

## Karyotype Analyses of Down Syndrome Children in East Priangan Indonesia

Meirina Gartika<sup>1\*</sup>, Abigail Chang Tian Ai<sup>1</sup>, Eka Chemiawan<sup>1</sup>, Willyanti Soewondo<sup>1</sup>

1. Department of Pedodontics, Dentistry Faculty, Universitas Padjadjaran.

### Abstract

Down Syndrome (DS), also known as Trisomy 21 is one of the most common genetic disorders in which there is an extra copy of chromosome 21. DS occurs in about 1 to 650-1000 live births and it can be caused by three types of chromosomal abnormalities: Non-disjunction, Translocation and Mosaicism. The aim of this study is to determine the percentage for each type of DS among patients who came to Rumah Sakit Gigi dan Mulut Fakultas Kedokteran Gigi Universitas Padjadjaran (RSGM FKG UNPAD).

This was a descriptive cross-sectional study. The sample for this research was 75 individuals suspected of DS who have given their blood for karyotype testing, however 5 samples were later excluded as the karyotype results either showed other chromosomal abbreviation than that of trisomy 21 or were normal or the blood sample failed to be cultured.

The results of this study showed that the valid total of 70 samples, 67 samples (95.71%) were Non-disjunction DS and 3 samples (4.29%) with Mosaicism. The overall male:female ratio of DS samples was found to be 1.92:1.

In conclusion, Non-disjunction was diagnosed in the majority of the samples similar with other cytogenetic studies on DS worldwide. However, Translocation DS was not reported and the percentage of Mosaic DS was slightly higher than other researches.

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### Introduction

Down syndrome (DS) is one of the most common genetic disorders, where there is an extra copy of chromosome no. 21. DS occurs in about 1 to 650-1000 live births.<sup>1,2</sup> The live birth prevalence rate of DS has increased over the past few decades mainly due to two factors. Firstly, the rise in maternal age at birth, women aged over 35 years are at a much higher risk of giving birth to a DS baby. Secondly, is the improvement of medical and rehabilitation care in infancy. Both the survival and life expectancy of individuals suffering from DS has increased significantly, more than half of them will live up to more than 50 years.<sup>3,4</sup>

There are three basic types of DS which is, non-disjunction DS, translocation DS and mosaic DS. In varies studies conducted in other countries, it shows that the prevalence of non-disjunction DS cases in different racial or ethnic populations accounts for approximately (95%), translocation (4-2%) and (3-1%) for mosaic Down syndrome.<sup>1,5,6,7</sup> In spite of the considerable number of children with DS in Indonesia, it still lacks reliable national empirical data on the percentage of each type of Down syndrome. DS subjects are at an increased risk for certain health problems and children with DS are ten times more likely than other children to develop leukemia, suffer from congenital heart defect and have higher mortality rate from infectious diseases if the infections are left untreated. In case report by Avci<sup>8</sup> on a 1-year-old girl with DS shows that spontaneous chylothorax may be associated with DS, so pleural effusion with DS must be promptly diagnosed and aspirated. Children with DS generally experience developmental delays.<sup>9,10,11</sup>

\*Corresponding author:

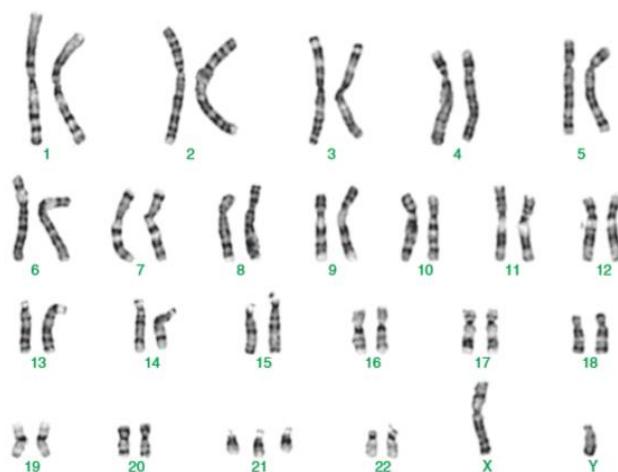
Meirina Gartika  
Department of Pedodontics, Dentistry Faculty, Universitas  
Padjadjaran  
E-mail: meirina.gartika@fkg.unpad.ac.id

