forces resisting its movement (Kardos 1996).

2.3.3 Primary tooth eruption variation between ethnic groups

Primary tooth eruption dates have already been extensively investigated. Previous studies have shown that although there is considerable individual variation between individuals, there is generally very little variation in primary tooth eruption times between different populations (Roche *et al* 1964: Lavelle 1979: Magnusson 1982: Ramirez *et al* 1994). Moreover, it has also been shown that gender makes little difference in the timing of the eruption of the primary dentition (Hitchcock *et al* 1983: Ounsted *et al* 1987). Therefore, large population studies which measure factors related to primary tooth eruption can be cautiously applied to other population groups and to boys and girls equally.

2.3.4 Primary tooth chronology

The *Chronology of the Human Dentition*, produced by Logan and Kronfield (1933) which was later modified by McCall and Schour was used uncritically as a standard of dental development from the 1940's until it was modified by Lunt and Law in 1974. In the light of these changes many of these original papers may need to be revised.

2.3.5 Relationship between somatic growth and eruption of the primary dentition

Infante and Owen (1973) in a study of 273 caucasian children, demonstrated a significant association between the total number of teeth present and the height, weight and head circumference in boys and between number of teeth and height in girls. In their study they scored an anterior tooth as emerged if 1mm of the tooth surface was exposed and a posterior tooth when all four cusp tips were exposed. However, they did not compare the measurements of height and weight against population norms nor against standard growth charts. Nevertheless, they concluded that the timing of tooth emergence was significantly related to general somatic growth and perhaps to nutritional status.

2.3.5.1 Prematurity

The timing of the eruption of the primary dentition is related to prematurity and birth weight. Fadavi *et al* (1992) examined 31 premature children aged from 15 months to 5 years of age. The subjects were grouped into three age ranges and the number of teeth

that had pierced the oral mucosa were counted. The average number of erupted teeth for the different age groups was then plotted and compared to a norm, composed of the subjects reported in the study by Infante and Owen (1973). In addition, the length of time the subjects had been intubated was compared to the number of erupted teeth. The authors reported delayed eruption of the primary dentition in six out of eight premature children under two years of age with low birth weights. Moreover, no association was found between either birth weight or intubation period and the number of erupted primary teeth. They concluded that that low birth weight premature children had delayed eruption of the primary dentition in the first 24 months of life.

2.3.5.2 'Catch-up growth'

The study by Fadavi *et al* (1992) also showed that, after 24 months, the children appeared to *'catch up'* and achieve norms comparable to healthy children of the same age.

Loevy *et al* (1989) evaluated dental eruption in premature children with low birth weights and the effect of oral intubation on the palate and compared dental eruption patterns to those of Infante and Owen. In this study, 7 out of 28 subjects were found to have delayed eruption but oral intubation was not related to a high arched palate. However, the authors in this study also reported that delays in dental eruption were corrected during the second year.

2.3.6 The influence of malnutrition on eruption of the primary dentition

2.3.6.1 'Failure to thrive'

The term *failure to thrive* (FTT) is applied to infants and young children who do not achieve the normal or expected rate of growth and is classically subdivided into two areas, 'organic' and 'inorganic'. The differential diagnosis of children who have 'failure to thrive' is shown in Table 3 below.

Table 3

The Differential Diagnosis Of Children Who Have 'Failure To Thrive'

•normal child of short stature	•severe developmental delay
•idiosyncratic growth pattern	•chronic infection
•breast feeding failure	•gastrointestinal disorders
•formula feed errors	•metabolic disease
•non-organic failure to thrive	•congenital heart disease

from Marcovitch (1994)

However, it is now clear that malnutrition is the primary cause of failure to grow appropriately in all cases of non-organic failure, and in the majority of organic cases.

2.3.6.2 Poor nutrition and primary tooth eruption

The eruption of the primary dentition has been shown to be influenced by poor nutrition. In 1992, a study of 44 children from an inner-city area of South London found that there was a significant difference between the number of erupted teeth of infants who were failing to thrive compared to matched controls (Reilly *et al* 1992). The criteria for subject selection in this study were: full-term singleton birth, birthweight above the third percentile, and weight for age at or below the third percentile aged 12 months and sustained for three months; and an erupted tooth was recorded if the cusp tip had penetrated the oral mucosa at the time of the examination. Furthermore, since this study used weight for age parameters to measure FTT it can be assumed that these subjects were suffering from acute rather than chronic malnutrition.

2.3.6.3 Chronic versus acute malnutrition

Alvarez *et al* (1990) studied the effects of malnutrition on eruption of the primary dentition and on dental caries in Peru. In 1990 they examined 1481 children between 1 year and 13 years of age and measured the children's nutritional status using anthropometry. In this study, the subjects were further classified as *wasted* (weight for height <90% of the 50th percentile), *stunted* (height for age <95% of the 50th percentile), or *wasted and stunted.* Stunting was used to measure chronic malnutrition, whilst wasting was indicative of current or acute malnutrition. The authors reported that *wasting* and the *wasting and stunting* were found to have the greatest delay in

primary tooth eruption. *Stunting* appeared to have a lesser effect on the eruption. This suggested that acute malnutrition (i.e. low weight for height) has a more pronounced effect than chronic malnutrition (i.e. low height for age).

In a second study in 1993, Alvarez *et al* carried out a longitudinal investigation into dental caries in the primary teeth of children who had suffered from infant malnutrition. This study not only showed that these children were more susceptible to dental caries but also that by the age of two years, children who had not been malnourished had significantly more teeth than those who had. Moreover, in this study they showed that retarded linear growth had the most pronounced effect on primary tooth eruption (Alvarez *et al* 1993). Therefore, it is not clear if it is decreased weight or decreased height which is the most significant measure of malnutrition in children in respect of the developing primary dentition.

2.3.7 Malnutrition in children with liver disease

Children with liver disease have a particular predilection for malnutrition. They suffer from defective protein and fat metabolism caused by both malabsorption and metabolic disturbance (Whitington & Balisteri 1991). It has been shown that severe malnutrition (weight and/or height more than 2 standard deviations below the mean) affects 50% of children with established cirrhosis (Pierro *et al* 1989: Beath *et al* 1993a).

Virtually all candidates for liver transplantation demonstrate at least mild nutritional depletion at the time they are assessed for transplantation. The standard treatment of

nutritionally depleted patients is critically dependent on the administration of adequate calories to ensure optimal use of protein sources. However, patients with liver disease are often protein restricted and so protein-calorie malnutrition, in particular, has been well reported in these patients. Malnutrition in children with liver disease is attributed to a high catabolic state, anorexia, malabsorption, and decreased protein synthesis and is exacerbated by haemorrhage, ascites, encephalopathy, cholangitis and infection (Moukarzel *et al* 1990).

2.3.7.1 Malnutrition in babies with liver grafts

Babies under one year of age who receive liver transplants have an even higher energy and protein requirement. In this age group, malnutrition secondary to chronic liver disease has been reported to be as high as 80% following liver transplantation. However, studies have shown that aggressive nutritional support can significantly improve the life expectancy (Moukarzel *et al* 1990 : Beath *et al* 1993b).

2.3.7.2 'Catch-up' growth following liver grafting

Children who receive liver grafts have their growth and development carefully monitored and receive early and aggressive nutritional support. Beath *et al* (1993b) in a study of 32 babies, transplanted under 1 year of age in Birmingham reported a 1 year survival of 88%. Moreover, within 12 months of liver transplantation they had started to 'catch-up' on their healthy peers. This finding was attributed to better nutritional

support and to the discontinuation of corticosteroids after three months, (Beath *et al* 1993b).

2.3.8 The influence of steroid therapy

It has long been accepted that steroid therapy increases susceptibility to oral infection and causes delayed wound healing (Mason 1970). However, it has been shown that corticosteroid therapy has no deleterious influence on the clinical parameters of periodontal disease, even with prolonged use (Safkan & Kanuuttila 1984). Indeed, there have been reports that topical steroids inhibit gingivitis (Tollefsen *et al* 1978 : Vogel *et al* 1983). Nevertheless, steroid therapy in children is known not only to cause delayed growth and development but has also been shown to cause delayed eruption of the permanent dentition (Luyk *et al* 1985).

2.3.8.1 Steroids and growth

Blodgett *et al* (1956) studied the effect of prolonged cortisone therapy on statural growth, skeletal maturation and metabolic status of children. They showed that the drug could, within a few weeks of onset of therapy, slow the rate of statural growth and skeletal maturation in children. However, they also reported that children who had been given growth suppressing doses of cortisone underwent a compensatory growth spurt when doses were either reduced below growth suppressing levels or were eliminated altogether.

Schunior *et al* (1990) in a more recent study examined the effects of irradiation, chemotherapy and prednisolone medication on growth and craniofacial proportion, using an animal model. The authors reported that although there was significant retardation of growth and craniofacial development attributed to the irradiation, these changes were exacerbated by prednisolone medication. Furthermore, there appeared to be a difference in the response between males and females. However, the authors also concluded that separate studies would have to be carried out to fully differentiate between the individual treatment modalities.

2.3.8.2 The effect of steroid therapy on the dentition

It has been reported that corticosteroid therapy was an aetiological factor in the development of enamel hypoplasia and delayed eruption of the permanent dentition in children with chronic renal failure. However, at the same time, it was also suggested that some of these findings might be equally attributable to the effect of vitamin D deficiency (Bublitz 1981).

There have been no direct reports of delayed eruption in the primary dentition attributable to steroid therapy but it is possible that this is due to the hitherto small number of infant transplant recipients and the wide variation in children with other medical conditions that require steroid therapy.

2.3.8.3 Steroid therapy in paediatric liver transplant recipients

Steroid therapy in paediatric liver transplant recipients is generally discontinued three months after liver transplantation and so the impact of steroid therapy on delayed eruption of the primary dentition of paediatric liver transplant recipients is likely to be negligible. However, studies that seek to elucidate the causes of delayed eruption in a population such as this should take concomitant steroid therapy into account.

2.3.9 The influence of the overlying soft tissues

The soft tissue which separates the crown of an unerupted tooth from the oral cavity might also influence eruption. The resilience, texture or thickness of the overlying oral mucosa may counteract the forces of eruption and thereby impede the emergence of the primary teeth in the same way that some permanent teeth require surgical exposure.

Di Biase (1971) investigated the nature of the overlying mucous membrane in 25 cases of delayed eruption of permanent teeth and compared biopsies from affected patients with sections of normal mucosa overlying second permanent molars that were nearing eruption. He reported that the mucosa was *'fleshy'* and histological examination revealed that the submucosa contained large areas of immature oedematous connective tissue. Histochemical staining disclosed the presence of diffusely distributed mucopolysaccharides but no procollagen. and that it was

composed of collagen and immature oedematous tissue which contained a heavy deposition of mucopolysaccharides.

2.3.9.1 Delayed eruption of the primary dentition

Delayed eruption of the primary dentition attributable to thickened overlying oral mucosa has also been reported. Galili *et al* (1974) described a case of a twelve month old child with delayed eruption of the primary dentition who was suffering from I-cell Disease, which is a variant of Hurler's syndrome. The authors also reported that the configuration of the gingival tissues followed the contour of the erupted crowns suggesting that the alveolar but not gingival emergence had occurred.

2.3.9.2 Drug induced thickening of the oral mucosa

Delayed eruption attributable to drug induced thickening of the oral mucosa has also been described. Phenytoin has been reported as causing overgrowth of the oral mucosa in an edentulous adult, (Dreyer 1978). Reich *et al* (1981) reported that a 19 month old infant had delayed eruption due to phenytoin therapy which had been prescribed soon after birth. However, the child in this report was '*small for her age*'.

Church and Brandt, (1983) reported the case of a four-year old child with delayed eruption of the primary dentition which was also attributed to phenytoin therapy. The child in this study was suffering from agenesis of the corpus callosum and had been

medicated with phenytoin since the first few months of life. Radiographic examination confirmed that the primary dentition was present and that the teeth had erupted through the alveolar bone. The primary teeth erupted normally after surgical removal of the overlying oral mucosa.

Appleton and Leach (1991) reported a case of delayed eruption of all permanent teeth in a 13 year old boy diagnosed as suffering from tonic-clonic seizures at the age of seven years and then eventually controlled using phenytoin at the age of 10 years. Radiography confirmed the presence of the permanent teeth.

Cyclosporin is also known to cause gingival overgrowth but there has never been a report of cyclosporin causing delayed eruption. However, now that cyclosporin is used in babies with liver transplants it should not be dismissed as a contributory factor in the delayed eruption of the primary dentition.

2.3.10 Summary

The development and eruption of the primary dentition is related to somatic growth and development but can be influenced by nutritional status, particularly in the first year of life. Medication with cyclosporin, which is known to cause localised overgrowth of the gingivae, and the effect of steroid therapy have never been investigated but may influence the eruption of the primary dentition in paediatric liver transplant recipients.

Reduction hepatectomy has made liver transplantation available to babies under one year of age. Clearly, the effect of liver transplantation on the developing dentition requires further evaluation. Moreover, the increasing number of babies and infants with liver transplants affords the opportunity to monitor the developing dentition for the first time.

2.4 Cyclosporin

Cyclosporin was first discovered in 1977, and was first used clinically in 1978 in renal allograft recipients (Borel *et al* 1977: Seymour & Jacobs 1992). Cyclosporin is a neutral hydrophobic cyclic peptide composed of 11 amino acids. It is produced from the fermentation of two fungi, *Trichoderma polyposum* and *Cylindocarpon lucidum*. Twenty years after its first introduction, cyclosporin is the first line drug in renal, bone marrow, pancreas, and heart and heart-lung transplants and the 96% five year success rate of solid organ and bone marrow grafts can be attributed to its use (Williamson *et al* 1994: Lee & Canafax 1996).

Cyclosporin is now used to treat other systemic diseases which have an immunological component. These include: type 1 diabetes mellitus, psoriasis, AIDS, Behcet's disease, multiple sclerosis, myasthenia gravis, erosive lichen planus, allergic encephalomyelitis, mycosis fungoides, systemic lupus erythematosus, primary biliary cirrhosis, schistosomiasis, chronic active hepatitis and rheumatoid arthritis (Wysocki *et al* 1983: Beveridge 1983 : Seymour & Jacobs 1992 : Williamson *et al* 1994).

Cyclosporin is a potent immunosuppressive agent that blocks interleukin-2 production from CD4+ cells, inhibits T-cell proliferation, binds immunophilin and inhibits calcneurin production (Lee & Canafax 1996). As such, cyclosporin directs its action towards modulating, at therapeutic doses, the action of T-lymphocytes on B-lymphocytes and by blocking the factors that control cell proliferation (Gelfand *et al* 1987: Seymour & Jacobs 1992: Schincaglia *et al* 1992: Mariani *et al* 1993).

Many side-effects of cyclosporin have been reported. These include: nephrotoxicity, hepatotoxicity, neurotoxicity, lymphoma, hypertrichosis, hirsutism, fibrosis of pulmonary, pericardial and renal tissues, convulsions, and hyperbilirubinaemia as well as gingival overgrowth, (Calne 1979, 1980: Iwatsuki *et al* 1983: Rateitschak-Plus *et al* 1983: Wysocki *et al* 1983: Beaman *et al* 1985: Von Graffenried & Krupp 1986: Modeer *et al* 1992: Williamson *et al* 1994).

2.4.1 Pharmacokinetics

Cyclosporin is insoluble, and in the early stages of its development it was difficult to promote its absorption. The Sandimmune formulation of cyclosporin is an oil in water macroemulsion in which the drug is suspended in olive or corn oil and dispensed as a soft gelatin capsule or in solution. It is highly lipophilic and is readily distributed across most biological membranes. Therefore, cyclosporin is extensively distributed throughout the body and the body fluids including saliva (McGaw *et al* 1987: Modeer *et al* 1992).

Cyclosporin is absorbed from the gastrointestinal tract into enterocytes in the upper small intestine. Systemic absorption of cyclosporin from the gastrointestinal tract depends on various factors, such as oil droplet size, bile, lipase, pH, surface area, and transit time and is facilitated by either the sodium-requiring transport systems for peptides or by uptake by the lymphatic system, (Drewe *et al* 1992: Lee & Canafax 1996).

Once cyclosporin reaches the blood stream it distributes out to the tissues that have a high percentage of fat or into red blood cells. The extent of the distribution depends upon the cyclosporin concentration, hematocrit level, and lipoprotein concentration. Thirty-three to forty-seven percent of cyclosporin is found in plasma, mainly bound to lipoprotein. The remainder is bound to erythrocytes, granulocytes and lymphocytes.

Cyclosporin in inactivated by gut and liver enzymes, to produce numerous metabolites. Some of the remaining cyclosporin, and most of the metabolites, are eliminated by biliary excretion and so very little is found in the urine. This metabolic destruction produces a reported terminal half-life of 19 ± 9 hours. This half-life is calculated from 24 hour concentration time profiles that can be influenced by the rates of resorption, distribution, elimination, and redistribution from tissues back into the blood (Lee & Canafax 1996).

2.4.1.1 Pharmacokinetic variability

Cyclosporin has a wide inter and intra-patient biopharmaceutic and pharmacokinetic variability and this has complicated the relationship between dose, blood level, and outcome, making it difficult to achieve the desired therapeutic response. The variable absorption is partly attributable to patient factors such as time post transplant, type of organ failure or disease, food, race, age, requirement of other drugs, diarrhoea, gastrointestinal motility, hepatic function and bile flow (Tan 1995: Schroeder *et al* 1995: Canafax 1996: Lee & Canafax 1996: Schroeder *et al* 1996). However, the

factor that is most responsible for blood level fluctuation is the highly variable oral bioavailability, which can range from 7 to 92%. The most probable cause for this variability in the pharmacokinetics of cyclosporin is its variable absorption in the small intestine (McMaster & Mirza 1994: Superina *et al* 1994). This hypothesis was reinforced by Dunn *et al* when they reported that intravenous cyclosporin therapy produced consistent distribution and elimination in paediatric patients irrespective of the transplanted organ, (Dunn *et al* 1996).

Suboptimal blood levels of cyclosporin are associated with an increased prevalence of acute rejection (Cooney *et al* 1986). Therefore, for maximal effectiveness, frequent blood level monitoring and appropriate dose adjustment is required to maintain trough levels in the desired range (Levy 1996).

2.4.2 Cyclosporin in liver transplantation

Liver transplant recipients are not only a very diverse group of individuals, ranging from small babies to adults but they also have a wide range of liver disorders. Therefore, no single cyclosporin therapy schedule fits all clinical situations. Liver transplantation presents a unique challenge to the use of cyclosporin because of the critical illness of the recipient, difficulties in resorption and metabolism by the grafted liver, and the lethal effects of infection. Moreover, the diversity of the patients who receive liver grafts and their stage of illness necessitates constant adjustment to the immunosuppressive therapy. Indeed, cyclosporin dosage is reduced, or even

discontinued, in patients who have fulminant hepatitis, infection or hepato-renal syndrome (McMaster & Mirza 1994).

Cyclosporin therapy in liver transplant recipients has been shown to have an even greater variation in the bioavailability compared to other organ transplantation. This is attributable to the fact that cyclosporin is lipid soluble and as such, requires both bile and bile salts to be absorbed in the small intestine. In addition, poor hepatic function further impairs cyclosporin metabolism, resulting in a shift in the balance between the parent drug and its metabolites (McMaster & Mirza 1994).

In the immediate early post-operative period following liver grafting the bioavailability of cyclosporin is extremely poor and even more variable. This is because biliary diversion in the first few weeks after transplantation and cholestasis are common complications. Moreover, recurrent hepatitis, biliary obstruction, and chronic rejection all cause cholestasis following liver transplantation and can even occur a long time after transplantation. The absorption of cyclosporin is further adversely affected by poor graft function (Cooney *et al* 1986: Trull *et al* 1993a, 1994b: Belli *et al* 1994a: Belli *et al* 1994b: Jamieson *et al* 1996:). Therefore, parenteral administration is usually required. However, intravenous cyclosporin has been shown to cause nephrotoxicity (Trull *et al* 1994).

2.4.2.1 Cyclosporin therapy regimen in OLT

The majority of liver transplantation programmes currently use triple therapy schedules of cyclosporin, prednisolone and azathioprine. Cyclosporin therapy is initiated at 2 to 4 mg/kg intravenously, and then the treatment is converted to oral cyclosporin at between 5 to 10 mg/kg of body weight. However, there is a discrepancy between centres in regard to the immunosuppressive therapy. Some use cyclosporin and azathioprine (dual therapy), others include a steroid (triple therapy), whilst some add antilymphocyte globulin (quadruple therapy) in the early post transplant period. Although the centres with the most intensive drug therapy have reported a reduction in acute rejection the diagnostic criteria have been so varied that comparison between the different centres has been impossible. However, intensive drug therapy has been associated with a higher prevalence of infection, particularly with cytomegalovirus (McMaster & Mirza 1994).

The development of rejection, infection and lymphoproliferative disease in liver graft recipients is directly related to the immunosuppressive protocol. In view of this, the liver transplantation programme in Birmingham uses intravenous cyclosporin combined with azathioprine and prednisolone in the early stages following transplant. The patient is then switched to oral cyclosporin. Imunosuppression is then maintained using dual therapy after cessation of steroids at 3 months post transplant (McMaster & Mirza 1994).

2.4.2.2 Cyclosporin therapy in babies and preschool children with liver grafts

Children, and babies in particular, require large doses of cyclosporin to achieve immunosuppression after liver grafting. This is because children have reduced bioavailability but increased plasma clearance (Whitington *et al* 1990: Dunn *et al* 1996).

Children and babies have a more limited absorptive surface area in the small intestine. The length of the bowel increases with somatic growth and is most rapid during infancy and in the preschool years, until adolescence when it becomes constant. The intestinal surface area increases by a geometric multiple of the length. Therefore, the intestinal surface available for drug absorption in childhood increases rapidly in early childhood and, as such, is an important factor in determining the bioavailability of cyclosporin in babies and preschool children. A further determinate factor in liver graft recipients is the amount of bowel length that is removed during surgery (Whitington *et al* 1990).

The importance of the length of the bowel was subsequently confirmed in a study by Dunn *et al* (1996) who suggested that the other pathological factors that are known to influence oral cyclosporin resorption, such as bile flow, may be less important than the bowel length in children under two years of age.

2.4.3 Neoral

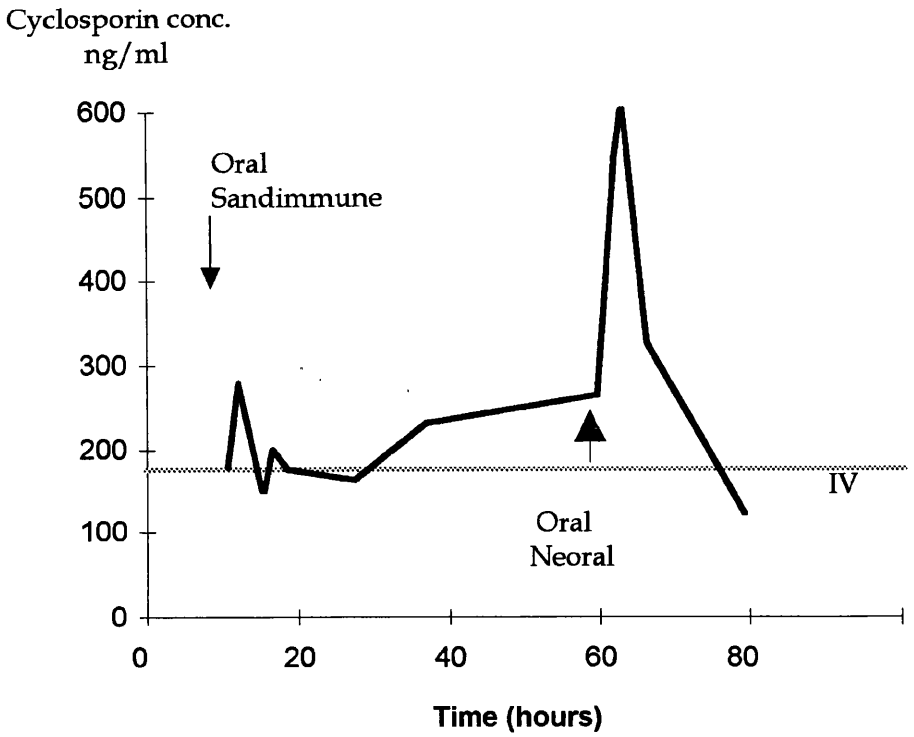
Sandimmune Neoral is a new preparation of cyclosporin which dispenses the drug as a microemulsion. One of the major benefits of cyclosporin in a microemulsion form (Sandimmune Neoral) is that the absorption is independent of bile and bile salts. Therefore, it is likely that the higher Cmax and area under the curve following oral administration of Neoral will alleviate many of the difficulties linked to the variable resorption of the drug. This will be of particular benefit to liver transplant recipients (McMaster & Mirza 1994).

