GROWTH AND CYTOGENETIC PROFILE AND VITAMIN D LEVELS IN CHILDREN WITH DOWN SYNDROME

A dissertation submitted to
The Tamil Nadu Dr. M G R Medical University in partial fulfillment of the degree of
MD in Pediatrics.

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VELLORE, TAMIL NADU, INDIA

CERTIFICATE

This is to certify that Dr. Anila Chacko is a bonafide MD Pediatrics Resident at the Department of Child Health, Christian Medical College, Vellore for the session 2007-2009. She has carried out this study entitled **"Growth and Cytogenetic Profile and Vitamin D levels in children with Down Syndrome"** at the Department of Child Health, under the guidance of Dr. Prabhakar D. Moses. This dissertation is hereby approved for submission to The Tamil Nadu Dr. MGR Medical University, Chennai, as partial fulfillment of the requirement toward the MD degree. This is an original study done by Dr. Anila Chacko and no part of it has been published or submitted to any university previously.

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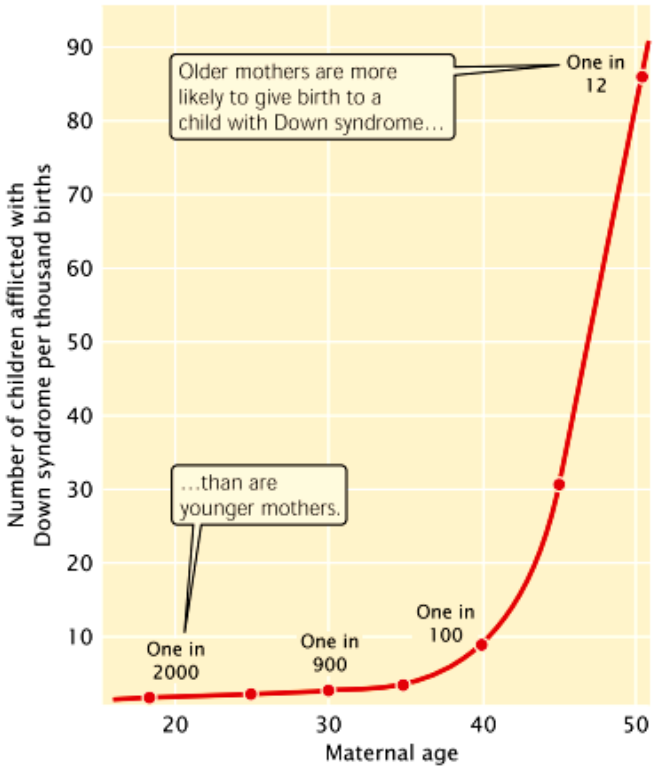
Introduction

Down syndrome is the most frequent genetic cause of mild to moderate mental retardation and associated medical problems and occurs in 1 out of 800 live births, in all races and economic groups. Down syndrome is named after John Langdon Down, the first physician to identify the syndrome in 1866. In 1959 Lejeune, Gautier and Turpins determined that Down syndrome was caused by trisomy 21. [1]

Down syndrome and chromosomal nondysjunction occur more often in the offspring of mothers conceiving at an older age. Today with the focus on education, employment, career and the need to be financially stable before having children, late child birth is becoming the norm. Hence the incidence of Down syndrome is likely to increase.

Figure 1 Down syndrome and increasing maternal age



The survival of children with Down syndrome has increased. More parents are now seeking care for these children. [2] Hence it is imperative that we anticipate an increase in incidence of children with Down syndrome and their presence at our clinics. Improving their quality of life and helping them to reach their maximum potential should be our goal.

Children with Down syndrome are shorter than their peers. Average height at most ages is around

the 2nd centile for the general population. For the majority the cause of growth retardation is not known. Some conditions leading to poor growth (congenital heart disease, sleep related upper airway obstruction, coeliac disease, thyroid hormone deficiency, deficiency of insulin like growth factor 1 and nutritional inadequacy caused by feeding problems) occur more frequently among those with the syndrome. [3, 4]

Adults with Down syndrome are prone for osteoporosis. Vitamin D deficiency is known to result in both deficient growth and osteoporosis. There is very little data on Vitamin D levels in children with Down syndrome and the effect it had on growth. We wanted to see if these children had reduced Vitamin D levels and consequently delayed growth as correction of Vitamin D deficiency is relatively easy.

Surprisingly, only one study in the world has looked at the Vitamin D status in children with Down syndrome. That was done on a group of 21 children in Spain, 16 years ago, which did not show any low levels of Vitamin D metabolites in any of these children. [5] Hence there was definitely scope for research in this area, before any final conclusion could be made.

Review of Literature - outline

- History
- Cytogenetic Profile of Down syndrome
- Growth in Down syndrome and Growth Charts for Anthropometric Assessment
- Vitamin D levels in Down syndrome
- Bone age
- Phenotypic features of Down syndrome and other associations-

  Heart disease

  Thyroid disease

  Hematology

  Hearing

  Ophthalmology

  Atlanto axial dislocation

  Developmental quotient of children

History

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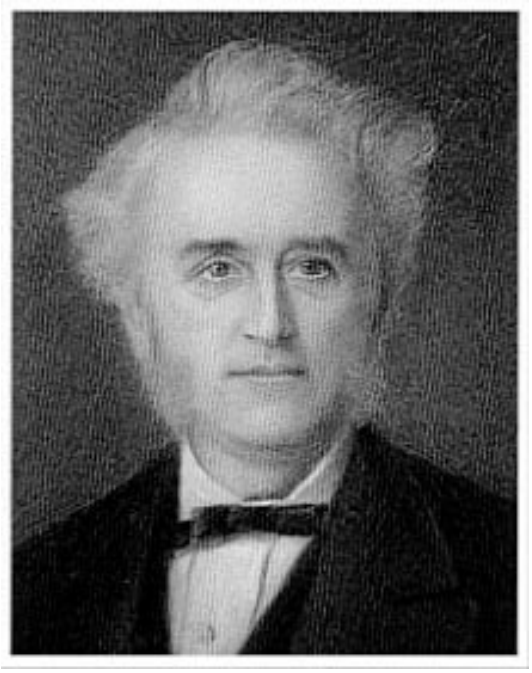
Fig. 2: John Langdon Down



John Langdon Down was the youngest son of a village grocer in Cornwall. Having first qualified in pharmacy he entered the London Hospital Medical School at the age of 25 where he was a triple gold medalist. He was appointed Medical Superintendent of the Royal Earlswood Asylum for Idiots in Surrey in 1856

In his definitive publication (in what he described the ethnic classification of idiots) in 1866, he wrote: "The very large number of congenital idiots are typical mongols. So marked is this that when placed side by side it is difficult to believe that the specimens compared are not children of the same parents. They present a close resemblance to one another in mental power". [51]

It was not until 1959 that Lejeune and colleagues discovered the extra chromosome 21 which was the underlying abnormality in Down's syndrome. Very little new was added to the clinical description of the condition apart from the description of single transverse crease in the palm noted by John Langdon Down's son Reginald in 1908 and the characteristic grey spots on the iris of the eye noted by Brushfield in 1924.[51]

Cytogenetic Profile

Trisomy is the gain of a single chromosome, represented as 2n +1. Approximately 94% of those who have Down syndrome have three full copies of chromosome 21, a condition termed primary Down syndrome. This usually arises from random **nondisjunction** in egg formation. Most children with Down syndrome are born to normal parents, and the failure of the chromosomes to divide has little hereditary tendency.

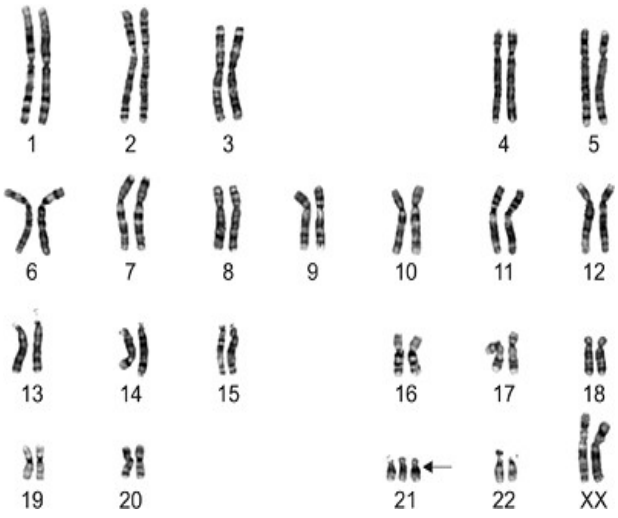


Fig. 3 Karyotype of a child with trisomy 21

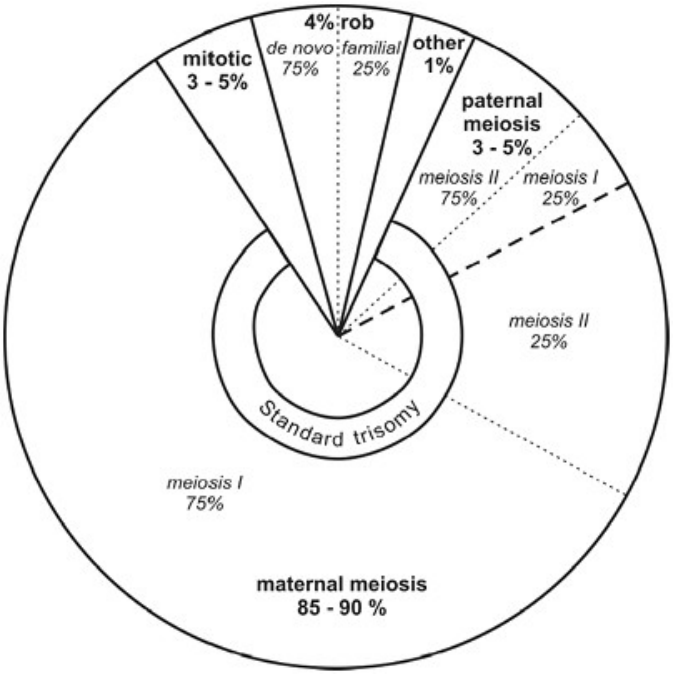
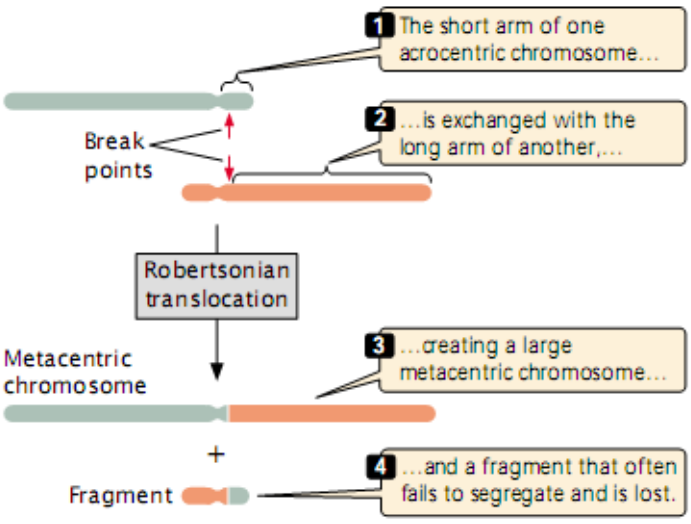


Fig. 4 Origins of trisomy21. Rob: Robertsonian
Fig 5 Robertsonian translocation



About 4% of people with Down syndrome have 46 chromosomes, but an extra copy of part of chromosome 21 is attached to another chromosome through a translocation. This condition is termed familial Down syndrome. It arises in offspring whose parents are carriers of chromosomes that have undergone a **Robertsonian translocation**, most commonly between chromosome 21 and chromosome 14: the long arm of 21 and the short arm of 14 exchange places. This exchange produces a chromosome that includes the long arms of chromosomes 14 and 21, and a very small chromosome that consists of the short arms of chromosomes 21 and 14. The small chromosome is generally lost after several cell divisions. About one-quarter of Robertsonian translocation DS is familial and three-quarters are de novo

Nondisjunction in a mitotic division may generate patches of cells in which every cell has a chromosome abnormality and other patches in which every cell has a normal karyotype. This type of nondisjunction leads to regions of tissue with different chromosome constitutions, a condition known as **mosaicism.** [6]

Cytogenetic analysis of Indian children

1. In the first large Indian study, **Verma** et al in 1979 found that translocation karyotypes were seen in 6.2% of children when the maternal age at conception was less than 30 years as against 1.1% when the maternal age was more than 30 years. Non-dysjunction was present in 92% and the rest were Mosaic.[7]

2. **Jyothy** et al studied cytogenetic data obtained from 1001 patients with Down syndrome (DS) and their parents over a 20 year period. The frequency of pure trisomy, mosaicism and translocation was 87.92, 7.69 and 4.39 per cent respectively. The origin of the extra chromosome 21 due to meiotic nondisjunction was 79.24 per cent maternal and 20.76 per cent paternal.[8] In another study published by the same author, the Translocation in Down syndrome is usually of Robertsonian type with the fusion of chromosome 21 to D or G group chromosomes. Most frequent forms are t (21; 21) and t (14; 21). The other less frequent translocations are t (13; 21), t (15; 21) and t (21; 22). [9]

3. **Sheth** et al looked at 382 clinically suspected children with Down syndrome. Free trisomy 21 constituted 84.8% of cases, translocation 8.9%, mosaic 3.9% and in 2.4% cases regular T21 was associated with structural or numerical changes. Translocation was parentally inherited in 26.5% cases and maternal transmission was twice as common as paternal. Males were more pronounced to be affected than females in all the groups. 91.6% of DS babies were born to younger mothers (20-35 yr) compared to 8.4% in elderly mothers (>35 yr). [10]

4. **Kava** et al studied 524 patients with Down syndrome over a period of 7.5 years. Results of cytogenetic abnormalities were available in 42.2%. Free trisomy (non-dysjunction) was present in 95%, 3.2% had translocation, and 1.8% were mosaics. [11]

Growth and Growth Charts

Short stature is characteristic of Down syndrome.

- Growth retardation commences prenatally
- After birth, growth velocity is most reduced between 6 months and 3 years of age
- Puberty generally occurs early and is associated with an impaired growth spurt
- These individuals reach their final height at relatively young ages. [12]

There is also a predisposition to overweight, particularly among adolescents and adults that may itself be related to the growth deficiency since it reduces energy requirements. In addition to being a risk factor for metabolic disorders, overweight is an aggravating factor for other conditions that affect this group, such as heart diseases and muscular hypotonia.[13]

Although growth is influenced by biological and environmental factors, racial variations certainly have a major role. For the majority, the cause of growth retardation is not known. Some conditions postulated to lead to poor growth are:

- congenital heart disease
- sleep related upper airway obstruction
- coeliac disease
- thyroid hormone deficiency
- deficiency of insulin like growth factor 1
- nutritional inadequacy caused by feeding problems. [3,4]

Growth Charts

Statural growth is a well known indicator of health during childhood. As growth and final height differ markedly between children with Down syndrome and healthy children, standard growth charts should not be used for children with Down syndrome.

The publication of growth charts specifically for children with Down syndrome in various populations, e.g. American, Sicilian, Dutch and French draws attention to the importance of constructing growth charts for Indian Down syndrome children.

The potential benefits of growth charts include:

- growth monitoring to detect any deviation in growth patterns
- evaluating the efficacy of measures aimed at promoting growth
- providing reassurance to parents
- evaluating the results of clinical research or intervention for individual patients
- comparing their growth with that of the normal population
- detection of the development of an additional disease which impairs growth such as hypothyroidism or coeliac disease. [12, 14]

Percentile distributions of anthropometric indexes - weight-for-age (W/A) and height-for-age (H/A) specific for children and adolescents with DS have been developed. The distribution compiled in the United States by Cronk et al is one of the most often cited in the literature and has distributions for weight according to sex and covering the age group from 1 month to 18 years. Other charts have been developed in Spain, Sweden, the United Kingdom and Ireland, Italy, Saudi Arabia, Brazil and Japan. [13]

1. Lopes et al [13] plotted height and weight of 138 children with Down syndrome on 3 growth charts (Cronk Charts, USA; Spanish charts for children with Down syndrome and the WHO charts for normal children). As can be seen from the following table, there was wide variation in the number of children categorized into the different categories (< 5th centile and >95th centile for height and weight) by different charts. There was poor agreement among the three charts. They emphasized the need for region specific growth charts for children with Down syndrome. They also summarized the findings from various studies around the world which is given below.

