

Down Syndrome : Report of a Cytogenetic Study and a New Proposed Practical Classification

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Abstrak

Sindrom Down merupakan kelainan genetik yang paling sering dijumpai, yang disebabkan oleh kelainan jumlah kromosom atau aberasi numerik, yaitu trisomi-21. Secara sitogenetik, kelainan ini dapat menampilkan berbagai aberasi kromosom. Klasifikasi sindrom ini yang ada sekarang didasarkan pada keadaan kromosomnya, terasa kurang praktis, terutama untuk konseling, sehingga dapat dikatakan tidak berguna. Makalah ini menyampaikan hasil pemeriksaan sitogenetik pada 280 penderita sindrom Down. Berdasarkan hasil pemeriksaan itu, diusulkan klasifikasi baru yang hanya terdiri dari 4 kelompok, yang lebih praktis sehingga lebih bermanfaat, yaitu: Sindrom Down reguler atau primer, sindrom Down translokasi atau sekunder dan sindrom Down mosaik atau tersier serta sindrom Down lain atau kuarternar. Empat kelompok sindrom Down menurut klasifikasi ini, hampir sejalan dengan 4 kelas sindrom Down menurut International Classification of Diseases yang terbaru dari WHO (WHO-ICD 10).

Abstract

Down syndrome is the most frequent genetic abnormality, with numerical chromosome aberration (namely trisomy-21) as the aetiology. Cytogenetically, this disease has many types of chromosome aberrations. The existing classification based on the chromosome picture, is not very practical, especially for counseling, and hence not very beneficial. This paper reports 280 cases of Down syndrome which had been examined cytogenetically. In correlation with the results, this paper proposes a new, more beneficial and practical classification of Down syndrome. This proposed classification consists only of 4 groups : Regular or primary Down syndrome, translocation or secondary Down syndrome, mosaic or tertiary Down syndrome, and other types or quarternary Down Syndrome. The existence of 4 groups of Down syndrome in this new proposed classification fits the newest International Classification of Diseases of the WHO (WHO-ICD 10) quite well.

Keywords : Down syndrome, cytogenetic study, cytogenetic classification.

INTRODUCTION

Down syndrome (DS) is the most frequent genetic abnormality with numerical chromosome aberration (namely trisomy 21) as the aetiology.¹ The syndrome was named after Dr. Langdon Down, who in 1866 reported a syndrome of mental deficiency. The facial appearance of the patient, resembles the face of the people of the Mongol race. The likeness is independent to the race of the patient. Once you have seen a patient with DS you will always recognize other cases, even in patients of other races. Because of the abnormal mongoloid-like 'facies', he named the syndrome mongolism or mongoloid idiocy.² Because the name sounded very racial, it was then named Down

syndrome.¹ Nearly a century went by before its nature could be concealed, although Waardenburg in 1932 and Penrose in 1939 had suggested the possibility of chromosome abnormalities as the aetiology.^{1,2}

Mittwoch in 1952 studied meiosis of male patients with DS, and found 24 bodies/chromosomes.² Her conclusion of human meiotic chromosome number as 24 and made the diploid number of 48 in human, made it farther from the nature of DS. It was Lejeune in 1959 who showed the actual existence of chromosome aberration in DS which later was also confirmed by others.²

Down syndrome can occur in the male as well as in the female, it can be found all over the world and it is not restricted to any race or any season. In general

the incidence is 1 out of 700 - 800 newborns, or 1 in 900 - 1000 of the general population.^{1,3} Epidemiologically, about 95% of the cases, are influenced by the maternal age at conception. Recently, a higher percentage of dependancy to mother age was also confirmed by Antonarakis et al.⁴ Using DNA polymorphisms, they got 188 DS patients with an extra chromosome-21 from the mother, compared to only 9 DS with an extra chromosome-21 which was paternally derived. The relative risk of a mother to give birth to a baby with DS increases slowly until the age of 30 years, rather fast after the age of 30 and it becomes much faster after 35 year of age, as shown in Figure 1.

From logical thinking, family with a DS child can be thought as has a kind of burden, and will not be happy or satisfied with their life. Branholm and Degerman⁵ showed the opposite of it. Most of the families with a DS child, as their study subjects, remain to have their satisfaction with life. Both researcher interpreted those satisfaction as a result of adequate adaptive resources.