Many studies have already confirmed that the Neoral preparation not only increases the bioavailability of cyclosporin following liver grafting but also produces better long-term results, even in patients with cholestasis (Trull *et al* 1993a, 1994b: Belli *et al* 1994a: Belli *et al* 1994b: Levy 1996: Grant *et al* 1996: Jamieson *et al* 1996). Moreover, the Neoral microemulsion preparation has also been found to have a significantly greater bioavailability in paediatric liver transplant recipients (Lin & Lee 1994: Superina *et al* 1994: Alberti *et al* 1995: Dunn *et al* 1996).

The variation in serum concentration that is commonly found in the Sandimmune cyclosporin formulation and the comparison between that and both Neoral and intravenous therapy is shown graphically in Figure 1 on the following page.

Figure 1

Typical Cyclosporin Concentration Time Curve.



Typical cyclosporin concentration curve following oral Sandimmune (first arrow) and oral Neoral (second arrow) administration. C max and AUC are higher and the curve follows a more predictable pattern following the Neoral administration. The horizontal grey line indicates the steady-state concentration of cyclosporin on continuous intravenous infusion (Superina *et al* 1994).

2.4.4 Cyclosporin-induced gingival overgrowth

Gingival overgrowth is the only known oral side-effect of cyclosporin therapy. It was first reported in the early eighties, and was initially compared to the gingival swelling caused by phenytoin and the calcium channel blockers (Calne 1980: Rateitschak-Plus *et al* 1983: Wysocki *et al* 1983: Tyldesley & Rotter 1984: Savage *et al* 1987: Starzl *et al* 1989a,b: Seymour 1991: Seymour & Jacobs 1992: Dongari *et al* 1993).

2.4.4.1 The prevalence of gingival overgrowth in organ transplant recipients

The reported prevalence of cyclosporin-induced gingival overgrowth in transplant recipients is variable at between 8% and 100% (Ross *et al* 1989: Seymour & Jacobs 1992: Thomason *et al* 1993: Dongari *et al* 1993: Somacarrera *et al* 1994).

There are some authors who have postulated that this variation in the prevalence of gingival overgrowth could be attributed to individual sensitivity either between individual subjects or between different fibroblast populations (Hassell & Stanek 1983: Wysocki *et al* 1983 : Daly CG, 1992). However, it could be postulated that this variability in cyclosporin-induced gingival overgrowth appears to mirror the inter and intra-variability that has already been discussed in respect of cyclosporin bioavailability.

2.4.4.2 Cyclosporin-induced gingival overgrowth in liver graft recipients

The prevalence of gingival overgrowth in liver graft recipients is shown in the following Table 4 overleaf.

In Table 4, it can be seen that the prevalence of cyclosporin-induced gingival overgrowth in the paediatric liver transplant population has been found to be between 86% and 100%. This is very much higher than the 53% reported in a recent study of paediatric heart transplant recipients (Lowry *et al* 1995). However, the studies shown in Table 4 had conflicting findings in respect to the effect of duration of cyclosporin therapy, dose and circulating trough concentration on the presence and severity of gingival overgrowth. Moreover the study populations were relatively small with wide age ranges and variable durations of cyclosporin therapy.

The largest study into gingival overgrowth in paediatric liver graft recipients is by Ross *et al* (1989) who examined the gingival changes in 21 patients following liver transplantation. They found that plaque index, gingival index, pocket depth and gingival width were all increased in the liver grafted group compared to a control group, but reported that there was no significant correlation between the circulating cyclosporin and plaque index, gingival index, or pocket depth.

	study information				oral findings	
AUTHORS	**Date**	**Number of Subjects**	**Age in months mean / (range)**	**Medical Diagnosis**	**Prevalence of Gingival Overgrowth**	**Other Comments**
Svirsky	1989	1	37	OLT	100%	
Zaia *et al*	1993	1	84	OLT	100%	
Funakoshi *et al*	1992	5	(53)	OLT	100%	no relation between gingival overgrowth and dose or therapy duration
Seow *et al*	1991	7	39 /(1-34)	OLT	86%	gingival overgrowth worsened with therapy duration
Ross *et al*	1989	21	100 (/ 24-192)	OLT	not reported	increased plaque index in subjects but no correlation between trough cyclosporin , therapy duration or concomitant nifedipine medication.

Table 4 The Prevalence Of Gingival Overgrowth in Previous Studies of Liver Graft Recipients

2.4.5 Pathogenesis of gingival overgrowth

Cyclosporin-induced gingival overgrowth is firm and pink, with focal lobulations and a stippled surface consisting primarily of a highly vascularised connective tissue with an overlying irregular, multi-layered, parakeratinised epithelium of variable thickness (Tyldesley & Rotter 1984: Rateitschak-Plus *et al* 1983: Wysocki *et al* 1993).

Wysocki *et al* (1983) described the excised hyperplastic gingival papilla of an adult renal transplant recipient as smooth, relatively blunt, with some areas exhibiting broad, blunt, papillary projections separated by shallow narrow crypts and increased numbers of fibroblasts. Irregularly arranged collagen fibre bundles, in the process of breakdown, and focal accumulations of inflammatory cells have also been described. Tyldesley and Rotter (1984) reported that the predominant feature in cyclosporin-induced gingival overgrowth was a proliferation of collagen fibres in the corium which were lightly distributed in a foamy basophilic ground substance.

These histologic findings have been largely confirmed in investigations using serology, immunology and electron microscopy. McGaw *et al* (1988) used serology to characterise the influence of cyclosporin on the individual components of the gingival connective tissue in four renal patients. They reported that cyclosporin-induced gingival overgrowth represents a net accumulation of collagen and other matrix materials in addition to a cellular overgrowth. Mariani *et al* (1993), when looking at the ultrastructural features of the attached gingivae in kidney transplant recipients medicated with cyclosporin, reported that there was a particular abundance of

amorphous substance together with a marked plasma cell infiltration. In a histologic, histochemical and immunohistologic examination of a patient medicated with cyclosporin for myasthaenia gravis, Savage *et al* (1987) also concurred that cyclosporin-induced gingival overgrowth is an overgrowth of fibrous tissue containing immuno-competent cells, including plasma cell aggregations, macrophages and T-helper cells.

Therefore, unlike other examples of drug-induced gingival overgrowth, cyclosporin appears to cause a significant increase in the number of inflammatory cells, collagen and matrix, rather than an increase in the fibroblast numbers alone. As such, the gingival swelling can be attributed to either an increase in the collagenous elements of the connective tissue matrix or to epithelial acanthosis and accumulation of extracellular substance. Consequently, the term gingival *overgrowth* appears to be more appropriate than gingival *hyperplasia* (Rateitschak-Plus *et al* 1983: Wysocki *et al* 1983: Tyldesley & Rotter 1984: Lambertenghi *et al* 1986: Seymour & Jacobs 1992: Barber *et al* 1992: Mariani *et al* 1993).

2.4.5.1 Effect of cyclosporin on gingival fibroblasts

Cyclosporin modifies the appearance of gingival fibroblasts (Yamasaki *et al* 1987) and causes a shift in the balance between their generative and degenerative activity (McGaw *et al* 1988). Moreover, it has been shown that there are heterogenous fibroblast subpopulations and so cyclosporin, or one of its metabolites, may act selectively on different subpopulations of fibroblasts. This could also provide an

explanation to the variation in the prevalence and severity of gingival overgrowth that is found between individuals medicated with cyclosporin (Hassell & Stanek 1983).

2.4.5.2 Effect of cyclosporin on other components of the gingivae

Non-cellular components of the human gingivae, such as the protein fibronectin, could be important in the differentiation of hyperplastic gingival lesions. It has been suggested that gingival overgrowth could be caused by cyclosporin creating an imbalance between tissue formation and degradation, thereby inducing connective tissue accumulation or inhibition of matrix breakdown (Wysocki *et al* 1983: Yamashaki *et al* 1987: Barber *et al* 1992: Schincaglia *et al* 1992: Seymour & Jacobs 1992: Romanos *et al* 1992: Williamson *et al* 1994).

2.4.5.3 Effect of cyclosporin on the gingival immune response

Cyclosporin has been shown to modify the immune response within the gingival tissues. It has even been suggested that the enormous infiltration of normal plasma cells together with the reversibility of gingival overgrowth is indicative of a hypersensitivity reaction (Lambertenghi *et al* 1986). Nevertheless, lymphokines, which can activate fibroblast activity, can be stimulated or repressed by cyclosporin. Cyclosporin has been found to selectively impair Interleukin I release from macrophages and also impair Interleukin II from helper T cells (Savage *et al* 1987). On the other hand, the level of Interleukin-6, which is produced in a variety of cells in

the presence of inflammation, such as that found in the gingival tissues when oral hygiene is poor, has been shown to be increased by the drug. These studies suggest that cyclosporin may exert a direct effect on cytokines to produce gingival overgrowth (Williamson *et al* 1994).

2.4.5.4 Relationship between gingival overgrowth and cyclosporin dose

The relationship between cyclosporin dose and gingival overgrowth has been extensively investigated. The varied prevalence and severity of gingival overgrowth in transplant recipients has been attributed to there being a 'threshold dose', below which the biological effect of the drug or one of its metabolites on the gingival tissues is dispelled (Seymour & Jacobs 1992: Wysocki *et al* 1983: Daly 1992). Daly (1992) concluded that a daily dose of 700mg was required in order to cause a mild gingival enlargement. However, this hypothesis was based upon observations on only one 65 year old patient with chronic active hepatitis.

Fu *et al* (1995) evaluated gingival overgrowth with variable dosage of cyclosporin using stone models taken from impressions of a rat mandible over a two week period. They found a significant positive relationship between gingival dimension and both the dosage and the duration of cyclosporin administration. In an in vitro study of the effect of cyclosporin on human fibroblasts, Coley *et al* (1986) reported that the effect of cyclosporin on fibroblasts not only varied amongst individuals but was also dose dependent.

However, Daley *et al* (1986) reported that they found no correlation between the mean daily oral dosage and the severity of gingival overgrowth. The relationship between cyclosporin dosage and gingival overgrowth was further disputed in a study of 23 renal transplant recipients in which it was concluded that gingival overgrowth was unrelated to cyclosporin dosage (Thomason *et al* 1993).

2.4.5.5 Gingival overgrowth and trough circulating cyclosporin concentration

Seymour *et al* (1987) reported a significant correlation between the mean plasma concentration scores of cyclosporin and the increase in gingival overgrowth in 24 adult renal transplant patients who were examined at three and six months post transplant. Furthermore, in a longitudinal study of 100 transplant recipients which included heart, liver and kidney patients, Somacarrera *et al* (1994) also suggested that the cyclosporin blood concentration was the key factor that influenced gingival overgrowth.

Nevertheless, there have been various studies which failed to show a relationship between gingival overgrowth and the trough circulating cyclosporin level. Daly *et al* (1986) carried out a prospective longitudinal study of 100 patients, 18 of whom were transplant recipients and 76 who were diabetics. Of these, 34 had serum trough concentrations recorded. They reported that they had not found a correlation between the presence of gingival overgrowth and the serum trough concentration of cyclosporin in the therapeutic range.

In 1991, Seymour and Smith, in a study of 27 adult renal transplant patients, also concluded that the whole blood concentration of cyclosporin was not a determinant for any increase in gingival overgrowth; a reversal of their reported findings from a previous study in 1987.

Other recent studies have also confirmed that the mean whole blood cyclosporin concentration was not related to the occurrence of gingival overgrowth (Pernu *et al* 1992). Indeed, King *et al* (1993) reported that gingival overgrowth was unrelated to cyclosporin dose, blood or saliva level in 66 renal transplant recipients.

Therefore, the relationship between daily dose and the plasma concentration of cyclosporin and the development and severity of gingival overgrowth is still controversial. Reasons for the conflicting results may be due to variation in research methodology, underlying medical condition, age of the patient, duration of therapy, concomitant medication and sample size. Moreover, the influence of the vehicle of delivery and the bioavailability on the pharmacokinetics of cyclosporin has not been taken into account in these publications.

2.4.5.6 Age and cyclosporin-induced gingival overgrowth

There have been reports that variation in the prevalence and severity of cyclosporin-induced gingival overgrowth is related to the age of the patient. Daly *et al* (1986) reported that all forty-seven of the diabetics in their study, who were under 20 years of age, had gingival overgrowth. This finding was highly significant when the gingival

overgrowth severity in this group was compared to a similar group of adults. They concluded that adolescents were at greatest risk of developing cyclosporin-induced gingival overgrowth and postulated that this may be attributed to either the added effect of growth hormone on the fibroblastic response or to a greater mitotic and secretory capability in young fibroblasts.

2.4.5.7 Effect of the duration of cyclosporin therapy on gingival overgrowth

Fu *et al* (1995) reported that gingival dimensions increased significantly with the duration of cyclosporin administration in rats. Cyclosporin-induced gingival overgrowth is seen to occur 2-6 weeks after the commencement of therapy and the changes associated with gingival overgrowth occur most rapidly during the first three to six months of therapy. Cyclosporin-induced gingival overgrowth has also been shown to be reversible, with a resolution of the gingival response once the drug has been discontinued. Moreover, the resolution of gingival overgrowth has also been documented following reduction in the daily dosage of cyclosporin (Daley *et al* 1992).

2.4.6 The effect of other oral parameters on cyclosporin-induced gingival overgrowth

2.4.6.1 Cyclosporin in saliva

Cyclosporin-induced gingival overgrowth has been related to the concentration of cyclosporin in saliva (McGaw *et al* 1987: Seymour *et al* 1987: Seymour & Jacobs 1992 : King *et al* 1993). Modeer *et al* (1992) demonstrated that the gingival tissues are

exposed to considerable concentrations of cyclosporin when the drug is administered in mixture form and therefore postulated that this topical effect of cyclosporin on the gingival tissues could have a role in the pathogenesis of gingival overgrowth. McGaw *et al* (1987) found a significant correlation between gingival overgrowth scores and whole saliva cyclosporin but no correlation was found when parotid or submandibular salivary cyclosporin samples were examined separately. They also found that the cyclosporin levels in whole saliva in the 30 renal transplant recipients consistently exceeded corresponding serum levels.

2.4.6.2 The influence of plaque on cyclosporin-induced gingival overgrowth

There has been a widely held belief that cyclosporin-induced gingival overgrowth is, in part, related to gingival irritants, such as dental plaque, dental calculus, imperfections in dental restorations, orthodontic appliances and the effects of mouth breathing (Wysocki *et al* 1983: Rateitschak-Plus *et al* 1983: Daley *et al* 1986: Tyldesley & Rotter 1984: McGaw *et al* 1987: Bartold 1987).

The hyperplastic gingivae and dental plaque share the same environment. This raises the possibility that plaque bacteria or one or more of their components are involved directly or indirectly in the hyperplastic response during cyclosporin therapy. Indeed, dental plaque together with components of the immune response may somehow modulate the normal mixture of fibroblast subpopulations by selecting populations which have a lower or higher synthetic activity.

Lipopolysaccharide (LPS) is the main structural component of the bacterial cell wall in gram-negative bacteria. Barber *et al* (1992) analysed the role of bacterial LPS in cyclosporin-induced gingival overgrowth. They found that although fibroblast proliferation was directly stimulated by cyclosporin it was inhibited by LPS. They believed that this inhibitory role of bacterial LPS may provide the explanation to the non-cellular nature of the hyperplastic response. Bartold (1989) found that Cyclosporin can also promote fibroblast proliferation *in vitro* without an alteration in synthetic activity and postulated that Cyclosporin may reverse the lipopolysaccharide inhibitive effect on fibroblast proliferation.

Daley *et al* (1986) in a study of 100 patients who were medicated with cyclosporin found that there was a correlation between oral hygiene and the presence of gingival overgrowth but only a weak correlation between the quality of oral hygiene and the severity of the gingival overgrowth. They concluded that the mild chronic irritation caused by dental plaque promotes the hyperplastic effect of cyclosporin on the gingiva. McGaw *et al* (1987) in a study of 30 renal transplant recipients found a significant correlation between gingival overgrowth and both plaque and gingivitis and proposed that dental plaque could act as a reservoir for cyclosporin.

However, not all studies have found that dental plaque is an aetiological factor in cyclosporin-induced gingival overgrowth. King *et al* (1993) in a study of 66 renal transplant recipients, 18 of whom were medicated with cyclosporin only, found that gingival overgrowth was only weakly related to plaque and calculus. Seymour *et al* (1987) in a study of 24 adult renal transplant recipients receiving either azathioprine or

cyclosporin found no significant difference for plaque scores compared to the gingival inflammation present between the two differing drug therapy groups. Tyldesley and Rotter (1984) reported that even frequent oral hygiene procedures were insufficient to eliminate the gingival changes.

It has been reported that transplant recipients medicated with cyclosporin have a significantly increased level of plaque and this might explain why gingival overgrowth is more prominent in areas where dental plaque has accumulated (Bartold 1987). As such, it has been suggested that the changes in the gingiva may be attributable to this change in plaque index (TD Daly *et al* 1986: Ross *et al* 1989: Pernu *et al* 1992). However, the prominence of plaque as an aetiological factor is controversial and it is difficult to compare the differing studies.

2.4.6.3 The synergistic action between cyclosporin and nifedipine

Calcium channel blockers are used in medical practice for various cardiovascular disorders such as; angina, cardiac arrythmia and hypertension. Their mode of action is blockage of the slow calcium channels in the cell membrane. The side effects of the calcium channel blockers include: headache, facial flushes, dizziness, oedema, nausea and dyspepsia; and are all attributable to excessive vasodilatation (Seymour 1991).

Nifedipine is a long-acting vasodilator that is widely used for cardiotherapy. It is an effective antihypertensive agent. Its main effect is to relax cardiac vascular smooth muscle by blocking transmembrane calcium flux through calcium channels The main

oral side-effect of nifedipine is gingival overgrowth. The prevalence of nifedipine-induced gingival overgrowth has been reported to be in excess of 10% (Nishikawa *et al* 1991).

Singer & Zebrowski (1988), in an in vitro study of the effect of nifedipine on human gingival fibroblasts, demonstrated that the drug caused an increase in cell number, enhanced and altered cell attachment and an increased production of matrix constituents. Salo *et al* (1990) investigated the effect of nifedipine on collagen and protein synthesis and on the cellular proliferation in human gingival fibroblasts in vitro. They reported that nifedipine had a specific effect on reducing gingival fibroblast cellular total protein and collagen synthesis but had no influence on cell proliferation.

Nifedipine induced gingival overgrowth has been reported as occurring within two months of therapy commencement (Seymour 1991). Nishikawa *et al* (1991) reported two patients with nifedipine induced gingival overgrowth who had been treated with the drug for more than three months. One of the subjects, a 61 year old woman, had reported that she had noticed gingival enlargement two months after starting nifedipine therapy. Moreover, two months after the nifedipine was discontinued spontaneous regression of the gingival overgrowth was observed.

Therefore, it appears that when nifedipine is discontinued, the gingival overgrowth improves moreover, it has even been reported that this improvement can be seen to occur in as little as 1 week (Lederman *et al* 1984).

Nifedipine is commonly used in transplant recipients to control hypertension and to ameliorate cyclosporin nephrotoxicity (Feehally *et al* 1987). As many as sixty percent of renal transplant patients are initially medicated with a combination of cyclosporin and nifedipine (Thomson *et al* 1993).

Several authors have reported that the effect of Cyclosporin and nifedipine on the gingival tissues appears to be additive (Thomason *et al* 1993: King *et al* 1993). Slavin and Taylor (1987) reported that concurrent medication of nifedipine and cyclosporin resulted in an increased rate of gingival overgrowth when compared to that caused by cyclosporin alone.

Thomason *et al* (1993) reported that the severity of gingival overgrowth was greater in patients medicated with a combination of cyclosporin and nifedipine. In their study, two groups of renal transplant recipients were examined, 32 were medicated with cyclosporin alone and 23 with combined cyclosporin and nifedipine therapy. They found that 11 of the 23 patients on combined therapy had clinically significant gingival overgrowth compared to only 12 out of the 32 patients who had cyclosporin alone. However, despite this difference between the two groups the results were not statistically significant.

The reported prevalence of clinically significant gingival overgrowth in subjects who were on both drugs ranges from 48% to 51% (McGaw *et al* 1987: Thomason *et al* 1993: King *et al* 1993). These studies have been carried out on adult renal transplant recipients who were found to have clinically significant gingival overgrowth when

medicated with a combination of cyclosporin and nifedipine and are significant when compared to previous reports of only a 27% prevalence in a similar population who were medicated using Cyclosporin alone.

The reason for the apparent synergistic action of cyclosporin and nifedipine might lie in similarities in their mechanism of action at the cellular level. Both drugs have been shown to inhibit the uptake of calcium by gingival fibroblasts. In addition, cyclosporin and nifedipine cause intracellular calcium depletion which may diminish the synthesis and secretion of collagenase, decreasing the rate of gingival connective tissue turnover. Moreover, both might also be associated with calcium ion exchange, folate uptake, increased testosterone metabolism, and alteration of host immune response. Both drugs may also have a similar relationship between dose and severity of the gingival overgrowth which also appears to be affected by plaque, (Dongari *et al* 1993).

There have been no reports of the effect of nifedipine on paediatric liver graft recipients. This group are generally only medicated with nifedipine in the first few months following transplantation, and thereafter only if hypertension persists. It is not clear if the synergistic action of nifedipine and cyclosporin is dependent on length of exposure time. The effect of both drugs on the gingivae appears to be reversed when the drug is discontinued. Nevertheless, any investigation into the effect of cyclosporin in paediatric liver graft recipients must take the possible effect of nifedipine into account.

2.5 Cytomegalovirus

The single most important pathogen in liver transplantation is cytomegalovirus (CMV) (Gorensek *et al* 1990: Stratta *et al* 1992). CMV infections most commonly occur 5-6 weeks following liver transplantation. The most frequent manifestation of CMV disease is graft hepatitis but other manifestations include gastrointestinal disease, pneumonitis and retinitis. It is likely that CMV promotes other opportunistic infections (Chatterjee *et al* 1978: Paya *et al* 1993: Hadley *et al* 1995).

2.5.1 Oral manifestations of CMV infection

Oral CMV infection is extremely rare but the virus can be cultured in the saliva from patients with disseminated CMV infection (Kanas *et al* 1987: Langford *et al* 1990). The most commonly associated oral manifestation of CMV infection is salivary gland inclusion disease which has been reported in 10 to 30 % of still births. Furthermore, CMV is thought to have a role in other salivary gland diseases such as Sjogren's Syndrome (Cawson & Eveson 1987). There also appears to be an association between CMV and oral ulceration in patients with AIDS (Kanas *et al* 1987: Langford *et al* 1990). It has been suggested that the virus may be implicated in the aetiology of recurrent apthous ulceration since CMV is already known to cause ulceration of the gastro-intestinal tract (Langford *et al* 1990: Epstein *et al* 1992). In addition, comparisons have been drawn between the epidemiology and immunology of Acute Necrotising Gingivitis (ANG) and CMV infection, suggesting that CMV may be an aetiological factor in ANG (Sabiston 1986).

2.5.2 CMV and gingival overgrowth

In a recent publication, a case was described of a CMV seronegative male who received a seropositive heart transplant and subsequently developed gingival overgrowth. Although the authors attributed the gingival overgrowth to CMV the patient's medication included cyclosporin and prednisolone. They reported that gingival biopsy confirmed squamous epithelial overgrowth with stromal and chronic inflammation which had CMV inclusions in the submucosal vascular endothelial cells and stromal fibroblasts. In situ hybridisation for CMV DNA confirmed that the virus was present in the stromal fibroblasts and in the vascular endothelial cells. CMV was also isolated from the throat, urine, blood and from a gingival biopsy. However, two months later when the gingival overgrowth had resolved the CMV culture was negative. The authors postulated that in this case gingival overgrowth occurred due to the infiltration of CMV infected cells, inflammatory cell infiltration and oedema, (Epstein *et al* 1992).