Table 1 Weight for age and height for age indexes of children and adolescents with Down syndrome classified according to three different reference distributions

	Children (2 to 9.99 years) (n = 98)*			Adolescents (10 to 17.9 years) (n = 40)†		
Index	**United States**	**Spain**	**WHO‡**	**United States**	**Spain**	**WHO§**
W/A index						
< P5 (%)	1.0	18.4	9.2	5.0	2.5	-
Between P5 and P95 (%)	83.7	77.6	86.7	95.0	80.0	-
> P95 (%)	16.3	4.1	4.1	0.0	17.5	-
H/A index						
< P5 (%)	0.0	20.4	55.1	2.5	2.5	60.0

Myrelid et al. also compared anthropometric data from Swedish children and adolescents with Down syndrome with the Down syndrome -specific distribution from the United States. The mean height of the Swedish Down syndrome subjects at 18 years of age was greater than that of the individuals with Down syndrome from the United States. In terms of weight, the mean weight of the Swedish adolescents with Down syndrome at 18 years corresponded to the 50th percentile for boys and the 25th percentile for girls on the distribution from the United States. The authors attributed these differences to ethnic diversity and the different sample sizes.

In Portugal, Fernandes et al. examined 196 children aged 0 to 48 months with DS and 96 siblings of these children who did not have DS. When they compared their results with the DS-specific distribution from the United States, the authors observed that the Portuguese children had a similar growth to those in the United States up to 24 months of age, but, from 24 to 48 months, they exhibited higher values for length and weight.

In a study carried out in Chile, Pinheiro et al. conducted research with 116 children and adolescents with DS, aged from 3 months to 18 years. These authors assess agreement between diagnoses of W/A and H/A indexes according to the DS-specific distributions from the United States and Spain. [13]

2. In India, Sachdev et al [15] in 1981 measured anthropometric indices of 139 children up to the age of 5 years. They found that the height curves in the first 9 months fell below the 50^{th} percentile of normal children and subsequently there was a marked fall below the 10^{th} centile. The weight curve fell slightly below the tenth centile and advanced almost parallel to it. Head circumference measurements were similar. Time taken to reach “normal height and weight” was between one and a half to two times for children with Down syndrome. However since then, there have been no further studies, especially for older children.

Vitamin D levels, Bone density

Vitamin D status has a profound effect on growth and development of children and has major implications for adult bone health. Overt cases of vitamin D deficiency represent only the tip of an iceberg of vitamin D insufficiency. Severe vitamin D deficiency is usually associated with 25-hydroxyvitamin D [25(OH) D] concentrations less than 5.0 ng/mL and results in rickets and osteomalacia. Less severe deficiency has been associated with numerous negative skeletal consequences, including secondary hyperparathyroidism, increased bone turn over, enhanced bone loss and fracture risk.

In assessing a person's vitamin D status the most commonly used and most sensitive index is 25(OH) D. 1, 25-dihydroxyvitamin D [1, 25(OH) 2D] can be normal, high, or low in vitamin D deficiency. Age, sex, pubertal status, latitude, season, race, and ethnicity influence serum concentrations of 25(OH) D. Unfortunately, assays for 25(OH) D still lack sufficient standardization as indicated by international comparative studies. The assays are not cross calibrated and differences of upto 38% have been reported in 25(OH) D estimations across different laboratories. Hence, serum 25(OH) D levels from different regions or countries cannot be compared satisfactorily.

Children with Down syndrome are shorter than their peers. The cause for this is unclear. Adults with Down syndrome are prone for osteoporosis. Vitamin D deficiency is known to result in both deficient growth and osteoporosis. There is very little data on Vitamin D levels in children with Down syndrome and the effect it had on growth.

1. Del Arco et al [5] in 1992 published their findings from 21 children with Down syndrome in Cantabria, Spain. The serum levels of the active Vitamin D metabolites 25-hydroxyvitamin D [25(OH)

D], 1, 25-dihydroxyvitamin D [1, 25(OH) 2D] and 24, 25 dihydroxyvitamin D [24, 25(OH) 2D]) were checked. Serum calcium, magnesium, phosphate, alkaline phosphatase, parathormone and osteocalcine were also determined.

In the Down syndrome group, the average values of the three Vitamin D metabolites were comparable to those of an age-matched group both in winter and summer. No child with Down syndrome showed values below the normal range, either in Vitamin D metabolites, or in the other parameters of calcium metabolism. This investigation showed that children with d Down syndrome do not require Vitamin D prescription when appropriate periods of sunlight exposure are provided.

2. The bone mineral density (BMD) of lumbar vertebrae of children with Down syndrome was studied by Kao. [17] The BMD was measured by dual photon absorptiometry (DPA). They showed that the BMD in Down's syndrome was significantly lower compared to that found in normal children ($P < 0.01$).

Hence we thought that there was need to see if children with Down syndrome had deficiency of Vitamin D. However the definition of hypovitaminosis D is not standardized. Different authors have used different cut off levels. Below are some of the significant Indian studies – some in normal children and some in adults.

1. Marwaha et al [18] studied a cohort of 5137 children and adolescents to assess the prevalence of vitamin D deficiency. They found clinical evidence of vitamin D deficiency in 556 children (10.8%). They compared biochemical variables (in 760 children) of the calcium–vitamin D axis between 2 socioeconomic groups and studied the effect of hypovitaminosis D on bone mineral density.

Concentrations of 25(OH) Vitamin D of 10–20, 5–10, and less than 5 ng/mL were classified as mild, moderate, and severe hypovitaminosis D as recommended by Lips.

The unadjusted mean serum concentration of 25(OH) D for the entire group was 11.8 +/_7.2 ng/mL.

Adjusted mean +/_ SE values of serum 25(OH) D for the lower socioeconomic score group was 10.4+/_ 0.4 and 13.7 +/_ 0.4 ng/mL for the upper socioeconomic group ($p < 0.01$). Age, sex, and SES independently influenced the variations in 25(OH) D concentrations.

Males had significantly higher mean serum concentrations than did females ($P<0.004$). According to the Lips classification, hypovitaminosis D was seen in 92.6% of the LSES group (severe: 11.2%; moderate: 39.5%; and mild: 42.1%) and in 84.9% of the USES group (severe: 4.9%; moderate: 25.5%; and mild: 57.6%). Thus severe hypovitaminosis D (<5 ng/mL) was seen in 8.6% of their study population.

Severe hypovitaminosis D (<5 ng/mL) was seen in 8.6% of their study population, in 23.5% of Finnish adolescents, and in 45.2% of Chinese adolescents in and 6.7% in summer. In other studies from Finland, using cutoffs of 8–10 ng/mL, the prevalence of hypovitaminosis D was 13.5%, which compares with 37% of children in the current study who had serum 25(OH)D concentrations <9ng/mL (lower limit of manufacturer's normal range).

Other studies have also noted low serum 25(OH) D concentrations among adults of Indian origin in both India and the United Kingdom. This value was significantly lower than that reported in studies from Europe and Brazil and marginally higher than that reported from China.

2. Pettifor in an editorial in Indian Pediatrics said: "The use of varying cut off points for vitamin D deficiency and insufficiency by different authors has made it difficult to compare the results of research published by different authors and has further complicated comparisons between different communities and populations.". He recommended a cut off value of 10 ng/ml to define vitamin D deficiency.[19]

3. Sharma et al from the All India Institute of Medical Sciences defined hypocalcemia in children as total serum concentration levels less than 8.0 mg/dl with normal concentrations of serum albumin.

They identified 29 patients over an 11 year period. [20]

4. Hypophosphatemia was assessed based on the different cut off points for different age groups. (1-3 yr 3.8-6.5mg/dl; 4-11yr 3.7-5.6mg/dl; 12-15yr 2.9-5.4mg/dl). [21]

5. Alkaline phosphatase levels considered normal were based on the different cut off points for different age groups (1-9 yrs: 145-420U/L; 10-11 years 130-560U/L ; 12-13 years Male 200-495U/L; Female 105-420 U/L; 14-15 years Male: 130-525 U/L; Female 70-230 U/L). [21]

Bone Age

"Bone age" of a child is the average age at which children reach a particular stage of bone maturation. At birth, only the metaphyses of the long bones are present. As a child grows the epiphyses become calcified and appear on the x-rays, as do the carpal and tarsal bones of the hands and feet. As sex steroid levels rise during puberty, bone maturation accelerates. The cartilaginous zones become obliterated and the epiphyses are said to be closed.

The most commonly used method is based on a single x-ray of the fingers, hand, and wrist. A hand is easily x-rayed with minimal radiation and shows many bones in a single view. The bones in the x-ray are compared to the Greulich and Pyle standard.

The Brush Foundation enrolled 1000 children from 1931 to 1942. They were examined at regular intervals and different anthropometric measurements, X-ray films, psychometric and psychological tests were administered. Based on this the Greulich and Pyle Atlas of skeletal maturation of the hand was published. [22]

They looked for a method which would provide more precise data about the development of the child than could be inferred from its height, weight and age alone. People belonging to different ethnic societies had different heights and weights at the same age and so a single height/weight for age chart, could not be used for all children. Children of different regions have different age of onset of puberty. Thus the age at which the maximum annual increment of height and weight occurs – the so called preadolescent spurt of growth, is different in different people groups. They thus looked for a dependable indicator of maturity, which would be independent of body size. The development status of the skeleton as disclosed by an X-ray film of the hand and wrist appeared to meet this need.

Phenotypic profile, Associations and Natural history: an Overview

The following abnormalities are seen in Down syndrome [23]

General: Hypotonia, hyperflexibility of joints, relative short stature with an awkward gait.

Central Nervous System: Mental deficiency

Craniofacial:

- Brachycephaly with a relative flat occiput and a tendency toward midline parietal hair whorl
- Mild microcephaly with upslanting palpebral fissures
- Thin cranium with late closure of fontanelles
- Hypoplasia to aplasia of frontal sinuses, short hard palate
- Small nose with low nasal bridge
- A tendency to have inner epicanthal folds.

Eyes: Speckling of iris (Brushfield's spots) with peripheral hypoplasia of iris; fine lens opacities by slit lamp examination (59%); refractive error.

Ears: Small; overfolding of angulated upper helix; sometimes prominent; small or absent ear lobes.

Dentition: Hypoplasia, irregular placement, fewer caries than normal.

Neck: Appears short.

Hands:

- Relatively short metacarpals and phalanges.
- Fifth finger: Hypoplasia of midphalanx of fifth finger (60%) with clinodactyly(50%), a single crease (40%), or both
- Simian crease (45%).
- Distal position of palmar axial triradius(84%)
- Ulnar loop dermal ridge pattern on all digits (35%)

Feet: Wide gap between first and second toes; plantar crease between first and second tos. Open field dermal ridge pattern in hallucal area of sole (50%).

Pelvis: Hypoplasia with outward lateral flare of iliac wings and shallow acetabular angle.

Cardiac: Anomaly in about 40%; atrioventricularis communis, ventricular septal defect, ASD and aberrant subclavian artery in descending order of frequency.

Skin: Loose folds in posterior neck (infancy). Cutis marmorata, especially in the extremities (43%).Dry hyperkeratotic skin with time (75%).

Hair: Fine, soft and often sparce; straight pubic hair at adolescence

Genitalia: Male: relatively small penis. Hypogonadism in terms of fertility (100%) and testosterone production.

Other abnormalities: Seizures (<5%), Strabismus (33%), Nystagmus (15%), keratoconus (6%), cataract (1.3%), low placement of ears; webbed neck; tracheoesophageal fistula, duodenal atresia, tetralogy of Fallot; atlantoaxial dislocation(12%),cryptorchidism (27% till 9 years and 14% after 15 years), syndactyly of second and third toes. The incidence of leukemia is about 1:95, or close to 1%. Thyroid disorders are common.

Down Syndrome and Heart Disease

Approximately 40% of children with Down syndrome have congenital heart disease. The Atlanta Down Syndrome Project evaluated 227 children with trisomy 21 with Congenital Heart Disease (CHD):

- 45% had an atrioventricular septal defect (AVSD; with or without other CHD)
- 35% had a ventricular septal defect (VSD; with or without other CHD)
- 8% had an isolated secundum atrial septal defect
- 7% had an isolated persistent patent ductus arteriosus
- 4% had an isolated tetralogy of Fallot
- 1% had other defects. [52]

Similar studies have shown comparable statistics. Left sided obstructive lesions such as coarctation and valvar aortic stenosis are rare, and transposition of the great arteries has not been reported in Down syndrome. All babies with Down syndrome should have an early screening echocardiogram.

Bhatia et al evaluated the utility of echocardiography in assessing the frequency and nature of cardiac malformations in children with Down syndrome. Fifty cases of chromosomally proven Down syndrome were studied. Twenty-two (44%) children had heart diseases.

Endocardial-cushion-defect was the commonest anomaly, followed by ventricular septal defect. The study further suggests that clinical examination of the cardiovascular system alone may not be sufficient in detecting heart disease. Two-dimensional echocardiography offers an excellent non-invasive tool for diagnosing cardiac malformations in Down syndrome. [24]

Thyroid Disorders

Down syndrome is one of the most common causes of mental retardation. Hypothyroidism might be one reason for growth retardation in Down syndrome. The reported prevalence of hypothyroidism has

varied between 3% and 54%. Thyroid autoantibodies are found in 13-34% of patients. Thus, both hypothyroidism and hyperthyroidism are more common in patients with Down syndrome than in the general population [25]

1. Gibson et al did a longitudinal study on thyroid disease in Down syndrome. The following definitions were used:

- Hypothyroidism: low thyroxine and TSH of 6 mu/ml or more
- Isolated raised TSH (IR-TSH): normal thyroxine and TSH of 6 mu/ml or more
- Euthyroid: normal thyroxine and TSH less than 6mu/ml
- Positive auto antibodies: titre greater than 1:64

81% had normal thyroid function and 19% had IR-TSH. Between the first sampling and the resampling about 5 years later, 18 out of 122 children were lost to follow up. Of the 103 individuals resampled, 92% had normal thyroid function, 9.7% had IR-TSH and two cases (2%) of definite hypothyroidism were identified. The cause of IR TSH was not determined. Autoantibodies were seen in nine at first testing and in seven at second testing. On both occasions a positive association was seen with IR-TSH but not with hypothyroidism. [26]

2. Tuysuz et al studied 320 children with Down syndrome between 5 days to 10 years. They concluded that the prevalence of congenital hypothyroidism was 1.8% in children with Down syndrome while 25.3% of them had compensated hypothyroidism. Besides congenital hypothyroidism cases, those with TSH levels between 11 and 20 mU/l may benefit from treatment with low-dose thyroxine. [27]

3. Unachak et al studied 140 patients aged from 3 days to 14 years.

- Ten patients (7.1%) were diagnosed with overt thyroid disease: congenital hypothyroidism 3.6%, acquired hypothyroidism associated autoimmune thyroiditis 1.4% and hyperthyroidism 2.1%.
- Sub-clinical hypothyroidism (SH) accounted for 32.9% of all cases; 10.7% showed a

spontaneous decrease to normal TSH levels and 13.6% had persistently elevated TSH levels with the median follow-up time of 6 and 12 months, respectively. [28]

4. Sharav et al showed that 60% had a TSH level higher than 5.7 mU/L in the presence of high or normal thyroxine levels. High TSH levels were predominant in patients under 4 years of age, ie, during the phase of active growth, and showed a declining trend with increasing age. All of these infants had delayed growth of all parameters including head circumference, height, and weight, as compared with normal infants, and growth was particularly retarded in patients with TSH levels greater than 5.7 mU/L. [29]

5. Karlsson et al studied 85 patients with Down syndrome in a longitudinal study between the ages of 1 year and 25 years. Two patients had thyrotoxicosis associated with high concentrations of TSH receptor stimulating antibodies. This agrees with earlier reports that Down syndrome children also run higher risks of thyroid hyper function compared with healthy subjects. [25]

Haematological Malignancies

A cohort of 120 children with Down syndrome with acute lymphoblastic leukaemia (ALL) were studied and treated at AIEOP centers in Italy between 1982 and 2004. [30] Ten-year event-free survival and survival were significantly worse compared with non-DS patients ($P < 0.001$). DS patients diagnosed since 1995 had a better outcome ($P = .06$) than those diagnosed in previous years, but still had worse outcomes than non-DS patients ($P = .04$). Event-free survival of DS patients at NCI standard risk was lower than that of non-DS patients ($P = .006$).

Presenting features of childhood ALL in DS differ from those in non-DS patients. Girls are more

commonly affected. There were no affected infants and the immunophenotype (T-lineage exceptionally rare). Furthermore, *TEL/AML1* accounts for about 20% of childhood ALL in most series worldwide but in their series only 1 case was positive. They are almost invariably characterized by BCP phenotype, and are often *TEL/AML1* negative. Treatment of acute leukemia in DS subjects are apparently more favorable in AML, but not in ALL. The unfavorable outcome could be attributed to the biology of the disease, to the DS host characteristics, or to the treatment applied.

Pui et al noted in 1993 that children with DS and ALL had a low frequency of adverse clinicobiologic features at diagnosis; however, these findings did not translate into a better outcome, apparently because of treatment-related toxicity.