The developmental delays experienced by DS children have significant implications in the field of dentistry as well. The most prominent is the abnormal dento-craniofacial growth such as dental anomalies and craniofacial size. Craniofacial size of DS children is shorter or smaller than normal children, forming a wide and short head, leading to smaller than normal dental arches and malocclusion.<sup>12</sup> Begzati's research in Kosovo shows that gingivitis in DS children is 65% of the subjects, and the prevalence of periodontal disease is 43%, while the OHI-index of grade 3 is found in 18,5% of subjects. More than half of subjects has one of malocclusions.<sup>13</sup> Immunological disorders of DS individuals will also affect their oral health, and the treatment plan for each patient has to be adjusted to suit their specific needs.<sup>14</sup>

## Materials and methods

The research type conducted was descriptive with cross-sectional study. The samples for this research came from Persatuan Orang Tua Anak Down Syndrome (POTADS) who have given their blood for karyotype testing. The samples are then segregated into the three classifications to determine the percentage of each karyotype. This segregation is determined through the genotype of somatic cells where the presence of XX genotype denotes a female and XY genotype for male, without regard to phenotypic manifestations. After a standard chromosomal analyses with Trypsin-Giemsa banding was performed on the cultured peripheral blood lymphocytes at metaphase stage the 46 chromosomes (23 pairs) are arranged. According to internationally accepted guidelines the chromosomes are arranged from largest to smallest, the position of their centromeres and oriented with the short arm (p) on top. Sex chromosomes are displayed last, this organized display is called a karyogram. While a karyotype is a description of words written from left to right without leaving any spaces, separating each item with a comma. The karyotype begins with the total number of chromosomes in a cell, followed by the notation of the sex chromosomes. An extra or missing chromosome is designated with a "+" and "-" sign respectively before the number of the chromosome.

1. Non-disjunction DS based on karyotype analysis is when there is an entire extra copy of chromosome 21 in every cell as shown in Figure 1. Making the total sum of chromosomes 47 instead of the usual 46 for each cell.



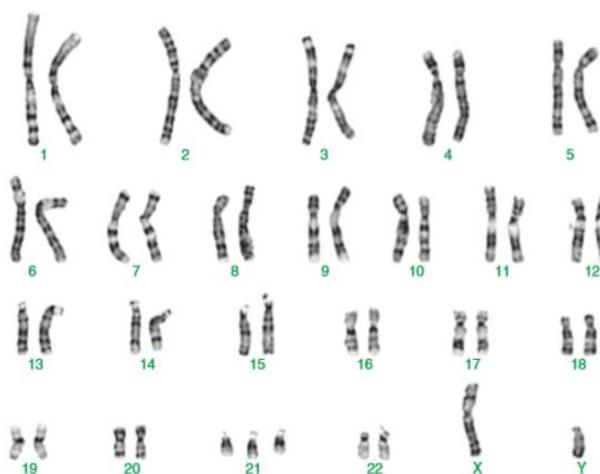
**Figure 1.** 47XY,+21 Non-disjunction Down syndrome.<sup>15</sup>

2. Translocation on the other hand, the karyotype analysis will show that a part of chromosome 21 is attached to another chromosome usually chromosome 13, 14, 15 or 22. However, the total number of chromosomes in each of the cells is still 46. Figure 2 shows a robertsonian translocation between chromosome 14 and 21.



**Figure 2.** 46,XX,rob14;21 Translocation Down syndrome.<sup>15</sup>

3. Mosaicism based on karyotype analysis shows that some of the cells have a total of 46 chromosomes and some 47. Those cells with 47 chromosomes contain an extra chromosome 21 similar to that of trisomy 21.



**Figure 3.** 47,XY,+21/ 46,XY Mosaic Down syndrome. A) shows only 2 copies of chromosome 21 while B) shows 3 copies of chromosome 21.<sup>15</sup>

## Results

A total of 75 samples of Down syndrome who visited RSGM and had given their blood for karyotyping was collected and analysed. Five were excluded from analysis, the reason being that two samples failed to be cultured to determine the type of DS while another two samples were found to have normal chromosomal count and one was determined to be tetrasomy 21 making the valid total to be 70 samples.

