

Mitotic index in Down syndrome – is it an indicator of immunological status

Priya Ranganath, Geetha Suresh, Amudha Subramaniam, Sayee Rajangam
Division of Human Genetics, Department of Anatomy, St. John's Medical College, Bangalore, India.

Down syndrome (DS) is a condition in which the genes on chromosome 21 occur in three copies. This is supposed to influence the growth in the tissues of the body and this could lead to a decreased mitotic index. In view of this, the present investigation was carried out using peripheral lymphocyte culture to find out whether there is a change in mitotic index in DS patients. Mitotic index in male and female patients was reduced to an average of 3.64 and 3.82 respectively. Thus, the index could be an indicator of the reduced immunological status of the individual with DS.

Key Words: Mitotic index, immunology in Down syndrome.

Introduction

Down syndrome (DS), a well known autosomal entity is because of trisomy 21 condition. The genes on chromosome 21, which occurs in three copies is supposed to influence the various tissues and systems in the body, resulting in the characteristic phenotype of DS.

Immune system disturbances are commonly observed in DS individuals including an increased frequency of autoimmune disorders, altered proportions of peripheral blood lymphoid subsets, and an increased susceptibility to infection.¹ In connection to this finding, few scientists have reported that in aged DS patients there could be decreased mitotic index.² These scientists have demonstrated low PHA response in older DS cases compared to normal controls. They have opined that this aspect may or may not correlate with reduced interleukin-

2 (IL-2) production and consequently reduced proliferative response to antigens and mitogens.

In view of the above, the present investigation was carried out, using peripheral lymphocyte culture, to find out whether mitotic index in DS has been affected or not.

Materials and Methods

The Division of Human Genetics is a research center for karyotyping patients suspected with chromosomal disorders. Here peripheral blood is routinely put up for T-lymphocyte culture. At a fixed hour of incubation division is arrested using colchicine. Then the cells are harvested and dropped to slides and stained. In this lab the chromosomes are stained for GTG bands and then analyzed.

Previously stained slides were selected; 25 belonged to DS patients and 25 belonged to controls, all below 3 years of age. One empty slide was taken, and leaving the extreme right end, which was used to number it, the slide was divided into 3 portions. The central point of each of these 3 portions was selected, co-ordinates recorded, so that in the same position, slides of DS and controls could be analyzed.

A minimum of 1000 cells were checked. Care was taken so that the selected cells are representatives of the culture. Without compromising on accuracy a less strenuous method was adopted. The number of cells

undergoing division in a particular field was counted under low power (10 x). If the number of cells undergoing division was 1 or 2, it was considered as low index. If the number was 5 or 6, it was considered as medium index; and if it was between 10 and 15, it was considered as high.

Results

Mitotic index is usually represented as the percentage of cells undergoing division to the total number of cells. Table 1 shows mitotic index in Down syndrome. In the male DS, it was found that in portions I and III, the mitotic index was decreased, but not in the portion II, and on an average, it was marginally decreased. In the female DS, it was observed that in portions I and II, the mitotic index was decreased but not in portion III, and on an average it was decreased. On the whole, in portions I and III, the mitotic index was reduced, but not in portion II. The reduction in female seemed to be more prominent.

Discussion

Immune system disturbances are commonly observed in DS individuals. Various publications are available in this context by different authors.

Immune system: Although infections are common in DS individuals, most of them do not have immunological disease,^{3,4} and detailed investigation of antibody-mediated immunity and cell-mediated immunity in DS has not identified critical immunodeficiencies. Abnormalities that are documented include many lesser immune system defects, often inconstantly present and of uncertain clinical significance, such as leukopenia and macrocytosis,⁵ which may date from prenatal life to old age.^{2,6} These immunological alterations are often age-related changes and may be one feature of a general,

early onset senescence in DS.⁴ Complex and sometimes conflicting descriptions of in-vivo or in-vitro immunological impairments usually make interpretation of clinical relevance difficult.