The definition of anaemia used for analysis was haemoglobin in the age group 6mth-6yrs haemoglobin is <10.5 gm/dl; 7yr-12yr <11 gm/dl, 12-18 yrs: female <12 gm/dl and male <13 gm/dl.[21]

Otolaryngologic Manifestations of Down Syndrome

Common anamolies of the ear, nose and throat associated with Down syndrome are: [53]

- **Orofacial:** Progressive enlargement of lips with age, macroglossia, high narrow palatal vault, delayed dental age and hypoplastic middle third of the face
- **Upper airway and special considerations: s**mall nasopharyngx and oropharynx, mild to moderate subglottic narrowing and obstructive sleep apnea syndrome
- **Otologic Disorders:** Pinna size is small. Diameter of the external auditory canal is also significantly decreased.
- **Conductive hearing loss** in Down syndrome is also caused by middle ear disease particularly otitis media. Conductive hearing loss can also be due to ossicular fixation or residual

mesenchymal tissue in the middle ear.

- **Sensorineural hearing loss**. A study has revealed progressive ossification along the outflow pathway of the basal spiral tract that leads to the cochlear nerve.
- Other studies have demonstrated temporal bone anomalies: mondini's cochlea, shortened apical cortical turns, shortened organ of corti, decreased spiral ganglion cells, widened semicircular canals and vestibules, anomalous lateral semicircular canals and vestibules and residual middle ear mesenchyme.

Ophthalmological manifestations in Down syndrome

Proper ophthalmologic evaluation is necessary in children with Down syndrome as it influences their educational development.

da Cunha et al studied a total of 152 children with Down's syndrome between two months and 18 years of age. [31]

- Ocular findings in decreasing prevalence were the following: upward slanting of the palpebral fissure with the outer canthus 2 mm or higher than the inner canthus (82%), epicanthal folds (61%), astigmatism (60%), iris abnormalities (52%), strabismus (38%), lacrimal system obstruction (30%), blepharitis (30%), retinal abnormalities (28%), hyperopia (26%), amblyopia (26%), nystagmus (18%), cataract (13%), and myopia (13%).
- Patients younger than five years had a higher prevalence of hyperopia than those in other age

groups; patients between five and 12 years old had a higher prevalence of astigmatism; and patients older than 12 years of age had more iris abnormalities, strabismus, and cataract. Myopia and myopic astigmatism were more common in the patients with cardiac malformations.

Gonzalez et al examined 60 children with Down syndrome and 60 controls. Children with Down syndrome had a significantly higher incidence of refraction errors as a whole (p 0.001), myopia (p 0.01), hypermetropia (p 0.02), astigmatism (p 0.001) and strabismus (p 0.001). [32]

Atlantoaxial dislocation

Cervical spine instability associated with Down syndrome has been of concern since it was first reported by Spitzer in1961. Between 10% and 30% of individuals with Down syndrome show radiographic evidence of an increased atlanto dens interval (ADI). Determination of the ADI provides indirect information about the space available for the cord (SAC).

Pueschel et al reported that 14.6% of 404 patients with Down syndrome had an ADI greater than 4.5mm, but only 1.5% of the group had symptoms and were ultimately treated with surgical stabilization of the cervical spine. Increased ADI in the Down syndrome population has not been directly correlated with a concomitant increase in neurologic compromise.

Radiographs of the cervical spine in the Down syndrome population must be evaluated by standards specific to that population and not by traditional standards derived from radiographs of the cervical spine in the general population.

MRI may be helpful in determining the presence of cord compression in flexion and is most significant when signal changes exist within the cord. When the anterior aspect of the cervical spinal cord is compressed by the odontoid process during flexion of the neck, impending myelopathy is of concern.

Progressive rotary subluxation of the atlantoaxial junction will significantly narrow the spinal canal and may impose marked, irreversible cord injury. Somatosensory evoked potentials have been used to detect cord compromise in patients with Down syndrome who show abnormal radiographic findings of the cervical spine but are clinically asymptomatic. [33]

Down syndrome and social quotient

Bhatia et al [34] studied 40 consecutive children with Down syndrome. Developmental Quotient (DQ) of children in the study group was evaluated by a clinical psychologist using Gessel's developmental schedule, Seguin Form Board, Vineland social maturity scale, DASSI-II (Bayley's scale), Malin's Intelligence Scale (for Indian children) and Stanford-Binet test. 50% had Developmental Quotient of 51-70 followed by 32.5% with DQ 36-50, 10.0% with DQ 30-35 and 7.5% with DQ above 70.

Various studies have shown that children with Down's syndrome showed low scores on motor, adaptive and social development at all ages as compared to normal children. This is also seen in relation to feeding, socialization, toilet training and sleep. [34]

The Vineland Social Maturity Scale measures the different social capacities of an individual. It provides an estimate of social age (SA) and social quotient (SQ) and shows a high correlation (0.8) with intelligence. It is designed to measure social maturation in eight social areas: Self help general, self help eating, self help dressing, self direction, occupation, communication, locomotion and socialisation. The scale consists of 89 test items grouped into year levels. It can be administered to children between the age groups of 0 – 15 years. Social quotient = 100 X (social age in months/chronological age in months) [35]

Aims

The main aims of the study were:

1. To study the prevalence of Vitamin D deficiency by assessing 25(OH) Vitamin D levels in children with Down syndrome.
2. To assess the effect of 25(OH) Vitamin D deficiency on height, bone age and other biochemical markers in these children.

Since there were very few recent clinical studies published on children with Down syndrome in India, we also aimed to look at

1. Assessment of the phenotypic and cytogenetic profile of Children with Down syndrome and presence of common associated malformations, deficiencies and associated illnesses.
2. An evaluation of their social quotient.

Materials and Methods

Study setting: The study was conducted in the Department of Child Health, Christian Medical College, Vellore – a tertiary care medical centre in South India.

Study Period: The duration of the study was from October 2007 to September 2008.

Study Design: Prospective descriptive study

Instuments: Questionnaire

Inclusion Criteria: Children clinically suspected to have Down syndrome between the ages of 5 months- 16 years and who were cytogenetically proven were enrolled in the study.

- Informed consent was obtained from the parents.
- Relevant history and detailed clinical examination was carried out.
- Measurements were taken by the Primary investigator or trained nurses. Height was measured on the infantometer in those less than 2 years or those who could not stand. In older children the stadiometer was used. Weight was checked on a sensitive electronic weighing scale to the closest 10 grams. The head circumference was measured using non stretchable tapes to the nearest millimeter taking the maximum occipitofrontal diameter.
- The height and weight (and head circumference for children less than 3 years) were plotted on 2 growth charts – growth charts of "normal" Indian children (Agarwal) as recommended by the Expert group of the Indian Academy of Pediatricians [36] and growth charts for children with Down syndrome as described by Cronk. [37]
- Blood was collected for the following investigations: complete blood count, thyroid function test and thyroid stimulating hormone, calcium, phosphorus, alkaline phosphatase, 25(OH) Vitamin D levels (by Automated Chemiluminescent Immunoassay) and karyotyping (Annexure

1)

- X-ray left hand and wrist was taken for bone age and lateral neck X-ray in neutral position to look for atlanto-axial dislocation in children older than 3 years. Echocardiogram, eye checkup and hearing assessment were done. Developmental assessment (social quotient) was done using the Vineland Social Maturity Scale.
- The data was entered in a spreadsheet and analyzed by SPSS software version 11.

The following definitions were used in our study:

1. Hypertelorism: Canthal index: Inner canthal distance/outer canthal distance X 100

   It is normally 38 in males, 38.5 in females. It is increased in hypertelorism. [38]

2. Upslanting eyes: upward slanting of the palpebral fissure with the outer canthus 2mm or higher than the inner canthus [31]

3. Epicanthal folds: An epicanthal fold is skin of the upper eyelid -- from the nose to the inner side of the eyebrow -- that covers the inner corner (canthus) of the eye.

4. Prominent malformed ears: Abnormal helix, low set ears

5. Clinodactyly: Short incurved fifth finger because of hypoplasia of mid phalanx of fifth finger. [38]

6. Sandal gap: Wide gap between the first toe and the second toe. [38]

Results – an Overview

- *Demography*
- *Cytogenetic profile of children with Down*
- *Maternal history*
- *Anthropometry*
- *25 (OH) Vitamin D levels; calcium, phosphorus and alkaline phosphatase*
- *Bone age*
- *Phenotypic profile*
- *Heart disease*
- *Thyroid*
- *Hematology*
- *Atlantoaxial dislocation*
- *Ophthalmology and Hearing*
- *Social quotient*
- *Socioeconomic status*

Demography

Sixty two children suspected to have Down syndrome were interviewed. Six were excluded:

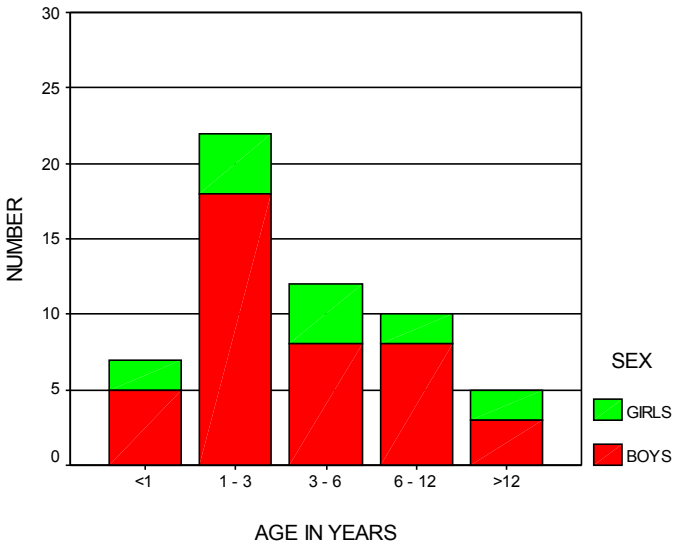
- two had cytogenetic profile of Down syndrome but were not willing for further tests
- three had clinical features of Down syndrome but did not give blood for further testing and confirmation
- The sixth child's cytogenetic analysis did not show Down syndrome

Thus, 56 children - 42 boys (75%), and 14 girls (25%) were included in the analysis. The table and graph below describe the age and sex distribution of the children.

Table 2 Overview of age of the children included in the study

Mean age	4.33 years
Median age	2.87 years
Standard deviation	4.03
Minimum age	5 months
Maximum age	14.9 years

Figure 6 Age and sex distribution of the children included in the study



The commonest age group was 1 – 3 years, followed by 3-6 years. Boys outnumbered girls 3:1.

Cytogenetic Profile

All children included in the study had cytogenetic confirmation of Down syndrome. The frequency of the different Cytogenetic abnormalities is given in the table below. Since the association between the Cytogenetic abnormalities and the social quotient has not been looked at before, we also analysed this separately.

Table 3 Cytogenetic profile of the children included in the study

Cytogenetic analysis	Frequency	Percentage
Trisomy 21	50	89.3
Translocation	4	7.1
Mosaic	2	3.6
Total	56	100

Translocation

3 children in the study had t (21; 21) translocation and one had t (14; 21)

- 46XXder (21,21) (q10,10)+21[20] Robertsonian translocation
- 46 XXder (14;21)(q10:q10) +21, trisomy due to Robertsonian translocation (14,21),
- 46XY t(21.21), DS along with homologous Robertsonian translocation
- 46XYder(21,21)(q10,q10)+21[20])

Mosaicism

Of the 2 mosaic children,

- one had 47XY+21 (13) 46 XY (7) and
- the other had 47XY+21 (17) 46 XY (3).

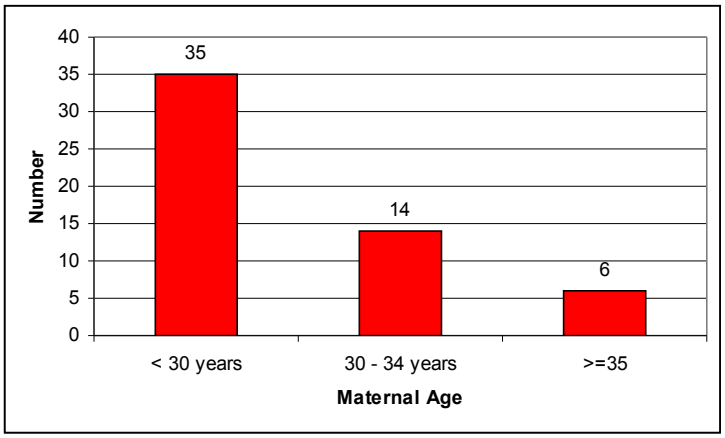
We analysed the cytogenetic findings with the various other aspects of our study. A synopis of our findings is given below. In view of the small numbers, the significance is not clear.

- Boys constituted 76% of the trisomy (38/50), 50 % of the translocations (2/4) and 100% of the mosaics (2/2).
- All 3 patients with atlantoaxial dislocation had trisomy 21.
- Of the 3 patients who had acute lymphoblastic leukaemia, 2 had trisomy (4% of the trisomy) and one had translocation (25% of the translocations).
- Of the 10 children whose parents had consanguineous marriage, 8 had trisomy and one each had translocation and mosaic.
- On the Cronk charts for weight, 50% of the translocations (2/4) and 100 % of the mosaics (2/2) had weight above the 50^{th} centile, while only 24% of the children with trisomy (12/50) were above the 50th centile. This was not statistically significant.
- There was no statistical significance or trend between the different cytogenetic abnormalities and the other anthropometric measurements (height, weight and head circumference) as checked on both the charts
- Of the children who were evaluated for hearing abnormalities, 49% of the children with trisomy (17/35), 75% of those with translocation (3/4) and 100% of the mosaics (1/1) had hearing abnormalities.
- There was no significant relationship between the cytogenetic abnormalities and cardiac abnormalities, bone age and ophthalmological abnormalities.

Maternal History

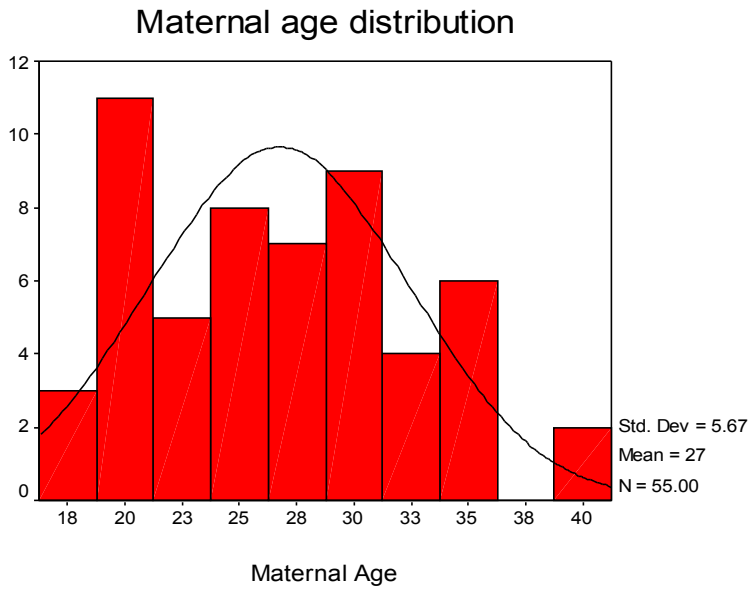
Maternal age at delivery

Figure 7 Maternal age at delivery categorized in groups



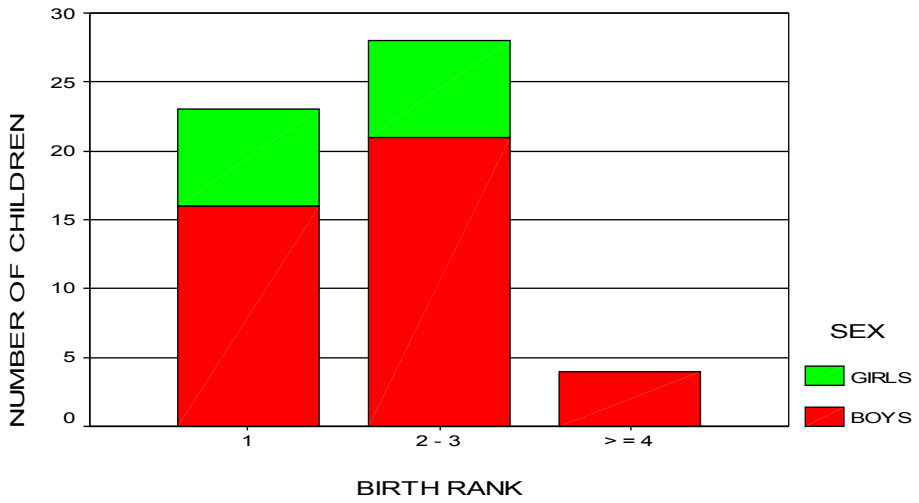
35.4% of the mothers were older than 30 years with 10.9% older than 35 years.