However, this patient's immunosuppression was '*significantly reduced*' at the same time and so the observed resolution of gingival overgrowth and conversion to CMV negative may have been coincidental.

2.5.3 CMV in liver transplantation

The serological CMV status determines the risk of infection following liver transplantation. If both the recipient and donor are seronegative there is little risk of

infection provided CMV-negative blood products are used. If either the donor or the host is seropositive the risk of CMV infection is high (Whitington & Balisteri 1991). As a consequence of CMV transmission from CMV sero-positive donors liver transplants are matched, where possible, by CMV status (Hersman *et al* 1982: Meyers *et al* 1986: Arnold *et al* 1991: Sutherland at al, 1992: Paya *et al* 1993: Hadley *et al* 1995).

CMV is a very common virus, 45% to 80% of the population developing CMV antibodies at some time in their lives. Prevalence depends on age, sex and socio-economic background but there is a predilection for the immunosuppressed and for organ transplant recipients in particular (Naraqi *et al* 1978: Sabiston 1986: Stratta *et al* 1992). The reported prevalence of CMV infection following liver transplantation is as high as 40% and following kidney transplantation as high as 73% (Gorensek *et al* 1990: Naraqi *et al* 1978: Chatterjee *et al* 1978). The most severe CMV infections occur in bone marrow transplant recipients (Meyers *et al* 1986: Stratta *et al* 1992). Congenital CMV infection has also been reported in the offspring of liver transplant recipients (Laifer *et al* 1995).

Stratta *et al* (1992), in a review article of CMV infection, associated with liver transplantation, reported that the prevalence of CMV infection in liver transplantation ranges from 35% to 60% with higher prevalences attributable to seropositive allografts and much lower prevalences attributable to reactivation and transfusion of blood products.

Infection most commonly occurs within the first few months following liver transplantation coinciding with the immediate period of maximal immunosuppression. (Naraqi *et al* 1978: Hersman *et al* 1982). The most common site of infection following liver transplantation is the transplanted liver graft. CMV hepatitis occurs in 4% of recipients of liver transplantation and may be severe enough to warrant retransplantation. In a retrospective cohort study, CMV infection occurred in 33% of recipients and CMV disease occurred in 19% (Hadley *et al* 1995). Over half of the liver transplant recipients who have CMV infection progress to CMV disease (Stratta *et al* 1992). CMV disease and recurrent CMV disease in particular, is usually associated with extrahepatic sites such as the gastrointestinal tract and the lungs (Naraqi *et al* 1978: Meyers *et al* 1986: Stratta *et al* 1992: Wiens *et al* 1993: Hadley *et al* 1995). The clinical management of CMV disease consists of early detection, variable reduction in immunosuppression, optimal nutritional and metabolic support, and drug therapy (Stratta *et al* 1992: Boudreaux *et al* 1993: Chen *et al* 1994: Schmidt *et al* 1995: Winston *et al* 1995).

2.5.4 Diagnosis of CMV infection

Diagnosis of CMV infection is made on the basis of the presence of any one of the following in the absence of clinical symptoms:-

- Isolation or identification of CMV from any site, including blood, urine, sputum or stool.
- Positive seroconversion displayed by either the presence of CMV-IgM or of a four-fold increase in CMV-IgG titres using complement fixation.
- Objective clinical findings *(Stratta et al 1992)*

The diagnosis of CMV disease is made when CMV infection is invasive or symptomatic in nature. This is then confirmed histologically or by positive CMV culture from a deep tissue specimen (Stratta *et al* 1992: Sido *et al* 1993). The development of CMV disease is related to viral load and also to the degree of immunosuppression. (Meyers *et al* 1986: Stratta *et al* 1992: Wiens *et al* 1993: Hadley *et al* 1995:)

Therefore, CMV infection following transplantation is due to either reactivation of latent virus, primary infection, or reinfection.

2.5.5 Risk factors for CMV

Risk factors for CMV following organ transplantation have been identified. These include type and intensity of immunosuppression, use of antilymphocyte globulin, cytotoxic drugs, corticosteroids, age, sex, race and underlying disease (Gorensek *et al* 1990). CMV and the immune system seem to exert reciprocal effects on each other. Indeed, CMV seems to act as an immunosuppressant and as such may interact with the allograft to induce dysfunction or provoke rejection (Stratta *et al* 1992: Briggs 1972: Naraqui *et al* 1978). Therefore, not only can CMV status act as an indicator of the net state of immunosuppression but also the presence of CMV disease can signify over immunosuppression (Stratta *et al* 1992).

The risk of CMV disease or infection is higher with primary rather than secondary infection (reactivation). Donor availability currently precludes the matching of

donor/recipient according to CMV status, thus children who are seronegative at transplant are at particular risk of CMV disease. In the Liver Unit at Birmingham Children's Hospital, 75% of children are seronegative at transplantation. The prevalence of CMV infection following liver transplantation is 38% (Davison *et al* 1993).

Thus, both cyclosporin and cytomegalovirus appear to influence or be influenced by the immune response. The paper by Epstein *et al* (1992) suggests that CMV influences cyclosporin-induced gingival overgrowth. It is an interesting hypothesis that should not be disregarded since concomitant CMV infection may be confounding studies that seek to determine the mechanism of cyclosporin induced gingival overgrowth.

2.6 Literature Summary

Liver transplantation is not only increasingly successful but is now available to babies under one year of age. Therefore, the population of liver graft recipients is going to include an ever larger number of infants and children. As such, liver transplantation presents new challenges to both the medical and dental professions. To date there have been very few studies into the effect of liver transplantation on the oral tissues of children with liver grafts.

2.6.1 Intrinsic green pigmentation

The majority of the children who receive liver grafts suffer from extra-hepatic biliary atresia but although intrinsic green pigmentation of the dental tissues in this population has already been reported the prevalence in the primary and permanent dentitions is, as yet, unknown.

The largest studies into intrinsic green pigmentation were carried out over thirty years ago and were mainly focused on subjects with rhesus incompatibility. The intensity of the pigmentation in these subjects has been reported not only to be variable between individuals and between teeth but also to improve with time. This information could be used to reassure the parents of children with liver grafts who have intrinsic pigmentation but these previous studies based their findings on subjective rather than objective measurement.

Although it has already been suggested that the deposition of the green pigment ceases on receipt of a liver graft, it is still unclear whether or not the pigmentation found in children with EHBA and metabolic liver disease is deposited in utero. The chronology of the development of the primary dentition could be used to date the onset of the staining and, by so doing, give an indication of the true physiological and pathological status of the foetal liver.

2.6.2 Enamel hypoplasia

Hyperbilirubinaemia and malabsorption of vitamins A and D might result in defects of the enamel and dentine and, indeed, enamel hypoplasia has been reported in relation to liver disease and liver transplantation. However, there are no epidemiological data on the prevalence of enamel hypoplasia in paediatric liver graft recipients.

2.6.3 Delayed eruption of the primary dentition

Delayed eruption of the primary dentition has already been reported in paediatric liver graft recipients but the study sample was small and if the criteria for the diagnosis of delayed eruption had included an elapse of a six month period beyond the eruption dates of Lunt and Law (1974) only one subject would have been affected.

Delayed eruption of the primary dentition has been found to be both related to somatic growth and adversely affected by malnutrition. Paediatric liver graft recipients have a particularly high nutritional demand but they also receive aggressive nutritional

support. Moreover, the somatic growth of children who receive liver grafts has been shown to 'catch-up' one year after liver transplantation and so one would expect any delay in the eruption of the primary dentition to be quickly reversed.

Paediatric liver graft recipients make up the youngest population in the world to be medicated with cyclosporin. Other drugs which cause gingival overgrowth have been reported in association with delayed eruption of both the primary and secondary dentitions. However, the likelihood that cyclosporin might also act as an aetiological factor in delayed eruption of the primary dentition has never been investigated.

The prevalence and aetiology of delayed eruption of the primary dentition in paediatric liver graft recipients is still to be investigated. The increasing number of babies receiving liver grafts will facilitate such an investigation for the first time.

2.6.4 Cyclosporin-induced gingival overgrowth

Cyclosporin therapy in liver graft recipients has to be tailored to each individual patient. The dose is measured according to age, liver disease, severity of disease and to the presence of complications such as infection. Cyclosporin is already known to have large inter and intra-individual bioavailability but this is even more pronounced in liver graft recipients. Moreover, the bioavailability in babies and in pre-school children is even more variable due to poorer absorption and the maintenance of optimal levels has to keep pace with growth.

The prevalence of cyclosporin-induced gingival overgrowth in paediatric liver graft recipients has been found to be high, between 86% and 100%. However, the previous studies into gingival overgrowth in this population have not only been few in number but they have also had very small samples. Since liver grafts are now available to very young children the effect of other variables on the prevalence and severity of gingival overgrowth such as the age of the child, the age at transplantation and the duration of cyclosporin therapy require investigation for the first time.

2.6.5 Cytomegalovirus

Infection is the major cause of morbidity and mortality following liver transplantation and the single most important pathogen is cytomegalovirus.

It has been shown that CMV and the immune system exert reciprocal effects on each other. Cyclosporin has also been shown to modify the immune response within the gingival tissues. It has been suggested that cytomegalovirus might have an influence on cyclosporin-induced gingival overgrowth.

Since there is already known to be a fine balance between the immune response and CMV infection in liver graft recipients, this population appears to offer the best opportunity to test this hypothesis.

3.0

Aims of the Investigation

The Null Hypotheses

3.1 Aims of the investigation

The aims of this clinical study are:

1. To report on the prevalence of intrinsic green pigmentation, enamel hypoplasia, delayed eruption of the primary dentition and cyclosporin-induced gingival overgrowth in children with liver grafts.
2. To measure the association between the trough cyclosporin concentration and concomitant nifedipine therapy on the presence and severity of gingival overgrowth.
3. To report on the severity, variability and distribution of intrinsic green pigmentation in the primary and permanent dentitions of paediatric liver transplant recipients.
4. To measure any change in the severity of the intrinsic pigmentation.
5. To evaluate the role of the previous liver disease, the presence of malnutrition, and medication with cyclosporin in the aetiology of delayed eruption of the primary dentition.
6. To investigate the effect of age, the age at transplantation and the duration of cyclosporin therapy on the prevalence and severity of gingival overgrowth.
7. To evaluate the influence of cytomegalovirus on cyclosporin-induced gingival overgrowth.

3.2 The null hypotheses

The null hypotheses in this study are as follows:

1. Children with liver grafts do not have an increased prevalence of intrinsic green pigmentation, enamel hypoplasia, delayed eruption of the primary dentition or cyclosporin-induced gingival overgrowth.

2. The distribution of the intrinsic green pigmentation is neither incremental nor deposited in utero and there will be no improvement in the severity of the green pigmentation with time.

3. Delayed eruption of the primary dentition is not related to the previous liver disease, malnutrition or cyclosporin therapy.

4. There is no association between the trough cyclosporin concentration and gingival overgrowth in paediatric liver graft recipients.

5. Cyclosporin-induced gingival overgrowth is not affected by the age of the child, their age at transplantation, or by the duration of cyclosporin therapy.

6. Cytomegalovirus does not influence the effect of cyclosporin on the gingival tissues.

4.0

Clinical Studies into the Oral Condition of Paediatric Liver Graft Recipients

Material, Method and Results

4.1 Introduction

This study was approved by the Medical Ethics Committee at Birmingham Childrens' Hospital.

A total of five clinical studies were undertaken:

- a cross-sectional study to investigate the prevalence of intrinsic green pigmentation, enamel hypoplasia, delayed eruption of the primary dentition and cyclosporin-induced gingival overgrowth in children with liver grafts.
- a clinical evaluation of the severity and chronology of intrinsic green pigmentation
- a controlled study to investigate the aetiology of delayed eruption of the primary dentition in liver graft recipients
- a report on the prevalence and severity of cyclosporin-induced gingival overgrowth when the following variables were rendered constant:

    (a) age at examination

    (b) age at transplantation

    (c) duration of cyclosporin therapy

- an investigation to examine the influence of cytomegalovirus on cyclosporin-induced gingival overgrowth

The children in each of the studies were examined by the author after verbal consent had been obtained from their parent. In four of the studies, the children were examined by the author when they attended the Liver Unit for their regular out-patient medical review. In the remaining study, the evaluation of the aetiology of delayed eruption of the primary dentition, examinations were carried out on children who were attending both the Liver Unit out-patient clinic and associated gastroenterology and dietetic clinics.

All of the studies were prospective except the final evaluation into the effect of CMV infection on cyclosporin-induced gingival overgrowth. In this study the CMV status was matched retrospectively after the dental examination had already been performed. In this way the investigator was blind as far as the CMV status until after the dental examinations had been performed.

The Liver Unit is a supra-regional centre and so children and their parents travel from all over the country to attend the out-patient clinic. Therefore, collection of the data for these studies was dependent upon the liaison between the author and Graham Gordon, the co-ordinator of the Out-patient Liver Unit Clinic. It was he who then directed the children and their parents towards the dental clinic, between visits to the paediatricians, phlebotomist, dietician, physiotherapist and social worker!

The Liver Unit has a data base, which became computerised during the course of this study, to which the author was given unlimited access. This data base contains all of the laboratory investigations that are carried out on each liver graft recipient. The cyclosporin concentrations are closely monitored and blood samples are generally taken during the out-patient visits, the results are later logged onto this data base. This allowed the author to retrospectively match the cyclosporin concentrations to the dental examinations.

4.2

Cross-Sectional Study into the Oral Condition of Paediatric Liver Graft Recipients

4.2.1 Study sample

Fifty-five children (32 girls and 23 boys) were examined over an 18 month period. The mean age was 56 months (range 8 months to 194 months, median 42 months).

4.2.1.1. Diagnosis prior to transplantation

The diagnosis prior to transplantation for children in the study is shown in Table 5 following.

Table 5

Diagnosis Prior to Liver Transplantation

Diagnosis prior to liver transplant	*Number of Children*
Extrahepatic biliary atresia	24
Fulminant hepatitis	12
Alpha 1 antitrypsin deficiency	6
Tyrosinaemia	5
Cryptogenic cirrhosis	3
Hepatoblastoma	2
Allegilles syndrome	1
Primary oxalosis	1
Wilson's disease	1

4.2.2 Methodology

4.2.2.1 Data collection

Children were examined in the dental unit either in the dental chair or on their mother's knee, where appropriate. A dental light source was used whenever possible but, for a minority of the children, a fibre-optic pen torch was used.

The following information was collected:

Medical and transplant history :

- *age and sex of patient*
- *age at transplant*
- *time post transplant*
- *drug history* :in respect of cyclosporin, nifedipine and steroid therapy.
- *serum cyclosporin concentration*: the mean calculated from the blood sample taken on the day of the examination and on the concentrations recorded from the previous two out-patient visits.

The Dental Examination:

- *teeth present* :all erupted or partially erupted teeth were charted as present. A tooth was considered to be delayed when there was a delay of greater than six months outwith the normal eruption dates according to (Lunt and Law 1974) which is shown in Table 6
- *hypoplastic defects* :using the Thylstrup and Fejerskov Index (1978) which is shown in Table 7
- *intrinsic staining* :scored as being present or absent.
- *gingival overgrowth*: measured using an Index of Severity of Gingival Overgrowth, shown in Table 8. A score was allocated which related to the degree of tooth coverage caused by gingival overgrowth. All teeth were examined and the final score was that which reflected the most severe gingival overgrowth found in each subject.

Table 6

Eruption Times Of The Primary And Permanent Dentitions

	Tooth	Eruption Date
		months
MAXILLA	**A**	10 (8-12)
	B	11 (9-13)
	C	19 (16-22)
	D	16 (13-19)
	E	29 (25-33)
MANDIBLE	**A**	8 (6-10)
	B	13 (10-16)
	C	20 (17-23)
	D	16 (14-18)
	E	27 (24-30)
		Years
MAXILLA	**1**	7 - 8
	2	8 - 9
	3	11-12
	4	10-11
	5	10-12
	6	6 - 7
	7	12-13
	8	17-21
MANDIBLE	**1**	6 - 7
	2	7 - 8
	3	9 -10
	4	10 -12
	5	11 -12
	6	6 - 7
	7	11-13
	8	17-21

Lunt & Law 1974

Table 7

Thylstrup & Fejerskov Index

Score	Clinical Description
1	narrow white lines
2	pronounced white lines
3	cloudy areas
4	entire surface opaque
5	opacity and pits
6	horizontal bands of pits
7	outer enamel lost <half surface
8	outer enamel lost >half surface
9	changes in anatomy

Table 8

Index Of Severity Of Gingival Overgrowth

Score	Clinical Description
0	No observable overgrowth
1	Overgrowth of gingival tissue extending to 1/3 of the crown
2	Overgrowth of gingival tissue extending to middle of crown
3	Overgrowth of gingival tissue extending over 2/3 of the crown
4	Overgrowth of gingival tissue completely covering the crown

4.2.2.2 Statistical analysis

Data were analysed using the SPSS PC statistics package. The association between the level of gingival overgrowth and the trough cyclosporin concentration was examined using Analysis of Variance (ANOVA). The association between the presence of gingival overgrowth and concurrent nifedipine medication was examined using the Chi-square Test. Pearson's Product Moment Correlation Coefficient was used to quantify the association between the duration of cyclosporin therapy and the level of gingival overgrowth. A level of $p<.01$ was accepted as statistically significant.

4.2.3 Results

The mean time that the children in the study had been in receipt of a liver graft was 1year 5months (range 1 month to 5 years). Their mean age at transplantation was 3yrs 4 months (range 6 months to 14 years 6 months). Table 9 gives the composite findings related to delayed eruption, enamel hypoplasia, intrinsic staining and gingival overgrowth.

Table 9

Dental Findings In Children With Liver Grafts

Stage of Dental Development	Number of children
primary dentition	42
permanent dentition	2
mixed dentition	11

Dental Finding	Teeth affected	Number of children examined	Number of affected children
Delayed eruption	Primary	42	7
	Permanent	13	0
Enamel hypoplasia	Primary (incisors)	42	3
	Permanent (incisors)	13	3
Intrinsic staining	Primary (all teeth)	42	25
	Permanent	13	1*
Gingival overgrowth	___	55	30

* early mixed dentition

4.2.3.1 Delayed eruption of the primary dentition

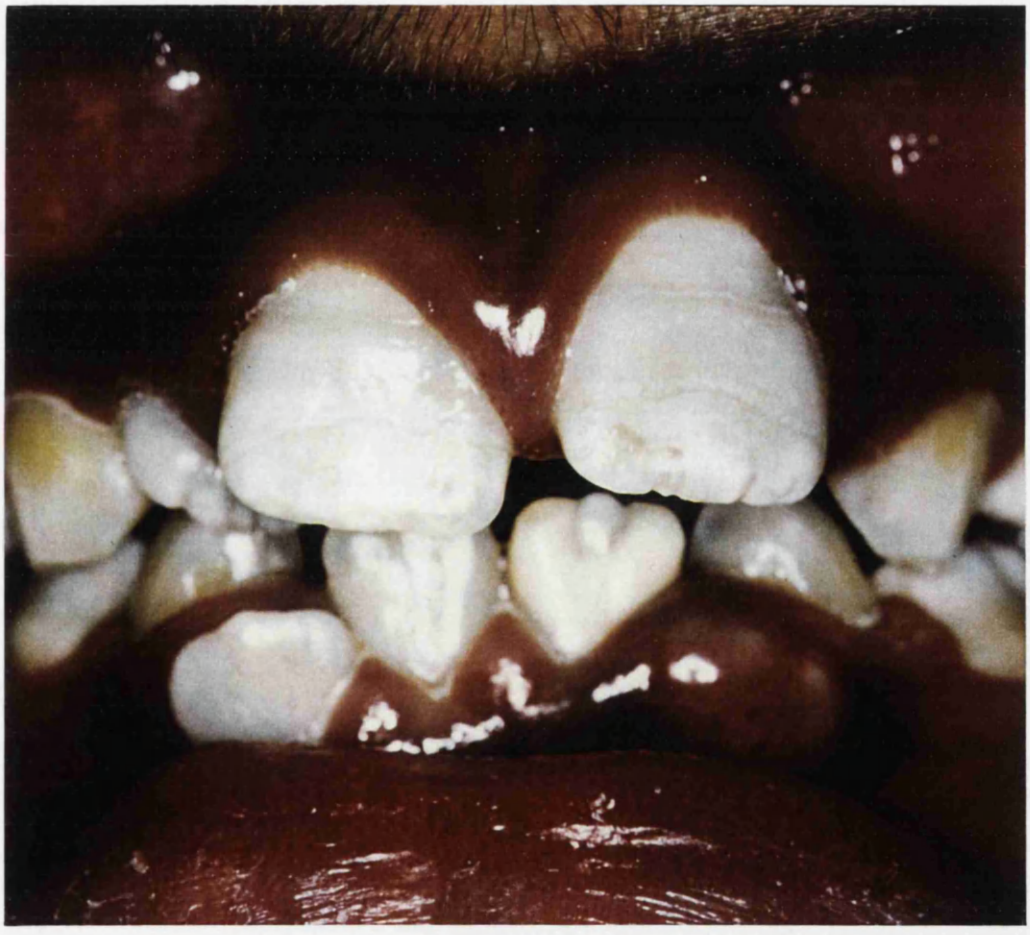
There were 17 children under 36 months of age. For 7 (41%) of these infants, eruption of various primary teeth was delayed, that is delay of greater than 6 months outwith normal eruption dates (Lunt and Law 1974: Proffit 1983). For example, three of these children were 20 months of age and only had some or all of their primary central incisors. There was no delay in eruption found in children above 3 years of age. All seven of these children had suffered from biliary atresia and six of them had been medically diagnosed as having 'failed to thrive'.

4.2.3.2 Enamel hypoplasia

Six of the fifty-five patients (11%) had evidence of enamel hypoplasia. Three of these had enamel hypoplasia affecting permanent central incisors, two scored "1" on the Thylstrup and Fejerskov Index and one scored "2". The hypoplastic areas corresponded to the stage of development of these teeth at the time of transplantation. The other three children had hypoplastic defects scoring "1" on the primary central incisors. The two children with hypoplasia of the permanent dentition were both transplanted following mineralisation of the affected permanent teeth and the hypoplasia appeared to be attributable to enamel mottling, especially since both of these children had always lived in a fluoridated area. An illustration of one of these children is shown in Figure 2 overleaf.

Figure 2

A 7 year old child, 3 years post OLT with enamel hyploplasia of the permanent incisors which scored '2' on the Thylstrup & Fejerskov Index.



4.2.3.3 Intrinsic green pigmentation

Twenty-five patients (49%) had intrinsic green pigmentation of the primary dentition but 23 of these had previously been diagnosed as having biliary atresia. All of the primary teeth in the 25 children were found to be affected but the primary first and second molars were more deeply stained than the incisors and canines. One patient had intrinsic staining affecting permanent incisors but she had been transplanted at the age of 3. This was relatively late compared to the others with biliary atresia and so she had remained jaundiced for much of her infancy.

4.2.3.4 Gingival overgrowth

Thirty (55%) of the fifty-five children had evidence of gingival overgrowth. Serum cyclosporin levels ranged from 0 to 342 ng/ml with a mean 152 ng/ml. No significant relationship was found between the circulating cyclosporin level and the presence of gingival overgrowth using analysis of variance ($F= 0.69$, $p =0.78$). There was a significant inverse correlation using Pearson's Product Moment Correlation Coefficient between the duration of cyclosporin therapy and gingival overgrowth $r =-0.33$ ($p>.01$).

Fourteen patients were taking nifedipine in addition to cyclosporin at the time of the dental examination. The mean time they had been taking nifedipine was 4 months (range 1 to 12 months). No significant relationship was found between the presence of gingival overgrowth and concomitant nifedipine medication (Chi-square 0.17, $p=0.77$).

Of the 34 children who had gingival overgrowth, 11 had a severity score of '1', 17 scored '2' and 6 scored '3'. These findings are shown as frequencies in Figure 3 overleaf. The mean trough cyclosporin concentration in relation to the severity of the gingival overgrowth in this sample is shown in Figure 4 overleaf.