Now we know that there are many types of chromosome aberrations in DS. Cytogenetically DS or trisomy-21 was classified in several groups. The classification is not practical, especially for counselling. In correlation with the newest International Classification of Diseases of the WHO (WHO ICD 10), the existing classifications are not well matched. A more practical DS classification should be made and used.

The purpose of this study is to compare the cytogenetic condition found in the DS patients in Jakarta to DS patients in general population (literature), and to put forward a new classification of DS matching with the WHO-ICD 10, which is more practical and hence more beneficial especially for genetic counseling.

MATERIALS AND METHODS

Patients clinically diagnosed as suspected Down syndrome were subjected to cytogenetic examination at our laboratory of the Section of Medical Genetics,

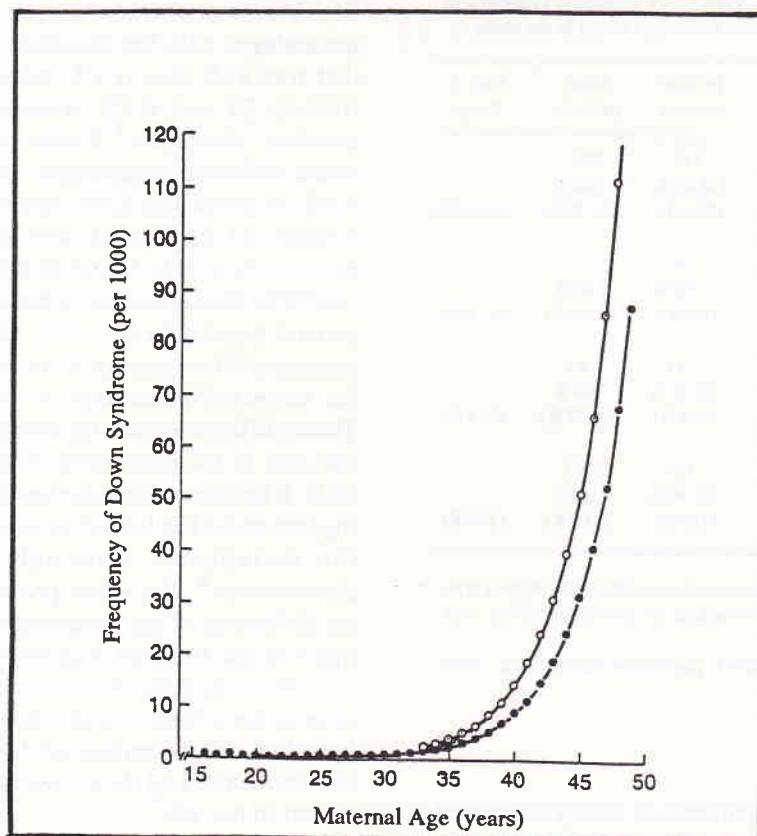


Figure 1. The relative incidence of (risk to have) Down syndrome according to maternal age (from Epstein),⁷ circle = at the time of amniocentesis, solid dot = at birth

Department of Medical Biology Faculty of Medicine, University of Indonesia. There were 283 patients suspected of Down syndrome, examined cytogenetically over a period of 6 years (1989 - 1994).

The cytogenetic examination performed was chromosome analysis. The method used was the modified method of Moorhead. The cell preparation was stained and then examined morphologically.

RESULTS

Three from the 283 patients showed normal 46,XY or 46,XX karyotype. The remaining 280 patients, consisting of 125 females and 155 males, were cytogenetically confirmed as trisomy 21. As predicted, most of them were 47,XY or 47,XX with an additional free chromosome-21 (229 patients or 81.79%). Four patients (1.43%) had Robertsonian translocation to G chromosome (3 patients) and to D chromosome (one patient). The rest (47 patients or 16.78%) had mosaicism or mixoploid (46,XX/47,XX,+21 or 46,XY/47,XY,+21) (Table 1).

Table 1. Number and percentage* of the Down syndrome according to sex and type of chromosome aberration found in the study

Type of chromosome aberration	Male patients	Female patients	Total patients	Exp. † %-age
47,XX or XY, +21	117 51.09% (75.48%)	112 48.91% (89.6%)	229 100% (81.79%)	(93-96%)
46,XX or 46,XY, +21 +t(D:21) or t(G:21)	2 50% (1.29%)	2 50% (1.6%)	4 100% (1.43%)	(2-5%)
46,XX/47,XY,+21 or 46,XY/47,XY,+21	36 76.59% (23.23%)	11 23.41% (8.8%)	47 100% (16.78%)	(2-4%)
Total	155 55.36% (100%)	125 44.64% (100%)	280 100% (100%)	(100%)

* percentages without brackets are percentage of the total in each type of aberration and percentages in brackets are percentage of the total female/male group.