Figure 8 Maternal age at delivery of children with Down syndrome



The mean maternal age at the time of delivery was 27 years.

Figure 9 Birth rank of children grouped according to the sex of the children



23 of the children were the first born, 28 were second/ third born, and 4 children were of the birth rank 4 or greater. 1 child was adopted and details were not known.

- Five (9.1%) of the mothers had previous spontaneous abortions.
- The mean age of the maternal grandmother at time of mother's birth was 24 years. However some of the parents were not sure about the exact age.
- 8 of the 55 children (15%) had consanguineously married grand parents. 1 child was excluded as he was adopted.
- 10 of 55 children (18%) had consanguineously married parents
- The mean birth weight of the children was 2.5kg. The minimum weight was 1.4kg and the maximum weight was 3.92kg.
- Antenatal scan was done in 39 (69.6%) of the 55 children. In none of the scans were features of Down syndrome picked up. None of the mothers underwent any screening for Down syndrome. None of them knew antenatally that their child had Down syndrome.

Anthropometric Measurements

IAP growth charts

Figure 10 Distribution of height on the

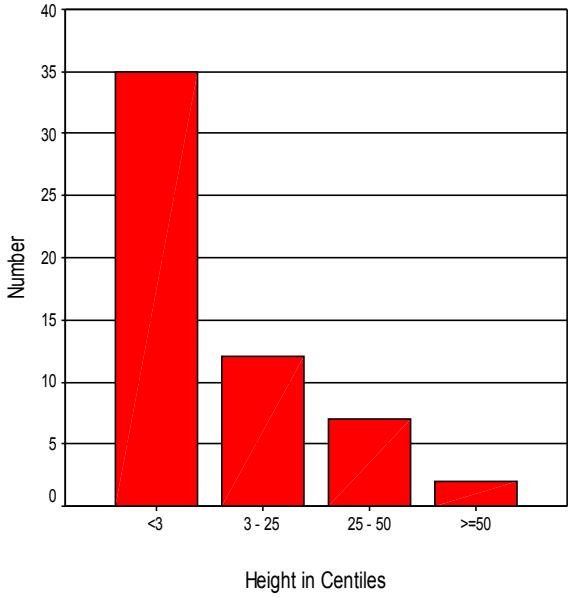
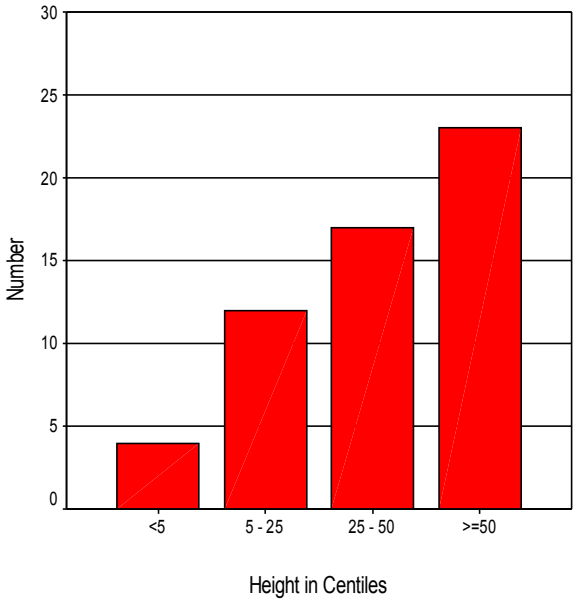


Figure 11 Distribution of height on the Cronk growth charts



On the IAP charts, the distribution was as follows: 62.5% <3rd centile, 21.4% between 3rd -25th centile, 12.5% between 25th - 50th centile and 3.6% >= 50th centile.

On the Cronk charts, the distribution was as follows: 7.1% <5th centile, 21.4% between 5th -25th centile, 30.4% between 25th - 50th centile and 41.4% >= 50th centile.

Thus, there is hardly any correlation between the two charts.

Height Vs Age

The children were clubbed into 2 groups based on their age (< 3years and >=3 years). There was no statistical difference between the children in the two age groups with relation to their height – (IAP and Cronk charts). There was no statistical difference between the two sexes on comparison of the height of the children either by the IAP or the Cronk chart.

Figure 12 Distribution of weight on the IAP growth charts

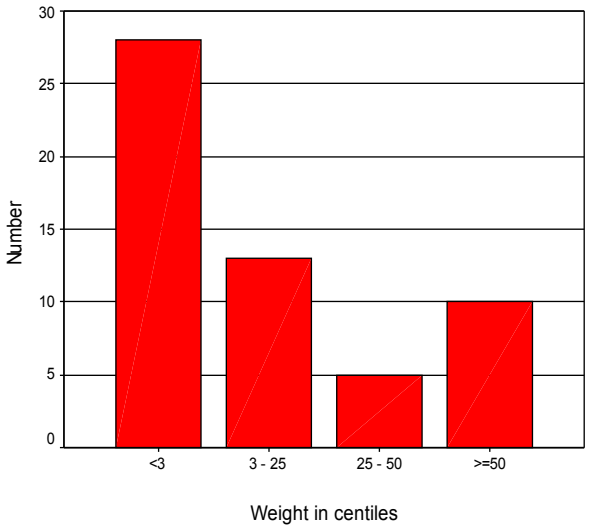
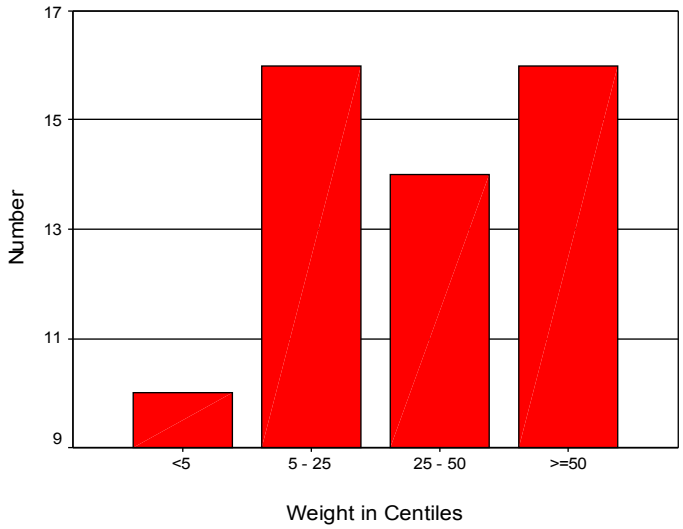


Figure 13 Distribution of weight on the Cronk growth chart



On the IAP charts, the distribution of weight was: 50% <3rd centile, 23.2% between 3rd -25th centile, 8.9% between 25th - 50th centile and 17.9% >= 50th centile.

On the Cronk charts, the distribution of weight was: 17.9% <5th centile, 28.6% between 5th -25th centile, 25% between 25th - 50th centile and 28.6% >= 50th centile.

Weight Vs Age

Table 4 Weight according to the IAP growth chart vs. age

Count

		IAP weight Centiles		
		<25	>=25	Total
AGE IN YEARS	< 3	26	3	29
	>=3	15	12	27
Total		41	15	56

There was no difference between the age groups when the Cronk charts were used to plot weight. However, when the IAP chart was used, it was found that 90% of the children less than 3 years were below the 25th percentile as opposed to 56% of those above three years. This was statistically significant (p 0.004). Thus children with Down syndrome appear to increase their weight with age.

Figure 14 Weight as per the IAP chart vs. sex of the child

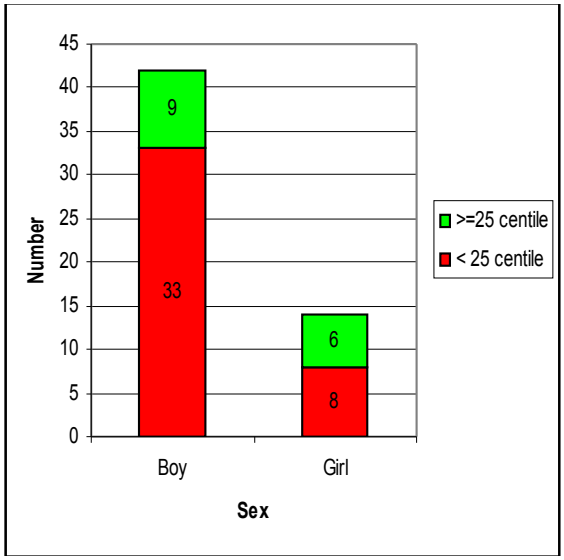
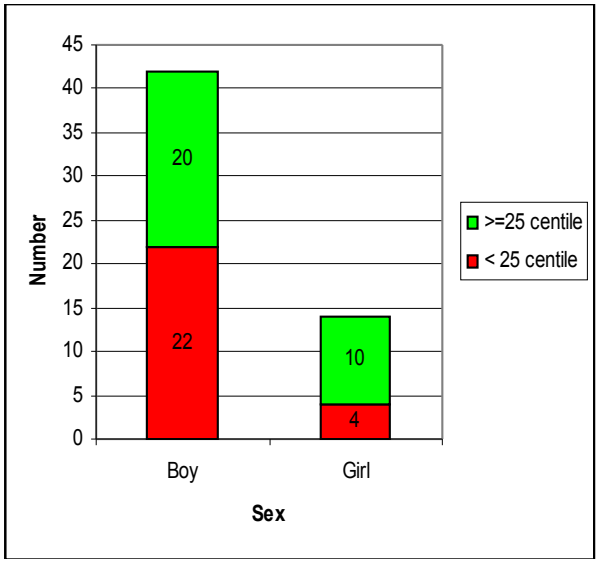
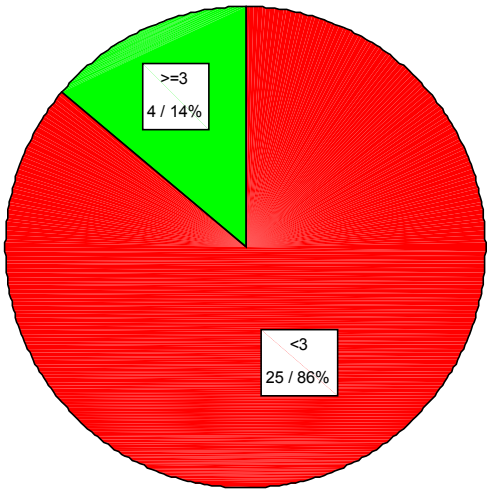


Figure 15 Weight as per the Cronk chart vs. sex of the child



As seen in the two figures above, when the weight was plotted on the two charts, it was seen that a higher percentage of girls were above the 25th percentile as compared to the boys. This was statistically significant in the Cronk chart ($p<0.05$), though this has to be interpreted with caution as one third of the cells had an expected value less than 5.

Figure 16 Prevalence of the children with microcephaly.

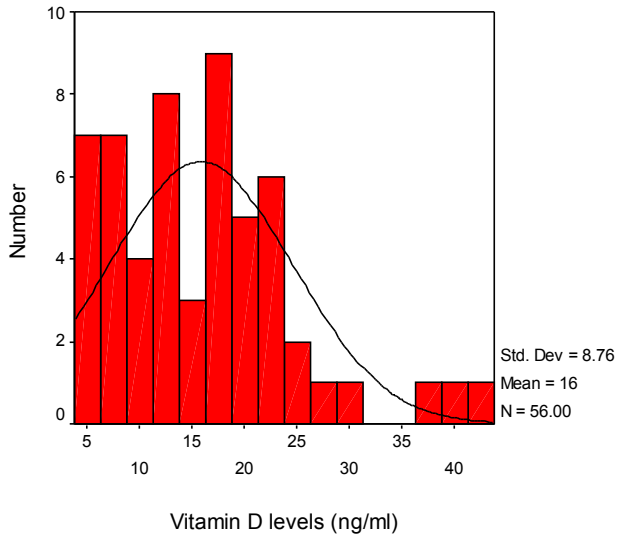


The head circumference was plotted on IAP growth charts for children less than 3 years. 86% of them were microcephalic (head circumference <3rd centile). There was no difference between the two sexes.

25(OH) Vitamin D levels

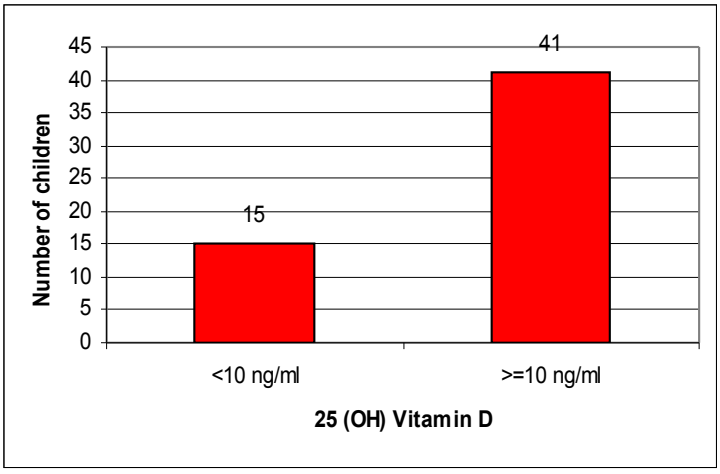
Pettifor in an editorial in Indian Pediatrics had stated “The use of varying definitions for vitamin D deficiency has made it difficult to compare the results of research by different authors and has further complicated comparisons between different communities and populations.” He recommended that vitamin D deficiency should be defined as 25(OH) D values <10 ng/mL. [19]

Figure 17 Distribution of serum 25 (OH) Vitamin D levels



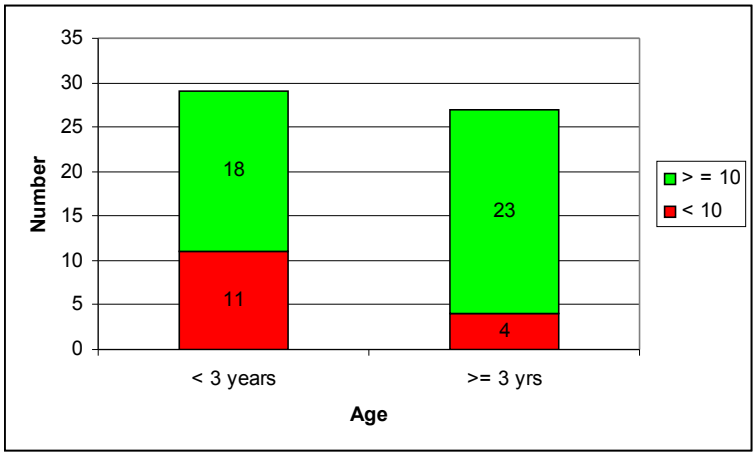
The mean serum 25(OH) vitamin D level in the study population was 16ng/ml**.**

Figure 18 Prevalence of 25 (OH) Vitamin D deficiency (<10ng/ml)



The prevalence of 25(OH) vitamin D level <10ng/ml was 26.8% and >=10ng/ml was 73.2%.

Figure 19 Prevalence of 25(OH) Vitamin D < 10 ng/ml in the different age groups.



25(OH) Vitamin D deficiency was more in children less than 3 years (p 0.051). This is probably because of low exposure to sunlight in this age group.