Illustrations of two of the children examined as part of this study are shown in Figure 5

Figure 3

Gingival Overgrowth Severity in Paediatric Liver Graft Recipients

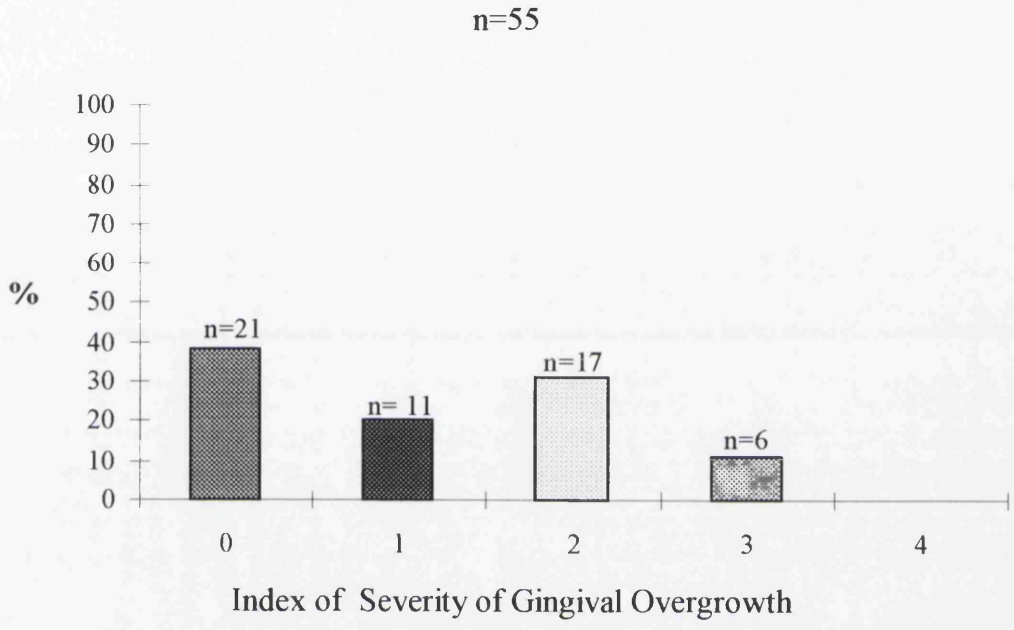
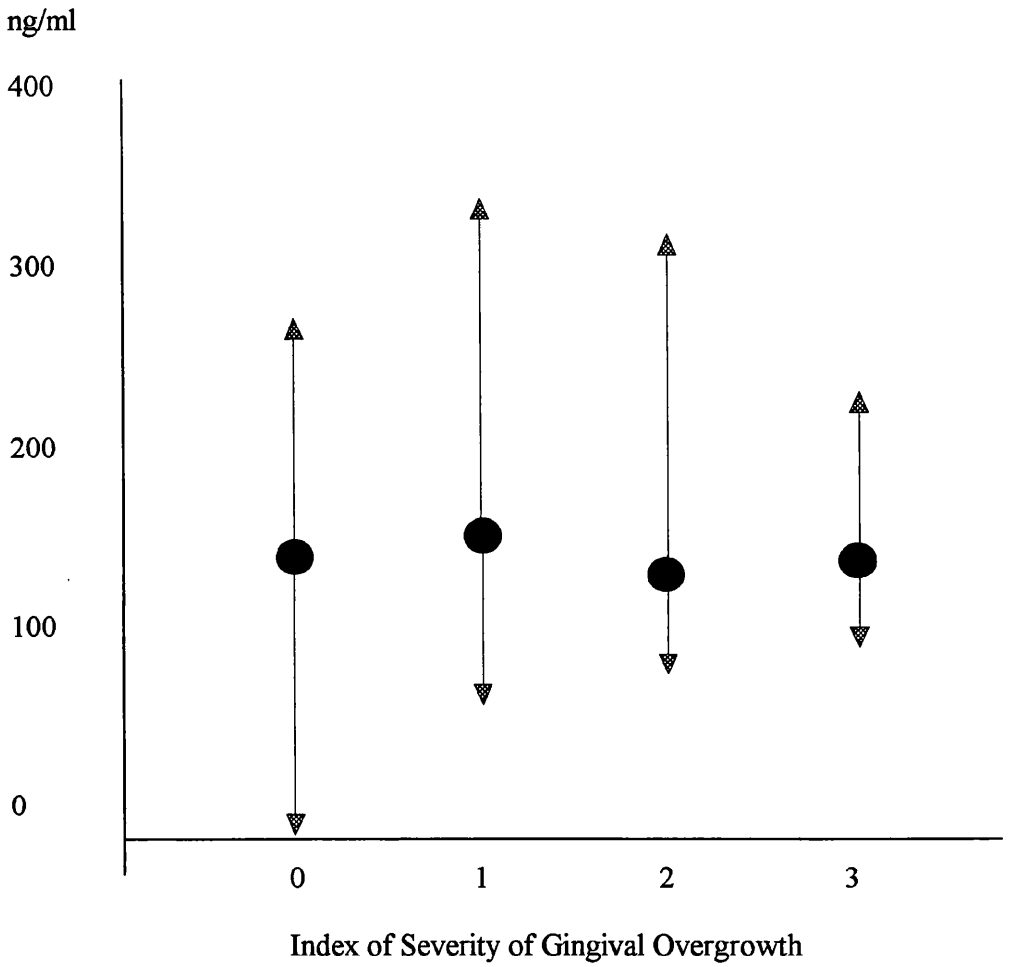


Figure 4

Trough Cyclosporin Concentration Compared to Severity of Gingival Overgrowth



The black circle represents the mean trough cyclosporin concentration within each group of children who scored '0', '1', '2' and so on using the Index of Severity of Gingival Overgrowth scale. The grey arrows represent the trough cyclosporin concentration range within each group.

Figure 5

Some of the children examined as part of this clinical study.

(A) 5 year old child, 3 years post OLT

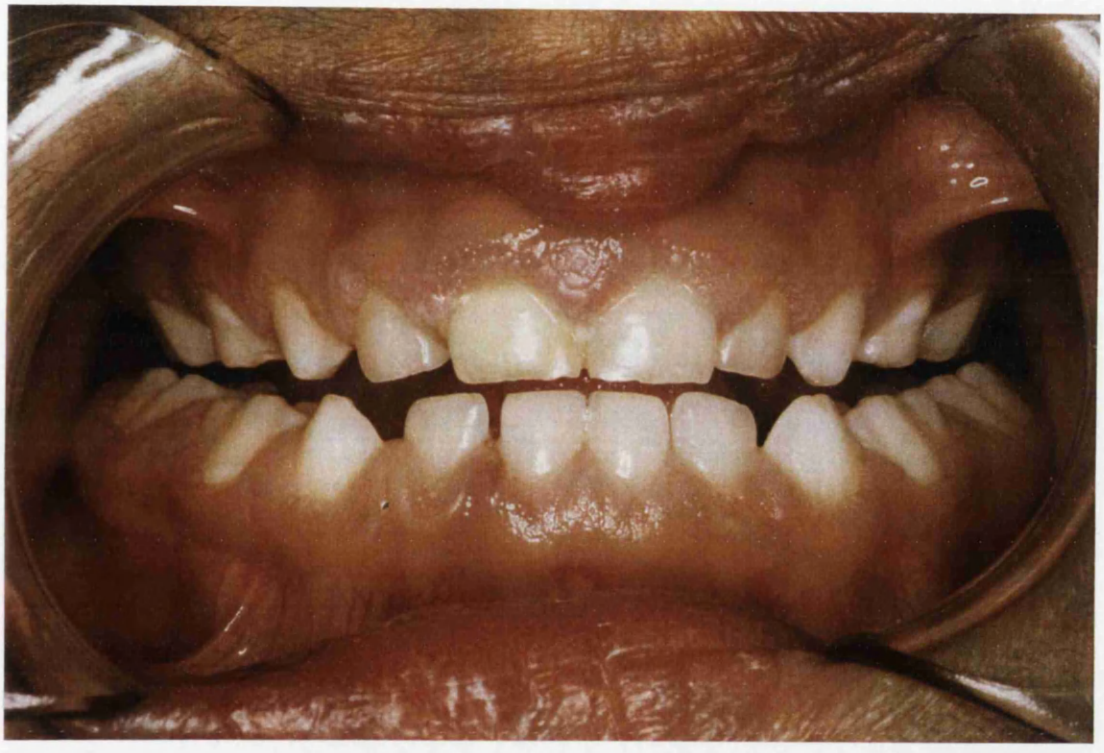
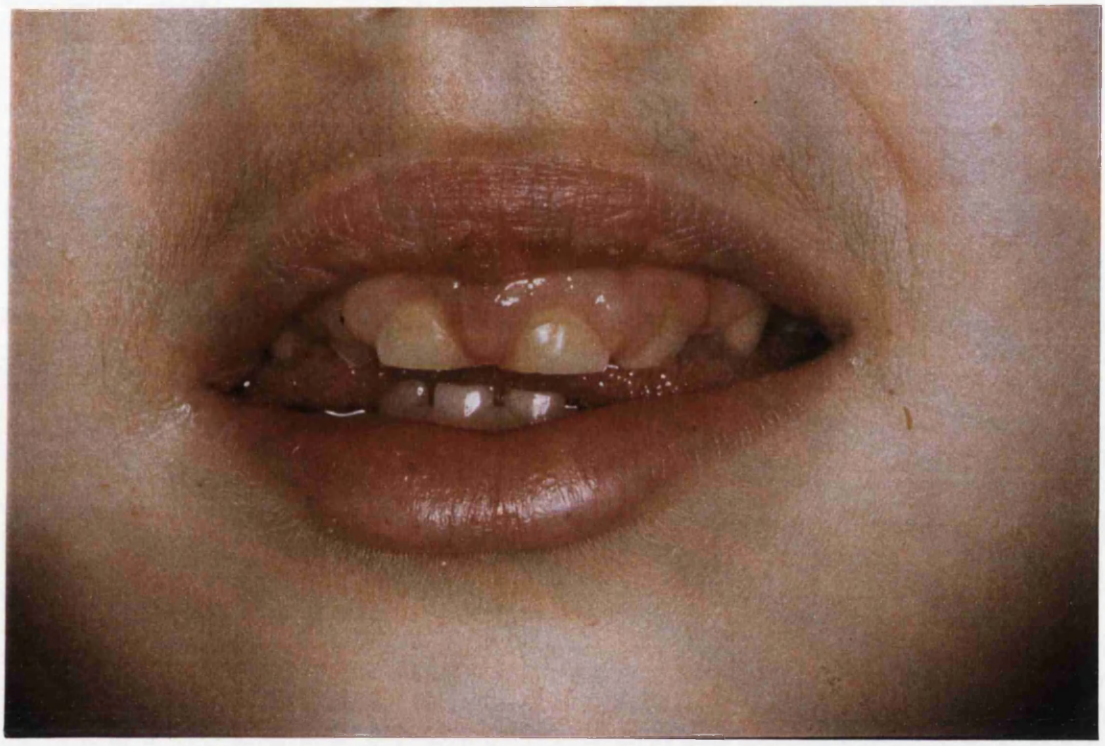


Figure 5

(B) 4 year old child, 2 years post OLT



4.2.4 Reproducibility of the gingival overgrowth severity evaluation

4.2.4.1 Method

During the course of this study the author arranged to re-examine as many children as possible within three months of their first examination. Despite the distance travelled and their infrequent attendance at the Liver Unit, reproducibility data from a sample of 13 children were obtained. This represented 24% of the original study sample.

4.2.4.2 Sample

Table 10 below summarises the demographic information in the study sample.

Table 10

Reproducibility Study Sample

sex M:F	9 : 4
mean age	63 months
duration of cyclosporin therapy	23 months
age at OLT	30 months
mean cyclosporin conc.	177 ng/ml

4.2.4.3 Result

Cohen's Kappa statistic was calculated to measure agreement between the gingival overgrowth severity scores that were obtained at the two examinations. The reproducibility in the gingival overgrowth severity scores was 0.45, indicative of moderate agreement (Landis and Koch 1977).

4.3

An Investigation into Intrinsic Dental Pigmentation in Children with Liver Transplants

4.3.1 Introduction

The aim of this study was to examine the severity, distribution and chronology of intrinsic green pigmentation both in the primary and permanent dentitions and to measure whether the colour of the intrinsic pigmentation reduced with time.

This study was divided into three discrete investigations:

1. A clinical study in which the severity of the intrinsic pigmentation was measured in both liver graft recipients with full primary dentitions and in liver graft recipients with permanent incisors and first molar teeth. A colour scale which was specially developed for the purpose of this study was used.
2. A longitudinal investigation evaluated the severity of the green pigmentation over a three year period.

The reproducibility of the data obtained using the colour scale was also reported.

4.3.2 Clinical study into the severity of the green pigmentation

4.3.2.1 Methodology

The dental examinations were carried out over a four year period, a total of 97 children with liver grafts were examined. Of these, only 37 were found to have intrinsic pigmentation of the dental hard tissues. These children came from different parts of the country and some attended the Liver Unit out-patient clinic infrequently.

The following study groups of children were identified:

- children with a full primary dentition
- children in the early mixed dentition

Children with intrinsic pigmentation who did not satisfy the criteria for inclusion into the primary dentition study group above but who continued to be examined in order to obtain further data which could be used both in the evaluation of the change of colour with time and in the examination of the reproducibility of the Intrinsic Green Pigmentation Severity Scale.

The following information was collected for both study groups:

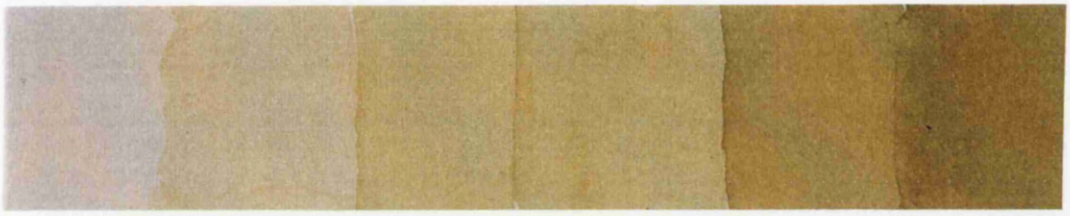
- diagnosis prior to transplant
- the severity of the 'greenness' was measured on a newly developed colour scale which is shown in Figure 6 overleaf. Using this colour scale each individual tooth in each subject was given a 'severity of greenness' score ranging from '0' to '5'.

The intrinsic green pigmentation severity scores for each primary tooth in each child who had a full primary dentition were then summed together to give an overall score.

In the mixed dentition group, the intrinsic green pigmentation severity score for each erupted permanent molar and permanent incisor in each child was noted but not summed.

Figure 6

Severity Of Intrinsic Green Pigmentation Colour Scale



0 1 2 3 4 5

- *3 parts vermilion* in 1 part water*
- *4 parts sap green** in 1 part water*
- *1 part cadmium yellow*** 1 part water*

1 part water added to the above to give the colour code for score '5'

an additional 1 part water was added to produce score '4'

then an additional 1 part of water was added to produce score '3' and so on.
(Daler-Rowney watercolour codes: *588; **375; ***620)

4.3.2.1.1 Study sample: intrinsic green pigmentation in primary teeth

Twenty children (9 males and 11 females) with intrinsic green stain were examined. Their mean age was 56 months (range 30 months to 82 months, median 56 months). The children in this sample had received a liver graft at a mean age of 18 months (range 7 months to 54 months, median 11 months). Seventeen of the children had suffered from extrahepatic biliary atresia, two had alpha-1-antitrypsin deficiency and the remaining child had sclerosing cholangitis.

4.3.2.1.2 Study sample: intrinsic green pigmentation in permanent teeth

Seven children (3 males and 4 females) were examined. Their mean age was 107 months (range 82 months to 138 months, median 108 months). The children in this sample had received a liver graft at a mean age of 36 months (range 7 months to 114 months, median 23 months).

4.3.2.2 Results

4.3.2.2.1 Intrinsic green pigmentation in the primary dentition

The mean green pigmentation score was 38. The scores ranged from '8' to '62'. The results confirmed that there was indeed a wide degree of variation between individual children.

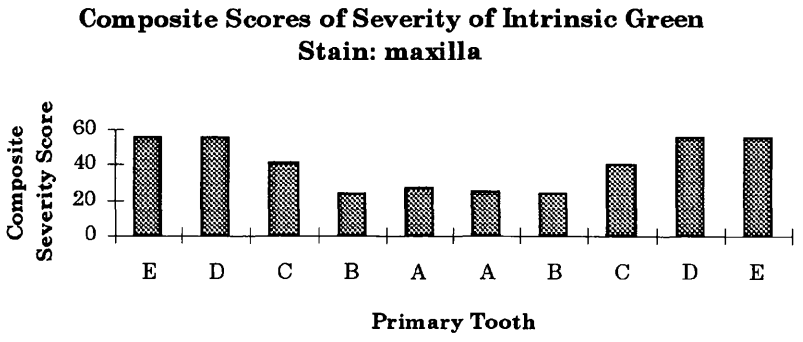
Children with extrahepatic biliary atresia did not appear to have any more severe green pigmentation than children who had suffered from alpha-1-antitrypsin deficiency or sclerosing cholangitis. Seventeen of the children had EHBA and the mean pigmentation severity score was 38. Of the remaining 3 children, two had alpha-1-antitrypsin deficiency and had a mean pigmentation severity score of 40. The remaining child had sclerosing colangitis and also had a mean score of 40.

A greater degree of variation in the severity of the green pigmentation was found within individual children. In some of the subjects, the cervical margins of the primary incisor teeth were more deeply stained than the incisal edge. The primary molar teeth were found to be more severely stained than the canines and incisors. The primary molars had a mean score of '3', compared to the canines which had a mean score of '2' and the primary incisors which had a mean score of 1.2. To highlight this intra-individual variation further, the sums of all of the children's scores of severity in relation to each primary tooth are shown in the following Figure 7 (a) and (b).

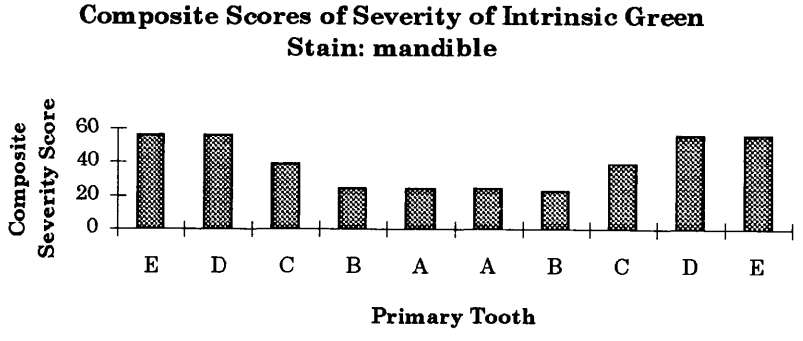
Figure 7

Composite Intrinsic Green Stain Severity Scores In Relation To The Affected Primary Teeth

(a)



(b)

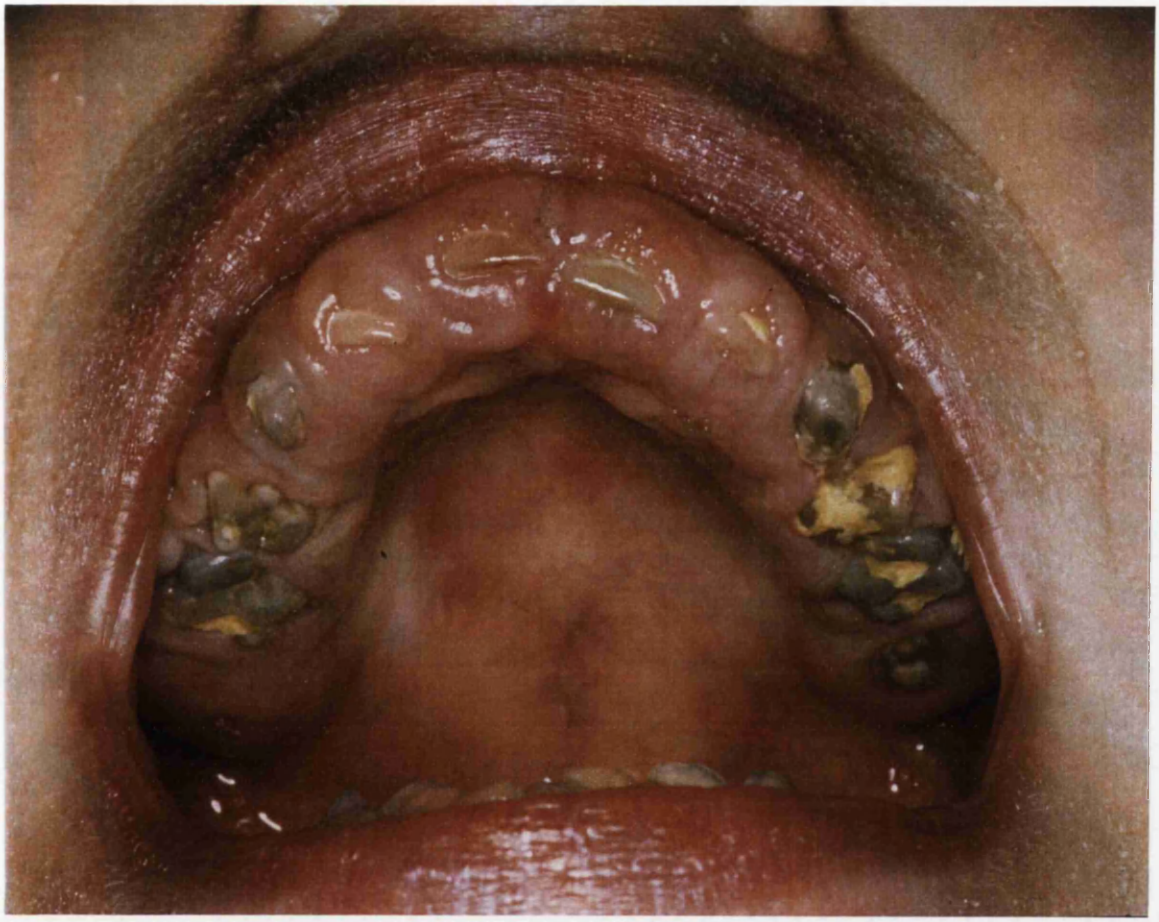


4.3.2.2.2 Intrinsic green pigmentation in the permanent dentition

There was greater variation in the severity of the green pigmentation amongst the seven liver graft recipients who were in the early mixed dentition. The intrinsic pigmentation ranged from all of the teeth scoring '1' to all scoring '5'. However, in these the molar teeth were found to be only slightly more severely stained than the incisors. One of the affected children is shown in Figure 8 overleaf.

Figure 8

Photograph of a child in early mixed dentition with intrinsic green pigmentation



This 7 year-old girl previously suffered from biliary atresia but was not transplanted until she was 3 years old. She had the worst intrinsic staining, oral hygiene and gingival overgrowth in the entire study

4.3.3 Changes in intrinsic green pigmentation in the primary dentition

4.3.3.1 Methodology

Nine children with intrinsic green pigmentation were re-examined three years after their first examination. The teeth that were present were again scored using the Intrinsic Green Pigmentation Severity Scale. A total of 93 teeth were examined, these were predominately primary canine and primary molar teeth.

4.3.3.1.1 Study sample

There were 3 males and 6 females. Their ages at the first examination ranged from 10 months to 5 years 7 months, mean 2 years 10 months. All of the children had extra hepatic biliary atresia. The children had received a liver graft between the ages of 6 to 16 months of age.

4.3.3.1.2 Data analysis

The Wilcoxon Rank Pair test was used to compare between the summed severity scores of each individual at the two examinations.

4.3.3.2 Results

The statistical analysis failed to show any difference between the intrinsic green pigmentation scores obtained at the two examinations. Therefore, the results of this study suggest that the severity of the green pigmentation does not improve with time.

4.3.4 Reproducibility using the intrinsic green pigmentation scale

Ten children in the primary dentition, were re-examined within 6 months of the initial examination. Cohen's Kappa statistic was calculated to enable the measure of agreement obtained on the data derived from the colour scale to be measured against that which would have been obtained at random.

The study examined both the reproducibility of the severity of the intrinsic pigmentation in the primary incisors and the reproducibility of the severity of the intrinsic pigmentation in the primary molars. Any child who had caries or hypoplasia in the relevant teeth was excluded.