† expected percentage in a general population (corrected), from Epstein.⁷

DISCUSSION

Only 3 (1.06%) out of 283 patients clinically diagnosed as suspected DS were not confirmed cytogenetically as DS. Our clinical accuracy is high compared to Cortes et al⁶ from Chili with 11% (22 out of 201 patients) misdiagnosed as DS.

As known very well, DS can occur in the male as well as in the female. This means that the incidence of this abnormality is the same in male and in female. The number of DS in this study is very different in male and female patients (155 and 125 respectively), but the ratio of male to female patients with DS found in our report (1.24 : 1.0) is not too different from Chili⁶ with ratio of (1.18 : 1.0). In our study rough calculation of the X^2 for the difference between male and female is > 3 . This can be interpreted as significantly different. This can be the result of an actual difference of DS incidence in the general population, or a lower compatibility to survive in the female compared to male DS patients. Another possible explanation for the difference in this study might be the different attitude of our society toward male and female DS patients. Our society cares and hopes for betterment in the male more than they do for the female DS patients. Further study should be done to ensure which one of those possibilities is the right one.

Table 1 shows that the percentage of each group of DS is rather different from the expected in general population. Epstein in his review had 93-96% regular or free, 2-5% translocation and 2-4% mosaic trisomy 21.⁷ Verma et al from New Delhi have rather similar percentages with the expected in general population, that was 93% free, 4.1% translocation, 2.6% mosaic trisomy 21 and 0.3% trisomy 21 with other concomitant aberration.⁸ Cortes et al from Chili found rather different percentages : 89,9% classic or free 3.9% translocation, 2.8% mosaic trisomy 21 and 3.4% trisomy 21 associated with other aberration.⁶ The mosaic trisomy 21 found in this study is much higher (16.78%) than expected in corrected percentage of the general population (2 - 4%). Correlationally, the percentage of free trisomy 21 is lower in this study, than the corrected percentage in the general population. Those differences can be interpreted as laboratory errors due to the processing of the slide preparation of cells. It has been shown before that laboratory processing can cause the lost of an individual chromosome of the metaphase, although more of the small chromosome⁹. The other possibility that can explain the difference of the percentage found in this study is that it is due to racial or genetic reason.

From the theoretical point of view, mosaicism can arise in the mitotic cell division, post-zygotic non-disjunction or chromosome lost. In vitro study of chromosome lost from normal subjects has been shown in our lab.⁹

Mosaicism of DS can begin from a normal 46,XX or 46,XY zygote. During the early cleavage a cell divides and yields to daughter cells with trisomy 21 and monosomy 21, followed by death of the

monosomy 21 cells. The blastocyst then will consist of normal 46,XX or XY cells and trisomy 21 cells. Another possibility is that a mosaic DS can arise from a trisomy 21 zygote. Lost of one of the free chromosome 21 during mitotic division in the early period of embryo yield to daughter cells, one with trisomy and the other one with normal chromosomes (Figure 2). Which one of those 2 possibilities results in mosaic DS, should be investigated. The result of this study, a higher number of mosaicism in the male, needs explanation. An explanation for this maybe that non-disjunction (for zygote with 46 chromosome) or lost of chromosome during mitotic division (for 47,+21 zygote) happens more easily in zygote with Y chromosome.

Cytogenetic classification of Down syndrome

Hamerton² has proposed a cytogenetic classification of DS : primary, secondary, mixoploid or mosaic trisomy 21, double trisomy (21 with other type of trisomies), primary G (21) tetrasomy and normal phenotype subjects with translocation of chromosome

21 to either D [t(D;21)] or G chromosome [t(21;G)] (Table 2). Primary G tetrasomy and double trisomy are very rare. The inclusion of normal phenotype subjects with t(D;21) and t(21;G) in the DS classification provoked many questions.