Table 5. 25(OH) Vitamin D levels vs. sex of the child (p 0.6)

Count

		Sex		
		Boy	Girl	Total
25 (OH) Vitamin D	< 10 ng/ml	12	3	15
	>= 10 ng/ml	30	11	41
Total		42	14	56

Table 6. 25(OH) Vitamin D levels vs. height as plotted on IAP chart (p 0.75)

Count

		Height as per IAP charts			
		< 3 rd centile	3 - 50 th centile	>= 50th centile	Total
25 (OH) Vitamin D	< 10 ng/ml	9	5	1	15
	>=10 ng/ml	26	14	1	41
Total		35	19	2	56

Table 7. 25(OH) Vitamin D levels vs. height as plotted on Cronk chart (p 0.26)

Count

		Height as per Cronk Chart			
		<5th centile	5th to 50th centile	>=50th centile	Total
25 (OH) Vitamin D	< 10 ng/ml		10	5	15
	>=10 ng/ml	4	19	18	41
Total		4	29	23	56

Table 8. 25(OH) Vitamin D levels vs. weight as plotted on IAP chart (p 0.95)

Count

		Weight as per IAP chart			
		< 3rd centile	3 - 50th centile	> 50th centile	Total
25 (OH) Vitamin D	< 10 ng/ml	7	5	3	15
	>=10 ng/ml	21	13	7	41
Total		28	18	10	56

Table 9. 25(OH) Vit D levels vs. weight as plotted on the Cronk chart (p 0.41)

Count

		Weight as per Cronk chart			
		< 5th centile	5th - 50th centile	>= 50th centile	Total
25 (OH) Vitamin D	< 10 ng/ml	1	9	5	15
	>=10ng/ml	9	21	11	41
Total		10	30	16	56

Table 10. 25(OH) Vitamin D levels vs. socioeconomic score (p 0.24)

Count

		Socio economic score (Modified Kuppuswamy score)		
		< 15	>=15	Total
25 (OH) Vitamin D	< 10 ng/ml	4	11	15
	>=10 ng/ml	18	23	41
Total		22	34	56

Table 11. 25(OH) Vitamin D levels with relation to bone age (p 0.3)

Count

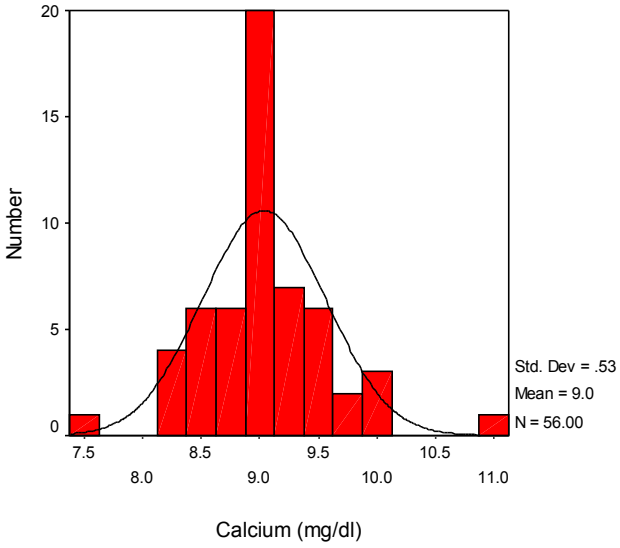
		Bone age			
		< 1sd	Normal	> 1 sd	Total
25 (OH) Vitamin D	<10 ng/ml	1	14		15
	>=10 ng/ml	7	30	3	40
Total		8	44	3	55

Inference: There was no significant relation ship between 25 (OH) Vit D levels and height,

weight, age, socioeconomic status and bone age.

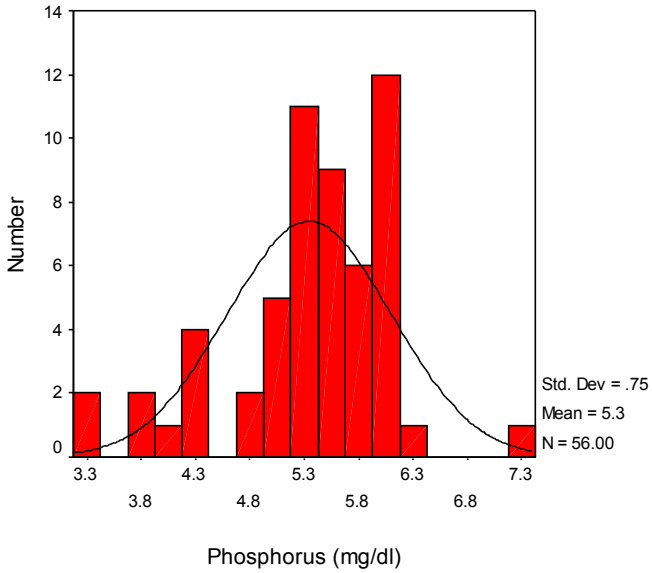
Biochemical Indices of 25(OH) Vitamin D deficiency

Figure 20 Distribution of serum Calcium levels



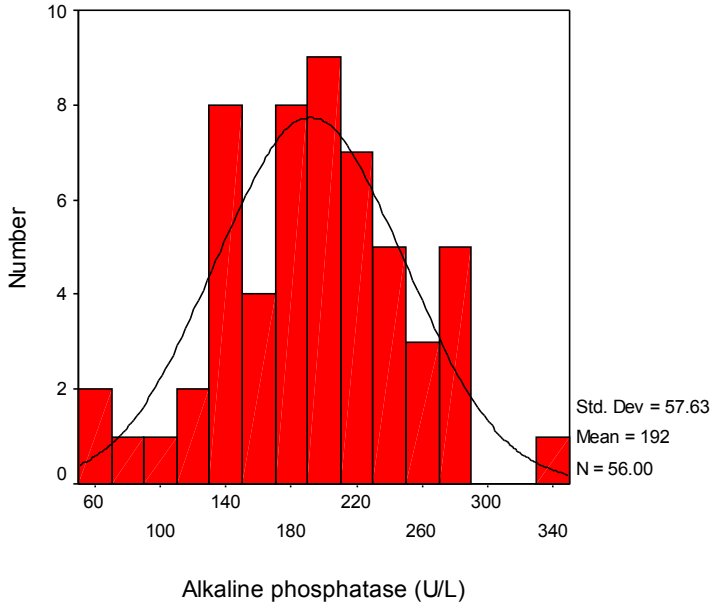
The prevalence of hypocalcaemia (serum calcium <8mg/dl) was 1.8%.

Figure 21 Distribution of serum Phosphorus levels



Hypophosphatemia was present in 2 children (3.6%).

Figure 22 Distribution of serum alkaline phosphatase levels



All children had alkaline phosphatase levels in the normal range.

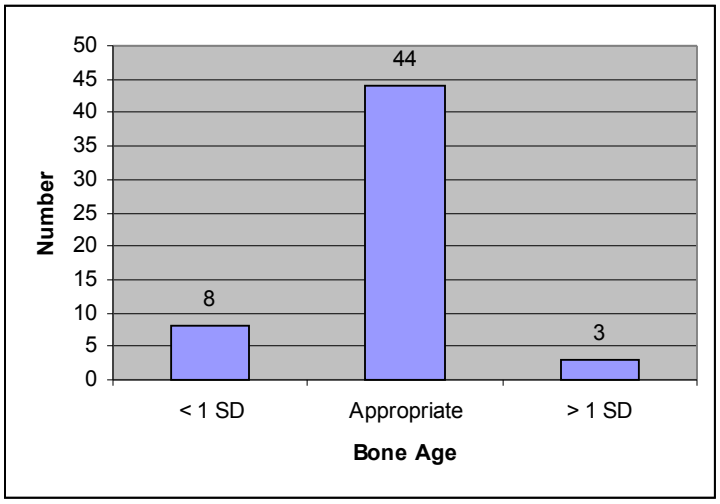
Table 12. Comparison of serum calcium, phosphorus and alkaline phosphatase levels in children with Vitamin D levels < 10 ng/ml and >=10 ng/ml

	25 (OH) Vit D	number	Mean	Std. Deviation	Std. Error Mean
Calcium	< 10 ng / ml	15	9.060	.3397	.0877
	>= 10 ng / ml	41	9.027	.5840	.0912
Phosphorus	< 10 ng / ml	15	5.527	.6660	.1720
	>= 10 ng / ml	41	5.210	.7736	.1208
Alkaline Phosphatase	< 10 ng / ml	15	186.87	43.876	11.329
	>= 10 ng / ml	41	193.34	62.307	9.731

The independent samples T test was used to compare the values in the 2 groups. There was no statistical difference in the biochemical indices in the 2 groups The significance (2 tailed) values for calcium was 0.837, Phosphorus 0.166 and alkaline phosphatase 0.713. There was no significant difference in age and sex between the two groups.

Bone age

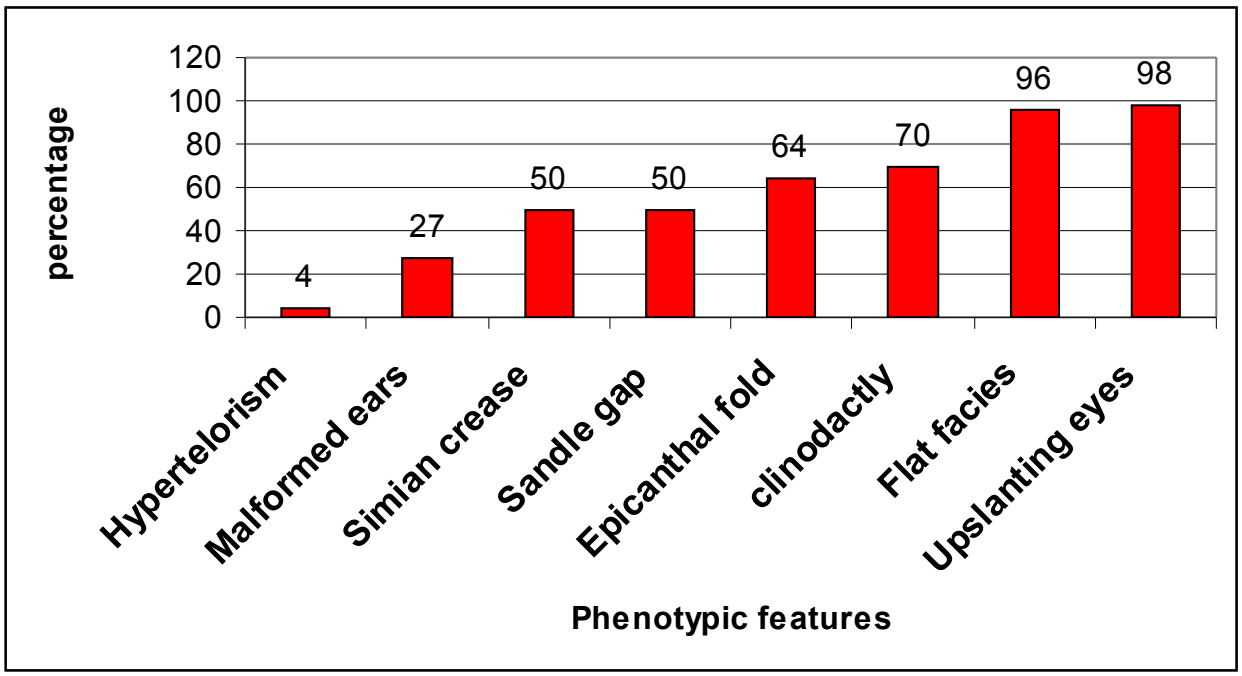
Figure 23 Bone age in children with Down syndrome



The bone age was assessed using X- Ray left hand and wrist. This was compared to Gruelich and Pyle standards. 55 children had bone age done.

Phenotypic Features

Figure 24 Phenotypic features in the children in our study

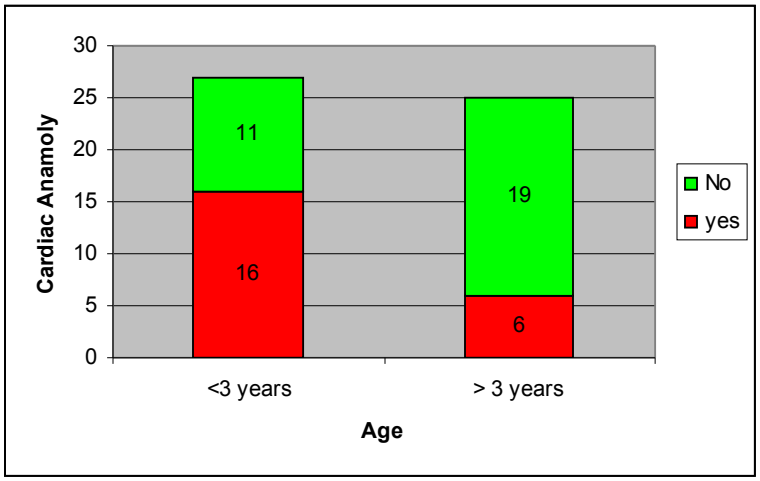


The figure above shows the phenotypic features in the order of increasing prevalence.

Cardiac abnormalities

52 children had echocardiograms done. Cardiac anomalies were detected in 22 (42%).

Figure 25 Prevalence of cardiac anomaly in various age groups



Younger children had more cardiac anomalies compared to older children (p 0.013).

Figure 26 Cardiac Anomalies on Echocardiogram vs. Weight (IAP chart)

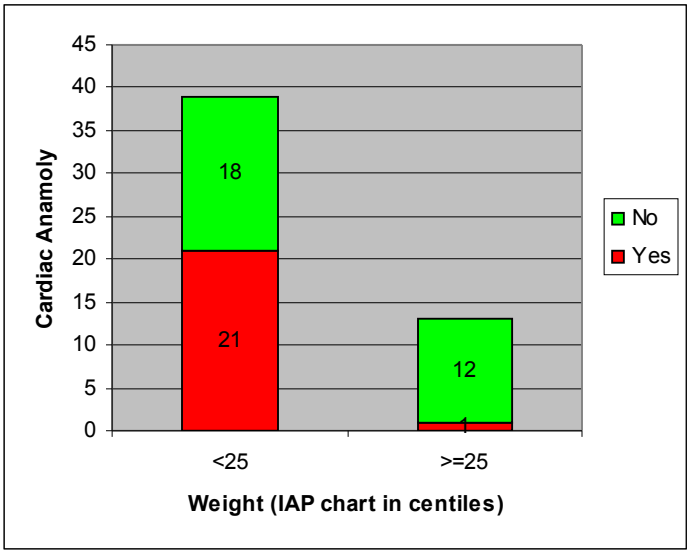
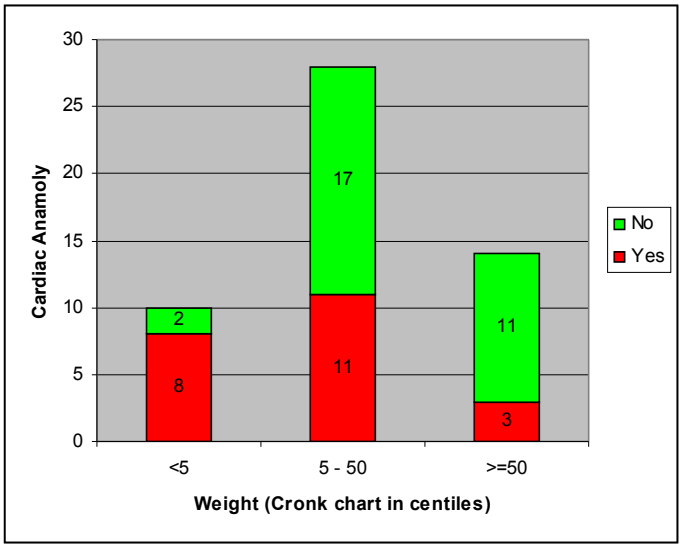


Figure 27 Cardiac anomalies on Echocardiogram vs. Weight (Cronk chart)



The figures above show that children with heart disease had a lower weight centile, both on the IAP and Cronk charts. This was statistically significant. (p 0.004 and 0.015)

Table 13 Correlation between clinical examination and Echocardiogram findings

Count

		Cardiac clinical examination		
		Abnormal	Normal	Total
Echo cardiogram	Abnormal	15	7	22
	Normal		30	30
Total		15	37	52

There were 7 children who were clinically normal, but were found to have echocardiographic abnormalities.

There was no correlation between presence of cardiac abnormalities and the

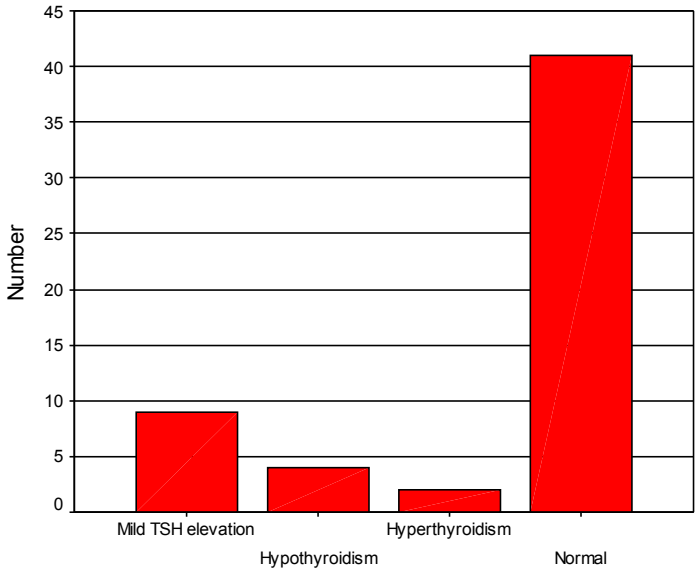
- sex of the child
- height of the child on the IAP and Cronk charts
- head circumference
- cytogenetic profile

- social quotient

Thyroid Abnormalities

Since thyroid abnormalities also affect growth, the thyroid function test (TFT) and thyroid stimulating hormone (TSH) were checked for all the children.