Cell-mediated immunity (CMI): Many abnormalities are observed, but clinical implications are difficult to discern. The thymus in DS is smaller than in chromosomally normal control individuals. Thymus histologic abnormalities include reduced cortical area, thymocyte depletion, loss of corticomedullary demarcation, enlarged cystic Hassal's corpuscles, and evidence of defective expansion of T-cell precursors.⁷ Whereas the total number of circulating T lymphocytes is normal or slightly reduced, the distribution of different T-cell subsets may differ from the distribution in controls. Some changes may be age-related observations such as the appearance of functionally defective natural killer (NK) cells or depletion of T lymphocytes, especially in males.⁸ Some but not all reports have demonstrated low phytohemagglutinin responsiveness in older DS individuals when compared with chromosomally normal controls, which may or may not correlate with reduced interleukin-2 (IL-2) production and, consequently, reduced proliferative response to antigens and mitogens.² Studies have suggested evidence of diminished cell surface expression of the alpha, beta-chains of the T-cell receptor in DS thymocytes, the T-cell receptor being necessary for antigen specific recognition as well as for low levels of expression of the CD3 antigen.^{9,10} Thus, Ugazio et al (1990) have suggested that reduced expansion of T-cell precursors may play an important role in DS immunodeficiency leading to an incomplete cell repertoire and abnormality of CMI. The defective proliferative response of T lymphocytes has been further investigated and was concluded that T cell activation deficiency is characterized by partial signal transduction through TCR/CD3 complex, in association with a selective failure of ZAP-70 tyrosine phosphorylation.¹¹

Humoral immunodeficiencies: They are generally agreed to be less striking than cell-mediated deficiencies in DS, but interpretation is difficult because of the variety of reported changes. There are no major abnormalities of the B-lymphocytes, and serum immunoglobulin levels are not grossly deranged, although there is a tendency

Table 1: Mitotic index in down syndrome

Mitotic index	I portion	II portion	III portion	Average
Male DS	2.8 ± 2.94	4.36 ± 4.33	3.76 ± 4.66	3.64 ± 3.03
Male control	4.08 ± 4.36	3.52 ± 2.87	4 ± 3.89	3.87 ± 2.61
Female DS	2.18 ± 2.27	4.82 ± 3.68	4.45 ± 6.17	3.82 ± 3.09
Female control	4.5 ± 3.44	5 ± 3.23	4.3 ± 3.65	4.6 ± 2.39
DS	3.29 ± 3.38	4 ± 4.88	3.21 ± 3.17	3.5 ± 3.1
Control	3.8 ± 4.97	2.53 ± 2.2	3.8 ± 4.16	3.38 ± 2.72

for high IgG and low IgM levels after early childhood. The antibody response to various viral protein antigens is inconsistent in DS subjects, and some studies have found reduced response, for example, to influenza virus, but other studies did not find such differences. However, there is no doubt that the prevalence of hepatitis B surface antigen (HBsAg) is higher in DS individuals, whether they are in institutional care or living with their family at home. DS individuals do respond to hepatitis B vaccination, albeit to a lesser degree,¹² so vaccination is indicated to avoid the high risk of becoming an HBsAg carrier. Hepatitis B carriers with DS were, in one study, at increased risk of developing autoimmune thyroid disease compared with hepatitis B carriers without DS.¹³

Nonspecific immunity: While the number of polymorphonuclear phagocytes is normal, decreased neutrophil chemotaxis, deficient leukocyte opsonization and phagocytosis, decreased leukocyte bactericidal activity, and low leukocyte chemiluminescence activity after phagocytic activation have all been reported in DS individuals.¹⁴ The gene encoding copper-zinc superoxide dismutase (SOD-I) is located on chromosome 21 and enzymatic activity shows a gene dosage effect leading to 150% activity in DS individuals. It is speculated that increased SOD-I leads to low levels of superoxide anions and reduced destruction of organisms which require superoxide radicals for efficient killing. Low serum zinc levels have also been proposed as a possible reason for impaired chemotaxis observed in DS individuals, since that mineral has a role in promoting chemotaxis as well as being a cofactor in T-cell responsiveness.² However, DS individuals do not usually develop clinical signs associated with zinc deficiency and, from published trials,¹⁵ almost all of which are open without controls, the case for convincing clinical benefit from zinc therapy is not proven.

In this study it has been seen that when the total number of DS patients is considered, the mitotic index is reduced, however, the average did not show any decrease. The decrease in female DS patients seemed to be more prominent. The decreased mitotic index could be an indicator of the reduced immunological status of the DS individual. This could be the reason of slow growth and repair of tissues and possibly increased rate of susceptibility to infections. In spite of the sample size,

this pilot study indicates that the interleukin production may have influenced the response of lymphocytes to PHA.

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