De Grouchy and Turleau¹⁰ also classified (although not explicitly) the DS cytogenetically, the groups being : free, translocation, mosaic, partial and association trisomy 21 (Table 2). All kinds of double trisomies belonged to the last group. Partial and association trisomy 21 are also very rare. So a more simple and practical cytogenetic classification of DS should be proposed and used.

A new proposed cytogenetic classification of Down Syndrome

As seen in table 1, most of the subjects with DS belong to free additional chromosome 21, so primary or regular DS still can be used efficiently. According to Epstein, about 95% of DS patients belong to this class.⁷

Primary or regular DS means that the chromosome condition is 47,XX,+21 or 47,XY,+21. All

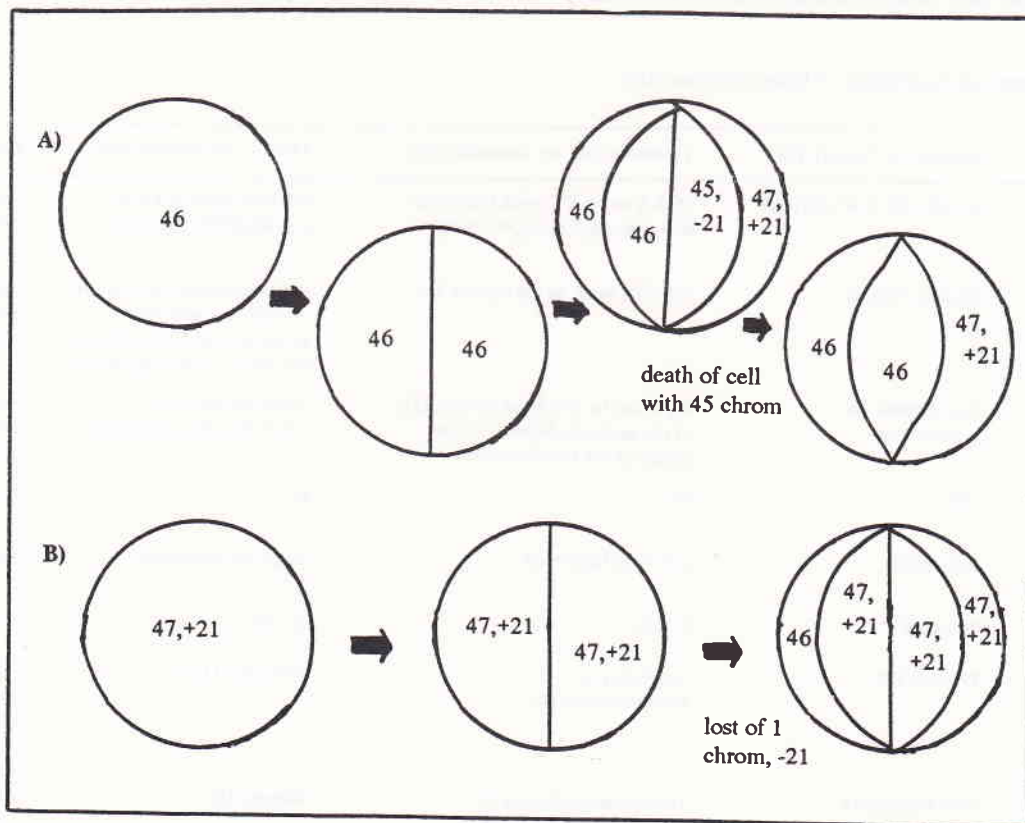


Figure 2. The tertiary or mosaic Down syndrome, began from :
 A) a normal 46,XX or 46,XY zygote and
 B) an already trisomy 21 zygote, (XX or XY)

patients with regular or primary DS have the similarity that their parents were normal cytogenetically. Still further, the recurrence risk of this abnormality is almost stable, doesn't increase drastically. The risk does increase but still correlate with the increasing age of the mother, which conform to general population. The clinical picture of these patients are typical, classical or standards.

Secondary or translocation DS also still can be used, although a little bit rare. Only 2 - 5% of the patients with DS can be categorized in this class.⁷ Although the nature of the translocations is different, there is still a similarity in that one of the parent has the same chromosomal translocation as the DS patient has, but the parent has no additional chromosome (balanced translocation). The recurrence risk of this type of DS is increased drastically. The risk could be very high up to 100% in 21-21 translocation in the mother. Patients belonging to this category have the same clinical picture as the regular or primary DS.