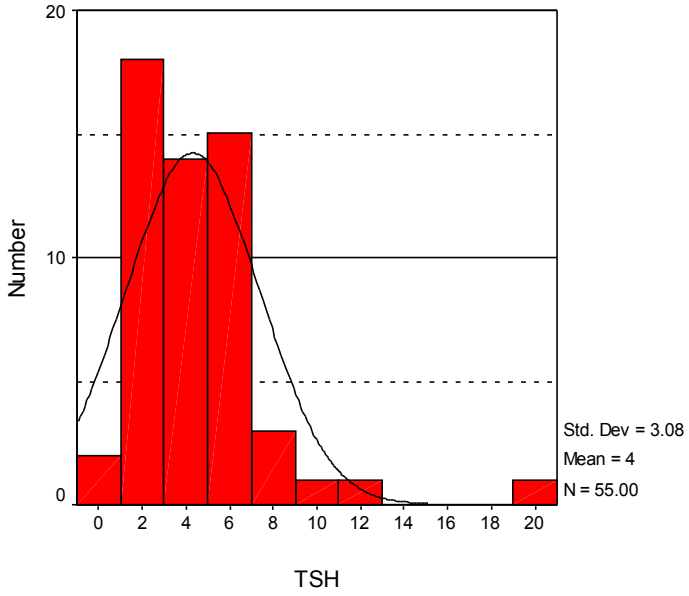
Figure 28 Thyroid profile in various age groups



The profile was as follows:

- Mild TSH elevation (TSH between 6 – 10 mU/L with normal TFT) was 16%,
- Hypothyroidism (TSH above 10 mU/L) was 7%,
- hyperthyroidism was 4%
- normal thyroid function was 73%

Figure 29 Distribution of TSH values



The mean TSH level was 4 mU/L.

Thyroid autoantibodies

Thyroid auto antibodies were checked for the following

a. Mild TSH elevation (3 / 9 children) all negative

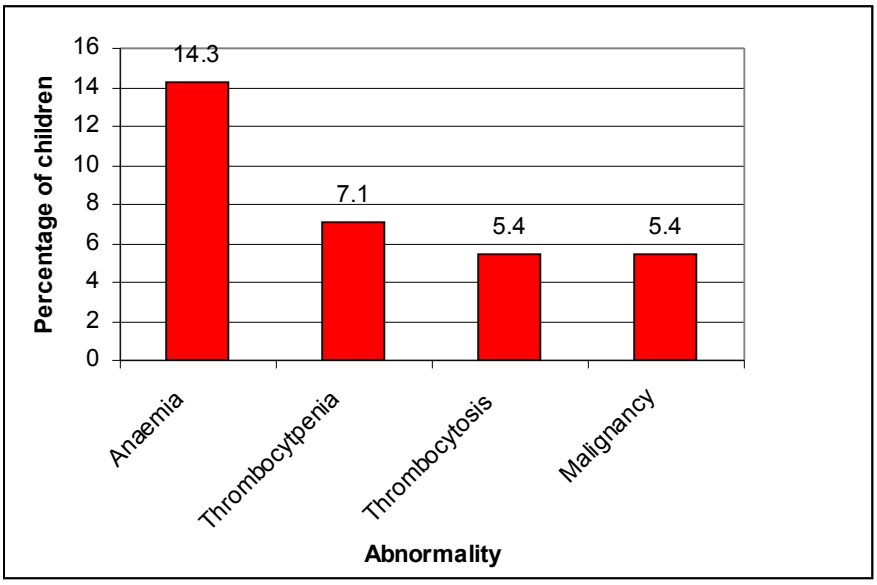
b. Hypothyroidism (3/4 children) all positive

c. Hyperthyroidism (2/2 children) all positive

There was no significant association between the thyroid status of the children and age (p 0.24), sex (p 0.52), height as plotted on the IAP chart (p 0.94), height as plotted on the Cronk chart (p 0.87), weight as plotted on the IAP chart (p 0.51), weight as plotted on the Cronk chart (p0.59), head circumference (p 0.62), bone age (p 0.53) and social quotient (p 0.79).

Haematological abnormalities

Figure 30 Haematological abnormalities seen in children with Down syndrome.



The incidence of anaemia was 14.3%.

Atlanto-axial dislocation

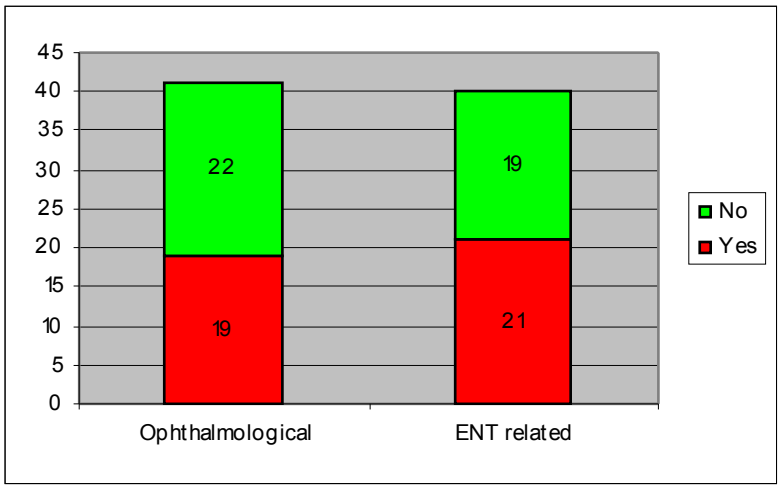
Atlanto-axial dislocation was looked for in children older than 3 years as per the AAP recommendations.

- 3/25 12% had evidence of atlanto-axial dislocation.
- 2 (67%) were boys and 1 (33%) was a girl.

Ophthalmologic and hearing evaluation

Of the 56 children, 41 had ophthalmologic evaluation and 40 had complete hearing assessment done. Ophthalmologic abnormalities were detected in 19/41 (46%) children and ENT related abnormalities were detected in 21/40 (53%) children.

Figure 31 Prevalence of ophthalmologic and ENT related abnormalities in children with Down syndrome.



The **ophthalmologic abnormalities** were as follows (some children had more than one abnormality): refractory error 13/41 (32%), nystagmus 4/41 (10%), cataract 3/41 (7%), squint 2/41 (5%), nasolacrimal duct obstruction 1/41 (2.4%) and brushfield spot 1/41 (2.4%).

ENT abnormalities: 10/40 (25%) had features of otitis media with effusion and 14/40(35 %) had hearing deficit either unilateral/ bilateral.

Table 14 Relationship between visual and auditory deficits (p 0.04)

		Auditory deficit		
		Yes	No	Total
Visual defict	Yes	5	11	16
	No	12	6	18
Total		17	17	34

Thirty four children had both evaluations. The interesting finding was that those who had auditory deficits did not have visual deficits and vice versa (p 0.04).

- **Table 15 Prevalence of visual deficits in different age groups. (p 0.035)**

Count

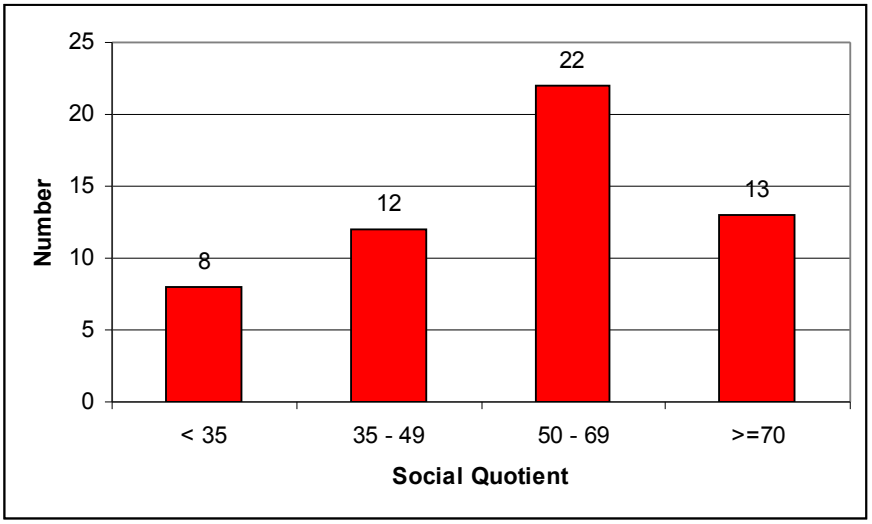
		Age		
		<3	>=3	Total
Visual deficit	Yes	5	14	19
	No	13	9	22
Total		18	23	41

As the age increased, the prevalence of visual deficits increased (p0.035). One possibility is that detection of visual deficits is easier as the child grows older. Still, prevalence of 61% visual deficits in children over three years is very high.

Social Quotient

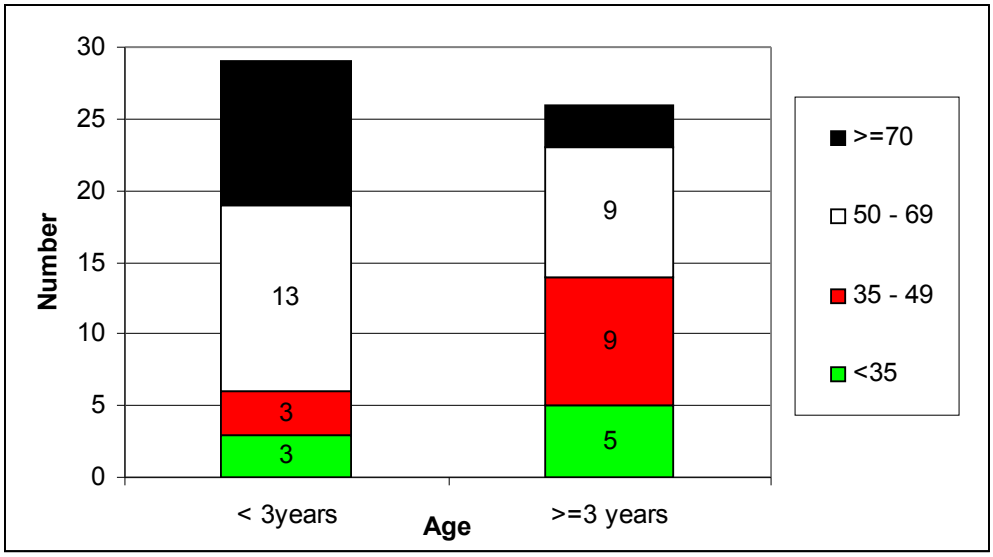
55 children who were evaluated for social quotient by administering the Vineland Social Maturity Scale

Figure 32 Social quotient in children with Down syndrome



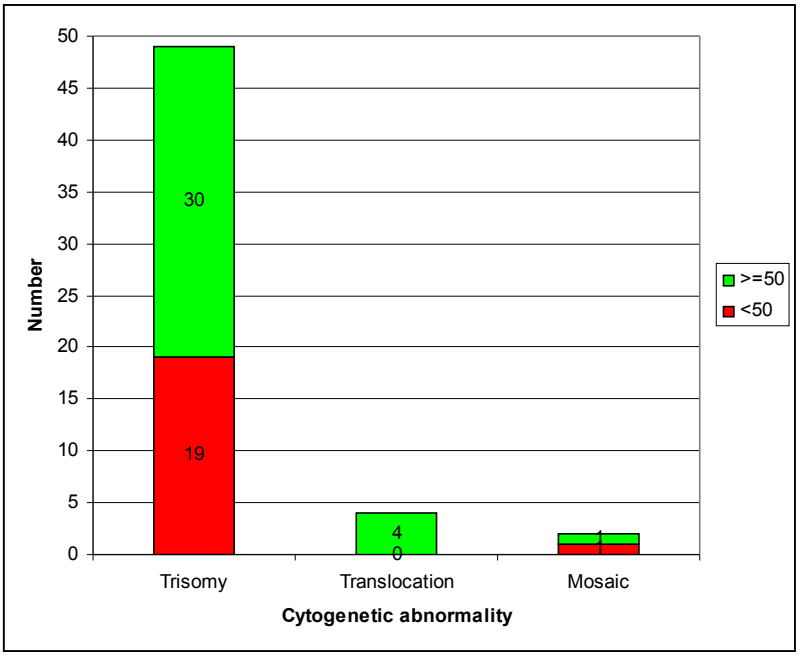
The average social quotient was 56.

Figure 33 Social quotient vs. age



Younger children have a larger percentage of high social quotient than older children. This was statistically significant (p value<0.05)

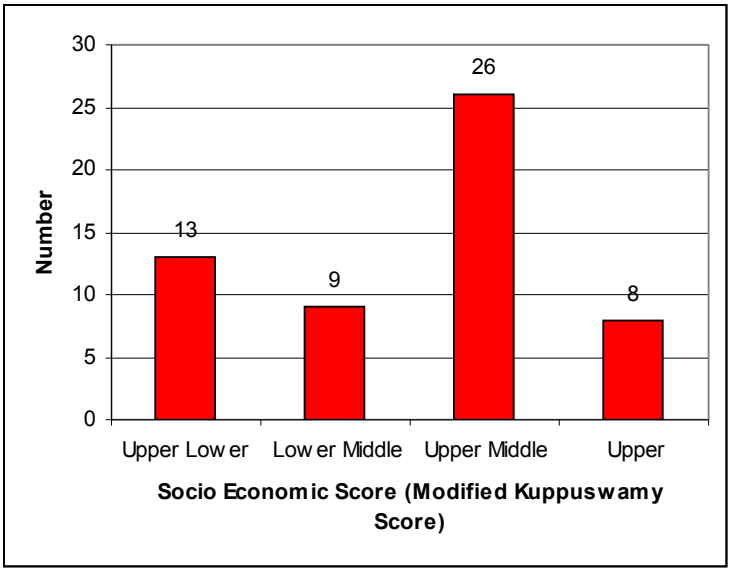
Figure 34 Social quotient vs. the cytogenetic abnormality



None of the children with translocation had social quotient less than 50, while 39% of the Trisomy and 50% of the Mosaics had low social quotient. This was not significant statistically.

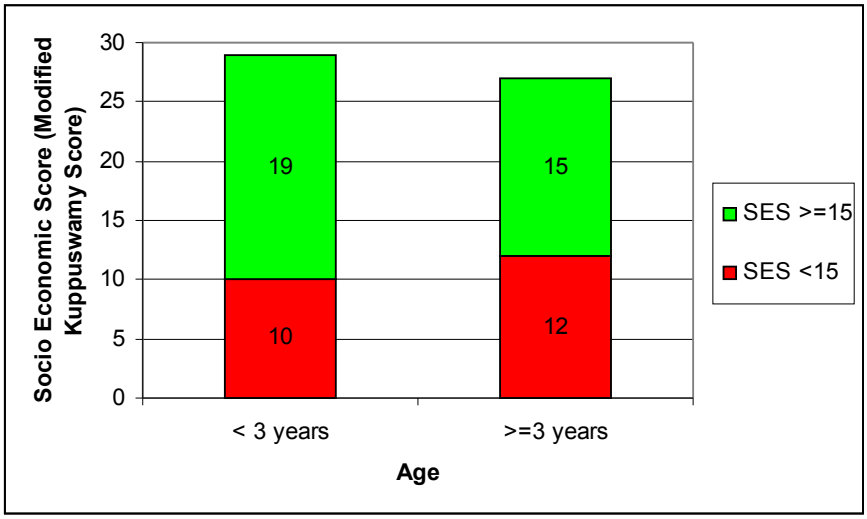
Socio economic Status

Figure 35 Socio economic status of the families as per the Modified Kuppuswamy score[50]



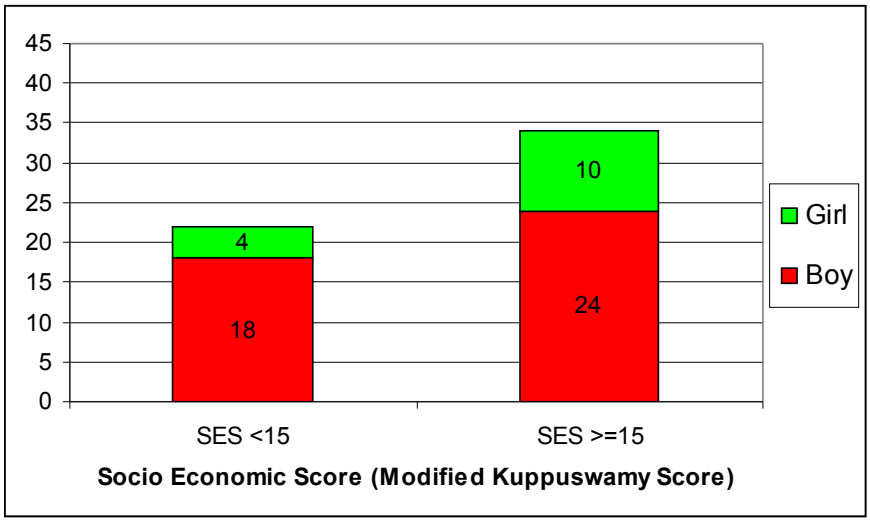
The two lower socioeconomic groups and the two upper groups were clubbed for analysis.