2.3.4.1 Reproducibility in primary incisors

The teeth of eight children were included. The kappa score in this study was found to be 0.33 indicative of poor agreement.

2.3.4.2 Reproducibility in primary molars

The teeth of 7 children were evaluated. The kappa score was found to be 0.6, indicative of substantial agreement.

4.4

Delayed Eruption of the Primary Dentition in Paediatric Liver Transplant Recipients

4.4.1 Introduction

This was a prospective controlled study into delayed eruption of the primary dentition in paediatric liver graft recipients. The aim of this study was to evaluate the contribution of the underlying liver disease, malnutrition and cyclosporin therapy in the aetiology.

4.4.2 Study sample

A total of 84 children between the age of 14 months to 36 months were examined by the author, after parental consent was obtained, between January 1994 and May 1996. Fourteen children were later excluded after they were found to be medicated with either corticosteroids or nifedipine. None of the subjects were medicated with phenytoin. The study sample contained 39 boys and 31 girls.

4.4.3 Methodology

All of the children were examined using dental mirror and a pen torch.

The examination into the prevalence and aetiology of delayed eruption of the primary dentition was evaluated using two methods:

- by determination of the prevalence of delayed eruption in paediatric liver graft recipients compared to control groups with and without underlying liver disease with malnutrition.
- by comparison between the number of erupted teeth in age- matched pairings between subjects from different study groups.

4.4.3.1 Measurement of the prevalence of delayed eruption in study and control groups

The children were grouped as follows:

I. Liver transplant recipients, medicated with cyclosporin, who have malnutrition

II. Liver transplant recipients, medicated with cyclosporin, who are not malnourished

III. Children with liver disease and malnutrition

IV. Children with liver disease with no malnutrition

V. Children with malnutrition with a cause other than liver disease.

A summary of the demographic information from each of the afore-mentioned study groups is shown in the following Table 11.

Table 11

Summary Of Descriptive Information In Each Group

Descriptive statistics of subjects in each group	*I*	*II*	*III*	*IV*	*V*
number in each group	7	17	16	21	9
mean age (months)	23	26	23	25	22
mean duration of cyclosporin therapy (months)	11	12	-	-	-
mean age at OLT	11	13	-	-	-
mean trough serum cyclosporin level (ng/ml)	151	178	-	-	-

4.4.3.2 The number of erupted teeth in age- matched subjects

Children from the following groups were then paired as follows:

a) Liver transplantation compared to liver disease (n=17).

b) Liver transplant recipients with and without malnutrition (n=7).

c) Liver disease with and without malnutrition (n=6).

d) Liver disease with malnutrition compared to subjects with malnutrition attributable to a cause other than liver disease (n=7).

4.4.4 Data collection

The nutritional status of the child was determined using Tanner-Waterhouse growth charts (1972) . A child was considered to be malnourished when they were on or below two standard deviations of the normal population height and growth curves, i.e. on or below the third percentiles, at the time of the dental inspection.

The mean of the last three circulating trough cyclosporin levels was noted for those subjects who had received liver grafts.

A primary tooth was scored as present if any part if the crown had emerged through the oral mucosa. A child was considered to have delayed eruption if there was more

than six months delay in primary tooth eruption compared to the modified chronological tables of Lunt and Law (1974). The appropriate section is summarised in the following Table 12.

Table 12

Chronological Table Of Primary Tooth Eruption

	Maxilla	Mandible
central incisor	10	8
lateral incisor	11	13
canine	19	20
1st molar	16	16
2nd molar	29	27

4.4.5 Statistical analysis

The prevalence of delayed eruption within groups I, II, III, IV and V was reported. The effect of liver transplantation on the eruption of the primary dentition was measured using the paired t-Test to compare the number of teeth present between subjects with liver grafts without malnutrition and liver disease without malnutrition.

The relationship between the number of erupted teeth and the mean circulating trough cyclosporin level in subjects who had received liver grafts was analysed using Multiple Regression Analysis.

4.4.6 Results

4.4.6.1 Prevalence of delayed eruption in study and control groups

The prevalence of delayed eruption of the primary dentition in liver graft recipients whether they were malnourished or not, was high, at 43% and 47% respectively.

The prevalence of delayed eruption in each group is summarised in Table 13 below and the relative frequency of delayed eruption within each group is shown diagramatically in Figure 9 overleaf.

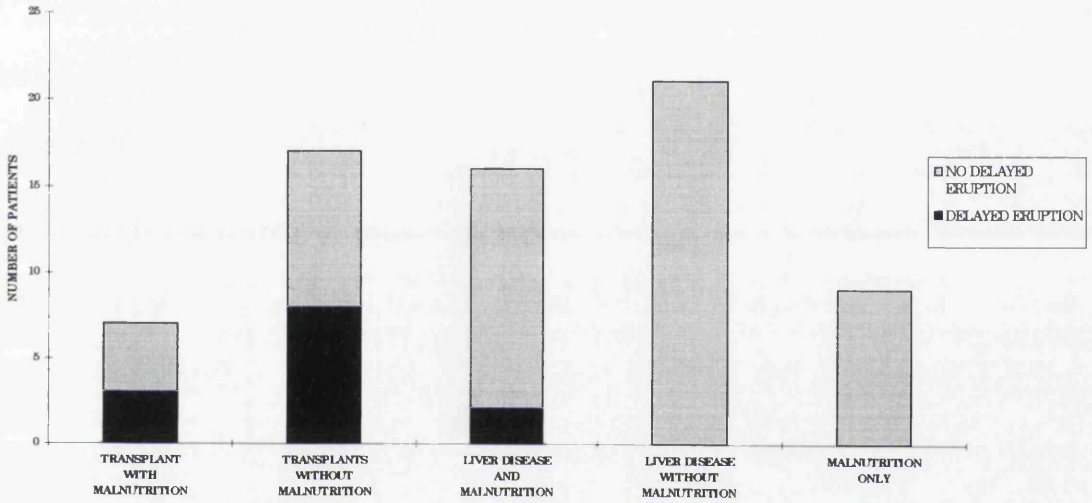
Table 13

The Prevalence Of Delayed Eruption In Each Study Group

	Group	*Number of Subjects*	*Number with Delayed Eruption*
I	OLT with malnutrition	7	3
II	OLT, no malnutrition	17	8
III	Liver Disease and malnutrition	16	2
IV	Liver Disease, no malnutrition	21	0
V	Malnutrition controls	9	0

Figure 9

The Prevalence Of Delayed Eruption Of The Primary Dentition In Each Group



4.4.6.2 Comparison between OLT recipients with and without delayed eruption

Those children with delayed eruption of the primary dentition had not only been in receipt of a liver graft for a much longer period of time but had also been transplanted at an earlier age than those without delayed eruption. However, the mean trough serum cyclosporin concentrations were lower in the delayed eruption groupings. Furthermore, the presence of malnutrition appeared to have no effect. The comparison between the paediatric liver graft recipients who did have delayed eruption of the primary dentition, compared to their peers who did not, in relation to age, age at transplant, duration of cyclosporin therapy and trough cyclosporin level is shown in Table 14 overleaf.

Table 14

Comparisons Between Liver Graft Recipients With and Without Delayed Eruption

GROUP	N	Delayed eruption	Mean age (months)	Age at OLT (months)	Cyclosporin therapy duration (months)	Mean trough serum cyclosporin (ng/ml)
OLT with malnutrition	3	**Y**	23	6	17	126
	4	**N**	24	16	8	169
OLT without malnutrition	8	**Y**	28	12	16	143
	9	**N**	25	14	9	209

4.4.6.3 Comparison between the number of erupted teeth in age-matched subjects

When the number of erupted teeth was compared in the age-matched pairings the children with liver grafts were found to have less teeth than those with liver disease. This was confirmed by the paired t-test which showed that there was a significant difference between the number of erupted teeth in children with liver grafts compared to children with liver disease, $t=2.46$, $p=0.013$ (one tailed).

Due to the small size of the remaining paired groups no further statistical analysis was performed. However, the following results were also found:

- In liver graft recipients, there was no difference in the number of erupted teeth when those who were malnourished were compared to those who were not.
- Children with liver disease and malnutrition had more teeth than those with liver disease who were not malnourished.
- The children with liver disease and malnutrition had less teeth than those with malnutrition attributable to another cause.
- There was no relation found between the number of erupted teeth present and the circulating cyclosporin level in the two groups of children who had received liver transplants.

The detailed comparison between the number of erupted teeth in the age-matched subjects is summarised in Table 15 overleaf.

Table 15 Comparison Between The Number Of Erupted Teeth In Age-Matched Groups

Liver transplant recipients compared to children with liver disease

	Age-matched group	number	mean age (months)	mean no. teeth	cyclosporin duration	age at OLT
(a)	**Liver transplant**	17	26	13	12 months	13
	Liver disease			16	-	-

Liver graft recipients with malnutrition compared to those without

	Age-matched group	number	mean age (months)	mean no. teeth	cyclosporin duration	age at OLT
(b)	**Liver transplant with malnutrition**	7	23	11	11.5 months	11.5
	Liver transplant without malnutrition			11	11 months	12

Children with liver disease and malnutrition compared to those without

	Age-matched group	number	mean age (months)	mean no. teeth	cyclosporin duration	age at OLT
(c)	**Liver disease with malnutrition**	6	23	14	-	-
	Liver disease without malnutrition			10	-	-

Liver disease with malnutrition compared to the malnutrition control group

	Age-matched group	number	mean age (months)	mean no. teeth	cyclosporin duration	age at OLT
(d)	**Liver disease with malnutrition**	7	21	8	-	-
	Malnutrition only			12	-	-

4.4.6.4 Relation between tooth number and the trough cyclosporin concentration

Multiple regression analysis just failed to show an association between the number of teeth and the trough cyclosporin concentration (R=0.47, F=4.1, significant F=0.06).

Therefore, the association between the trough cyclosporin concentration and the number of erupted teeth was re-analysed using ANOVA with age as the covariant. The association between the number of teeth and the trough cyclosporin level was highly significant when age was controlled statistically (F=5.1 p=0.01) in the children with liver grafts who were not malnourished.

There was no significant relationship found between the trough cyclosporin concentration and the number of erupted teeth in the seven children with liver grafts who were malnourished.

4.5

The Effect of Age, Age at Transplantation and Duration of Cyclosporin Therapy on Gingival Overgrowth in Children with Liver Grafts.

4.5.1 Introduction

In view of the variation in cyclosporin bioavailability with age and the large number of babies and infants who have received liver grafts, it is clear that cyclosporin-induced gingival overgrowth in relation to age, age at transplantation and duration of therapy merits further investigation. Although the cross-sectional study, which forms the first part of these clinical investigations, was the largest of its kind ever published, the variables in relation to age, age at transplantation and duration of cyclosporin therapy were not controlled.

The aim of this prospective study was to investigate the effect of the age of the child, their age at transplantation and the duration of cyclosporin therapy on the prevalence and severity of gingival overgrowth.

4.5.2 Study sample

A total number of 184 examinations were carried out in 97 children over a five year period. There were 52 females and 45 males and their ages ranged from 6 months to 17 years.

4.5.3 Methodology

A dental examination was carried out, by the author, on paediatric liver transplant recipients when they attended the Liver Unit for an out-patient review. Children who had no erupted teeth, or who were medicated with Neoral cyclosporin or FK506 were later excluded.

Children were examined in the dental unit using a dental light source, with the exception of babies who were examined on their parent's knee with either a dental light or a pen torch.

4.5.4 Data collection

The following information was collected:

- age and sex of patient
- age at transplant
- time post transplant
- concomitant nifedipine therapy
- serum trough cyclosporin concentration, calculated from that taken on day of examination and on the levels from the previous two blood tests.
- gingival overgrowth was scored as being present or absent in each subject. The severity of the gingival overgrowth was then scored according to the Index of Severity of Gingival Overgrowth as previously described.

The 184 examinations were then grouped as follows:

- Age-bands according to age at the time of the dental examination
- Age-bands according to age at the time of transplantation
- Time-bands by duration of cyclosporin therapy

Only the last examination recorded if the same subject was examined more than once within each study band.

4.5.5 Statistical analysis

Data were analysed using both the Microsoft EXCEL and SPSSPC statistics packages. The association between the level of gingival overgrowth and the trough cyclosporin concentration was examined using Analysis of Variance (ANOVA). A level of $p<.05$ was accepted as statistically significant. Within each study group, Pearson's Product Moment Correlation Coefficient was used to evaluate the association between the age of the patients at the time of the dental examination, their age at transplantation and the duration of cyclosporin therapy with the trough cyclosporin concentration and the severity of gingival overgrowth.

The two sample t-Test was applied, when there was a sufficiently large number of subjects, to measure the association between the trough cyclosporin concentrations in those subjects who had gingival overgrowth compared to those who did not. The null hypothesis was that there should be no difference between the two groups. A similar analysis was applied to assess the relationship between the presence of gingival overgrowth in those who were medicated with nifedipine compared to those who were not.

4.5.6 Results

A summary of the demographic information in each age band, age at liver transplantation and duration of cyclosporin therapy is shown in Table 16 overleaf.

AGE BANDS : ACCORDING TO AGE AT THE TIME OF THE DENTAL EXAMINATION

Age Band	number of subjects	M : F	No. with gingival overgrowth	No. on nifedipine	No. on nifedipine with gingival overgrowth	Mean Age at OLT in months
< 1 year	9	1:8	3 (33%)	5 (56%)	2	7
>1-2 yrs	21	8:13	10 (50%)	10 (50%)	6	10
>2-5 yrs	18	7:11	7 (39%)	2 (11%)	0	29
5-10 yrs	36	18:18	24 (67%)	4 (12%)	2	44
> 10 yrs	13	10:3	9 (69%)	4 (31%)	3	128

AGE BANDS :ACCORDING TO AGE AT TRANSPLANTATION

Age at OLT	number of subjects	M : F	No. with gingival overgrowth	No. on nifedipine	No. on nifedipine with gingival overgrowth	Cyclosporin duration
< 1 year	36	12:24	20 (55%)	13 (36%)	8	16 months
>1-2 yrs	13	7:6	9 (70%)	3 (23%)	2	25 ''
>2-3 yrs	11	6:5	5 (45%)	3 (27%)	2	20 ''
>3-5yrs	16	4:12	7 (44%)	2 (13%)	2	29 ''
>5-12 yrs	17	13:4	9 (52%)	5 (29%)	2	17 ''
> 12 yrs	4	4:0	3 (75%)	0	0	19

TIME BANDED :ACCORDING TO DURATION OF CYCLOSPORIN THERAPY

Duration	number of subjects	M : F	No. with gingival overgrowth	No. on nifedipine	No. on nifedipine with gingival overgrowth	Mean Age at Exam in months
< 1 year	26	5:1	9 (35%)	14 (54%)	5	36
>1-2 yrs	23	9:14	10 (43%)	3 (13%)	3	68
>2-3 yrs	16	6:10	15 (94%)	3 (20%)	3	64
>3-4 yrs	11	3:8	7 (64%)	1 (9%)	0	38
>4-5 yrs	9	7:2	6 (67%)	1 (1%)	1	79
over 5 years	12	4:8	12 (100%)	0	0	100

Table 16 Composite Table Of Age, Age At OLT And Duration Of Cyclosporin Therapy In Banded Groups

4.5.6.1 The effect of age on cyclosporin-induced gingival overgrowth

Analysis of variance failed to show any association between the severity of gingival overgrowth and the trough cyclosporin concentration in any of the age-banded groups.

The Pearsons Product Moment Correlation Coefficient showed that there was an inverse correlation between the trough cyclosporin concentration and the duration of cyclosporin therapy in all of the age-banded groups except in the babies under 1 year of age. The results are summarised in Table 17. There was no significant correlation found between the trough cyclosporin concentration and any of the other variables examined.

Table 17

Trough Cyclosporin Concentration Compared To Duration Of Therapy

age-band	*no. of subjects*	*Pearson Product Moment Correlation Coefficient*
1-2 years	20	$r = 0.65$ $p = 0.01$
2-5 years	18	$r = 0.71$ $p = 0.001$
5-10 years	36	$r = 0.15$ $p = 0.001$
> 10 years	13	$r = 0.63$ $p = 0.05$

There was no association found between the gingival overgrowth severity and any of the other variables. However, in the 5 years to 10 years age-band there was a significant difference between the trough cyclosporin concentration in those with gingival overgrowth compared to those without (Two-Sample *t-Test* $t= 2.09$, *two tailed critical* $t=2.03$, $p< 0.05$).

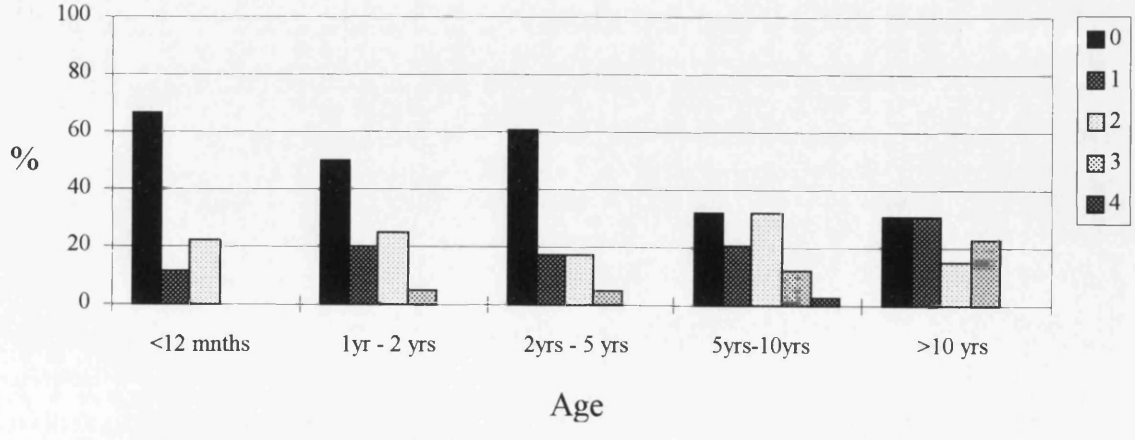
The relation between the gingival overgrowth severity and the mean trough cyclosporin concentration in each age-band is shown in Figure 10 overleaf.

Figure 10

Mean Trough Cyclosporin Concentration and Gingival Overgrowth Severity in Age-banded Groups

a) Trough cyclosporin concentration (ng/ml) in subjects grouped by age

b) Gingival overgrowth severity in subjects grouped by age



4.5.6.2 The effect of age at OLT on cyclosporin-induced gingival overgrowth

Analysis of variance failed to show any association between the severity of gingival overgrowth and the trough cyclosporin concentration.

The Pearson Product Moment Correlation Coefficient showed a significant inverse association between the trough cyclosporin concentration and the duration of cyclosporin therapy in subjects who were transplanted at between 3 and 5 years of age ($r = 0.64$ $p = 0.01$) and in those transplanted between 5 and 12 years of age ($r = 0.67$ $p = 0.01$).

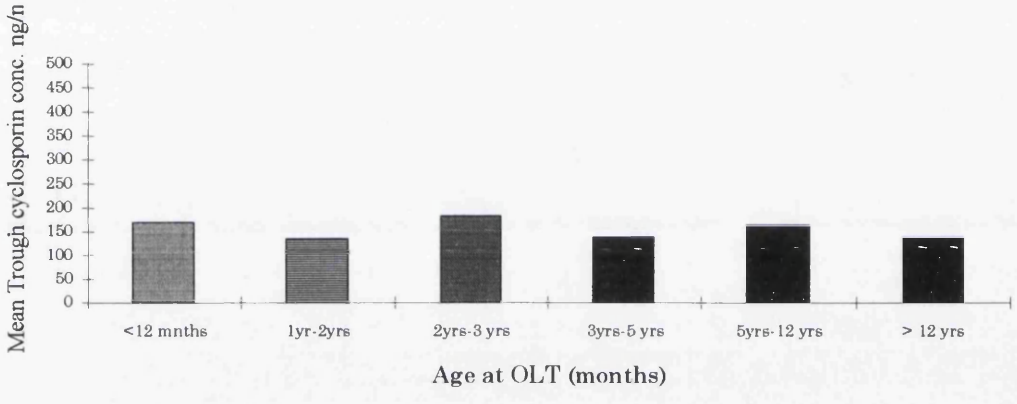
There was no association found between the severity of gingival overgrowth and any of the other variables.

The relation between the gingival overgrowth severity and the mean trough cyclosporin concentration in each OLT age-band is shown in Figure 11 overleaf.

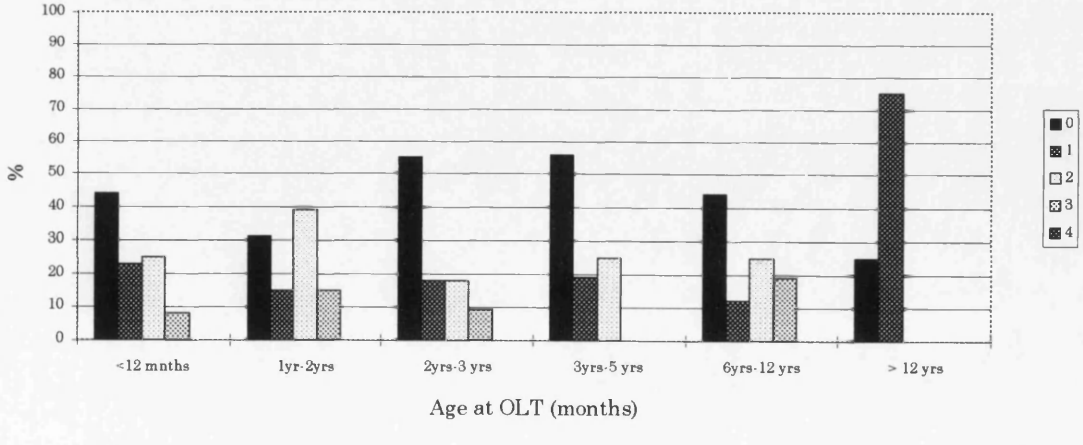
Figure 11

Mean Trough Cyclosporin Concentration And Gingival Overgrowth Severity In Groups Banded By Age At OLT

a) Mean trough cyclosporin concentration (ng/ml) in children grouped by age at OLT



b) Gingival overgrowth severity in children grouped by age at OLT



4.5.6.3 The effect of duration of cyclosporin therapy on gingival overgrowth

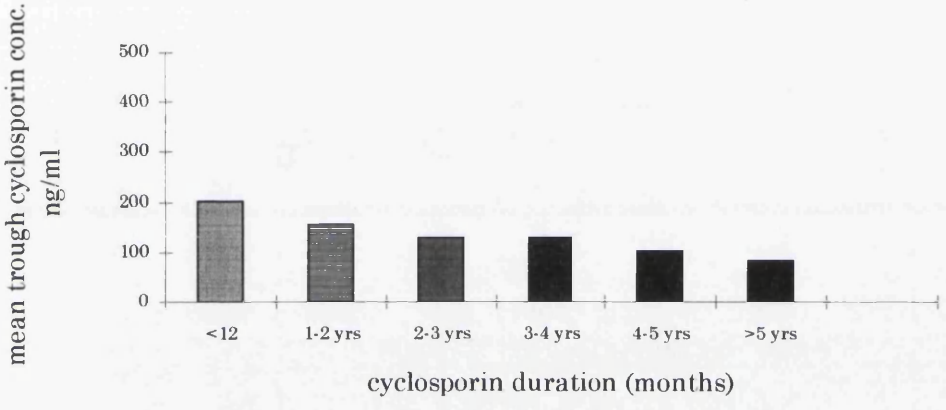
Analysis of variance failed to show any association between the severity of gingival overgrowth and the trough cyclosporin concentration in any of the groups, banded according to cyclosporin therapy. Furthermore, no association was found between either the severity of gingival overgrowth or the trough cyclosporin concentration and any of the other parameters within the groups. There was no difference found in the cyclosporin concentrations between those who had gingival overgrowth compared to those who had not when the t-Test was applied.

The relation between the gingival overgrowth severity and the mean trough cyclosporin concentration in each group, banded by duration of cyclosporin therapy, is shown in the following Figure 12.