Tertiary or mosaic DS can be proposed and used for DS patients with mosaicism or mixoploid condition of their chromosome number. Although this group of DS is also rather rare (almost the same as the secondary

DS),⁷ they still can be grouped into one. They have these similarities : their parents are cytogenetically normal and they still have cells with normal, disomic chromosome number (46). The recurrence risk of this condition is very low, may be much lower than the primary DS. Of all the patients with DS, mosaic DS has a broad range of clinical picture. They can appear clinically normal, but they can also appear as primary DS. The proportion and bodily distribution of the trisomic cells play an important role in this clinical picture.

Quarternary DS is a new category for any kind of cytogenetic condition other than those already categorized above. This kind of DS is clearly below 1% in frequency and might be under 5%. As shown in this study, from 280 patients with confirmed DS no patient can be categorized in this group. The chromosome aberration of a patient in this group should be stated clearly. For instance, a double trisomy of Klinefelter syndrome and DS, should be followed by its cytogenetic nature i.e. 48,XXY,+21.

All those characteristics of the DS for each group of the new proposed classification, which already illustrated, are briefly summarized in table 2.

Table 2. A new proposed classification of Down syndrome (DS)

DS classific.	Regular or Primary DS	Translocation or Secondary DS	Mosaic or Tertiary DS	Quarternary DS
Chromosome	47,XX,+21 or 47,XY,+21	46,XX or 46,XY, with translocation, e.g. 46,XY,-13,+t(13;21)	has 2 or more cell line e.g. 46,XX/47,XX,+21	other type, does not belong to other groups
Clinical picture	specific, typical	specific, same as the regular DS	variable appearance, can be normal as a non Down syndrome and can also be the same as the regular one	variable, same as the mosaic DS
Recurrence risk	low, depend on mother's age	high, can be 100% [as in ((21;21) of the mother], depend on the nature of the translocation	might be very low, lower than the regular DS	very low, lower than the tertiary DS
Dependency on mother's age	yes	no	no	no
Chromosome analysis in parent	no benefit	yes; very important	might be beneficial	might be beneficial
Frequency *	92 - 96%	2 - 5%	2 - 4%	< 1%
Hamerton's Classific. †	Primary DS	Secondary or Translocations DS	Mixoploid DS	- Primary G tetrasomy - Double trisomy
de Grouchy-Turleau's Classific. #	Free trisomy 21	Translocation trisomy 21	Mosaic DS	- Partial trisomy - Association trisomy

* from Epstein⁷

† from Hamerton²

from de Grouchy and Turleau¹⁰

This new proposed classification of the Down syndrome fit the newest International Classification of Diseases of the WHO (WHO-ICD 10), nearly 100%.¹¹ Down syndrome in this ICD are grouped in 4 classes that are Q90.0 for trisomy 21, meiotic non-disjunction, Q90.1 for trisomy 21, mosaic or mitotic non-disjunction, Q90.2 trisomy 21, translocation and Q90.9 for unspecified Down syndrome. The first and the fourth class in the ICD fit into the primary and the quaternary DS of the new classification. While the second and the third class in the ICD fit into the tertiary and the secondary DS of this new classification. The secondary and tertiary DS in this classification are the same as proposed by Hamerton² and also by de Grouchy and Turleau.¹⁰

There were no explanation why the WHO made their classification in such a way. Lack of a medical geneticist in the ICD-10 team especially in the sub-team for congenital malformation, and they didn't make any consultation to a medical geneticist, might be the explanation. As already discussed, the primary and secondary DS in this new classification, have very similar clinical picture, while the tertiary DS has a variable spectrum of clinical picture.

CONCLUSION

Two hundred and eighty patients from 283 (98.94%) clinically suspected as Down syndrome (DS) were proven cytogenetically as having trisomy 21. Only 1.06% was misdiagnosed as Down syndrome. The primary or regular DS was found in 229 patients or 81.79%, which was lower than expected (93 - 96%). The secondary or translocation DS was found in 4 patients (1.43%), and was slightly below the expected (2-5%). The tertiary or mosaic DS was higher (47 patients or 16.78%) than expected (2 - 4%). This result

need to be studied further, and combined to reports from other centres to get a higher number of patients with DS.

This new proposed classification has the same 4 classes of DS as the WHO-ICD 10 and fits it quite well. Furthermore, it is more practical and beneficial for counseling.

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