Figure 36 Age of the children vs. the socio economic score



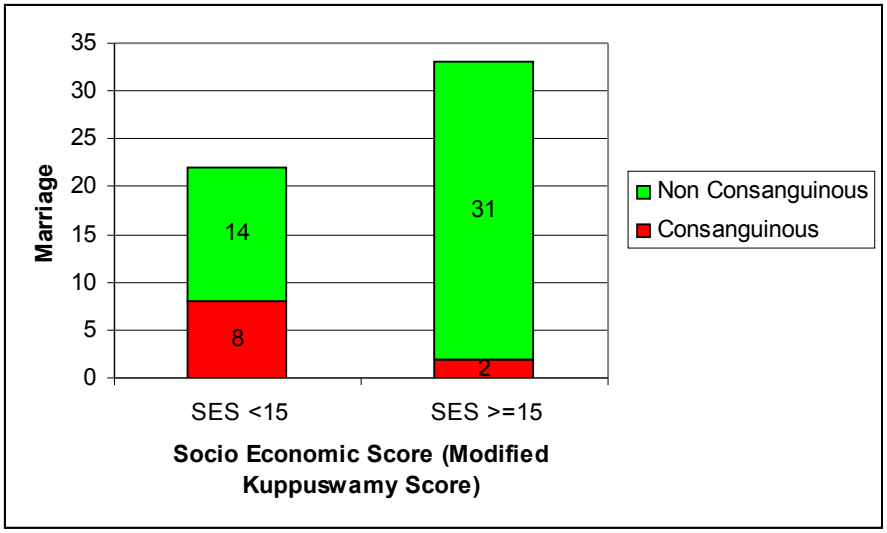
In the less than 3 years age group, only 34.5% of the children belonged to the lower socioeconomic group as opposed to 44.5% in the more than 3 years age group. Children belonging to poorer families are brought for treatment at a later age and this can affect their overall physical and mental development. This was however not statistically significant.

Figure 37 Sex of the children vs. the socio economic status



In the lower socio economic group, only 18% of the children were girls. Girls with Down syndrome belonging to the lower socio economic strata are not brought for treatment**.**

Figure 38 Socio economic status vs. consanguineous marriages



36% of the marriages in the lower socio economic groups are consanguineous vs. 6% in the higher socio economic group. This is statistically significant; p 0.004 (though one cell had an expected count less than 5).

There was no statistically significant difference in 25(OH) Vitamin D levels in different SES groups

Discussion - Outline

- *Demography*
- *Cytogenetic profile of children with Down*
- *Maternal history*
- *Anthropometry*
- *25 (OH) Vitamin D levels*
- *Bone age*
- *Phenotypic profile*
- *Heart disease*
- *Thyroid*
- *Hematology*
- *Ophthalmology and Hearing*
- *Atlantoaxial dislocation*
- *Social quotient*
- *Socioeconomic status*

Demography

56 children - 42 boys (75%), and 14 girls (25%) were included in the analysis**.** Thus the male: female ratio was 3:1.

- In the study by Kava [11] et al, the male: female ratio was 1.37:1 and in Sachdev's study the ratio was 1.84:1. [15]
- In other large series abroad, the male: female ratio seen in Myrelid's study [12] was 1.34: 1 and in Cronk's study, [37] it was 1.33:1.

Thus it is seen that our study had an unusually large number of boys. There was no statistical difference between the children in the different age groups with regard to their age and sex distribution.

For analysis, the children were grouped into 2 - less than 3 years and 3 years and greater. There were 29 children less than 3 years and 27 children 3 years and older.

Cytogenetic Profile

All children included in the study had cytogenetic confirmation of Down syndrome. The frequency of

- trisomy 21 was 89.3% (50/56)
- translocation was 7.1% (4/56)
- mosaicism was 3.6%. (2/56)

- Jyothy et al [8] analysed 1001 children. In their study, trisomy 21 was present in 87.9%, translocation in 4.93% and mosaicism in 7.69%.
- In Kava's study of 221 patients, trisomy 21 was present in 95%, translocation in 3.2% and mosaicism in 1.8%. [11]
- Sheth et al [10] analysed 382 children and found free trisomy 21 constituted 84.8% of cases, translocation 8.9%, mosaic 3.9% and in 2.4% cases regular T21 was associated with structural or numerical changes.

Translocation

3 children in the study had t (21; 21) translocation and one had t (14; 21).

This is similar to the data presented by Sheth [10] in which the homologous translocation (21q21q) is one of the most common chromosomal rearrangements in DS.

Maternal History

Maternal age at delivery

- 11% of the mothers were older then 35 years. In Verma's series, 33% of the mothers of children with Down syndrome were over 35 years. [39]
- The mean age was 26.75 years with standard deviation of 5.67 years. This is very similar to the mean age of 26.8 years in the study by Kava. [11]

Previous spontaneous abortions

Five (9.1%) of the mothers had previous spontaneous abortions. Hook EB stated that "the younger the mother and the more the number of abortions, the higher the relative risk of a Down syndrome live birth." [40]

Maternal grandmother's age

In our study, the mean age of the maternal grandmother at time of mother's birth was 24 years. (However some of the parents were not sure about the exact age). Malini et al [41] suggested that increased maternal grandmother's age at the time of mother's birth was a risk factor for Down syndrome. For every year of advancement of age of the maternal grandmother, the risk (odds) of birth of DS baby increases by 30%.

Grandparents consanguineously married

8 of the 55 children ie 15%, (1 child excluded as the child was adopted), had consanguineously married grand parents. Sayee et al found the incidence of grandparental and parental consanguinity to be 17.5%. They however concluded that consanguineous marriage did not predispose to Down syndrome. [42]

Parents consanguineously married

10 (18%) of the 55 children had consanguineously married parents. In Sayee's study, [42] the incidence of parental consanguinity was 17.2 %.

Antenatal detection of Down Syndrome

Antenatal scan was done in 39 (69.6%) of the 55 children. In none of the scans were features of Down syndrome picked up. None of the mothers underwent any screening for Down syndrome. None of them knew antenatally that their child had Down syndrome.

This was in contrast to other countries where second trimester screening for Down's syndrome is widely practiced. [43, 44]

Birth rank of the children

23 of the children were the first born, 28 were second/ third born, and 4 children were of the birth rank 4 or greater. 1 child was adopted and details were not known.

Mean birth weight

The mean birth weight of the children was 2.5kg. The minimum weight was 1.4kg and the maximum weight was 3.92kg. This corresponds to the study by Sachdev who stated that average birth weight varies from 0.5-1 SD below the control means. As per the figures depicted in their study, the 50th centile was approx. 3kg. [15]

Anthropometry

Growth monitoring is a screening tool to diagnose nutritional, chronic systemic and endocrine disease at an early age. Unfortunately, there are no growth charts for Indian children with Down syndrome. So, the height and weight of each child was plotted on the IAP growth charts and growth charts by Cronk for Children with Down syndrome. Head circumference for children less than 3 years was plotted on the IAP chart.

Height

The Kappa was estimated in order to evaluate agreement between the classifications of height for age according to the two charts. The kappa value was -0.120. Thus, there was poor agreement among the two charts

Weight

Children with Down syndrome are usually overweight. However in our study, at least three fourths of the children were below the 50^{th} percentile – irrespective of the growth chart used. The Kappa value between the two charts was 0.325. Thus, here too, there was poor agreement among the two charts

Head Circumference (age <3 years)

The head circumference was plotted on IAP growth charts for children less than 3 years. 86% of them were microcephalic (<3^{rd} centile). This corresponds to the study by Sachdev[15] where all children had head circumference below the 10^{th} centile.

Height Vs Age

The children were clubbed into 2 groups based on their age (< 3years and >=3 years). There was no statistical difference between the children in the two age groups with relation to their height – (IAP and Cronk charts).

Weight Vs Age

There was no difference between the age groups when the Cronk charts were used to plot weight. However, when the IAP chart was used, it was found that 90% of the children less than 3 years were below the 25th percentile as opposed to 56% of those above three years. This was statistically significant (p 0.004). Thus children with Down syndrome appear to increase their weight with age (table 3). Styles et al found that there was a high prevalence of overweight/obesity, particularly in adolescence and adult life. [3]

25(OH) Vitamin D levels

Pettifor in an editorial in Indian Pediatrics recommended a cut off value of 10 ng/ml to define vitamin D deficiency. [19] In our study, 26.8% of children had 25 (OH) Vitamin D levels below 10 ng/ml. Unfortunately there is a paucity of literature on the prevalence of Vitamin D deficiency in the general population in India.

- Tiwari et al [45] found the prevalence of hypovitaminosis (< 14 ng / ml) in Delhi in certain slums in children aged 9 months to 30 months to be 83%. However in other slums, prevalence was only 2%.
- Harinarayan et al [46] defined Vitamin D deficiency to be less than 20 ng / ml and found the prevalence in children to be between 63 and 82% in different populations in Andhra Pradesh.
- Marwaha et al saw hypovitaminosis D (< 9 ng/ml) in 36% of children. [18]

The demography of vitamin D deficiency

The prevalence of low vitamin D level is more in children less than 3 years (p0.051). This is probably because of low exposure to sunlight in this age group.

There was no statistically significant difference between the following parameters among the groups with vitamin D levels less than 10 ng / ml and those more than that.

- Sex of the child (p 0.6)
- Height as plotted on the IAP chart (p 0.75)
- Height as plotted on the Cronk chart (p 0.26)
- Weight as plotted on the IAP chart (p 0.95)
- Weight as plotted on the Cronk Chart) (p 0.41)
- Socio economic score (p 0.24)

Only one study (Del Arco et al in 1992) published their findings from 21 children with Down syndrome in Cantabria, Spain. The average values of the three Vitamin D metabolites were comparable to those of an age-matched group both in winter and summer. No child with DS showed values below the normal range, either in Vitamin D metabolites, or in the other parameters of calcium metabolism. They suggested that these children do not require Vitamin D when appropriate sunlight exposure is provided. Our study also shows that Vitamin D deficiency is not associated with delay in growth. [5]

25(OH) Vitamin D levels with relation to bone age

Bone age is the average age at which children reach a particular age of bone maturity.
It corresponds to the age of the child in normal children.

- In our study, 55 children had X-rays of the left hand and wrist done. The bones in the X-ray were compared to the Greulich and Pyle standard by a Radiologist. [22] The bone age was noted and features of rickets were looked for.
- There was no statistically significant difference in the bone age between the groups with 25(OH) vitamin D levels below and above 10 ng/ml (p= 0.3).

- There was one X-ray suspicious of rickets (X-ray reported as mild fraying of the distal ulnar and radial metasphyses - could be artefactual in view of suboptimal positioning). This child's 25(OH) vitamin D level was 21.5, with normal serum calcium, phosphorus and vitamin D levels). Otherwise, there were no radiological features suggestive of rickets.

Biochemical Indices of Hypovitaminosis D

- The prevalence of hypocalcemia (serum calcium less than 8mg/dl) was 1.8%.
- Hypophosphatemia was present in 2 children (3.6%).
- All children had alkaline phosphatase levels in the normal range.

Comparison of serum calcium, phosphorus and alkaline phosphatase levels in children with Vitamin D levels < 10 ng/ml and >=10 ng/ml

One of our aims was to see if children with low levels of Vitamin D had concomitant decrease in serum calcium and phosphorus and an increase in alkaline phosphatase levels. We divided the children into 2 groups – Vitamin D less than 10 ng/ml and >= 10 ng/ml. The following table gives the values in the 2 groups.

The independent samples T test was used to compare the values in the 2 groups. There was no statistical difference in the biochemical indices in the 2 groups. The significance (2 tailed) values for calcium was 0.837, Phosphorus 0.166 and alkaline phosphatase 0.713. There was no significant difference in age and sex between the two groups.

This is similar to the findings of Del Arco et al. [5]

Bone age

The bone age is an independent tool to assess the growth of a child irrespective of the anthropometric measures such as height and weight. The bone age was assessed using X-Ray left hand and wrist. This was compared to Gruelich and Pyle standards. [22]

55 children had bone age done.

- 80% were appropriate for age.
- In 15% bone age was delayed.
- In 5% bone age was advanced.

The bone age was significantly related to the weight of the child on both the IAP and Cronk charts with children with lower weights having delayed bone age. However, because of the small numbers, there were many cells with expected value less than 5 and so this has to be cautiously interpreted.

There was no correlation between the bone age and the

- age of the child
- sex
- height,
- head circumference
- cytogenetic profile
- Serum Vitamin D
- atlanto-axial dislocation
- socioeconomic score.

Phenotypic Features

Children with Down syndrome have characteristic phenotypic features. In our study, their prevalence was as follows:

- hypertelorism (3.6%)
- epicanthal folds (64.3%)
- upslanting eyes (98.2%)
- malformed ears(26.8%)
- flat facies (96.4%)
- simian crease (50%)
- clinodactyly (69.6%)
- sandal gap (50%)

Kava et al in their retrospective analysis noted mongoloid slant in 83.9%, ear abnormalities in 66.9%, epicanthic folds in 56.9%, and flat facial profile in50.9%, simian crease in 33.2%, clinodactyly in 36.1% and sandle sign in 46.2%. [11]

Cardiac abnormalities

52 children had echocardiograms done, cardiac anomalies were detected in 22 (42%) of them. This was similar to the data submitted by Bhatia et al (44%). [24]

The echocardiogram anomalies were

- 9/22 (40.9%) had atrial septal defects
- 8/22 (36.4%) had ventricular septal defects
- 6/22 (27.3%) had patent ductus arteriosus
- 2/22 (9.1%) had atrio-ventricular canal defects
- 2/22 (9.1%) had other defects- mitral valve and tricuspid valve prolapse; Pulmonary regurgitation with pericardial effusion

Younger children had more cardiac anomalies compared to older children. This was statistically significant. (p 0.013)

Kava et al [11]conducted a retrospective study on 524 patients over 7.5 years. Congenital heart disease was clinically diagnosed in 96 cases (18.3%). The nature of the cardiac defect was ascertained by color Doppler examination and/or 2D-echocardiography in 58 cases. The most common cardiac anomalies were ventricular septal defect (25.8%), tetralogy of Fallot (15.5%), and atrial septal defect (12.1%).

Bhatia et al [24] evaluated the utility of echocardiography in assessing the frequency and nature of cardiac malformations in children with Down syndrome. Fifty cases of chromosomally proven Down syndrome were studied. Endocardial-cushion-defect was the commonest anomaly, followed by ventricular septal defect.

Thyroid Abnormalities

Since thyroid abnormalities also affect growth, the thyroid function test (TFT) and thyroid stimulating hormone were checked for all the children.

In our study, the profile was as follows:

- Mild TSH elevation (TSH between 6 – 10 mU/L with normal TFT) was 16%,
- Hypothyroidism (TSH above 10 mU/L) was 7%,
- hyperthyroidism was 4%
- normal thyroid function was 73%

This fits in with literature where there is a wide variation in prevalence of hypothyroidism from 3 – 54% depending on the definition used. [47]

Thyroid autoantibodies

Thyroid auto antibodies were checked for the following

a. Mild TSH elevation (3 / 9 children)	all negative
b. Hypothyroidism (3/4 children)	all positive
c. Hyperthyroidism (2/2 children)	all positive

Thus it is worthwhile checking thyroid antibody status for all children with hypo and hyperthyroidism.

In a five year longitudinal study of 101 children with Down syndrome, Selikowitz found no differences in growth and development between those with slightly raised TSH, showing compensated hypothyroidism, and those with normal TSH. [48]

There was no significant association between the thyroid status of the children and

- Age (p 0.24) and sex (p 0.52)
- Height as plotted on the IAP chart (p 0.94)
- Height as plotted on the Cronk chart (p 0.87)
- Weight as plotted on the IAP chart (p 0.51)
- Weight as plotted on the Cronk chart (p0.59)
- Head circumference (p 0.62)
- Bone age (p 0.53)
- Social quotient (p 0.79)

Haematological abnormalities

- Anemia was detected in 8 (14%) of the children.
- Pre B ALL was identified in 3 (5.4%) of the children.
- Thrombocytopenia defined as platelet count less than 100 000/mm3 was detected in 4 (7.1%) of the children.
- Thrombocytosis as defined as platelet count > 450 000 was detected in 3 (5.4%) of the children.

Awasthi et al studied a total of 239 cases of Down syndrome of which of 15 had hematological manifestations at presentation. These comprised 4 cases of transient myeloproliferative disorder, 3 cases of TMD/acute leukemia, 4 cases of acute leukemia (AL), 2 of dual deficiency anemia, and 1 case each of myelofibrosis and idiopathic thrombocytopenia. [49]

The incidence of Pre B ALL in our study is probably higher as we are a tertiary care centre with a well set up Pediatric Oncology Department.

Ophthalmologic and hearing evaluation

Of the 56 children, 41 had ophthalmologic evaluation and 40 had complete hearing assessment done.

Ophthalmologic abnormalities were detected in 19/41 (46%) children and hearing abnormalities were detected in 21/40 (53%) children.