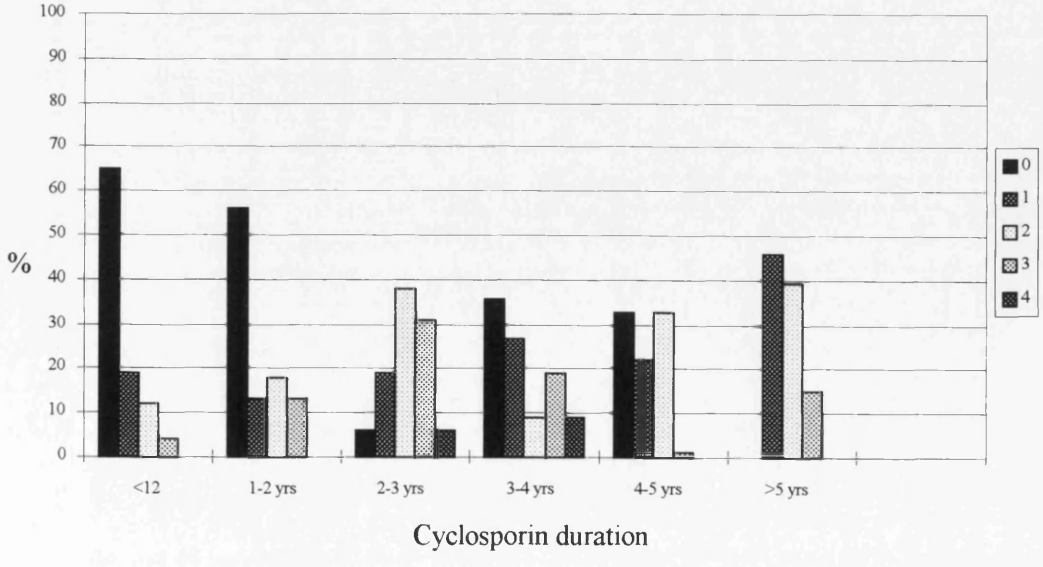
Figure 12

Mean Trough Cyclosporin Concentration And Gingival Overgrowth Severity In Groups Banded By Duration Of Cyclosporin Therapy

a) Mean trough cyclosporin concentration (ng/ml) in children grouped by duration of cyclosporin therapy



b) Gingival overgrowth severity in children grouped by duration of cyclosporin therapy



4.5.7 Effect of nifedipine on the presence and severity of gingival overgrowth

The association between nifedipine medication and the presence of gingival overgrowth was analysed in the group of subjects who were medicated with cyclosporin for less than 1 year. The two -Sample t-Test (assuming equal variance) failed to show any difference between gingival overgrowth severity in those medicated with nifedipine compared to those without ($t = 1.55$, one tailed critical $t = 1.67$, $p > 0.05$) .

The relation between gingival overgrowth severity and concomitant nifedipine medication was evaluated in the group of subjects who were aged between 13 months and 24 months. This group was selected because they were a large group with 50% with gingival overgrowth and 50% medicated with nifedipine. The contingency coefficient showed that there was no significant relationship ($p = .55$) between the severity of gingival overgrowth and nifedipine therapy. The data in relation to the nifedipine therapy and gingival overgrowth are shown in Table 18.

Table 18

Contingency Table Of Gingival Overgrowth Compared To Nifedipine Medication In Subjects Transplansplanted For Less Than 1 Year.

Gingival Overgrowth / nifedipine therapy	Y	N
0	4	6
1	3	1
2	2	3
3	1	0
4	0	0

4.5.8 The relation between gingival overgrowth and delayed eruption.

Of the 97 children who were studied, 47 were below 5 years of age. Sixteen had delayed eruption of the primary dentition according to the criteria from the previous paper. Within this group, the prevalence of gingival overgrowth was 78% .

4.6

The Influence of Cytomegalovirus on Cyclosporin-induced Gingival Overgrowth

4.6.1 Introduction

The aim of this study was to examine the effect of cytomegalovirus on cyclosporin-induced gingival overgrowth in children who have received liver grafts.

Since the susceptibility to CMV disease is known to be greatest following primary infection, the CMV status together with the diagnostic classification of primary infection, secondary infection and reactivation will be evaluated.

4.6.2 Study sample

Fifty-nine children taking cyclosporin (Sandimmune macromulsion preparation) following liver transplantation were examined in the dental surgery.

There were 29 males and 30 females, mean age 3yrs 9months (range from 9 months to 12 yrs). The mean time post transplant was 1 year 5 months (range 1month to 4yrs 7months). Thirty-one of the 59 children (53%) received grafts from seronegative donors and the remaining 28 children (47%) received grafts from seropositive donors. The CMV status prior to liver transplantation was available for 58 of the 59 patients. Forty-two (75%) of these children were CMV seronegative before liver transplantation and 16 (26%) were seropositive. There were 21 children who were CMV seronegative prior to transplant who received CMV seropositive transplants. Following liver transplantation, at the time of the dental examination, 32 (54%) children were CMV seronegative and 27 (46%) were CMV seropositive.

4.6.3 Methodology

The CMV status of the patient prior to liver transplantation was previously recorded on the Liver Unit Data Base and was matched retrospectively to the findings in the dental examination. It was possible to obtain an exact match between the CMV data and the dental findings since the dental examinations always coincided with the Liver Unit out-patient visit. Subjects in which there was found to be mis-matched data had been previously excluded.

4.6.3.1 Measurement of trough cyclosporin concentration

The circulating trough cyclosporin concentration was taken as the mean of the previous three blood tests.

4.6.3.2 Measurement of gingival overgrowth

Gingival overgrowth was scored as being present or absent and the Index of Severity of Gingival Overgrowth, which was used in the first study and was shown in Table 8 was used to record its severity.

4.6.3.3 CMV status

Patients were deemed to be CMV seropositive if either IgM and/ or IgG was detected in serum by enzyme-linked immunosorbent assay (ELISA). The presence of CMV inclusion bodies or an inflammatory cell infiltrate was not examined since in this study

population it was not appropriate to perform gingival biopsy. Reactivation of CMV was diagnosed where IgM was found in a child who previously had IgG. Primary infection was diagnosed when IgM was found in a previously IgG and IgM negative child. These diagnostic criteria are shown more fully in the Table 19 following.

Table 19

CMV Diagnostic Classification

CMV Status pre OLT	Diagnostic Classification	Immune Response
positive	reactivation	increasing IgM titres
positive	no reactivation	no IgM
negative	primary infection	increasing IgM titres
negative	secondary infection	IgM +ve for second time

4.6.4 Statistical analysis

The SPSS computer software package was used to perform the statistical analysis.

The analysis was performed as follows:

1) Association between gingival overgrowth and the diagnostic classifications of CMV infection.
2) Comparison between gingival overgrowth and CMV status after transplantation.
3) Comparison between gingival overgrowth and the status of the host before transplantation.
4) Comparison between host CMV status before and after transplantation in respect of gingival overgrowth.
5) Association between gingival overgrowth and the CMV status of the liver graft.
6) Comparison between subjects with latent or reactivated CMV infection compared to subjects who have never been exposed to CMV.

The association between the presence and severity of gingival overgrowth and CMV status was examined by the Chi-square test and by the Contingency Coefficient respectively.

ANOVA was used to evaluate any association between the circulating cyclosporin and the level of gingival overgrowth. The Student t-Test was used to examine the difference in circulating cyclosporin levels between those who had gingival overgrowth present and those who did not.

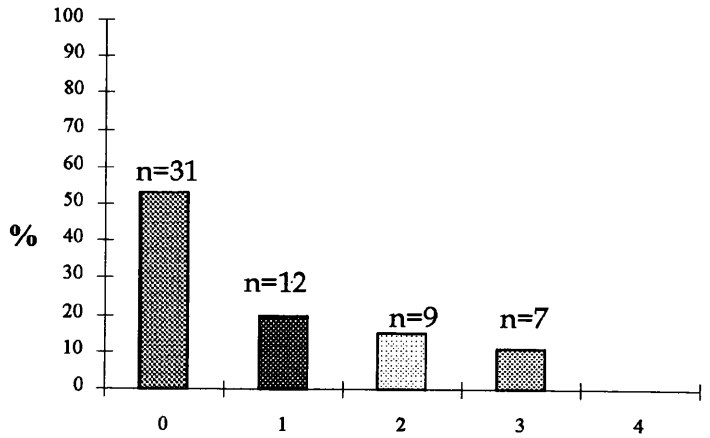
4.6.5 Results

4.6.5.1 Cyclosporin-induced gingival overgrowth

Thirty-one (53%) children had no gingival overgrowth. Of the 28 (47%) who did; twelve scored '1' on the Index of Severity, nine scored '2', and seven scored '3'. These findings, represented as the frequency at which each severity level of gingival overgrowth was found in the study population, are shown in Figure 13. There was no relation between CMV positive and CMV negative recipients and the presence of gingival overgrowth [Chi-squared = 0.2, p = 0.989]. The comparison between the CMV status of the recipients and the severity of gingival overgrowth is summarised in Table 20.

Figure 13

Gingival Overgrowth Severity



(n= 59)

Table 20

Comparison Between CMV Positive And CMV Negative Recipients

		CMV positive (27 recipients)	CMV negative (32 recipients)
mean serum cyclosporin (ng/ml)		157.6	166.6
No. children with gingival overgrowth		13 (48%)	15 (47%)
severity of gingival overgrowth:	**0**	14	17
	1	6	6
	2	4	5
	3	3	4
	4	0	0

4.6.5.2 Gingival overgrowth and the diagnostic classification of CMV infection

There was no relationship found between the severity of gingival overgrowth and a diagnosis of primary CMV infection, reactivation or secondary infection (contingency coefficient 0.38).

The comparisons between CMV status and the presence of gingival overgrowth are shown in Table 21 below.

Table 21

Gingival Overgrowth Compared To CMV Status

CMV status	number of children	number with gingival overgrowth
reactivation	1	0
no reactivation	6	3 (50%)
primary infection	17	8 (47%)
secondary infection	2	2 (100%)
negative	32	15 (47%)

4.6.5.3 Comparison between gingival overgrowth and CMV status after OLT

Irrespective of CMV status prior to transplantation, for all of the 59 children examined, no significant association was found between CMV status following transplant and either the presence (Chi-square 0.01 p= 0.99) or the level of severity of gingival overgrowth (contingency co-efficient 0.16).

4.6.5.4 Gingival overgrowth and the CMV status of the host before OLT

There was no relation between CMV status prior to transplant and either the presence (Chi-square 0.23 p= 0.98) or the severity of gingival overgrowth (contingency coefficient 0.16).

4.6.5.5 Gingival overgrowth and host CMV status before and after transplantation

Following transplantation, recipients who had been seronegative before transplantation (n=42) showed no significant association between their CMV status at the time of the dental examination and the presence of gingival overgrowth (Chi-square 0.002 p= 0.96). Similarly, in recipients who had been seropositive before transplantation (n=14) there was no significant association found between the presence of gingival overgrowth and the CMV status following transplantation (Fishers exact test *1 tailed*, 0.121).

4.6.5.6 Association between gingival overgrowth and CMV status of the liver graft

No relation was found between the CMV status of the donated liver and either the presence (Chi-square 0.23, p= 0.63) or the severity of the gingival overgrowth (contingency coefficient 0.16).

4.6.5.7 Comparison between subjects with latent or reactivated CMV infection compared to subjects who have never been exposed to CMV

When subjects who had latent or reactivation of CMV were grouped together and compared against those children who were never been exposed to the virus. The presence of gingival overgrowth was not related to either seropositive recipients of seropositive transplants (n=4) or seronegative recipients of seronegative transplants (n=21), (Chi-square 0.21 p=0.65). Moreover, there was no relation between any of the above and the severity of the gingival overgrowth (contingency coefficient 0.4).

4.6.5.8 Cyclosporin and gingival overgrowth

The mean circulating cyclosporin level was 162.51 ng/ml (n= 58, range 0 to 413ng/ml). There was no significant association between the level of circulating cyclosporin and either the presence or severity (ANOVA F=.52, p= 0.67) of gingival overgrowth. There was no difference in the circulating cyclosporin level between those who had gingival overgrowth and those who did not (Student t-Test, t=0.9, p= 0.67). The mean circulating cyclosporin level was 168 ng/ml in the 31 patients who were CMV seronegative following transplantation and was 157 ng/ml in the 27 patients who were seropositive following transplantation. There was no difference between the trough cyclosporin concentration of those who were CMV seropositive and those who were seronegative (Student t-Test, t= .54, p= 0.59).

Chapter Five

Discussion

5.1 Methodology

5.1.1 Study Design

The design of these clinical studies was constrained by the distance that many of the children with liver grafts had to travel to attend Birmingham Childrens Hospital. It was impractical to recall specific subjects for the purpose of a dental examination alone. Therefore, throughout the course of this study the examinations had to be carried out in conjunction with an out-patient appointment in the Liver Unit.

In the first year or so post-transplantation the children attend the Liver Unit relatively frequently. However, once their cyclosporin levels have been stabilised, in the absence of further complications, the children are managed by their local paediatric gastroenterologist. Indeed, many of these children only attend annually for what is generally described as their 'M.O.T'.

Initially, the author had hoped to examine all of the subjects longitudinally at 3, 6 and then 12 monthly intervals but since the children were all at different stages post-transplant, and therefore attending at varying frequencies, this was only partially successful in the younger age groups. Similarly, although separate cohorts of children were identified in relation to age, liver disease and age at transplantation, it eventually proved impossible to carry out the dental examination at the preordained times,

particularly in the older children. Therefore, it was more difficult to measure the changes in gingival overgrowth severity and intrinsic pigmentation longitudinally.

The repeat examinations to confirm the reproducibility of the data obtained using the gingival overgrowth and intrinsic pigmentation scales had to be carried out within a short time of the initial exam. Otherwise, the results could have been confounded with increased severity of gingival overgrowth, with duration of cyclosporin therapy, as reported by Seow *et al* (1991), and by fading of the intrinsic staining as suggested by Marsland and Gerrard (1953). Therefore, although it would have been more powerful to carry out the reproducibility studies using samples that more closely matched those of the main investigations, this was precluded by the supra-regional nature of the liver Unit in Birmingham.

5.1.1.1 Measurement of gingival overgrowth severity

The measurement of gingival overgrowth severity could be related to the study of Lowry *et al* (1995) into the oral effects of paediatric cardiac transplantation. Indeed, the authors were in correspondence throughout the period of this study in regard to the measurement of gingival overgrowth. Both studies had similar difficulties in establishing objective measurement of gingival overgrowth severity. These difficulties arose because both researchers found that it was impossible to take alginate impressions in the very young children, and so could not follow the objective criteria

for measuring gingival overgrowth severity that were published by Seymour *et al* (1985).

This study had an even younger sample population than the previously mentioned study by Lowry *et al* (1995). Therefore, the measurement of gingival overgrowth severity used in this study was a simple estimation of the amount of crown covered and was suggested by Professor Seymour himself in a personal communication.

This study found that the reproducibility of the gingival overgrowth severity data, obtained using the Index of Gingival Overgrowth Severity, showed only moderate agreement between dental examinations that were one month apart (Landis and Koch 1977). However, the majority of children in this study, were in the primary dentition. It is possible that the reason why only moderate agreement was found is that these results were a reflection of the difficulty in evaluating gingival overgrowth accurately in partially erupted, and worn, primary teeth. There may also have been an actual change in the gingival condition over the month time interval.

Seow *et al* (1991) only reported the gingival overgrowth as being *'mild to severe'* . On the other hand, Ross *et al* (1989) measured gingival width using a periodontal probe that was placed at the maximum tooth bulbosity on the buccal and lingual aspects perpendicular to the long axis of the tooth. However, the average age of their study

group was 8 years compared to a mean age of 4 years 8 months in the cross-sectional study and 5 years 3 months in the reproducibility study.

Funakoshi *et al* (1992) used a Gingival Enlargement Index, which measures interdental enlargement on the buccal and lingual aspects of the incisors and both posterior segments in both arches. Their study had five subjects with ages ranging from 3 years 4 months to 5 years 6 months and, as such, is the nearest comparable to this study. Although the use of the same scale was considered for this study it was rejected because not all the subjects in this study had a full dentition and because the scale was considered to be not sufficiently sensitive, since the gingival overgrowth could be seen to be present even when there was no obvious interdental enlargement.

Perhaps a three point, rather than a four point scale, would have been more efficacious to use in a population with a large number of children with primary teeth. A possible example of a three point scale that might be a more reproducible measure of gingival overgrowth in the primary dentition is as follows:

1. *gingival overgrowth detectable*
2. *gingival overgrowth covering between one half and over of the clinical crown but with the crown still visible*
3. *gingival overgrowth covering all of the of the clinical crown in a tooth that had previously emerged.*

However, before this scale can be used it must also be tested clinically to evaluate its reliability, sensitivity and reproducibility.

5.1.1.2 Measurement of enamel hypoplasia

The evaluation of enamel hypoplasia used the Thylstrup and Fejerskov Index (1978). This scale was chosen because it was simple to use and had been found to be effective in a pilot study of ten children with liver grafts that had been carried out in 1990. This index has been extensively used in epidemiological investigations of mottling, particularly in relation to fluorosis and is a well accepted reproducible system.

5.1.1.3 Measurement of intrinsic green pigmentation

The Intrinsic Green Pigmentation Colour Scale was developed to facilitate a more accurate recording of the intrinsic pigmentation than had previously been reported in this population.

Funakoshi *et al* (1992) merely reported that *'discolouration was observed'*. The publication by Seow *et al* (1991) gave a more detailed description, reporting that the colour ranged from yellow-brown to deep green with variation between patients. Marsland and Gerrard (1953) had suggested that the colour changed with time. Therefore, the author felt that the evaluation of the intrinsic green pigmentation had to

be as objective as possible to allow more accurate reporting and observation of any colour change. Since there were no known dental shade guides that had green pigmentation the development of a novel colour scale was the only option.

The evaluation of the reproducibility of the data based on the colour scale showed that the data from the primary incisor measurements were much less reproducible than that obtained from the primary molar teeth. A possible explanation for this finding is that a smaller proportion of the primary incisor crown is stained compared to the primary molars. However, the clinical crown of the primary incisors is often reduced in size due to attrition and gingival overgrowth and this might add to the difficulty in obtaining accurate measurements of intrinsic pigmentation.

5.1.2 Study samples

5.1.2.1 Cross-sectional study

The study sample in the cross-sectional study was far larger than other studies into paediatric liver transplantation, such as those of Ross *et al* (1989), Funakoshi *et al* (1992) and Seow *et al* (1991). The results were also representative of a much younger population than had previously been reported; not only was the mean age of the children 4 years 8 months but the median was only 3 years 8 months. Owing to the increasing availability of liver grafts to babies and infants this sample probably gave a more accurate overview of the oral effects of paediatric liver transplantation.

5.1.2.2 Intrinsic green pigmentation study

The number of children with intrinsic green pigmentation was surprisingly small since previous investigators had suggested that there was a very high prevalence of intrinsic green pigmentation in this population. This was particularly apparent in the difficulties that arose in finding a large enough group of liver graft recipients with intrinsic pigmentation of the permanent dentition.

However, the difficulty in collecting a sufficiently large sample of children with pigmented permanent teeth should not be interpreted as confirmation that the prevalence of green pigmentation in the permanent dentition is likely to be small.

Instead, the scarcity of numbers is more likely to be due to the fact that intrinsic green pigmentation is almost entirely confined to children with biliary atresia. It is only in the last few years, with the introduction of the split liver procedure, that babies with biliary atresia can be offered a liver graft. Therefore, this particular investigation was ahead of its time and, should it be repeated in five years time, suitable subjects might be more easily obtained.

5.1.2.3 Delayed eruption study

When this study was being designed the liver team and the dietetic department were convinced that there would be sufficient children with malnutrition to make up the control groups. However, collecting subjects who satisfied the criteria for inclusion into the malnourished groups was an extremely difficult and time-consuming part of this entire dissertation.

It is interesting to note that Reilly *et al* (1992) reported that less than 20% of the children in their study who were on or below the third percentile for weight at 12 months of age were referred to a paediatrician. Furthermore, their study into primary tooth eruption in failure-to-thrive infants found only 47 suitable subjects out of a population of 2608 (Reilly *et al* 1992).

Perhaps the inclusion criteria of on or below the third percentile were too severe, for both height and weight, but selecting only those children who were outwith two standard deviations of the normal will allow comparisons between this and future investigations. If the inclusion criteria had extended to all of those children who were diagnosed as 'failure to thrive' this would have produced a very diverse group of individuals indeed. Moreover, since it is not clear whether it is acute or chronic malnutrition that has the greatest effect on the developing dentition, only including subjects who were below both height and weight parameters eliminated any ambiguity.

The low prevalence of delayed eruption in the two control groups, namely those with liver disease and malnutrition and those with malnutrition of non-hepatic origin is surprising, in view of previous publications but might be attributable to the small sample sizes. However, the number of subjects in this study is comparable to some of these other publications, most notably is Alvarez *et al* (1989)who only had seven subjects who were actually 'stunted and wasted'.

5.1.2.4 Effect of age and duration of cyclosporin on gingival overgrowth

Despite the size of the sample in the cross-sectional study, it was dominated by pre-school children, and although it gave an accurate overview of the prevalence of gingival overgrowth in paediatric liver graft recipients it did not allow an accurate evaluation of the effect of age and of duration of cyclosporin therapy on the gingival tissues. Therefore, the fourth study was designed to give a more even age distribution

within the sample. That investigation clearly showed that there is an increasing prevalence of gingival overgrowth with age and with duration of therapy. It is likely that the reason why previous researchers have reported much higher prevalences of gingival overgrowth than was found in the cross-sectional study is that their samples were small and had predominantly older children. The mean age of the 21 children in the study by Ross *et al* (1989) was 8 years.

The study by Funakoshi *et al* (1992) contained five children who were aged between 3 years 4 months and 5 years 6 months and, as such, is more closely comparable to this investigation. However, Funakoshi *et al* (1992) found gingival overgrowth in all of their subjects compared to 39% in this study in the 2 to 5 year age band. Furthermore, the photograph in their publication was of the second most severe case in their sample and showed a child with severe gingival overgrowth comparable to that seen in this study in only one child throughout the course of five years. It can only be assumed that there is considerable individual variation between children of similar ages and underlines the necessity for largest possible sample size.

5.1.2.5 Effect of CMV on gingival overgrowth

The subjects in this investigation were part of a larger sample, selected at random from the authors data base after the presence and severity of the gingival overgrowth had already been recorded. In this way the author had no prior knowledge of the CMV status of the subjects. Each subject's name, hospital number and the date at which the

dental examination had been performed was then given to Dr. Davison who matched these with the CMV status recordings in the liver unit data-base.

The sample in this investigation is comparable to that in the cross-sectional study but had a better balance between males and females and between those with and without gingival overgrowth. The number of children who were CMV seropositive following transplantation was 46%. The prevalence of CMV infection following liver transplantation in children who were transplanted in Birmingham has already been reported to be 38% (Davison *et al* 1993).

After the CMV status data were added to the dental examination report it became clear that there were very few children with reactivation or secondary infection. The sample size was already considerable and due to its retrospective nature it would have been difficult to select further subjects who were known to have reactivation or secondary infection since the dates at which they had seroconverted might not have been easily matched to a dental examination and it would have been difficult to ensure that the investigators remained 'blind'. Furthermore, it was already very clear from the results that CMV infection had little significant influence on cyclosporin-induced gingival overgrowth and so a trawl of both data bases for suitable subjects was not carried out.

5.2 Enamel hypoplasia in paediatric liver graft recipients

This study found that the prevalence of enamel hypoplastic defects in paediatric liver graft recipients was only 11%. This is much less than had hitherto been expected, based on the evidence of the report of Seow *et al* (1991). This low prevalence is even more surprising given that only 15 % of the children in this study live in the West Midlands which is fluoridated.

The examination of the children in this study was carried out in good light but the teeth were not dried, nor were they always inspected with a probe. However, it was felt that the examination was sufficiently thorough to record clinically significant defects.

Seow, Shepherd and Ong (1991) reported that enamel hypoplastic defects were found in all of the subjects in their sample who had received liver grafts; a total of 7 with ages ranging from 1 year 8 months to 3 years 11 months. They made a diagnosis of enamel hypoplasia if there was any missing enamel or a break in the continuity of the enamel surface and reported that enamel hypoplasia was found on all of the erupted teeth of all of the subjects. They further reported that the hypoplastic defects were only present on those parts of the enamel formed after birth.