### Characteristics of Samples

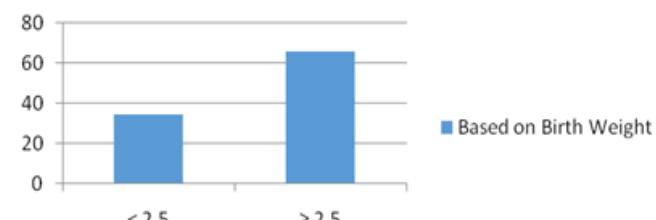
These samples can also be segregated into various demographic characteristics such as gender, birth weight, birth height, maternal age at birth of DS child and paternal age at birth of DS child as well as comorbidities associated with DS. The tables below describe each demographic characteristic separately.

Figure 4 described that 20.97% were born with a birth weight of less than 2.5 Kg and 79.03% were born with a birth weight of 2.5 Kg or more.

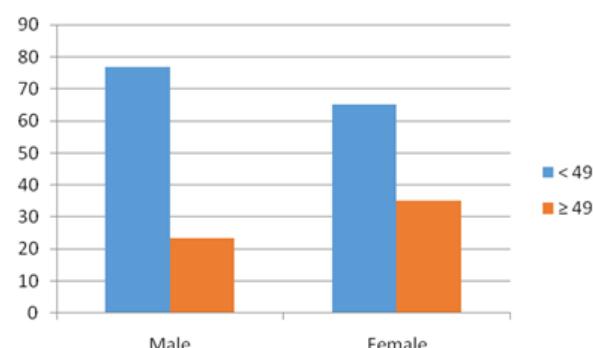
Figure 5 showed that 76.74% male subjects had a birth length of less than 49 cm and 23.26% were 49 cm or more. On the other hand, the female samples 65.00% had birth lengths less than 48 cm and 35.00% were 48 cm or more.

The distribution of the mother's age otherwise known as maternal age at the time of birth of the DS child in years is shown in Figure 6. The birth frequency of DS babies in younger mothers (<35 years) was 57.14% compared to older mothers ( $\geq 35$  years) 42.86%.

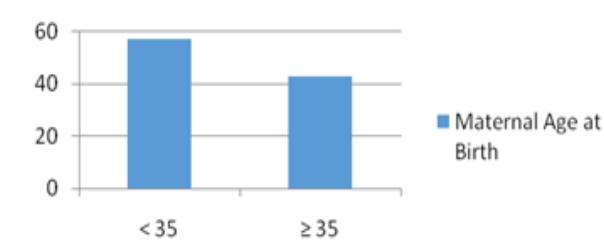
The distribution of the father's age otherwise known as paternal age at the time of birth of the DS child in years is shown in Figure 7. The birth frequency of DS babies in younger fathers (<40 years) was 68.57% compared to older fathers ( $\geq 40$  years) 31.43%.