The ophthalmologic abnormalities were as follows (some children had more than one abnormality):

- refractory error 13/41 (32%)
- nystagmus 4/41 (10%)
- cataract 3/41 (7%)
- squint 2/41 (5%)
- nasolacrimal duct obstruction 1/41 (2.4%)
- brushfield spot 1/41 (2.4%)

As the age increased, the prevalence of visual deficits increased (p0.035). One possibility is that detection of visual deficits is easier as the child grows older. Roizen [1]concluded that ophthalmologic disorders are found in about 38% of children less than 12 months, and 80% of those between 5 -12 years. The most frequent disorders found in children are refractive errors (35-76%), strabismus (27-57%), and nystagmus (20%).

Another interesting finding was that visual abnormalities were commoner in those who were below the 25th percentile in weight as per the IAP charts and it was opposite for those who were above the 25th percentile (p0.02).

47 children were evaluated for hearing abnormalities. Out of these, 7 children went to ENT outpatient clinic, but did not complete their evaluation and so were excluded. 21 of the remaining had abnormal findings. Roizen [1] stated hearing loss to be between 38 – 78%.

10/40 (25%) had features of otitis media with effusion

14/40(35 %) had hearing deficit either unilateral/ bilateral.

Thirty four children had both ENT and ophthalmological evaluations. The interesting finding was that those who had auditory deficits did not have visual deficits and vice versa. This was statistically significant (p 0.04)

Atlantoaxial dislocation and Down syndrome

Atlantoaxial dislocation was looked for in children older than 3 years as per the AAP recommendations. 3/25 (12%) had evidence of atlantoaxial dislocation. 2 were boys and 1 was a girl. One of the children had surgical stabilization of the spine.

This is similar to the data by Pueschel et al who reported that 14.6% of 404 patients with Down syndrome had an Atlanto Dens interval greater than 4.5 mm, but only 1.5% of the group had symptoms and was ultimately treated with surgical stabilization of the cervical spine. [33]

Social Quotient

Out of the 55 children who were evaluated for social quotient by administering the Vineland Social Maturity Scale

- 40% had a social quotient of 50-69
- 24% had a social quotient >= 70
- 22% had a social quotient of 35- 49

- 2% had social quotient of <35

The average social quotient was 56.

In the study by Bhatia, [34] out of the 22 children whose Developmental Quotient was evaluated by administering Gessel's developmental schedule, Seguin Form board, Vineland Scoial Maturity Scale, DASSI- II (Bayley's scale), Malin's Intelligence Scale (for Indian children) and Stanford- Binet test - 50% had a DQ between 51- 70.

Social quotient vs. age

Younger children have a larger percentage of high social quotient than older children. This was statistically significant (p value<0.05)

This implies that with early developmental assessments and intervention, the social quotient of older children can also be improved. Another explanation is that the VSMS administration is more suited to older children than younger ones.

None of the children with translocation had social quotient less than 50, while 39% of the Trisomy and 50% of the Mosaics had low social quotient. This was not statistically significant.

Socio economic Status

The modified Kuppuswamy score was used to assess the socio economic status of the family. It has three components – family income, education and occupation. The majority of parents in our study belonged to the upper middle class followed by the upper lower class. [50]

The two lower socioeconomic groups and the two upper groups were clubbed together for analysis. Thus the lower socio economic group had 22 (39%) children and the upper socioeconomic group had

34 (61%) children. Children from families belonging to the lower socioeconomic group are brought later for treatment.

There was no statistically significant difference in 25(OH) Vitamin D levels in different SES groups. This is contrary to Marwaha et al [18] in their study on normal Indian children found that children of LSES had more vitamin D deficiency (42.3% using a cutoff as < 9ng/ml) as compared to USES group (27%)

Summary

A prospective descriptive study was conducted in the Department of Child Health, Christian Medical College, Vellore – a tertiary care medical centre in South India from October 2007 to September 2008. The aims were to:

3. Study the prevalence of Vitamin D deficiency by assessing 25(OH) Vitamin D levels in children with Down syndrome.
4. Assess the effect of Vitamin D deficiency on height, bone age and other biochemical markers in these children.
3. Assessment of the phenotypic and cytogenetic profile of children with Down syndrome and presence of common associated malformations, deficiencies and associated illnesses.
4. An evaluation of their social quotient.

a. 56 cytogenetically proven Down syndrome were enrolled in the study.
b. Frequency of regular trisomy was 89.3%, translocation was 7.1% and mosaicism was 3.6%
c. Prevalence of 25(OH)vitamin D deficiency using a cut off value of 10ng/ml was 26.8%
d. There was no significant effect of vitamin D deficiency on height, bone age or other associated biochemical markers
e. The prevalence of phenotypic features was as follows: hypertelorism (3.6%), epicanthal folds (64.3%), upslanting eyes (98.2%), malformed ears (26.8%), flat facies (96.4%), simian crease (50%), clinodactyly (69.6%) and sandal gap (50%).

Prevalence of

Mild TSH elevation 16%

Hypothyroidism 7%

Hyperthyroidism 4%

Ophthalmologic abnormalities were detected in 46% and ENT related abnormalities in 53%.

Pre B Acute lymphoblastic leukaemia was identified in 5.4% of the children.

Cardiac anomalies were detected in 42%. The common cardiac abnormalities were atrial septal defect, ventricular septal defect and patent ductus arteriosus.

12% (3/25) had evidence of atlanto-axial dislocation.

64% of children had social quotient <70.

Conclusions

1. The prevalence of 25(OH) Vitamin D deficiency in children with Down syndrome was 26.8%.
2. There was no significant effect of 25(OH) Vitamin D deficiency in the height, bone age and other biochemical markers in these children.

Limitations of the study

- The population prevalence of vitamin D deficiency in children in India is unknown.
- Various studies have used different cutoff values for vitamin D deficiency and insufficiency.
- There were no age and sex matched controls of normal children for the 25(OH) vitamin D levels, anthropometric measurements, biochemical indices etc.
- There are no standard Indian Down syndrome charts for reference.

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Annexure 1. Steps of Peripheral blood karyotyping for Down syndrome

1. **Setting up:**

   i. Check the hospital number on the form, tube and enter into the register

   ii. Check whether the sample has a clot in it as this may interfere with the result.

   iii. Prewarm the media- RPMI 1640(Ros Well Park Memorial Institute 1640).

   iv. Use 2 tubes for each patient- label them A and B

   v. Incubate the tubes with the media at 37C for 15 mins.

   vi. PHA(Phytohemagglutinin) is taken from the fridge and allowed to thaw. The PHA is a mitogen, and it stimulates the division of T cells

   vii. To 5ml of prewarmed media, add 300 microlitres of PHA and then the blood sample as per age(mentioned below).

   viii. Keep in the incubator. Daily mix the sample. Harvesting is done from 69-72 hours.

Age	Sample of blood to be taken
3months- 6months	250microlitres to one tube
	300microlitres to second tube
6months- 10months	250microlitres to one tube
	500microlitres to second tube
1-3 years	500microlitres to one tube
	300microlitres to second tube
4 years onwards	500microlitres to both tubes

2. **Harvesting:** There are 3 steps- **mitotic arrest**, **hypotonic treatment** and **fixation**.

i. To the sample at 69 hours, colcemid is added (this arrests cells in metaphase). This is incubated for 1 hour.

ii. The sample is centrifuged for 10 minutes. After the spinning is over, the supernatant is discarded.

iii. To the pellet at the bottom of the tube, potassium chloride is added along the sides of the tube- drop by drop, tapping gently, upto the 10ml mark. This is kept in a water bath for incubation at 37C for 10 minutes. This causes cell swelling and the chromosomes to spread out.(Hypotonic treatment)

iv. 10 minutes later, prefix the tubes with fixative- methanol: acetic acid (3:1); add 10 drops to each tube. (Acetic acid lyses the RBCs and Methanol hardens the cell wall so that it will not break.).

v. Centrifuge again for 10 minutes.

vi. A brown pellet is formed at the bottom of the tube. The supernatant is discarded. The pellet is washed twice with 1ml of fixative (added drop by drop) for 1 minute. The supernatant is drained. Tap the tube and then add 10ml of fixative gradually.

3. **Making the tube ready for slide making**:

Freeze the above tube for 20 minutes.

Take the tube out to thaw at room temperature for 15 minutes.

Centrifuge for 10 minutes. Discard the supernatant.

Add 5ml of fixative again and centrifuge it. Remove the supernatant.

A white pellet is obtained at the bottom of the tube.

4. Slide making:

<u>Optimal Conditions for slide making</u>

Optimal temperature of the room: 20-22C

Relative Humidity : 50- 60%

Angle of slide : 20-30

Drying seconds : 35-40 sec

Aging time : 90-96C for 20 minutes

Warmer temperature : 37C

Nature of water : Deiodinised distilled water- chilled water

Method of slide cleaning- Soak the slide in methanol. Wipe dry. Resoak in methanol. Rotate the slides in cold water, and when the slides are taken out, a thin film of water is seen.

Stir the white pellet with fixative at a temperature of 20-22C, humidity of 50-60%.

Add one drop of the solution to the prepared slide at an angle of 20-30. Wipe the back of the slide and the sides.

Let the slide dry for 35-40 sec on a warmer (temp 37C)

Label the slide after it is dry.

View under a Phase Contrast Microscope to see the metaphases. A good metaphase is thin, long, well spread with minimal overlaps and is non refractile.

Keep the slide in an oven at 90- 96C for 20 mins. The slide is now ready for banding.

5. **Banding:**

Trypsin is prewarmed for 15 minutes. Dip the slides in trypsin. Slides on day 1 are dipped for 10-12 sec, 2 seconds added for each subsequent day.

Trypsin denatures the chromosomes so that the dark and light bands can be differentiated.

The slides are washed in ice cold saline, then Leishmann stain is added. The slide is stained for 2 minutes. The stain is washed off with cold tap water. The slide is dried and viewed under a microscope. The metaphases appear spread out and clearly seen.

6. **Capturing and Analysis**:

The slide is viewed under a microscope. The Ikaros software (Multisystem Germany) is used to separate out the chromosomes. A minimum of 20 metaphases are studied.

Karyotypes are described according to ISCN 2005- International System for Human Cytogenetic Nomenclature 2005 by Shaffer LG, Tommerup N., as per the Recommendation of International Standing Committee of Human Cytogenetic Nomenclature, Published by Cytogenetic and Genome Research, by Karger S. and Basel 2005.

Christian Medical College, Vellore Department of Child Health

Growth and cytogenetic profile and vitamin D levels in children with Down syndrome

Information sheet

You are being requested to participate in a study to see if children with Down syndrome have reduced Vitamin D levels. We hope to include about 40 children from this hospital in the study.

What is Down syndrome?

Down's syndrome is caused by the presence of an extra chromosome - one of the tiny bundles of DNA found in all of our cells. Normally we have 46 chromosomes in every cell in our body, 23 from each of our parents. In Down's syndrome, an extra copy of chromosome number 21 is included. The baby will have 47 chromosomes in each body. The extra chromosome causes characteristic physical features, usually including slanting eyes, a flat face, a large tongue and broad hands with little fingers that curve inwards. Further information on this syndrome is available at http://www.downs-syndrome.org.uk/

Why is this study being conducted?

Children with Down syndrome are short. The cause for this is not clear. Other children (children without Down's syndrome) with reduced Vitamin D levels are also short. We want to see if this growth retardation in children with Down syndrome is because of reduced Vitamin D levels.

If you take part what will you have to do?

You will be asked questions regarding the child and his parents. The child will be examined in detail which will include an ear, eye and heart check up. In addition to the usual blood tests, 1-2 ml of blood will be taken for this study to check the Vitamin D levels and related body elements and enzymes. You will not receive any remuneration if you decide to participate in the study.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

Will we get the results of this blood test?

The results of the blood tests will be available and you can receive a copy of the values.

What happens if there is an abnormality in the blood tests?

If there is a deficiency in the Vitamin D levels, the child will be given appropriate treatment.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal. You will not be identified by name in any publication or presentation of results. Your medical notes will be reviewed by people associated with the study.

If you have any further questions, please ask Dr. Anila Chacko (tel: ****** or email: ******)

CONSENT TO TAKE PART IN A CLINICAL STUDY

Title: Growth and cytogenetic profile and vitamin D levels in children with Down syndrome

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____, son/daughter of _____,

Relationship with the patient _____

(Please tick boxes)

- Declare that I have read the information sheet provide to me regarding this study and have clarified any doubts that I had. [ ]
- I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]
- I understand that the study staff and institutional ethics committee will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]
- I understand that my identity will not be revealed in any information released to third parties or published [ ]
- I voluntarily agree to take part in this study [ ]

Name:
Signature:
Date:

Name of witness:
Relation to participant:
Date:

Clinical profile of patients presenting with Down syndrome

1. Name: 2. Hospital No: 3. Date of examination: 4.Case No.

5. Date of birth: 6. Age: 7. Sex: (i) boy (ii) girl

8. Birth rank (including abortions and stillbirths and deaths): (i) 1 (ii) 2 (iii) 3 (iv) >= 4

9. Previous abortions: (i) Yes (ii) No

10. Permanent family planning measures: (i)Tubal ligation (ii) vasectomy (iii) hysterectomy

11. Mid parental height

12. Maternal grandmother age at time of mother's birth: Sure of age yes /no

13. Any of the grandparents consanguineously married? (i) Yes (ii) No

14. Relatives/Siblings / parents with Down syndrome: (i) Yes (ii) No

15. Consanguineous marriage: (i) Yes (ii) No

16.a. Antenatal scan done: (i) Yes (ii) No b.Any abnormality detected: (i) Yes (ii) No
If yes, what?

17. Down syndrome detected antenatally? (i) Yes (ii) No
Antenatal tests- Maternal serum alfafetoprotein,
HCG,
Oestradiol
Amniocentesis
Chorionic villus biopsy

18. Maternal age at the time of birth: Sure of maternal age yes /no

19. Cried immediately at birth (i) yes (ii) No 20. Birth weight: (TermGA/Preterm GA)

21. Height cm: centile 22. Weight kg: centile

23. Head circumference: (i) Normal (ii) Microcephaly (iii) Macrocephaly

24. Face (i) Hypertelorism : Inner canthal: Outer canthal ratio
(ii)Flat facial profile (i) Yes (ii) No
(iii)Upslanting palpebral fissure (i) Yes (ii) No
(iv)Epicanthal folds (i) Yes (ii) No
(v)Prominent malformed ears (i) Yes (ii) No

25. Hands and feet: Simian crease (i) Yes (ii) No
Short broad hands (i) Yes (ii) No
Clinodactyly (i) Yes (ii) No
Sandle gap (i) Yes (ii) No

26. Cardiovascular findings:

27. Ophthalmology findings:

28. Hearing levels:

29. Social Quotient:

30. Hall's Criteria for principal features in newborn:

1. Hypotona
2. Poor Moro reflex
3. Hyperflexibility of joints
4. Excessive skin on back of neck
5. Flat facial profile
6. Slanted palpebral fissure
7. Anomalous auricles
8. Dysplasia of pelvis
9. Dysplasia of midphalanx of 5^{th} finger
10. Simian crease

31. Complete blood count (i) Anemia/ (ii) thrombocytopenia/ (iii) Polycythemia/ (iv) Evidence of malignancy/ (v) No evidence of malignancy

32. Serum Calcium level _____ (i) Normal (>7mg/dl) (ii) Abnormal (<7mg/dl)

33. Serum Phosphorus level _____

34.25(OH) Vitamin D level _____ Normal (25(OH) Vitamin D 9- 37.6 ng/ml

35. Thyroid function tests (i) Normal/ (ii) Abnormal

36. ECHO:

37. Xray C Spine for children older than 3 years:

37. Xray left hand and wrist for bone age/ rickets:

38. Cytogenetics? Yes/ no Cytogenetic analysis:

SES (Modified Kuppuswamy score)

(A)Education

1. Professional or honours	7
2. Graduate or post graduate	6
3. Intermediate or post high school diploma	5
4. High school certificate	4
5. Middle school certificate	3
6. Primary school or literate	2
7. Illiterate	1

(B)Occupation

1. Profession	10
2. Semiprofession	6
3. Clerical, shop owner or farmer	5
4. Skilled worker	4
5. Semi skilled worker	3
6. Unskilled worker	2
7. Unemployed	1

(C)Family income per month (Rounded off to the nearest 50)

1. >/13500	12
2. 6750-13499	10
3. 5050-6749	6
4. 3375-5049	4
5. 2025-3374	3
6. 676-2024	2
7. <- 675	1

Total Score		Socioeconomic class
26-29		Upper (I)
15-25	Middle	Upper Middle (II)
11-15		Lower Middle (III)
5-10	Lower	Upper Lower (IV)
<5		Lower