The findings in this study and those by Seow *et al* (1991) highlighted above, appear to be conflicting. However, a possible explanation of the discrepancy might lie in the fact that all of the subjects in the study by Seow *et al* with one exception, had heights at or below the third percentile. Alvarez *et al* (1993) found that subjects who had suffered

from chronic malnutrition not only had delayed eruption but also had a high prevalence of dental caries and the authors attributed the latter to the deleterious effect of malnutrition on enamel formation in early life. Based on this knowledge, it is possible that the aggressive nutritional support that is undertaken in Birmingham has effectively reduced the likelihood that defects of the enamel would occur.

A recent investigation by Noren *et al* (1993) suggested that enamel hypoplasia could be attributed to oral intubation. Therefore, it is possible that the high prevalence of enamel hypoplastic defects reported by Seow *et al* (1991) is related to oral intubation. However, the subjects in this study also receive frequent general anaesthetics for endoscopy and liver biopsy.

Moreover, in view of the fact that the largest proportion of the enamel of the primary dentition is calcified in utero, any report of widespread postnatally acquired defects of the enamel of primary teeth must be considered dubious. Indeed, in a study into the prevalence of enamel hypoplasia in Mexican children, Goodman *et al* (1987) found that only 6.1% of the completely erupted and unworn primary teeth were hypoplastic.

Defects on the enamel of the permanent teeth could be expected to be more common. Indeed, the position of the enamel hypoplastic lesions in the three children with affected permanent incisors corresponded to the stage of dental development at the time of transplant. However, the photograph of the most severely affected child has an appearance that is more suggestive of endemic enamel fluorosis than of chronological enamel hypoplasia.

It is clear that further study is required, to evaluate the true effect that paediatric liver transplantation has on the enamel of children who receive a liver graft between birth to six years of age.

5.3 Intrinsic green pigmentation

5.3.1 Prevalence of intrinsic green pigmentation in paediatric liver graft recipients

Intrinsic green pigmentation is commonly found in paediatric liver graft recipients. This study confirmed that the prevalence of intrinsic green pigmentation in this population is in excess of 50%. The high prevalence of intrinsic pigmentation is not due to the liver transplantation procedure itself. Instead, it is a reflection of the high number of children suffering from biliary atresia who are now more likely to survive due to the increasing availability, and better survival rate, of paediatric liver transplantation.

5.3.2 Variation in the severity of the intrinsic pigmentation

Seow *et al* (1991) reported that the intrinsic pigmentation in the primary teeth of liver graft recipients ranged from yellowish-brown to deep green, with varying colour intensity in each patient. The present study also confirmed that some children are more severely affected than others and that the primary molar teeth do indeed appear to be more severely pigmented than the primary incisors. The pigmentation in the primary incisors of the subjects in this study was found to be mainly concentrated around the cervical area of the crown. In contrast, a larger proportion of the crown of the primary molar teeth was pigmented and this pigmentation was more evenly distributed. These findings were consistent with the report by Rosenthal *et al* (1985) in which they described the green pigmentation as becoming more vivid and noticeable as the depths of the cavity preparation extended.

The findings in this present study are also very similar to the variation and extent of the intrinsic pigmentation that was reported by Tank in 1951 in an investigation into green pigmentation of the primary dentition in two children with haemolytic disease of the new-born.

These clinical findings suggest that the green pigment was laid down in an incremental pattern. Moreover, the pigmentation in the primary incisors appears to have been predominantly deposited around the cervical portion of the crown, an area that is formed in the immediate postnatal period. However, this hypothesis needs to be tested further in vitro.

5.3.3 The chronology of intrinsic green pigmentation in primary teeth

The area of pigmentation in the cervical area of the crown of the exfoliated teeth that were presented in this study is calcified shortly after birth, according to Lunt and Law (1974). Therefore, the present study found no evidence that bilirubin was deposited in utero.

Nevertheless, many of the children in this study also appeared to have intrinsic green pigmentation from the incisal edges to the gingival margins. However, this appearance might have been due to the reflection of green pigmentation from both the cervical area of the crown and the surface of the root. Indeed, Seow at al (1991), Van Cleynen and Demars-Fremault (1990), and Shapiro *et al* (1975) all presented photographic

evidence showing severe intrinsic green pigmentation in primary incisor roots. This explanation might also be the reason why it was difficult to accurately measure the severity of the pigmentation in the primary incisors of the subjects in the present study.

5.3.4 Variation in the green pigmentation with time

The results in the present study show that the severity of the intrinsic green pigmentation in the primary teeth did not lessen with time. This suggests that the green pigment is bound to the dental tissues and adds further weight to the hypothesis that it is deposited incrementally rather than by diffusion.

However, Thursfield (1912) clearly reported that primary teeth which had been a vivid yellow in colour had later become green and that the tint varied considerably between *bright* and *dull*. At the same Royal Society meeting in 1912, Langmead (1912) also reported that the pigmentation of the primary teeth in a jaundiced child was seen to change from yellow to green. The ensuing discussion at the Royal Society has also been published, and chromogenic bacteria were one of the explanations that were put forward for the colour change.

In addition, Marsland and Gerrard (1953) noted that the pigmentation was always brightest when the tooth first erupted and then faded as the child grew older. They even noted that at the time the tooth had exfoliated it often appeared to be free of pigment altogether. This latter finding was confirmed in the present study since, when

the exfoliated primary teeth were received they did indeed appear to be much less severely stained than expected.

Nevertheless, the present study has shown that it is difficult to accurately quantify the severity of the pigmentation in primary incisors even with the use of a colour scale. However, the study sample used to evaluate colour change in this study, was predominantly composed of primary molar teeth that were not close to exfoliation. Therefore, it is possible that this and the other previous studies, rather than reporting conflicting results, have demonstrated that the severity of the pigmentation in the primary incisors becomes more severe during root formation and then fades during root resorption. As such the green colour in the primary incisors is partially reflected from the root. This same colour change is less likely to be seen in pigmented primary molar teeth since a larger proportion of the clinical crown is pigmented.

5.3.5 Intrinsic green pigmentation in the permanent dentition

The intrinsic pigmentation in permanent incisors and first molars in the present study showed more homogeneity than was found in the subjects with primary teeth. The evaluation of intrinsic pigmentation in permanent teeth was too limited to put forward any other hypotheses. However, based on the evidence presented in the study as a whole it is likely that the green pigmentation in permanent teeth will be almost entirely confined to those with biliary atresia. It is likely that the deposition of the pigment will be incremental in nature corresponding to the jaundiced period and will not fade with time.

5.3.6 Further study

It is clear that a larger study to carry out a histologic examination of primary incisor teeth is required since the incremental pattern of deposition of the intrinsic green pigment needs to be confirmed.

The previous publications are predominantly clinical in nature and there is no published evidence to show how the pigment is incorporated into the enamel and dentine during matrix formation. There are many questions that have still to be answered. In vitro investigations will be useful to determine if the pigment is truly biliverdin, if it can bind to hydroxyapatite, if it can diffuse through the dentine and enamel and if it is water soluble whilst present in the tooth tissue. In addition, the storage media and sectioning methodology have still to be ascertained.

Nevertheless, in the light of the findings in this study, it is likely that the permanent teeth in children with liver grafts who suffered from biliary atresia will have intrinsic green pigmentation. Therefore, the efficacy of operative techniques and new and existing dental materials to camouflage the green pigmentation will soon need to be explored.

5.4 Delayed eruption in the primary dentition

5.4.1 Prevalence

Both the cross-sectional study and the controlled study have confirmed that prevalence of delayed eruption of the primary dentition in paediatric liver graft recipients is in excess of 40%. The only other study which reported delayed primary tooth eruption in this population was Seow *et al* in 1991.

5.4.2 Delayed eruption in relation to malnutrition

The present study has shown that children with liver grafts and malnutrition not only had a similar prevalence of delayed eruption but also had the same number of teeth as those with liver grafts who were not malnourished. Therefore, these findings suggest that the high prevalence of delayed eruption of the primary dentition in paediatric liver graft recipients cannot be attributed to poor nutrition alone.

Moreover, if malnutrition had been the most significant factor in the aetiology of delayed eruption in the liver transplant groups, the malnourished controls would have been expected to have had a higher prevalence of delayed eruption or much fewer teeth. These findings are surprising in the light of previous publications but it is possible that the influence of nutritional status on the eruption of the primary dentition has been over-estimated.

Almost every study into the relation between dental development and nutritional status has shown that primary tooth eruption is significantly delayed in malnourished children (Alvarez *et* al 1989a: Alvarez *et al* 1992b: Reilly *et al* 1992). However, these studies based their findings only on the comparison between the number of teeth present in the malnourished population sample and the age-matched controls.

In the present study, the subjects were also assessed according to whether or not they met the criteria for a diagnosis of delayed eruption. These criteria, for the diagnosis of delayed eruption, required an elapse of a six month period beyond the eruption dates of Lunt and Law (1974). Therefore, it is conceivable that the subjects with malnutrition in the previous studies may indeed have had fewer teeth but might not always have been diagnosed as having delayed eruption, had the same diagnostic criteria been applied.

Furthermore, although other investigators have reported that there is no clear evidence that mild-to moderate protein malnutrition influences primary tooth eruption, it has been suggested that severe malnutrition may have an effect (Delgado *et al* 1975).

5.4.3 'Catch-up' growth in primary tooth eruption

The subjects in this study did not appear to exhibit 'catch-up' growth in relation to primary tooth eruption following receipt of a liver graft.

Dental development has previously been reported in association with somatic 'catch-up' growth. Loevy *et al* (1989) demonstrated that delays in dental eruption are corrected during the second year of development in children who were born with very low birth weights. These findings were confirmed by a later study by Fadavi *et al* (1992) who showed that low-birth-weight, premature children have delayed eruption of the primary dentition only in the first 24 months of life.

'Catch-up' in somatic growth has already been reported in paediatric liver graft recipients within twelve months of liver transplantation (Beath *et al* 1993b). Both the present study and this study, by Beath *et al* (1993b) were conducted in the Liver Unit in Birmingham and, as such, the two study samples contain many of the same subjects. Therefore, one would have expected to find delayed eruption only in the children who had most recently received a liver graft. However, in this investigation the children with delayed eruption had been in receipt of a liver graft for longer than those who did not have delayed eruption.

5.4.4 Delayed primary tooth eruption and liver disease

The subjects with liver grafts had a much higher prevalence of delayed eruption than those with an underlying liver disease. Moreover, liver graft recipients were found to have significantly fewer teeth than those with liver disease, when the number of erupted teeth was compared between age-matched subjects. These findings suggest

that the underlying liver disease has only a small influence on the aetiology of delayed eruption of the primary dentition in liver transplant recipients.

Nevertheless, none of the children with liver disease who were well nourished were found to have delayed eruption, whereas those who were malnourished had a higher prevalence of delayed eruption than the control group. Therefore, children with liver disease might only have an increased propensity for delayed eruption of the primary dentition when the disease is associated with severe malnutrition.

It has already been shown that children with liver disease have a higher than average nutritional requirement and they already suffer from defective protein and fat metabolism. The combined effect of these two factors is likely to magnify the severity of the malnutrition found in these subjects (Moukarzel 1990: Beath *et al* 1993 b).

Therefore, there are two possible explanations for the higher prevalence of delayed eruption in subjects with liver disease and malnutrition:-

- Children with liver disease who are malnourished probably suffer from a nutritional deficiency from birth, and so, this has an earlier and more prolonged effect on the development of the dentition.
- The malnutrition found in children with liver disease is likely to be more severe than malnutrition caused by breast-feeding failure, errors in the formula, chronic

infection and congenital heart disease and, therefore, exerts a greater effect on the developing primary dentition compared to an acute episode of malnutrition.

It could be argued that the children in the groups with liver disease were less ill than those with liver grafts. However, many of the subjects examined in the liver disease control groups had been either referred or scheduled for liver transplantation themselves. The shortage of donated liver grafts means that there are many children who are severely ill still awaiting transplantation.

5.4.5 Delayed eruption and the age of the child at liver transplantation

The liver transplant recipients with delayed eruption were found to have been transplanted at a younger age compared to those who did not have delayed eruption, irrespective of nutritional status. Those with delayed eruption were transplanted at mean ages of 6 months and 12 months respectively compared to 16 and 14 months in the non-transplanted controls. The only reasonable explanation for this finding is that the delayed eruption might be related to factors directly related to the transplantation process itself.

5.4.6 Delayed eruption and cyclosporin therapy

The common factor in all the subjects with liver grafts was the cyclosporin medication. There was an association between mean circulating trough cyclosporin level and the number of teeth present in the children with liver grafts. Therefore, a possible alternative explanation to the high prevalence of delayed eruption in paediatric liver transplant recipients is that cyclosporin has a direct effect on the overlying soft tissues to prevent the emergence of the primary dentition.

The possible link between malnutrition in subjects with liver disease and the relation to delayed eruption in the primary dentition has already been discussed. However, the reason why 'catch up' in primary tooth eruption was not evident in this study could be due to the effect of cyclosporin on the mucosa overlying teeth that were already delayed in their eruption. In other words, cyclosporin might not have had a direct effect on dental development but magnified an already retarded dental development prior to transplantation by making emergence of the teeth more difficult once these teeth had regained their eruptive potential.

In the two previous reports of delayed eruption of the primary dentition, in subjects medicated with an agent known to cause gingival overgrowth, the subjects were known to be *'small for her age'* and *'severely handicapped'* (Reich *et al* 1981: Church & Brandt, 1984). In the light of the findings in this study, it is possible that these were also examples of drug induced thickening of the overlying mucosa causing

delayed eruption in subjects with an existing predisposition to retarded dental development.

5.4.7 Delayed eruption and the duration of cyclosporin therapy

The children with delayed eruption had been transplanted for a longer period of time than those who did not.

An evaluation of the association between the length of time a subject had been transplanted and the number of erupted teeth present would have been valuable. However, this would only have been possible if all the subjects were exactly the same age since there will always be a positive correlation between age and the number of erupted teeth. Nevertheless, in this study, the association between the presence of delayed eruption and the length of time post transplant is striking and must merit further consideration.

5.4.8 The effect of previous steroid and nifedipine therapy

If steroid therapy had any effect of the eruption on the primary teeth in this study this would have been expected to be most apparent soon after receipt of a liver graft. Blodgett *et al* (1956) in a study of the effects of prolonged corticosteroid therapy on structural growth and skeletal maturation in children, clearly demonstrated that

although the steroids stopped growth when given in high doses, a reduction in dosage was almost immediately followed by an upsurge in the growth rate. The findings in this study, therefore, discount short term corticosteroid medication as an aetiological factor in delayed eruption of the primary dentition.

Nifedipine is also known to cause gingival overgrowth but there have been no reports of an association with delayed eruption. Furthermore, other reports have shown that the effect of nifedipine on the gingival tissues is reversible when drug therapy is discontinued. Nishikawa *et al* (1991) reported a case of a 61 year old woman who underwent spontaneous regression of the gingival overgrowth two months after her medication was changed. Furthermore, Lederman *et al* (1984) have reported that if the administration of nifedipine is discontinued, oral signs may improve in as little as one week.

Therefore, if the previous steroid or nifedipine therapies had been contributory factors in the aetiology of delayed eruption of the primary dentition in this population one would, again, have expected to find the highest prevalence in those subjects who had been transplanted for the shortest duration. The findings in this study, therefore, do not support the hypothesis that steroids or nifedipine were aetiological factors.

5.4.9 Further study

The effect of cyclosporin on the overlying soft tissues merits further investigation.

Cyclosporin-induced gingival overgrowth has never been reported before in association with unerupted teeth. Further study into the effect of cyclosporin on the overlying mucosa or fibroblasts might prove to be valuable in ascertaining the mechanism by which cyclosporin affects the gingival tissues.

5.5 Cyclosporin-induced gingival overgrowth

5.5.1 Prevalence

This study has shown that the prevalence of cyclosporin-induced gingival overgrowth in a cross-section of paediatric liver transplant recipients is 55%. This is in keeping with a similar study of paediatric heart transplant recipients in which 10 out of 19 subjects had *clinically significant* gingival overgrowth and used similar methodology (Lowry *et al* 1995).

When the prevalence of gingival overgrowth is reported in relation to the age at which the children were examined the prevalence was lowest in the children under 1 year of age (33%), increasing to 69% in children who were over 10 years of age. The trough cyclosporin concentrations throughout the age-banded groups was similar.

The gingival overgrowth in this study was most commonly observed to cover only between 1/2 and 1/3 of the clinical crown and the severity of gingival overgrowth was similar within the different age groups.

The prevalence and severity of gingival overgrowth was much less than had hitherto been expected, based on the previous published studies into gingival overgrowth in

both adult and child liver graft recipients who all reported 100 % prevalences (Ross *et al* 1989: Svirsky 1989: Seow *et al* 1991: Funakoshi *et al* 1992).

The individual variation in absorption offers an explanation as to why this study differs so markedly from these other studies. It is clear that the variation in cyclosporin bioavailability is so great in this population that only large sample sizes will give a true reflection of the prevalence and severity of cyclosporin-induced gingival overgrowth. The current study is the largest one ever undertaken, with the most accurate data on cyclosporin levels.

5.5.2 Other factors in the aetiology of cyclosporin-induced gingival overgrowth

The effect of other factors such as fibroblast population mix, the presence of plaque, and individual immune response should not be discounted. These are also likely to contribute to the variation in the prevalence and severity of gingival overgrowth.

It was unrealistic to measure plaque and gingival inflammation using specific indices in this young child sample. Furthermore, in view of the diversities between paediatric liver graft recipients and their relative sparcity in number, clinical studies into the effect of these other oral health parameters such as plaque indices and probing depths are probably best studied in another, more accessible, paediatric transplant population.

5.5.3 Gingival overgrowth in infants

The lower prevalence of gingival overgrowth in infants under 1 year of age is probably not associated to their relatively short exposure time to cyclosporin. Cyclosporin-induced gingival overgrowth has been shown to occur as early as 2-6 weeks after the commencement of therapy (Daly *et al*: 1986). Instead, the lower prevalence of gingival overgrowth in this age group can probably be attributed to their decreased bioavailability of cyclosporin (McMaster *et al* 1994: Whitington *et al* 1990: Whitington *et al* 1991). The difficulty in maintaining suboptimal therapeutic levels in children possibly relates to a lower exposure to cyclosporin in this group.

5.5.4 Effect of age at OLT on gingival overgrowth

There were two groups of children in this study who appeared to have the highest prevalence of gingival overgrowth: those who received liver grafts in their second year (70%), and those who received liver grafts when they were over twelve years of age (75%).

There are two possible explanations for these findings. In the 12 year and over group, the increased prevalence can be attributed to the onset of puberty. Daly *et al* (1986) also reported a higher prevalence of gingival overgrowth in this group, irrespective of sex, and attributed this to fibroblast susceptibility to growth hormone.

The high prevalence of gingival overgrowth in the children who were transplanted in their second year of life is more difficult to explain. However, like the pubertal group, this is also a time of rapid growth and it is interesting to note that this is the period of time when many of the primary teeth are erupting and when there is plaque accumulation for the first time.

These findings suggest that there is a 'low prevalence window' of cyclosporin-induced gingival overgrowth for transplantation after the age of 3 years but before the onset of puberty. This theory might explain why the previous studies into paediatric liver transplantation reported disparate results in respect of the effect of duration of cyclosporin therapy, since it is possible that the discrepancy lies not in the duration of cyclosporin itself, but on the effect that cyclosporin exerts on the gingival tissues at different ages.

Clearly further study is required, not only to confirm these findings but also to determine the underlying cause.

5.5.5 The effect of duration of cyclosporin therapy

There appears to be an increasing prevalence of cyclosporin-induced gingival overgrowth with increasing duration of drug therapy. However, the statistical analysis in the cross-sectional study showed that there was a weak inverse correlation between gingival overgrowth severity and the duration of cyclosporin therapy.

This study also found that there was an inverse relation between the trough cyclosporin concentration and the duration of therapy. Uncorrected cyclosporin dose is known to decrease with increased body size (Whitington *et al* 1990). Therefore, these results might only be a reflection of the falling concentration of cyclosporin with age, especially when the cyclosporin therapy regimen falls behind somatic growth allowing the serum concentration to fall below optimal levels.

It is already clear that maintaining an optimal concentration of cyclosporin is difficult, but this is exacerbated in a growing child. Resolution of gingival overgrowth with reduced or discontinued cyclosporin therapy has been reported (Daly 1992). Therefore, it might not be unreasonable to conclude that, in some children, the reduction in gingival overgrowth severity over time is related to growth out-stripping cyclosporin therapeutic levels.

5.5.6 The relation between trough cyclosporin concentration and gingival overgrowth

A significant difference in the trough cyclosporin concentration between those who did and those who did not have gingival overgrowth was found in those who were examined whilst they were aged between 5 - 10 years.

It is clear that cyclosporin is associated with gingival overgrowth but there is conflicting published information on the relation between the trough concentrations and gingival response (Ross *et al* 1989: Morisaki *et al* 1990: Funakoshi *et al* 1992). Therefore, the general lack of correlation between the trough cyclosporin

concentration and gingival overgrowth in the present study is not altogether surprising.

Although the trough cyclosporin concentration is the most commonly used in clinical practice, it is now accepted that it is a very poor guide to the therapeutic efficacy of the drug. It has been shown that there is little correlation between cyclosporin dose, trough levels, and total exposure to the drug (Lindholm *et al* 1993).

5.5.7 Neoral

The Neoral microemulsion formulation has been demonstrated to have more consistent absorption and a closer correlation between blood trough values and drug exposure (AUC) in children (Lin & Lee 1996). Therefore, trough cyclosporin levels might be a more meaningful determinant of individual bioavailability in this new formulation than has been found in Sandimmune (Levy 1996).

However, the bioavailability and accuracy of the trough values of Neoral compared to those of Sandimmune have already been examined in a study of heart transplant patients in Harefield Hospital. The investigators confirmed that the pharmacokinetic profile of Neoral was indeed more consistent in size and shape and that the Cmax concentration was significantly higher than Sandimmune, but they also found that the trough levels in both preparations were the same (Mikhail *et al* 1994). In addition, they reported that no significant difference was seen in the correlation between trough

levels and AUCs in either formulation. They, therefore, concluded that the use of trough levels as an indicator of total exposure to the drug will be no more reliable in Neoral than they proved to be with Sandimmune. They also recommended that the increased predictability of absorption of Neoral should allow further investigation to establish whether peak, average or trough levels correlate best with the absence of rejection and toxicity.

5.5.8 The possible effect of Neoral on the gingival tissues

There have been conflicting reports in the medical literature as to whether Neoral has the same side effects as the Sandimmune formulation. Numerous studies have reported that despite the higher AUC and Cmax of the Neoral preparation that there is no increased toxicity (Levy 1996: Barone *et al* 1996: Chang & Choc 1996].

However, some authors have also warned that the increased exposure to cyclosporin might increase drug related adverse effects (Grant *et al* 1996). Bennett *et al* (1996) reported nephrotoxicity, arteriolopathy, microcalcification and fibrin thrombi in the glomeruli in renal graft recipients whose medication had been changed to Neoral. They suggested that the two cyclosporin preparations were not equivalent in terms of safety or efficacy.

A reduction in dosage of cyclosporin of between 6% to 20% has been recommended following transition to the Neoral preparation but this has been shown to provide insufficient immunosuppression, especially in renal transplant recipients. In this group,

the Neoral dose needs to very similar to that of Sandimmune in order to achieve comparable drug exposure and maintain trough values (Levy 1996). The current British National Formulary recommends that Neoral is substituted milligramme for milligramme.

The findings in this study suggest that there is likely to be an increased prevalence and severity of gingival overgrowth associated with the increased bioavailability of cyclosporin. Furthermore, it is clear that, until a valid means of measuring the cyclosporin bioavailability is developed, the true association between cyclosporin concentration and gingival overgrowth will continue to elude dental researchers.

5.5.9 The effect of concomitant nifedipine medication on gingival overgrowth

Although nifedipine is also known to cause gingival overgrowth, this study found no association between nifedipine medication and either the presence or the severity of gingival overgrowth. This lack of association between the presence or degree of gingival overgrowth and nifedipine medication either on its own or with cyclosporin is possibly a reflection of the very short length of time that nifedipine is prescribed for the children with liver grafts. However, it is interesting that Lowry *et al* (1995) also failed to show that concomitant nifedipine therapy had an effect on either the presence or the severity of gingival overgrowth in their study of paediatric heart transplant recipients.