**Figure 4.** Distribution of DS samples based on Birth Weight (Kg)



**Figure 5.** Distribution of DS samples based on Gender and Birth Length (cm).

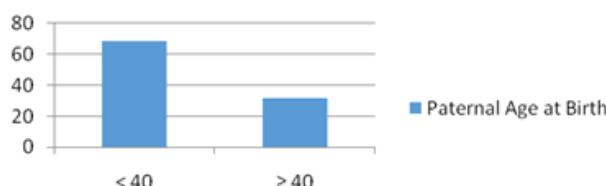


**Figure 6.** Distribution of Maternal Age at Birth of DS child.

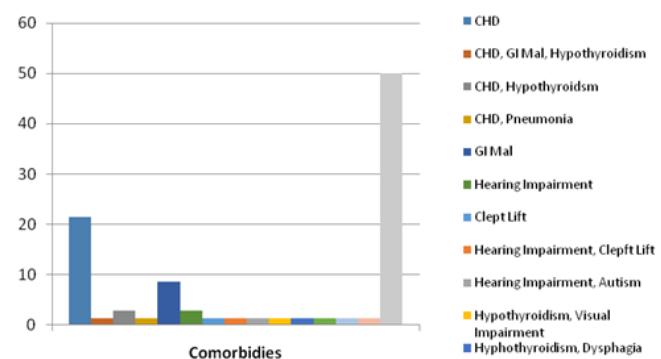
Figure 8 illustrated the comorbidities associated with DS that were reported in the samples. Fifteen samples (21.43%) were reported to have Congenital Heart Defect (CHD) making it the highest comorbidity associated with DS. One sample (1.43%) was found to suffer from CHD, Gastrointestinal malformation (GI Mal), and hypothyroidism. While two samples (2.86%) suffered from CHD and Hypothyroidism. CHD and pneumonia was diagnosed in one DS child (1.43%). GI Mal was the second highest comorbidity with six samples (8.75%) followed by hearing impairment two samples (2.86%). Only one sample (1.43%) each was reported with cleft lip, hearing impairment and cleft lip, hearing impairment and autism, Hypothyroidism and visual impairment, Hypothyroidism and dysphagia, Epilepsy, Tuberculosis as well as Asthma. On the other hand, thirty five samples (50.00%) did not report any associating disorder with DS.

Figure 9 showed the birth order of the DS child in the family. The samples of 34.33% were the first child born of the family, followed by 65.67% of those being other than the first child.

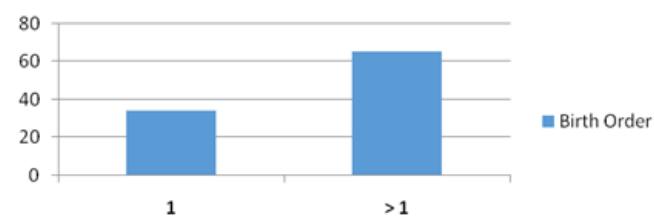
Figure 10 described the mother's obstetric history of the DS sample. The range of the number of times the mother was pregnant (Gravida) is one to seven times, the most (33.85%) were pregnant 2 times followed by 3 pregnancies (26.15%), 1 pregnancy (16.92%), 4 pregnancies (10.77%), 5 pregnancies (6.15%), 7 pregnancies (4.62%) and the least being 6 pregnancies (1.54%). Figure 10 described the mother's obstetric history of the DS sample. The range of the number of times the mother was pregnant (Gravida) is one to seven times, the most (33.85%) were pregnant 2 times followed by 3 pregnancies (26.15%), 1 pregnancy (16.92%), 4 pregnancies (10.77%), 5 pregnancies (6.15%), 7 pregnancies (4.62%) and the least being 6 pregnancies (1.54%). The number of times the mother gave birth (Parity) ranged from one to five.



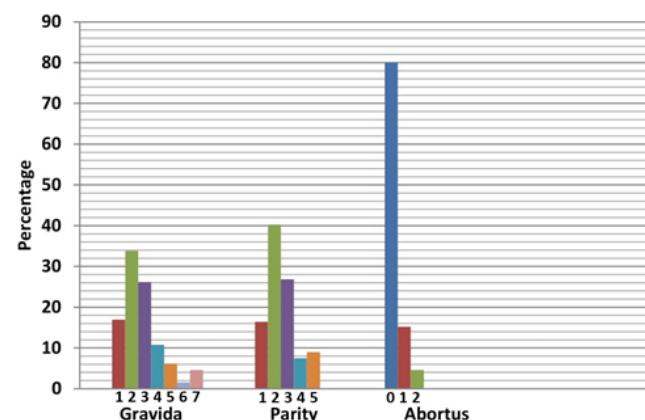
**Figure 7.** Distribution of Paternal Age at Birth of DS child.