5.6 Cytomegalovirus

Forty-seven percent of the children had gingival overgrowth but for the majority of these, this was only mild.

In this investigation, the circulating cyclosporin level could be considered to be a constant variable. There was no relationship found between the circulating cyclosporin level and either the presence or the severity of the gingival overgrowth in the sample of 59 children. Moreover, there was no significant difference between the circulating cyclosporin levels of the 31 children who had no gingival overgrowth compared to the 28 who did. Furthermore, the cyclosporin levels were similar in the CMV seropositive and CMV seronegative groups of children.

If CMV was in part responsible for gingival overgrowth a significant difference in the prevalence of gingival overgrowth between those who were CMV seropositive and those who were not would be expected. This study found no such evidence. Moreover, the gingival overgrowth was not found to be more severe in those who were CMV seropositive.

Moreover, since there is an increased risk of CMV disease following primary infection (Davison *et al* 1993) it is not unreasonable to assume that those patients who had primary CMV infection following transplantation would be more susceptible to the effect of cyclosporin on the gingival tissues, if CMV was a contributory factor.

However, this investigation also failed to show either an increased prevalence or an increased severity of gingival overgrowth in subjects with primary infection.

Therefore, although Epstein *et al* (1992) suggested that CMV might exert an influence on cyclosporin-induced gingival overgrowth this was not confirmed by this investigation.

5.7 Further study in liver graft recipients

5.7.1 The demand for dental care

The increased survival rate of paediatric liver graft recipients will doubtless lead to a future dental demand for correction of discoloured and hypoplastic teeth and gingival overgrowth. In addition, parents of these children require preventive advice and therapy and dental counselling in respect of the need for aesthetic treatment in later years. This must place a higher demand on the dental profession but the type of service provision, demand and cost of providing the specialist care will need to be assessed.

5.7.2 Monitoring of the effect of Neoral on gingival overgrowth in children

Further study will be required to clarify the effect of the microemulsion preparation of cyclosporin on the gingiva. This will be particularly relevant in the light of the increase in liver transplantation in babies under one year, since Neoral is especially targeted for use in this group. If the prevalence and severity of gingival overgrowth is indeed strongly related to bioavailability, this group of children in particular are likely to encounter more severe problems in relation to delayed eruption, prolonged teething and poor nutrition related to difficulties in mastication.

5.7.3 Clarification of the need for antibiotic prophylaxis

The published guidelines on the oral care of patients who have received liver grafts recommend steroid cover and prophylactic antibiotic therapy in accordance with American Heart Association guidelines for invasive procedures. These guidelines appear to be based on the experience of treating patients with bone marrow and renal transplants rather than those who have received liver grafts. No serious consequence of bacteraemia caused by oral flora to any solid tissue grafts has ever been reported. Greenberg and Cohen (1977) in a study of the oral and systemic complications in twenty-seven renal transplant recipients, reported no deaths due to infection. Therefore, even the provision of antibiotic prophylaxis, although sensible, has still to be evaluated. Moreover, the majority of paediatric liver transplant recipients only receive steroid therapy during the first three months post transplantation. The steroid therapy is discontinued to alleviate the risk of infection. Therefore, the need to provide steroid cover is also still to be clarified.

Chapter Six

Conclusion

6.1 Combined conclusion of the five investigations

This is the largest investigation into the oral effects of paediatric liver transplantation that has ever been conducted. The results have shown that paediatric liver graft recipients have a high prevalence of intrinsic green pigmentation (45%), delayed eruption of the primary dentition (40%), and cyclosporin-induced gingival overgrowth (55%). However, the prevalence of enamel hypoplasia was only 11% and this was lower than had hitherto been expected, based on the reports of previous investigators.

In this study, no association was found between the trough cyclosporin concentration and either the presence or the severity of gingival overgrowth in paediatric liver graft recipients. This finding was attributed to both the questionable reliability of the trough cyclosporin concentration and to variation in cyclosporin bioavailability in this population. The results also failed to show that concomitant nifedipine therapy adversely affected cyclosporin-induced gingival overgrowth. The most likely explanation for this result is that children with liver grafts are often only medicated with the drug for a short period of time.

This study confirmed that, not only was there wide inter and intra-individual variation in the severity of the intrinsic green pigmentation in the primary dentition, but primary molar teeth were also found to be more severely pigmented than the primary incisors. There was also wide variation in the severity of the green pigmentation in permanent teeth but there was less intra-individual variation. The clinical findings

suggested that the deposition of the pigment could be incremental in nature and occurred post-natally.

Contrary to earlier reports, no improvement in the severity of the intrinsic green pigmentation in primary teeth was observed. The previous studies used subjective rather than objective measurements of the severity of the pigmentation. Nevertheless, this study highlighted the difficulties in evaluating the severity and extent of intrinsic pigmentation, particularly in primary incisors.

Malnutrition alone was not found to be a significant factor in the aetiology of delayed eruption of the primary dentition. However, there was evidence that malnutrition due to liver disease contributes to the high prevalence of delayed eruption in those children who later receive a liver graft. The results of this study also strongly suggest that cyclosporin therapy is a significant aetiological factor. It is proposed that cyclosporin causes thickening of the overlying mucosa and thereby inhibits the emergence of a previously delayed primary tooth.

The prevalence of gingival overgrowth was found to vary with the age of the child. The prevalence was lower in babies but higher in adolescents and around the time that the primary dentition is erupting. The results of this study also showed that the prevalence of cyclosporin-induced gingival overgrowth varied according to the age at which a child received a liver graft. Although the prevalence of gingival overgrowth

was found to increase with the duration of the cyclosporin therapy, no significant statistical association was found. These findings could be attributed to the poorer bioavailability of cyclosporin in infants and the adverse effect of puberty in adolescents. Nevertheless, the results in this investigation also provide the most likely explanation for the disparate reports of gingival overgrowth prevalence in previous studies, since many of them included samples that mixed children and adolescents with adults.

This study showed that cytomegalovirus did not influence cyclosporin-induced gingival overgrowth.

6.2 The null hypotheses

On the basis of the results in this study only two of the null hypotheses could be entirely rejected:

1. Children with liver grafts did have a high prevalence of intrinsic green pigmentation, delayed eruption of the primary dentition and cyclosporin-induced gingival overgrowth but they did not have a high prevalence of enamel hypoplasia.
   Null hypothesis not entirely rejected.

2. The clinical findings suggest that the intrinsic green pigmentation was deposited incrementally post natally. There was no improvement in the severity of the green pigmentation with time.
   Null hypothesis rejected.

3. Delayed eruption of the primary dentition was found to be related to the previous liver disease, malnutrition and cyclosporin therapy.
   Null hypothesis rejected.

4. There was no association between the trough cyclosporin concentration and gingival overgrowth in paediatric liver graft recipients.
   Null hypothesis accepted.

5. Prevalence of cyclosporin-induced gingival overgrowth was affected by the age of the child, their age at transplantation, the duration of cyclosporin therapy.
   Null hypothesis rejected.

6. Cytomegalovirus does not influence the effect of cyclosporin on the gingival tissues.
   Null hypothesis accepted.

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Oral findings in children with liver transplants

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Summary A report is presented of a prospective study into the oral manifestations of liver disease in children. Fifty-five children who had received a liver transplant were examined; their ages ranged from 8 months to 16 years 2 months, and 37 of them were under 5 years of age. The following information was noted: the teeth that were erupted, the presence and severity of enamel hypoplasia and of gingival hyperplasia, and the presence of intrinsic discoloration. Results showed that seven of the 17 children under 3 years of age had delayed eruption of teeth. Six children had enamel hypoplasia, three in permanent incisors and three in primary incisors. Intrinsic discoloration was found in the primary dentition of 25 children (23 of whom had biliary atresia prior to receiving the transplant) and in one child's permanent incisors. Thirty-four children had gingival hyperplasia. There was a significant inverse relationship between the length of time since transplantation and the severity of gingival hyperplasia. Analysis of variance failed to show any association between serum cyclosporin levels and the severity of gingival hyperplasia. There was no significant relationship between current nifedipine medication and the presence of gingival hyperplasia.

Introduction

Liver transplantation is an accepted treatment for acute liver failure, chronic liver failure, metabolic liver disease and malignancy. Indeed, the only contraindications to liver transplantation are severe cardiopulmonary disease and irreversible organic brain disease [1]. Liver grafts are matched by ABO blood group, liver size and, when possible, by cytomegalovirus status. Orthotopic liver transplantation is a technique in which the recipient's liver is removed and a donor's liver is implanted in the original site [2]. To overcome the shortage of child donors, reduction hepatectomies have recently been developed. This is a procedure in which part of an adult liver is cut down to fit a child. Reduction hepatectomies now make up 46–50% of the total grafts in children and have enabled babies under 1 year of age and 10 kg in weight to be offered liver transplantation for the first time [3,4].

Since 1967 more than a thousand liver transplantations have been carried out in North America and Europe. This increase in numbers is due to earlier diagnosis and referral, improved patient selection and operative techniques, and to the successful development of immunosuppressive agents such as Cyclosporin A and FK506. [1,5,6]. Indeed, 1- and 2-year actuarial survival rates following transplantation in world centres are now in excess of 85% [1].

Children with liver disease require careful dental management due to their inadequate drug and protein metabolism and tendency towards prolonged bleeding. Intrinsic tooth discoloration, particularly in relation to biliary atresia, has also been reported [7,8]. However, as liver transplantation becomes increasingly common, problems associated with dental management will also become more prevalent. So far, published studies have not included pre-school children and sample sizes have been small. Furthermore, research has largely been confined to the effects of Cyclosporin A medication on the gingival tissues [5,9]; other possible oral manifestations associated with liver transplantation have not been investigated.

The aim of this paper is to report on delayed eruption of teeth, intrinsic staining, enamel hypo-

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plasia and gingival hyperplasia in a group of children who had received orthotopic liver transplants.

Methods

The Liver Unit in The Children's Hospital, Birmingham, serves the West Midlands Region, the largest in the UK, with a catchment population of over five million. It is a supra-regional centre and as such accepts referrals from all over the UK. The Dental Department works closely with the Liver Unit team and is involved with the patients as soon as they present for medical assessment prior to placement on the liver transplant waiting list.

Fifty-five children (32 girls and 23 boys) were examined over an 18-month period. Their mean age was 4 years 8 months (range 8 months to 16 years 2 months, median 3 years 6 months). The children were examined in the hospital's dental unit by one of the authors (M.T.H.) when they attended the Liver Unit for review. Other children who had recently received transplants and who were still in-patients were also included. The following details of medical and transplant history were noted: age and sex of the patient; age at transplantation; diagnosis prior to transplantation; length of time since transplantation; drug history, particularly cyclosporin, nifedipine and steroid therapy (generally, all patients are prescribed both nifedipine and prednisolone for the first 3 months after transplantation only); and mean serum cyclosporin level, calculated from data obtained from blood samples taken on the day of examination and on the previous two visits.

The following information was recorded at the dental examination:

Teeth present. All erupted or partially erupted teeth were charted as present. A tooth was considered to be delayed in its eruption when there was a delay of more than 6 months beyond the normal eruption age, based on the data presented by Proffit [10] (Table 1).

Table 1. 'Normal' ages (months) for eruption of primary teeth (according to Proffit [10])

	Maxillary	Mandibular
Central incisor	10	8
Lateral incisor	11	13
Canine	19	20
First molar	16	16
Second molar	29	27

Table 2. Criteria for scoring enamel hypoplasia (Thylstrup & Fejerskov [11]).

Clinical finding	Score
Narrow white lines	1
Pronounced white lines	2
Cloudy areas	3
Entire surface opaque	4
Opacity and pits	5
Horizontal bands of pits	6
Outer enamel lost < half surface	7
Outer enamel lost > half surface	8
Changes in anatomy	9

Hypoplastic defects. The Thylstrup & Fejerskov Index [11] was used. The criteria are outlined in Table 2.

Intrinsic staining of the teeth. In these patients the intrinsic stain is green in colour and although not readily matched to current shade guides it is readily distinguishable as 'green'. Although a colour scale of the severity of the 'greenness' was developed and used to score each tooth it became clear that when intrinsic staining did occur in the primary dentition all the primary dentition was affected to some degree and so, for the purposes of this study, intrinsic green staining was scored as being present or absent.

Gingival hyperplasia. Recently published methods of measuring gingival hyperplasia [12] necessitate taking impressions and making study casts, but this was found to be unacceptable to the very young children in our sample. Therefore a simplified method was used; the diagnostic criteria are shown in Table 3.

Table 3. Criteria for scoring gingival hyperplasia.

Clinical finding	Score
No hyperplasia	0
Hyperplasia extending to 1/3 of the crown	1
Hyperplasia extending to middle of crown	2
Hyperplasia extending over 2/3 of the crown	3
Hyperplasia completely covering the crown	4

Statistical analysis

Data were analysed using the SPSSPC statistics package. The association between the level of

gingival hyperplasia and circulating cyclosporin levels was examined using analysis of variance. The association between the presence of gingival hyperplasia and concurrent nifedipine medication was examined using the chi-squared test. Pearson's Product Moment Correlation Coefficient was used to quantify the association between the length of time since transplantation and the severity of gingival hyperplasia.

Results

The mean length of time between transplantation and examination was 1 year 5 months (range 1 month to 5 years). The mean age at transplantation was 3 years 4 months (range 6 months to 14 years 6 months). The findings related to delayed eruption, enamel hypoplasia, intrinsic staining and gingival hyperplasia are shown in Table 4.

Table 4. Dental findings in 55 children with liver transplants.

	No. of children	
	Examined	Affected
Delayed eruption		
Primary teeth	42	7
Permanent teeth	13	0
Enamel hypoplasia		
Primary incisors	42	3
Permanent incisors	13	3
Intrinsic staining		
Primary teeth	42	25
Permanent teeth	13	1*
Gingival hyperplasia	55	34

*Early mixed dentition.

Delayed eruption

There were 17 children under 3 years of age. Eruption of various primary teeth was delayed in seven (41%) of these infants (i.e. delay of more than 6 months beyond 'normal' eruption age [10]). For example, one child aged 22 months had no erupted primary teeth and three had only primary central incisors erupted at 20 months of age. All seven of these children had suffered from biliary atresia and six of them had been medically diagnosed as having 'failed to thrive'. No delayed eruption was found in children above 3 years of age.

Enamel hypoplasia

Three children had evidence of enamel hypoplasia in permanent central incisors (two score '1' and one score '2'); the hypoplastic areas corresponded to the stage of development of these teeth at the time of transplantation. Three other children had hypoplastic defects (score '1') in primary central incisors. Enamel hypoplasia was not found in any other teeth, but it should be noted that the children were still in the primary dentition stage or had only just erupted their mandibular and maxillary permanent incisors.

Intrinsic staining

Twenty-five children had intrinsic green staining of the primary dentition; 23 of these had previously been diagnosed as having biliary atresia. All the primary teeth of the 25 children were affected but the primary first and second molars were more deeply stained than the incisors and canines. One patient had intrinsic staining affecting permanent incisors; she had received a transplant relatively late compared to the others who had biliary atresia and had therefore remained jaundiced for much of her infancy.

Gingival hyperplasia

Thirty-four of the 55 children had gingival hyperplasia (11 score '1', 17 score '2' and six scored '3') There was a significant inverse correlation between the length of time since transplantation and the severity of gingival hyperplasia (Pearson's Correlation Coefficient $r = -0{\cdot}33$, $P < 0{\cdot}01$)

Serum cyclosporin levels ranged from 0 to 342 ng/ml, mean 152 ng/ml; there was no significant relationship between the circulating cyclosporin level and the severity of gingival hyperplasia (analysis of variance, $F = 0{\cdot}69$, $P = 0{\cdot}78$).

Fourteen patients were taking nifedipine in addition to cyclosporin at the time of the dental examination. The mean time they had been taking nifedipine was 4 months (range 1–12 months). There was no significant relationship between the presence of gingival hyperplasia and concomitant nifedipine medication (chi-squared = 0·17, $P = 0{\cdot}77$).

Discussion

Delayed eruption of teeth was noted in seven of the 17 children under 3 years of age. Previous research

indicating that protein calorie malnutrition leads to a delay in primary tooth eruption [13] offers an explanation as to why delayed eruption was found to be more prevalent in children who had suffered from biliary atresia, since these children are frequently undernourished. However, the association between nutritional status and delayed eruption in liver transplant patients requires more detailed research. Another factor that may be relevant is Cyclosporin A-induced fibroblast stimulation within the gingival mucosa, but this has not been investigated.

Palliative treatments such as the use of teething rings, hard and fibrous foods on which to gnaw, teething gels and systemic analgesia were recommended to the parents. Forewarning parents, especially of children with biliary atresia, of the likelihood of delayed eruption helped to allay parental anxiety.

The position of the enamel hypoplastic lesions in the affected permanent incisors of three children corresponded to the stage of dental development at the time of transplantation. The intrinsic green staining that affected the erupted permanent teeth of one of the children also corresponded to the timing of severe metabolic disturbance but, since all surfaces of the affected teeth were stained, this was probably due to the original liver disease, which was biliary atresia. Therefore, further long-term follow-up is required to examine the association between liver transplantation, enamel hypoplasia and intrinsic staining.

It is known that Cyclosporin A is associated with gingival hyperplasia, but there is conflicting published information on dose response [5,9,14], and so the lack of correlation between circulating Cyclosporin A levels and gingival hyperplasia in the current study is not altogether surprising.

Nifedipine is also known to cause gingival hyperplasia. Patients often receive nifedipine for the first few months following their liver transplantation to offset the hypertensive effect of Cyclosporin A. Indeed, Seymour [15] reported that 51% of renal transplant patients treated with Cyclosporin A and nifedipine developed gingival overgrowth. The lack of association between the presence or degree of gingival hyperplasia and nifedipine medication either on its own or with Cyclosporin A is possibly a reflection of the very short length of time that nifedipine was prescribed for these children with liver transplants.

The significant inverse correlation between the severity of gingival hyperplasia and length of time after transplantation could be due to the gradual decrease in Cyclosporin A dose, but this is not supported by the results of our study. Other authors have suggested that patients build up a resistance to stimulation of gingival fibroblasts or that the degree of gingival hyperplasia depends upon the sensitivity of the individual [5,15], but no study has yet been undertaken to examine this more carefully. However, our study has demonstrated that the effect of Cyclosporin A on the gingivae may involve other factors.

Prior to liver transplantation it is important to carry out a full dental examination and to treat potential sources of infection aggressively. High-speed suction during operative dental procedures to guard against ingestion of blood has also been advocated, because blood is metabolized by the liver [2,6]. Preventive care and careful parental counselling is of great importance if good oral health is to be maintained. In our experience, this is best achieved when liver transplantation is first contemplated, because dental care is then seen by the family as being an integral part of the child's treatment. Children who already attended their local dentist regularly were advised to continue.

Following liver transplantation, patients should be examined regularly and receive topical fluoride application and reinforcement of preventive advice. It is also useful to liaise with their dietitian, because many are on high carbohydrate supplementation. Some authors [6] have recommended prophylactic antibiotic therapy in accordance with American Heart Association guidelines in which the standard prophylaxis for low-risk patients is Penicillin V. However, no serious consequence of bacteraemia caused by oral flora to solid tissue grafts has been reported. Therefore it would seem more appropriate to treat these patients the same as any other with immunosuppression, and prescribe antibiotics only when required. Stringent preventive therapy to avoid dental sepsis altogether, and rigorous treatment prior to transplantation, are the most effective measures. Intermittent steroid therapy is discontinued by 3 months after liver transplantation and therefore steroid cover is generally not indicated for dental procedures.

Recipients of liver transplants are becoming ever younger and more numerous, and dentists will increasingly be called upon to manage these patients. Therefore it is important that they should be aware of problems which might arise, such as delayed eruption of primary teeth, and of the need for stringent preventive care and advice both before and after

transplantation. Dentists can play a vital role not only in co-operating in the general medical management but also in helping these children and their parents adjust to normal life following recovery.

Manifestations orales chez des enfants hépato-transplantés

Résumé. Une étude prospective des manifestations orales chez des enfants souffrant de maladie hépatique a été entreprise chez cinquante cinq enfants ayant reçu un transplant hépatique. Leur âge s'étalait de 8 mois à 16 ans 2 mois et 37 d'entre eux avaient moins de trois ans. Les informations suivantes ont été notées: les dents ayant fait leur éruption, la présence et la sévérité des hypoplasies, des hyperplasies gingivales, des décolorations intrinséques. Les résultats ont montré que 7 des 17 enfants âgés de moins de 3 ans avaient des retards d'éruption. Six enfants avaient des hypoplasies de l'émail, trois sur les incisives permanentes et trois sur les incisives temporaires. Des décolorations intrinséques ont été retrouvées dans la dentition temporaire de 25 enfants (23 d'entre-eux avaient eu une atrésie biliaire avant la transplantation), et sur les incisives permanentes d'un enfant. Trente enfants avaient une hyperplasie gingivale. Il y avait une relation significative inversée entre l'ancienneté de la transplantation hépatique et la sévérité de l'hyperplasie. L'analyse de variance n'a montré d'association entre le taux de cyclosporine dans le sang et la sévérité de l'hyperplasie gingivale. Il n'y avait pas de relation significative entre le traitement par nifédipine et la présence d'hyperplasie gingivale.

Orale Manifestationen bei Kindern mit Lebertransplantaten

Zusammenfassung. 55 Kinder, nach Lebertransplantationen wurden untersucht. Das Alter bewegte sich zwischen 8 Monaten bis 16 Jahre und 2 Monate. 37 davon waren weniger als 3 Jahre alt. Die folgenden Befunde wurden registriert: der Stand des Zahndurchbruches, das Vorhandensein und der Schweregrad von Schmelzhypoplasien, eventuele Verfärbungen und Gingivahyperplaisen. Man fand dass 7 der 37 Kinder unter 3 Jahren einen verzögerten Zahndurchbruch aufwiesen, ebenso hatten sie Hypoplasien, bei 3 Kindern in den bleibenden Frontzähnen und bei 3 in den Milchfrontzähnen. Verfärbungen wurden an den Milchzähnen von 25 Kindern und bei einem Kind an den bleibenden Frontzähnen gefunden. Von den Kindern mit betroffenen Milchzähnen litten 23 vor der Transplantation an einer Gallenatresie. Bel 30 Kindern wurde eine Gingivahyperplasie diagnostiziert. Eine signifikante umgekehrten Relation, wurde festgestellt, zwischen der Zeitspanne seit der Transplantation und dem Schweregrad der Gingivalen Hyperplasie. In einer weiteren Analyse fand man keine Beziehung zwischen dem Zyklosporinspiegel im Serum und dem Schweregrad der Gingivahyperplasie. Es war keine signifikante Beziehung zwischen der laufenden Nifedipine Medikation und der Gingivahyperplasie festzustellen.

Manifestaciones orales en niños con transplantes del higado

Resumen. Se presenta un reporte de un estudio prospectivo sobre las manifestaciones orales en niños que han recibido transplantes del hígado. Se examinó un total de 55 niños (de 8 meses a 16 años y 2 meses de edad; 37 de elos menores de 3 años de edad) que habían recibido transplante del hígado. La siguiente información fue anotado: dientes erupcionados, presencia y severidad de hipoplasia del esmalte y de hiperplasia gingival, y la presencia de pigmentaciones intrínsecas. Los resultados mostraron que 7 de los 17 niños menores de 3 años de edad presentaron erupción dental retardada. Seis de los niños presentaron hipoplasia del esmalte, tres en los incisivos permanentes y tres en los incisivos temporales. Pigmentaciones intrínsecas fueron observadas en la dentición temporal de 25 niños y en los incisivos permanentes de un niño (23 de ellos tenían atresia billar antes de recibir el implante). Treinta niños presenraron hiperplasia gingival. No hubo relación inversamente proporcional entre el tiempo transcurrido despues del transplante y la severidad de la hiperplasia gingival. El análisis de varianza no mostró asociación entre los niveles de ciclosporina sérica y la severidad de la hiperplasia gingival. No hubo relación significativa entre la medicación con nifedipina y la presencia de hiperplasia gingival.

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