**Figure 8.** Reported Comorbidities associated with DS samples.



**Figure 9.** Birth Order of DS sample



**Figure 10.** Mother's Obstetric History.

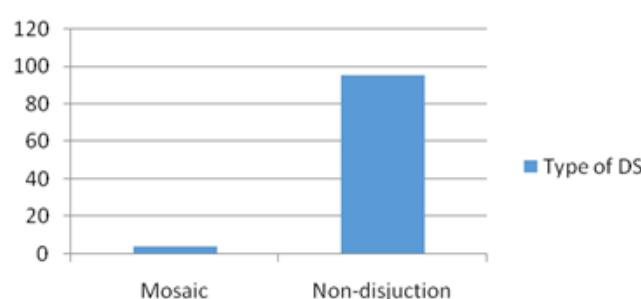
The majority gave birth twice (40.30%), proceeded by 3 births (26.87%), 1 birth (16.42%), 5 births (8.96%) and 4 births had the lowest percentage (7.46%). The distribution for amount of time the mother experienced a miscarriage (Abortus) was none (80.00%), once (15.38%) and twice (4.62%).

#### Type of Down Syndrome based on Karyotype

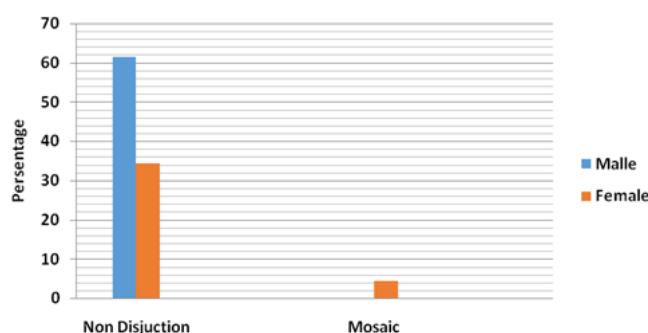
The results from this research for the percentage for each type of DS based on karyotype are showed in Figure 11. Figure 11

showed the percentage for each type of DS found among the samples. A total of sixty-seven samples (95.71%) of the sample population was found to be Non-disjunction DS while only a mere three samples (4.29%) were Mosaic DS.

On the other hand, Figure 12 described the distribution of the types of DS based on gender. For non-disjunction, there were forty-three males (61.43%) and twenty-four females (34.29%). Both genders found in non-disjunction makes up 95.71% of the total population. Mosaic DS however for this study there were only three males (4.29%) reported and no females.



**Figure 11.** Number and Percentage for types of Down syndrome.



**Figure 12.** Distribution of Types of DS based on Gender.

## Discussion

The research was conducted to document the prevalence of the types of DS among DS patients who visited RSGM based on the demographic characteristics. Low birthweight defined by the World Health Organization (WHO), as weight at birth of less than 2,500 grams. Thus, the average birth weight of the DS samples is 2.76 Kg with a standard deviation of 0.41 Kg demonstrating that on average for this study the DS child is born with a normal birth weight according to UNICEF.<sup>16</sup> However, the mean birth weight of DS

samples was lower than other researches by Myrelid<sup>17</sup> and Morris<sup>18</sup> on the birth weight (2.9-3.0 Kg) of DS infants.

In Figure 5 the distribution for Birth length was based on gender as birth length in boys are generally longer than girls.<sup>19,20</sup> For both male and female samples in this study, a majority of them were below the average birth length for Indonesian babies. Myrelid<sup>17</sup> in his research determined the average birth length for boys and girls were 48 cm. This study however showed that a majority of the DS samples were less than the mean of 48 cm.

Maternal age at birth of all studied DS children ranged from 20 to 45 years, with a mean of 31.63 years. For this study, a majority of the mothers were of a younger age than 35 years when they gave birth to the DS child. Older maternal ages (mean age of 35.39-36.27 years) were reported in a previous study by Jaouad<sup>21</sup> and Belmokhtar<sup>22</sup> However, other studies in India reported a much younger maternal age.<sup>23</sup>

It is well known that risk of birth of a child with DS increases with maternal age due to increased frequency of maternal non-disjunction. In this study, the majority of children with DS were born to younger women, who were traditionally considered to be at low risk as more number of pregnancies occurs in this reproductive age group. The factors proposed to explain this phenomenon are the customary young age at marriage, which is widely prevalent in Indonesia, reduced meiotic recombination and methylenetetrahydrofolate reductase (MTHFR) gene polymorphism.<sup>24</sup>

Paternal age at birth of all studied DS children ranged from 22 to 51 years, with a mean of 34.19 years. There is no clearly accepted definition of advanced paternal age. A frequently used criterion is any man aged 40 years or older at the time of conception hence, for this study advanced paternal age was defined as 40 years and above. Advanced paternal age has been associated with an increased risk of new gene mutations.<sup>25</sup>

Among the more commonly associated comorbidities of DS in this study are Congenital Heart Defect, Gastrointestinal Malformation, Hearing Impairment and Hypothyroidism. Congenital heart disease was diagnosed in 21.43% of DS cases. The most common cardiac defects were ventricular septal defect, patent ductus arteriosus and atrial septal defect.

Congenital cardiac defects were reported to be the most frequent congenital anomalies associated with DS from 18.3% to 59% in a previous study in Egypt and United Kingdom.<sup>5,26</sup>

Malformations of the gastrointestinal tract in this study are the second most frequent anomalies (8.57%) associated with DS similar with Kava's research in India.<sup>23</sup> Gastrointestinal atresia (duodenal, intestinal, and anal) and Hirschsprung disease are the most common malformations. For this study, hearing impairment was low (7.27%) compared with prior studies in India and China (19-70%).<sup>27,28</sup>

The association between thyroid dysfunction and DS is well recognized. In this research, it was found that a number of the DS children had hypothyroidism in association with other comorbidities. Thyroid dysfunction had been reported by El-Gilany<sup>5</sup> and Claret<sup>29</sup> ranging from 4-18%. They commented that transient elevations of thyroid stimulating hormone (TSH) were common in children with DS whether or not TSH values were initially normal or elevated. Claret, Corretger and Goday<sup>29</sup> determined that Hypothyroidism dissolves spontaneously in 39 cases (73.6%), in a mean time of  $13.2 \pm 11.1$  months.

The birth order of children with DS ranged from one to five. The samples of 34.33% were the first child of the family and the remaining 65.67% ranged from second to last child of the family. This agrees with a previous study done in Dubai(UAE) where a majority of the samples were last or second last child.<sup>30</sup>

Parity with the highest frequency was two births (40.30%) instead of five births (8.96%), which is the most live births reported in this study. This is contradictory to one study reported by Doria<sup>31</sup> on the parity and risk of DS, suggesting an increased risk with increasing parity. The range of recorded miscarriages in this research is 0-2 with zero miscarriages having the highest percentage (80.00%), (15.38%) for one miscarriage and (4.62%) two miscarriages. This result is similar with a study by Grande<sup>32</sup>, reporting that there is no significant difference in the chromosome abnormality rate between sporadic and recurrent miscarriage but the chromosome abnormality rate increased significantly with maternal age. For birth order, gravida, parity and abortus data for three samples of the 70 samples were missing as it

was either not in their medical records and were unable to be contacted through phone calls.

The data reported for distribution of types of DS in this study differed from other similar studies conducted in other countries. There was no Translocation DS report in this study, while the Non-disjunction DS (95.71%) was similar with other reported studies by Devlin, El-Gilany, Flores, and Zhao.<sup>1,5,6,7</sup> The occurrence of Mosaic DS (4.29%) was slightly higher than the standard quoted rates of 1-3%. This increase may be due to the absence of Translocation DS in this study and the inclusion of adult cases into this research as mosaic variants often do not have very prominent dysmorphic features of DS and Mosaic DS child usually goes undiagnosed until later in life. The absence of Translocation DS may be because this study only has 70 samples, or that the possibility of translocation DS occurring in this region is much lower than other studies that had been done in other countries. The gender ratio for Non-disjunction DS was 1.79:1, and only males were reported for Mosaic DS as shown in Figure 12.

## Conclusions

It can be concluded that of the 70 confirmed cases of DS, Non-disjunction was diagnosed in the majority of the samples similar with other cytogenetic studies on DS worldwide. However, Translocation DS was not reported.

## Declaration of interest

Authors report that there is no conflict of interest. This work was supported by Academic Leadership Grant Universitas Padjadjaran (Contract No. 872/UN6.3.1/LT/2017).

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